A NEW ANTIBIOTIC, 2-HYDROXY-5-IMINOAZACYCLOPENT-3-ENE

Sir:

A new pyrroline antibiotic has been isolated from the culture broth of strain No. MG11-A1 which was classified as *Streptoverticillium parvisporogenes*[1]. The antibiotic was assayed by the cylinder plate method using *Escherichia coli* K-12 ML1629 as a test organism. In this communication, we report the isolation, properties, structural elucidation and the mode of action of the antibiotic.

The strain No. MG11-A1 was shake-cultured at 27°C for 2 days in a medium containing 2% glucose, 2% soybean meal and 0.1% $CaCO_3$. This was inoculated to the same medium at 4.0% and the antibiotic was harvested after 2 days culture at 27°C. The culture broth was adjusted to pH 3 with oxalic acid, kept for 16~17 hours at 4°C, and filtered. The antibiotic in the filtrate (pH 7.0, 1,400 ml) was adsorbed on a column of Amberlite IRC-50 (70% Na^+ form) and eluted with 1 N HCl. Eluted fractions were immediately adjusted to pH 2~3 with 1 N HCl. The active eluates were concentrated to dryness and extracted with methanol. Evaporation of the methanol extract yielded a crude powder (2,305 mg). An aqueous solution of the crude powder was adjusted to pH 7.0 and passed through a carbon column. Eluted fractions were immediately adjusted to pH 2~3. The active eluates were evaporated to dryness yielding a partially purified powder (230 mg). Purification on cellulose (Avicel) columns developed with 1-propanol - 0.001 N HCl (9: 1 in volume) and evaporation of active fractions resulted in colorless crystals of the hydrochloride (74 mg). Recrystallization from 1-propanol yielded colorless needles of the hydrochloride (33 mg).

The hydrochloride melted at 108°C with decomposition. $[\alpha]_D^{24}$ 0° (*c* 0.8, water). Anal. Calcd. for $C_4H_6N_2O \cdot HCl$: C 35.69, H 5.20, N 20.82, O 11.90, Cl 26.39. Found: C 35.13, H 5.21, N 20.39, O 12.79, Cl 26.86. UV: $\lambda_{max}^{H_2O}$ nm (ε) 205 (1.13×10^4), 240 (1.58×10^3); PMR (D_2O, TMS as the external reference) (ppm): δ 6.56 (d, J=1.5, H-2), δ 7.08 (d, J=6, H-4), δ 7.76 (dd, J=6, 1.5, H-3); CMR (D_2O, TMS)(ppm): δ 86.5 (C-2), 123.5 (C-4), 152.1 (C-3), 166.4 (C-5). The IR spectrum of the antibiotic is presented in Fig. 1. The antibiotic showed intense high-resolution MS ion peaks at *m/e* 98.0463 (M^+, calcd. for $C_4H_6N_2O$: 98.0479), 56.0270 (C_3H_4O: 56.0262), 54.0350 (C_3H_4N: 54.0344) and 43.0292 (CH_3N_2: 43.0295).

Thin-layer chromatography of the antibiotic on Avicel SF (Funakoshi, Lot No. 008390) with 1-propanol - 0.001 N HCl (8 : 2) indicated an Rf of 0.52. By high-voltage paper electrophoresis with 3,000 V for 20 minutes in formic acid - acetic acid - water (1 : 3 : 36 in volume), the antibiotic moved to the cathode with Rm (relative mobility to alanine) 1.60. The antibiotic gave positive RYDON-SMITH, 2,3,5-triphenyltetrazolium chloride and pentacyanoaquoferriate reac-

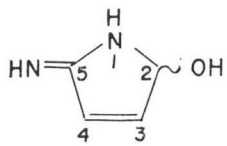


Fig. 1. The IR spectrum of 2-hydroxy-5-iminoazacyclopent-3-ene hydrochloride in KBr.

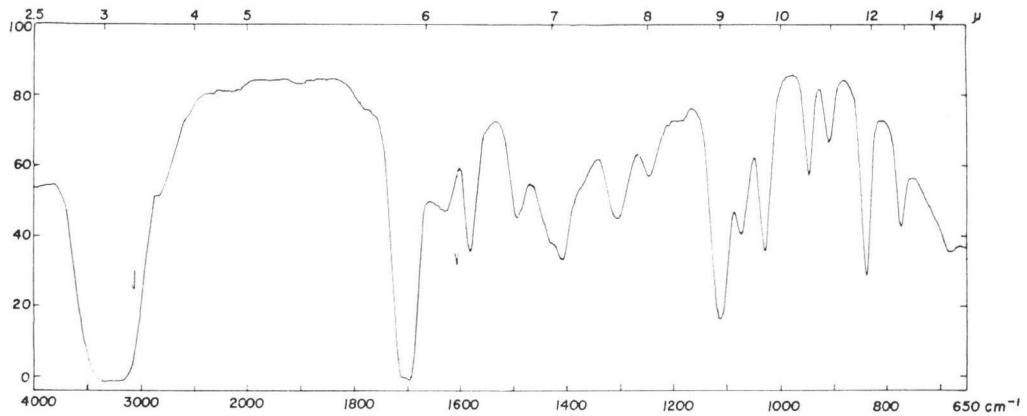


Table 1. The antimicrobial spectra of 2-hydroxy-5-iminoazacyclopent-3-ene hydrochloride.

Test organisms	Minimum inhibitory concentration (μg/ml)
Staphylococcus aureus FDA209P	50
Staphylococcus aureus Smith	50
Staphylococcus aureus ApO1	100
Staphylococcus epidermidis 109	50
Micrococcus flavus FDA16	50
Sarcina lutea PCI1001	100
Bacillus subtilis NRRL B-558	100
Escherichia coli NIHJ	100
Escherichia coli K-12	100
Escherichia coli ML 1629	100
Escherichia coli ML1630	100
Escherichia coli W677	50
Escherichia coli JR66/W677	50
Klebsiella pneumoniae PCI602	100
Shigella flexneri 4b JS11811	50
Salmonella typhi T-63	50
Proteus vulgaris OX19	50
Providencia sp. Pv16 (641)	100

Fig. 2. Effect of 2-hydroxy-5-iminoazacyclopent-3-ene hydrochloride on the growth of *E. coli* K-12.

Logarithmically growing cells were incubated at 37°C for 2 hours with various concentrations of 2-hydroxy-5-iminoazacyclopent-3-ene hydrochloride. The survivors were counted by plating on nutrient agar plates.

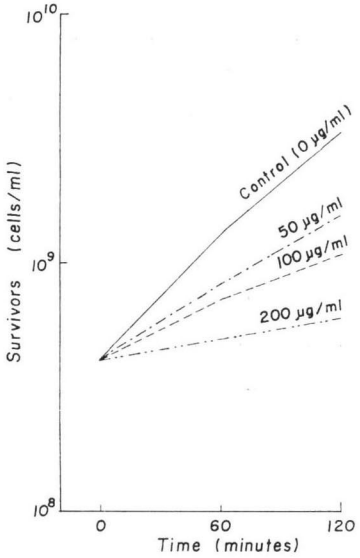
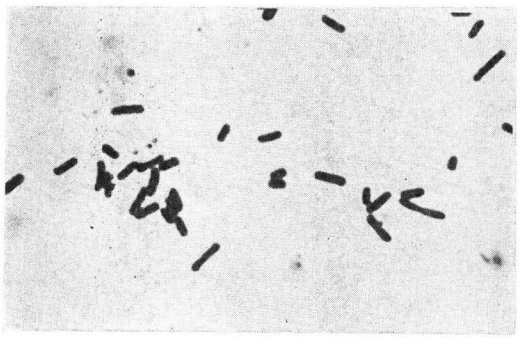


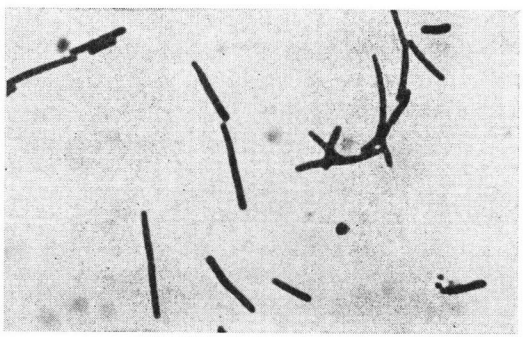
Fig. 3. Morphological change of *E. coli* K-12 exposed to 2-hydroxy-5-iminoazacyclopent-3-ene hydrochloride.

Smears were prepared from 4 hours cultures, stained with crystal violet, and photographed with a microscope. Magnification 1,000×.

(A) Control

(B) 2-Hydroxy-5-iminoazacyclopent-3-ene hydrochloride, 200 μg/ml.





tions, and negative ninhydrin reaction. The antibiotic was stable at pH 2 for 30 minutes at 60°C, but unstable at pH 7 and pH 9. The culture filtrate completely lost its activity after storage at 4°C for 20 hours at pH 7 or pH 9.

The PMR and CMR data of the antibiotic are very similar to those of the iminopyrroline moieties of the clazamycins[2] and of the pyrrolinone moiety of N-(2,4-dinitroanilino)-5-hydroxypyrrol-2-(5H)-one[3]. Therefore, the antibiotic structure was assigned the 2-hydroxy-5-iminoazacyclopent-3-ene (racemic).

The antibiotic is weakly active against Gram-positive and Gram-negative bacteria. Minimum inhibitory concentrations of the antibiotic determined by the agar streak method using 0.5%

peptone agar at 37°C for 17 hours are shown in Table 1. The acute intravenous LD_{50} to mice was 100~200 mg/kg.

The antibiotic almost completely inhibited the growth of *Escherichia coli* K-12 in a nutrient broth at a concentration of 200 μg/ml (Fig. 2). When *E. coli* K-12 was exposed to the antibiotic at 100~200 μg/ml, most of cells were elongated (Fig. 3). Preliminary experiments indicated that the incorporation of [^{3}H]thymidine, [^{3}H]uridine, [^{14}C]leucine and [^{14}C]N-acetyl-D-glucosamine into growing cells was not significantly affected by the antibiotic at concentrations of 100~200 μg/ml.

FEULGEN-positive nuclear material in the elongated cells seemed to be uniformly distributed. Consequently, it appears that 2-hydroxy-5-iminoazacyclopent-3-ene activity on bacteria is associated with cell wall physiology resulting in the cell elongation.

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