Communications to the Editor

A NEW ANTIBIOTIC, TAUTOMYCIN

Sir:

An unidentified strain of *Streptomyces* isolated from a soil sample collected in Jiangsu Province, China has been found to produce a new antibiotic which exhibits strong toxicity against a variety of eukaryotic cells including fungi, yeasts and animal cells. The antibiotic was designated as tautomycin because it exists as a tautomeric mixture in solution.

Fermentation was carried out at 28°C for 96 hours in a jar fermentor containing 18 liters of a medium which is composed of glucose 2%, soluble starch 1%, meat extract 0.1%, dry yeast 0.4%, soybean flour 2.5%, NaCl 0.2% and K_2HPO_4 0.005%. The filtered broth (36 liters) was extracted with EtOAc at pH 4 and the extracts were concentrated to dryness giving 4.8 g of oily residue. It was applied onto a silica gel column developed with the solvent, $CHCl_3$ - MeOH (2:1). Active fractions were collected and further purified by HPLC (Nucleosil $5C_{18}$, MeOH - H_2O, 8:2 plus 1% diethylamine - formic acid, pH 7.5). It was finally purified with a ODS-H-4251 column by the solvent, MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 7.3) (8:1:1). As shown in Fig. 1, the purified product gives two peaks on the column. Each peak was collected and analyzed by rechromatography in the same condition. Immediately after the separation each fraction showed almost a single peak. However, a counter peak appeared and increased gradually. Equilibrium was reached after standing overnight at room temp. The ratio of the mixture was approximately 6:4, as shown in Fig. 1. From this experiment, we concluded that the antibiotic is a tautomeric mixture.

Tautomycin is a hygroscopic amorphous white powder which decomposes gradually above 160°C. It is optically active, $[\alpha]_D^{20}$ +3.4° (*c* 1, $CHCl_3$) and soluble in MeOH, Me_2CO, EtOAc, $CHCl_3$ and benzene, but hardly soluble in hexane

Fig. 1. HPLC profile of tautomycin.
Column; Senshu Pak ODS-H-4251 (10× 250 mm), solvent; MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 7.3), 78:12:10, flow rate; 1.4 ml/minute, detection; UV 220 nm.

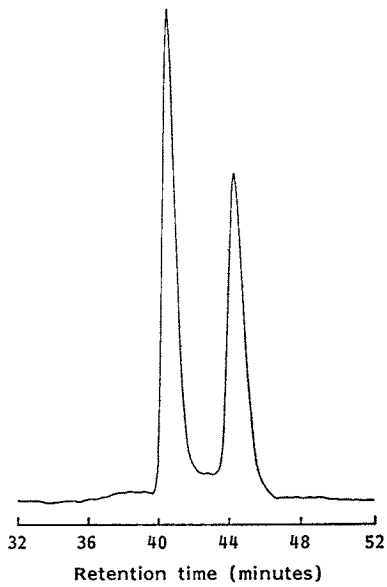


Fig. 2. UV spectrum of tautomycin (MeOH).

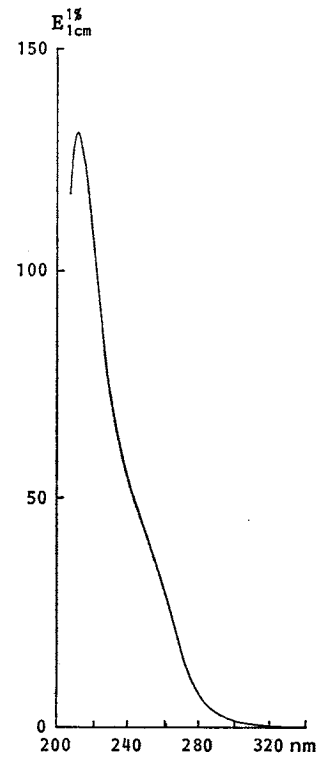


Fig. 3. IR spectrum of tautomycin (KBr).

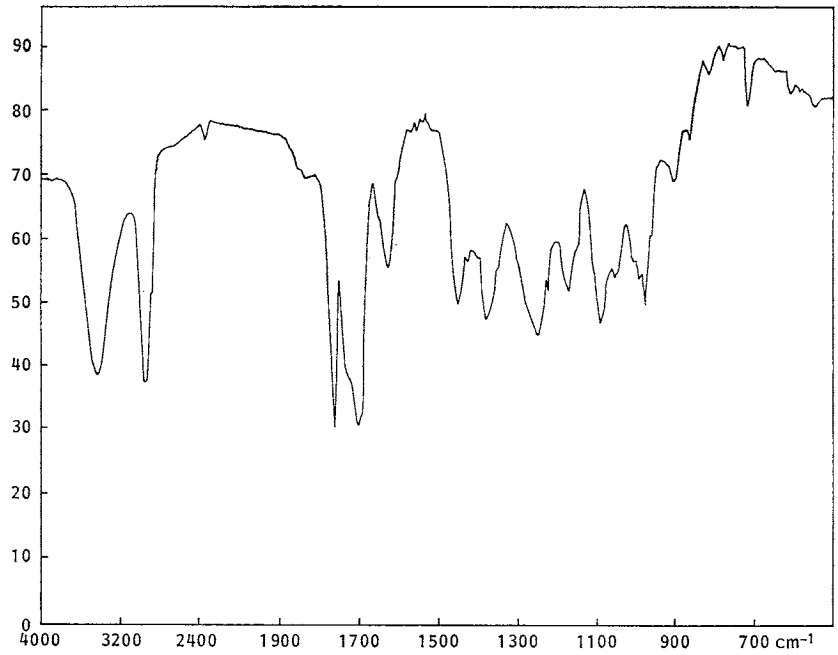
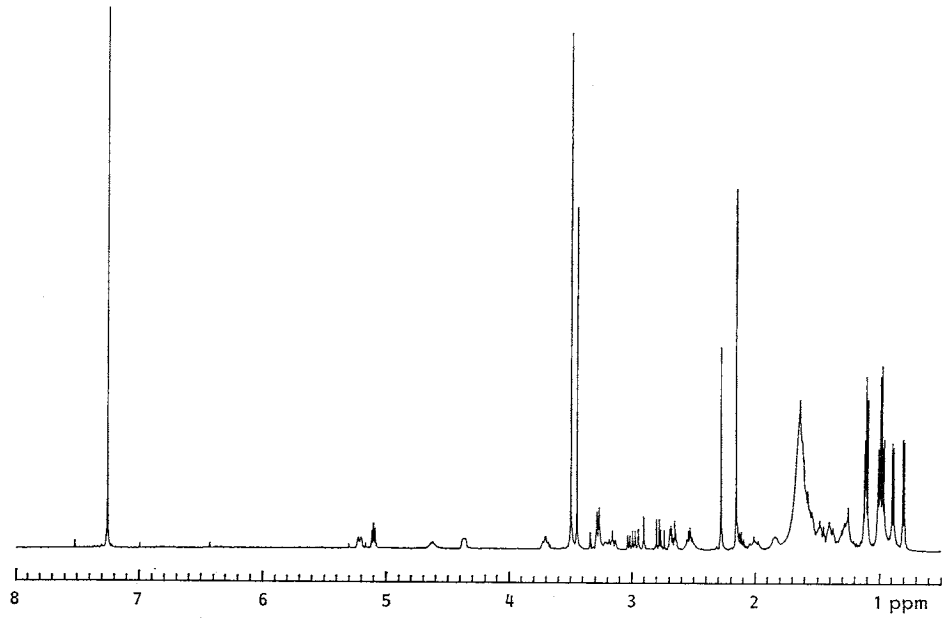


Fig. 4. ^1H NMR spectrum of tautomycin ($CDCl_3$).



and water. From elemental analysis and high resolution fast atom bombardment mass spectroscopy (HRFAB-MS) (triethanolamine matrix) $[(M+H+C_6H_{15}O_3N)^+$: 916.6078, found: 916.6158], the following molecular formula has been established. Calcd for $C_{42}H_{70}O_{12}\cdot 1\frac{1}{2}H_2O$: C 63.56, H 9.20, O 27.23. Found: C 63.36, H 8.69, O 26.72. It shows end absorption in UV region (λ_{max}^{MeOH} 210 nm, $E_{1cm}^{1\%}$ 132 nm) with a shoulder around 250 nm (Fig. 2). IR spectrum is shown in Fig. 3. Main absorption bands appear at the following wavelengths: 3400, 2900, 1755, 1695,

Table 1. Antimicrobial activity of tautomycin.

Test organism	MIC (μg/ml)
Botrytis cinerea IFO 5365	125
Colletotrichum lagenarium IFO 7513	125
Pyricularia oryzae IFO 5994	125
Rhizoctonia solani IFO 6258	125
Aspergillus niger	125
Alternaria mali IFO 8984	500
Glomerella cingulata IFO 9767	500
Xanthomonas oryzae IFO 3312	32
X. citri IFO 3781	32
Staphylococcus aureus IFO 12732	>500
Escherichia coli BE1186	>500
Bacillus subtilis IFO 3513	>500

The conventional agar dilution method was used. Medium: Potato - sucrose agar for yeasts and fungi. Bouillon agar for bacteria.

1620, 1445, 1375, 1240, 1155, 1085, 970, 900 and 715 cm^{-1}. The absorption at 1755 cm^{-1} suggests the presence of lactone. ^1H NMR spectrum is shown in Fig. 4. It gave a positive reaction to anisaldehyde - H_2SO_4 and 2,4-dinitrophenylhydrazine tests, but was negative to ferric chloride and ninhydrin tests. On paper electrophoresis, it migrates slightly to an anode at pH 3 and 8.

Tautomycin induced morphological change (blebbing) of human erythroid leukemia cell K562 at the concentration of 0.1~1 μg/ml. It also inhibited the spreading of human myeloid leukemia cell HL60 induced by phorbol ester at the concentration of 0.03~0.3 μg/ml.

It is inhibitory to various fungi and yeasts and limited species of Gram-negative bacteria (Table 1). It showed an excellent protective effect against cucumber gray mold in pot test at the dose of 6 ppm. It is highly toxic to mice: LD_{50} is about 7.5 mg/kg when administered orally and less than 5 mg/kg intraperitoneally.

Comparing with the lipophilic antifungal antibiotics having no nitrogen in the molecule, *e.g.*, oligomycin[1], venturicidin B[2], cytovaricin[3], algacidin[4] *etc.*, tautomycin is clearly different in UV spectrum, optical rotation and molecular formula. Moreover, no antibiotic has been reported which has the molecular formula corresponding to $C_{42}H_{70}O_{12}$.

XING-CHUN CHENG†
TSUYOSHI KIHARA
HIROO KUSAKABE
JUNJI MAGAE
YUMIKO KOBAYASHI
REN-PIN FANG†
ZHER-FU NI†
YIN-CHU SHEN†
KEIDO KO
ISAMU YAMAGUCHI
KIYOSHI ISONO*

Riken, The Institute of Physical and Chemical Research,
Wako-shi, Saitama 351-01,
Japan
†Shanghai Pesticide Research Institute,
Shanghai, China

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