A NEW ANTIBIOTIC, MEDERMYCIN

Sir:

Medermycin, an antibiotic active against gram-positive bacteria including staphylococci resistant to several antibiotics was obtained from cultured broths of a *Streptomyces* K73 recently isolated from a soil sample collected in Tokyo.

Streptomyces K73 is a chromogenic organism similar to *Streptomyces antibioticus*, *S. bikiniensis* and *S. tanashiensis*. Intensive taxonomical studies indicated a particular resemblance with *S. tanashiensis* IFO 12919, a strain that produces the antibiotic luteomycin[1]. Although there are some different cultural features, we have designated K73 as a strain of *S. tanashiensis*[2].

For the production of medermycin, K73 was cultured at 28°C with aeration and agitation in the following medium: starch 3.0%, peptone 0.5%, meat extract 0.5%, dry yeast 0.3% and NaCl 1.0%. The potency of medermycin, assayed by the use of a sensitive strain, *Staphylococcus aureus* FDA 209P, reached a maximum value at approximately 38 hours. The cultured broth was filtered at pH 2.0 by the aid of Celite 545 and the dark brown broth filtrate adjusted to pH 4.0 was passed through a column of non-ionic resin, Amberlite XAD II. Medermycin was adsorbed to the resin and eluted with 70% aqueous acetone. The active eluate fraction was evaporated *in vacuo* and the aqueous residue was extracted at pH 2.0 with a half volume of chloroform to remove a part of dark pigment. The aqueous fraction was adjusted to pH 7.5 and extracted repeatedly with a half volume of ethyl acetate. Medermycin in the ethyl acetate extract was then transferred into an aqueous solution by extraction with a small volume of dil.HCl and this solution was lyophilized after adjusting the pH 5.5. To prepare pure medermycin, the lyophilized preparation was chromatographed on phosphonomethyl cellulose (PPM cellulose). A small amount of crude preparation was applied to a column of PPM cellulose buffered at pH 4.0 with 0.02 M acetate buffer, and the column was developed with the same buffer.

When the buffer was changed to pH 5.0, a minor component was eluted. Medermycin, the major component was eluted by 0.02 M phosphate buffer pH 6.0. Medermycin was collected and the above ethyl acetate extraction procedure was repeated through the liophilization step to produce an orange-colored product.

Medermycin was recrystallized two or more times from *n*-butanol. Chemical, physicochemical and biological characterization of medermycin were performed on this preparation.

Medermycin is a mono-basic substance of pKa 9.5. Its stable orange crystals were isolated as a monohydrochloride of m.p. 180°C (decomp.) and of $[\alpha]_D^{22}+170°$ (*c* 1, methanol).

The mono-hydrochloride is soluble in water and lower alcohols, slightly soluble in benzene, chloroform, ethylacetate and ethyl ether, but insoluble in hexane, cyclohexane and petroleum ether. It gives a color reaction characteristic of quinone groups Acidic to neutral pH range aqueous solutions of this antibiotic are orange-colored, but alkaline solutions are dark violet. Medermycin can be regarded as an indicator type antibiotic. Although medermycin base was too unstable for mass spectrographic analysis, the molecular weight and molecular formula was derived from studies with the mono- and diacetyl derivatives.

Mono-acetyl medermycin signaled at 2.12 ppm in NMR spectrum. It has a parent peak at *m/e* 501 in the mass spectrum. A molecular formula of $C_{26}H_{31}NO_9$ is proposed for this mono-acetyl derivative.

Analysis: Calcd. for $C_{26}H_{31}NO_9 \cdot 1.5H_2O \cdot HCl$:
C 55.27, H 6.24, N 2.48.
Found: C 55.53, H 5.95, N 2.32.

Diacetyl medermycin signaled at 2.16 and 2.52 ppm in NMR spectrum and it had the parent peak at *m/e* 543 in the mass spectrum. A molecular formula $C_{28}H_{33}NO_{10}$ is proposed for the diacetyl derivative.

Analysis: Calcd. for $C_{28}H_{33}NO_{10} \cdot HCl$:
C 57.98, H 5.91, N 2.42.
Found: C 58.74, H 6.20, N 2.53.

These data suggest that the molecular formula for unacetylated medermycin base is $C_{24}H_{29}NO_8$.

Analysis: Calcd. for $C_{24}H_{29}NO_8 \cdot HCl$:
C 58.12, H 6.10, N 2.82.
Found: C 57.73, H 6.39, N 2.35.

Fig. 1. UV absorption spectrum of medermycin in ethanol

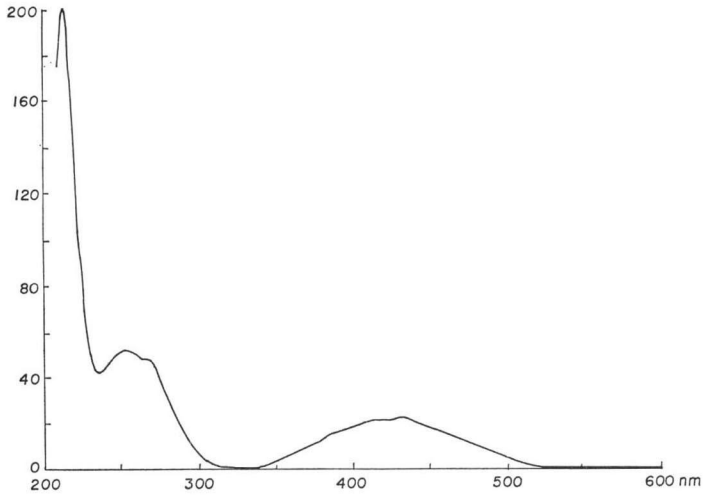
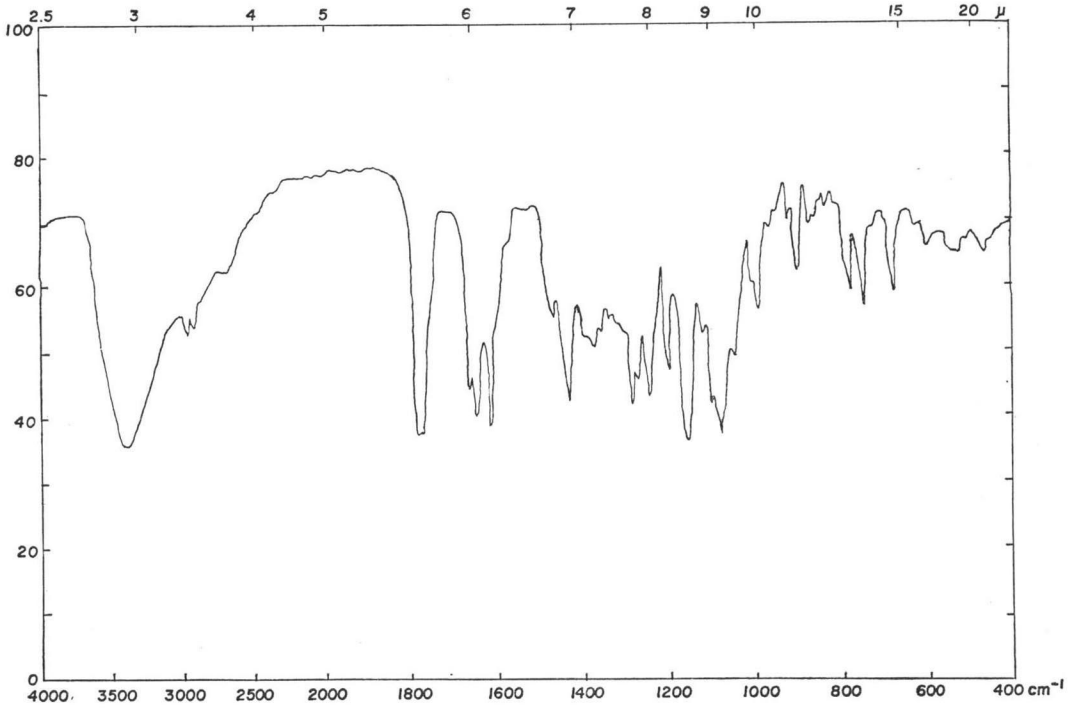


Fig. 2. IR absorption spectrum of medermycin in KBr



The UV absorption peaks of medermycin in ethanol are at 215 nm ($\log \varepsilon$ 4.71), 253 nm ($\log \varepsilon$ 4.12) and 269 nm ($\log \varepsilon$ 4.07) (Fig. 1).

The IR spectrum of medermycin is illustrated in Fig. 2. The existence of a quinone group is indicated in the absorption peaks at 1620, 1645 and 1660 cm^{-1}.

Thin-layer chromatography of medermycin was carried out on plates of silica gel and microcrystalline cellulose. The silica gel plates were developed with chloroform and ethanol, 1:1, and medermycin had an Rf value of 0.25. Cellulose plates were developed with *n*-butanol, acetic acid and water, 4:1:5, and an Rf value of 0.55 was observed.

As summarized in Table 1, medermycin

Table 1. Antimicrobial spectrum of medermycin

Test organisms	MIC (mcg/ml)
Staphylococcus aureus FDA 209P	0.2
Staphylococcus aureus FS 3271 (Tc, Cp, Sm-R)	0.8
Staphylococcus aureus FS 3839 (Tc, Sm, Sp-R)	0.4
Staphylococcus aureus FS 3877 (Tc, Cp, Km, Sp, Em, Im, Om, Lcm-R)	0.4
Staphylococcus aureus FS 3891 (Tc, Km, Sp, Em, Om, Lcm-R)	0.4
Staphylococcus aureus FS 3904 (Tc, Km, Sp, Em, Om, Lcm, Lm, Cp-R)	0.2
Staphylococcus epidermidis IFO 3762	0.4
Sarcina lutea PCI 1001	0.4
Bacillus subtilis PCI 219	0.4
Bacillus cereus IFO 3001	0.4
Escherichia coli NIHJ	100.0
Escherichia coli K12	50.0
Aerobacter aerogenes IAM 1063	100.0
Serratia marcescens IAM 1022	100.0
Proteus vulgaris IFO 3045	50.0
Proteus morganii IFO 3848	50.0
Proteus mirabilis IFO 3849	100.0
Proteus rettgeri	100.0
Pseudomonas aeruginosa IFO 3901	100.0
Shigella flexneri 2a	12.0
Salmonella infantis	50.0
Salmonella enteritidis	100.0
Xanthomonas oryzae IAM 1657	6.0

MIC values were obtained by the agar dilution method with nutrient agar containing 0.5 % glucose.

exhibits a high order of activity against such gram-positive organisms as *Staphylococcus aureus* FDA 209P and *Staphylococcus epidermidis*, five resistant staphylococci, *Staphylococcus aureus* FS 3271, FS 3839, FS 3877, FS 3891 and FS 3904, *Sarcina lutea*, *Bacillus subtilis* and *Bacillus cereus* (MIC ranges 0.2 to 0.8 mcg/ml). The gram-negative bacteria *Xanthomonas oryzae* and *Shigella flexneri* were inhibited at concentrations of 6 and 12 mcg/ml respectively, but the other eleven gram-negative bacteria required 50 mcg/ml or more.

Antitumor activity of medermycin was evaluated by the effect against YOSHIDA sarcoma cells intraperitoneally transplanted into rats 72 hours before administration of the antibiotic. No prolongation in survival times was observed for treated rats as compared with those of controls, at medermycin dosages of 0.5~1.5 mg/kg/day for 5 days. Another test for antitumor activity was carried out by the method of SATO.[8] One dose of medermycin was injected intraperitoneally and subsequently sarcoma cells were removed every day puncture, stained and observed. By this test procedure, a weak antitumor activity was recognized only at 24 hours in rats dosed at 1.0 and 5.0 mg/kg.

Acute LD_{50} toxicities of medermycin in mice by intravenous, intraperitoneal and oral administrations were 8.8, 7.8 and 620 mg/kg respectively.

Medermycin is in many respects somewhat similar to quinoid antibiotics kinamycins A and C[4,5], but it is most closely related to luteomycin[6,7] and antitumor substance No. 289[3,9]. Luteomycin and substance No. 289 have been regarded as chemical analogs, but this has not been confirmed by determination of their chemical structures.

Comparative studies were carried out with a preparation of antitumor substance No. 289

donated by Dr. H. UMEZAWA. Our tests indicated that medermycin resembled substance No. 289 in chemical characteristics other than the differences depicted in their IR spectra. Medermycin is, however, apparently differentiated in view of its very weak antitumor activity. In view of the slight differences in the molecular formulae for medermycin and antitumor substance No. 289, it is probable that medermycin and luteomycin are both chemical analogs of substance No. 289.

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