A NEW ANTIBIOTIC, CYCLAMIDOMYCIN

Sir:

Cyclamidomycin is a new antibiotic isolated from a streptomyces, No. MA 130-A1, related to *Actinomyces lavendocolor*[1] or *Actinomyces pseudovenezuelae*[2]. After we had completed the study, we read the publication by CORONELLI *et al*[3]. on pyracrimycin A. Cyclamidomycin and pyracrimycin A are identical, though they are produced by different species of Actinomycetes: pyracrimycin A is produced by *Streptomyces eridani*. The properties and structure determination of cyclamidomycin are briefly presented in this report.

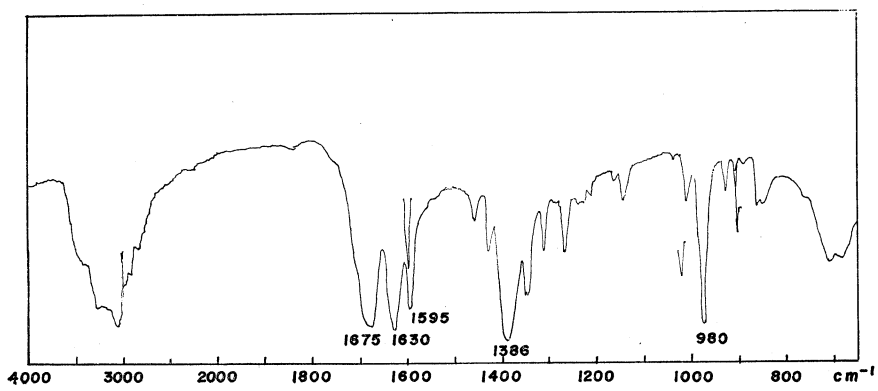
Using shake culture of strain No. MA 130-A1 in a medium containing 2.0 % starch, 2.0 % soybean meal, 0.5 % dry yeast, 0.25 % NaCl, 0.32 % $CaCO_3$, 0.0005 % $CuSO_4 \cdot 5H_2O$, 0.0005 % $MnCl_2 \cdot 4H_2O$, 0.005 % $ZnSO_4 \cdot 7H_2O$, cyclamidomycin accumulated for 2~4 days. The antibiotic was absorbed on a column of Amberlite IR 120 (H^+ form) and eluted with 0.5 N ammonia water. The active eluate was poured into a column of Dowex 1X1 (OH^- form) and eluted with water. The active fraction was concentrated to yield crude cyclamidomycin. The purification was accomplished by column chromatography of Cellulose powder (Whatman, CF 11) developing with *n*-butanol-H_2O (90:10).

Under paper electrophoresis, 3,300 V for 20 minutes in formic acid - acetic acid - water (25 : 75 : 900), cyclamidomycin moved 12.9 cm to the cathode with an Rm (L-alanine 1.0) of 1.29 by bioautography (*Klebsiella pneumoniae*). The existence of two minor active components (Rm 0.5 and 1.90) besides cyclamidomycin in the eluate of Amberlite IR 120 column was observed in the paper electrophoresis experiment. On thin-layer chromatography with Silica Gel (Eastman Chromagram Sheet 6061), cyclamidomycin gave one spot at Rf 0.30 using a solvent system of *n*-butanol - acetic acid - water (2 : 1 : 1).

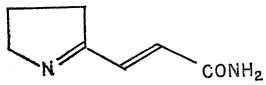
Cyclamidomycin was isolated as colorless crystals, m.p. 215~217°C (decomp.). Anal. calcd. for $C_7H_{10}N_2O$: C 60.85; H 7.30; N 20.28, found: C 59.76; H 7.32; N 19.74. The molecular formula is shown by high-resolution mass spectrum at *m/e* 138.081 (calcd. 138.079). The ultraviolet absorption spectrum shows a maximum at 238 mμ ($E^{1cm}_{1\%}$ 1820). The infrared absorption spectrum is shown in Fig. 1. The antibiotic is soluble in water, and practically insoluble in methanol, ethanol, *n*-butanol, ethyl acetate and chloroform. A pKa′ value of 5.70 was found by titration with an equivalent weight of 138.

Reduction of cyclamidomycin with $NaBH_4$ gave dihydrocyclamidomycin. Periodate-permanganate oxidation of cyclamidomycin or dihydrocyclamidomycin gave γ-aminobutyric acid. Hydrogenation of cyclamidomycin over PtO_2 in methanol produced tetrahydrocyclamidomycin, which gave an N-monoacetate ($C_9H_{16}N_2O_2$; *m/e* 184.122; m.p. 103°C) by acetylation with acetic anhydride in methanol. Ozonolysis of dihydrocyclamidomycin showed the presence of proline

Fig. 1. Infrared absorption spectrum of cyclamidomycin (KBr)



in the reaction mixture by paper electrophoresis. A novel amino acid was isolated in good yield by drastic acid hydrolysis (6N HCl, 105°C, 18 hours) of tetrahydrocyclamidomycin and assigned the structure (3-pyrrolidin-2-yl)-propionic acid ($C_7H_{13}NO_2$; *m/e* 143), which gave an N-monoacetate ($C_9H_{15}NO_3$; *m/e* 185.103; m.p. 133°C) by treatment with acetic anhydride in methanol. From the foregoing results, the structure of cyclamidomycin is as follows:



Cyclamidomycin in aqueous solution (1mg/1ml, at 60°C for 2 hours) was stable at pH 2~10, but unstable in more acidic solution (pH<1).

The antimicrobial activity of cyclamidomycin determined by the agar dilution method is shown in Table 1. Cyclamidomycin released nucleotides from the bacterial cells at relatively low concentrations*. The LD_{50} of cyclamidomycin to mice injected intravenously was 125 mg/kg. When cyclamidomycin was subcutaneously or intraperitoneally injected into mice infected with *K. pneumoniae* S-1802 (10 MLD) immediately and 6 hours after the infection, all mice died within 24 hours at the dose of 25, 12.5, 6.25, 3.13 and 1.56 mg/kg/injection.

Table 1. Minimum inhibitory concentration of cyclamidomycin

Microorganism tested	Minimal inhibitory concentration (mcg/ml)
Staphylococcus aureus FDA 209 P	25
Staphylococcus aureus Smith	25
Micrococcus flavus	12.5
Sarcina lutea PCI 1001	6.25
Bacillus subtilis NRRL B-558	25
Escherichia coli NIHJ	12.5
Escherichia coli K-12	12.5
Escherichia coli K-12 ML 1629*	12.5
Shigella flexneri 1a, Ew 8	6.25
Salmonella typhosa	6.25
Proteus vulgaris OX 19	6.25
Proteus rettgeri GN 311	6.25
Klebsiella pneumoniae PCI 602	3.12
Pseudomonas aeruginosa A3	50
Pseudomonas fluorescens	6.25
Candida albicans 3147	>100
Mycobacterium smegmatis ATCC 607	12.5

* Carrying a multiple drug resistant R factor

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* The mode of action of cyclamidomycin will be described in detail.