OXAZINOMYCIN, A NEW CARBON-LINKED NUCLEOSIDE ANTIBIOTIC

Sir:

As previously reported[1], a new antibiotic oxazinomycin (I) was obtained from the culture filtrate of strain No. 460 which was isolated from a soil sample collected at Tanesashi, Aomori Prefecture.

Studies on the morphological and physiological properties of the strain No. 460 revealed its resemblance to *Streptomyces rimosus* ATCC 10970 except for the following points. The strain No. 460 does not produce dark brownish pigment in tryptone-yeast extract broth and reduces nitrate to nitrite. The most significant difference between these two strains is utilization of carbohydrates tested in the medium of PRIDHAM and GOTTLIEB.[2]

Strain No. 460 utilizes only glucose but *S. rimosus* utilizes arabinose, xylose, glucose, fructose, inositol, mannitol, raffinose and cellulose. Thus, strain No. 460 was concluded to be a new species and named *Streptomyces tanesashinensis* nov. sp.

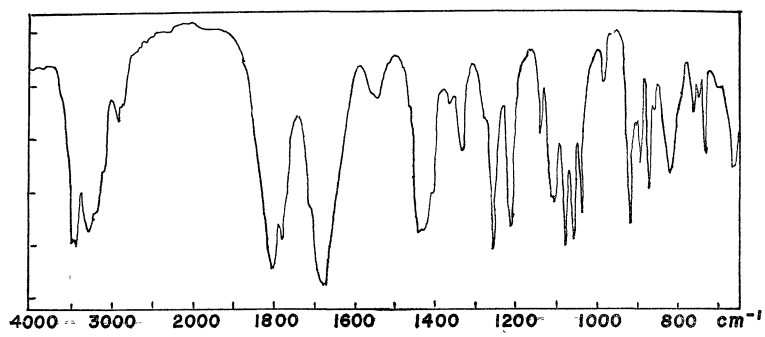
After fermentation of strain No. 460 for 40 hours in a medium containing 3% glucose, 0.5% peptone, 0.3% dry yeast, 0.5% meat extract, 0.5% NaCl and 0.3% $CaCO_3$ (pH 7.0), the production of oxazinomycin reached a maximum in the culture liquid. It was adsorbed on an activated carbon column and eluted with 30% aqueous acetone. The methanol extract from the concentrated matter of the active eluate was passed through a cellulose column packed and developed with water-saturated *n*-butanol. The active fractions were concentrated and purified on a silica gel column using 90% aqueous acetone as eluent. The yellow powder obtained after concentration of the active eluate was dissolved in a small volume of methanol, a few drops of water added and allowed to stand in a refrigerator for 2~3 days to yield colorless crystals.

Its physico-chemical properties are summarized in Table 1. There has been no antibiotic with properties similar to oxazinomycin except showdomycin[3]. But these two antibiotics differed from each other in their physico-chemical properties, especially in the following three points; the difference of the number of oxygen atom in the molecular formula (showdomycin: $C_9H_{11}NO_6$), a characteristic absorption band at 1799 cm^{-1} as shown in Fig. 1 (showdomycin: absent) and a sharp doublet of one proton observed at 7.9 δ (showdomycin: 6.8 δ), a typical vinylic or aromatic proton in the NMR spectrum in dimethylsulfoxide-d_6. Oxazinomycin gave a red-violet color with anthrone

Table 1. Physico-chemical properties of oxazinomycin

Nature	Acidic, colorless crystal, pKá 6.96
Solubility	Sol. in H_2O, MeOH and EtOH
M. P.	164~166°C (dec.)
Elementary analysis (%)	C 44.02, H 4.61, N 5.70 (measured)
	C 44.02, H 4.52, N 5.71 (calculated)
Formula	$C_9H_{11}NO_7$
M. W.	m/e 245.19
$[\alpha]_D^{20}$	+19.7° (*c* 1.0, H_2O)
UV max. ($E_{1cm}^{1\%}$)	231~232 mμ (168) in H_2O

Fig. 1. Infrared spectrum of oxazinomycin in KBr disk.



reagent, which was similar to that for deoxysugars, but gave a negative test with orcinol reagent. Although the presence of sugar by acid hydrolysis was not successfully established, three protons previously found in the 4.5~5.0 δ and a broad singlet of one proton at 3.35 δ were absent due to deuterium exchange. For the examination of mass spectrum of the antibiotic, the trimethylsilyl derivative was prepared to detect a peak corresponding to the tristrimethylsilyloxazinomycin (*m/e* 461). In the infrared absorption spectrum of oxazinomycin treated with diluted sodium hydroxide solution at room temperature, the characteristic absorption bands at 1799 and 1760 cm^{-1} disappeared at pH 9.0. It was interpreted to indicate ring opening of the base moiety of oxazinomycin in alkaline solution. As for a possible structure of oxazinomycin, it was thus proposed to be a carbon-linked nucleoside with an oxazine ring differing from the five membered maleimide ring in showdomycin (I and II).

In the NMR spectrum of oxazinomycin in dimethylsulfoxide-d_6-D_2O, seven protons are all assigned as shown in Fig. 2. The H_a proton signal at 4.5 δ as a quartet coupled with H_b and H_f with coupling constant 3.3 and 1.2 cps, respectively.

In view of the facts that H_f proton of oxazinomycin in dimethylsulfoxide-d_6 shifted to 1.1 ppm downfield from that of showdomycin in the NMR spectrum and the hydrolysis of oxazinomycin by alkaline treatment, the structure (I) seems more reasonable than the structure (II).

In order to confirm the structure of oxazinomycin, X-ray analysis has been carried out. Crystals are monoclinic, space group C 2, with cell dimensions a=15.35, b=6.54, c=11.74 Å and β=121.2°. From these results combined with chemical properties, it would be expected that the crystal structure was similar to that of showdomycin[4] through its hydrogen bonding network of sugar moiety. Thus, structure factors were calculated using the atomic parameters of the sugar ring found in showdomycin (R=0.50), and successive FOURIER and least-squares refinements resulted in choice of the structure (I) for oxazinomycin as shown in Fig. 3. At this stage, the R value

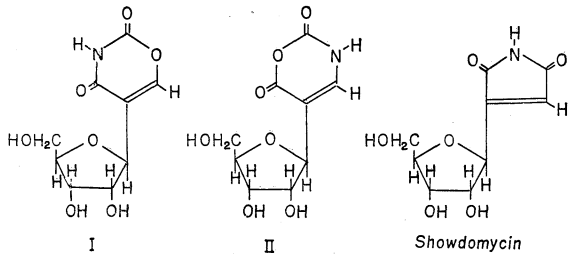


Fig. 2. NMR spectrum of oxazinomycin in DMSO-d_6 at 100 MHz.

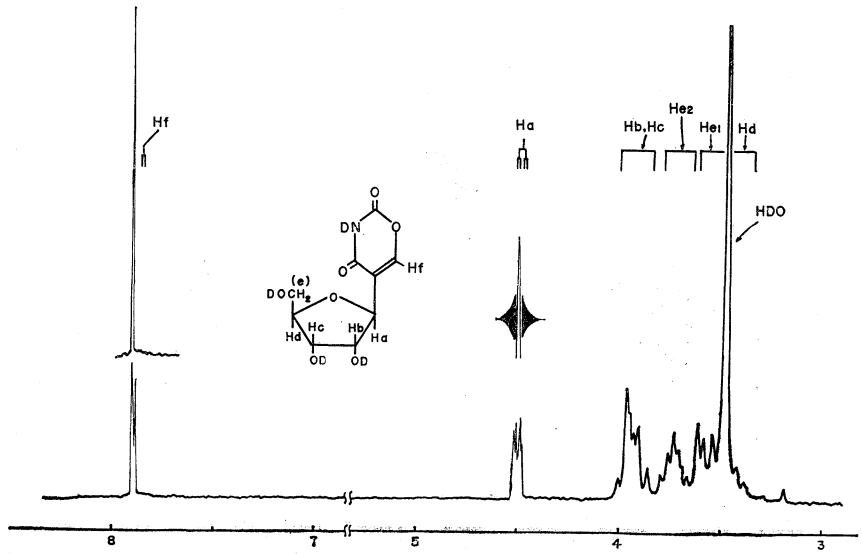
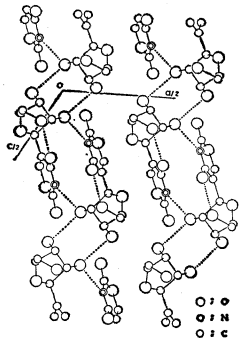


Fig. 3. Crystal structure of oxazinomycin viewed down the b axis.



The dashed lines represent hydrogen bonds

reached 0.15. The further study of X-ray analysis will be presented in detail elsewhere.

The minimal inhibitory concentrations against gram positive and negative bacteria, yeast and fungi determined by agar streak dilution method were as follows (μg/ml): *Staphylococcus aureus* FDA 209 P, 1.6; *S. aureus* 193, 12.5; *S. aureus* 52-34, 3.2; *Mycobacterium smegmatis* ATCC 607, >100; *Escherichia coli* NIHJ, >100; *E. coli* K-12, >100; *Proteus vulgaris*, >100; *Aeromonas liquefaciens*, >100; *Pseudomonas aeruginosa*, >100; *Candida albicans*, >100; *Trichophyton interdigitale*, >100.

A preliminary test of antitumor activity of oxazinomycin was examined on RFVL mice implanted with the EHRLICH ascites carcinoma in ascitic form. Ten mice in each group weighing 20~22 g were given intraperitoneal inoculation of 0.1 ml fresh ascitic fluid containing 10 million tumor cells per ml. Once daily treatments of oxazinomycin were started 24 hours after inoculation and treatments were continued for 5 consecutive days. The effectiveness of the antibiotic evaluated by the survival per cent of intraperitoneal doses of 1.25, 2.5 and 5.0 mg/kg was 70, 70 and 90 %, respectively.

Acute toxicity was examined in RFVL mice for a 7 day observation period and the LD_{50} for mice was 10~20 mg/kg given intraperitoneally and 100~120 mg/kg given intravenously.

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