Communications to the Editor

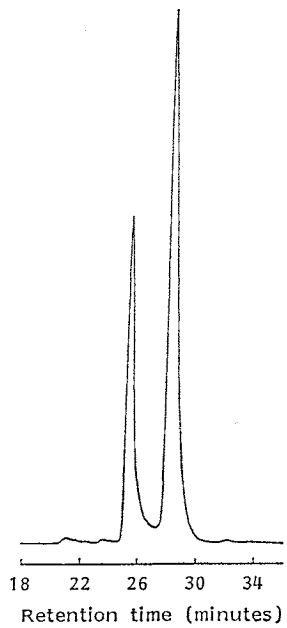
A NEW ANTIBIOTIC, TAUTOMYCETIN

Sir:

A strain RS-1223 of Streptomycete isolated from a soil sample collected in Zhejiang Province, China was found to produce a new antifungal antibiotic, together with streptothricins. The antibiotic exhibited strong toxicity against a variety of eukaryotic cells including fungi and animal cells. The strain was identified as *Streptomyces griseochromogenes* by taxonomic study and the antibiotic was designated as tautomycetin.

Fermentation was carried out at 28°C for 72 hours in a jar fermentor containing 18 liters of a medium 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 (30 liters) was extracted with EtOAc at pH 4 and the extracts were concentrated to dryness, giving 10.1 g of oily residue.

Fig. 1. HPLC profile of tautomycetin.

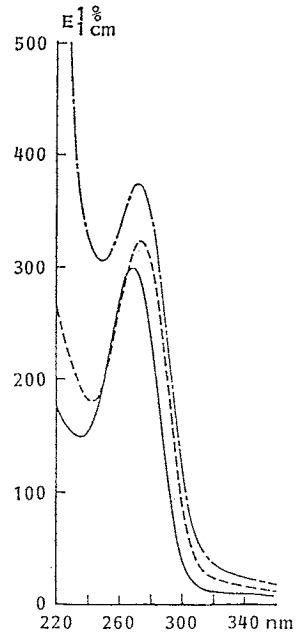


Column: Senshu Pak ODS-H-2031 (10×250 mm), solvent: MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 7.3), 75 : 15 : 10, flow rate: 1.4 ml/minute, detection: UV 220 nm.

It was applied onto a silica gel column, which was developed stepwise with the solvent system, $CHCl_3$ - MeOH (10 : 1, 4 : 1, 2 : 1, 1 : 1). Active fractions were collected and further purified by HPLC using a reverse phase column (Nucleosil $5C_{18}$, MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 4.0) (75 : 15 : 10)). As shown in Fig. 1 the purified product gave two peaks in HPLC. Each peak was collected and analyzed by rechromatography (Nucleosil $5C_{18}$, MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 7.3) (75 : 15 : 10)). 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 0°C. The ratio of the mixture was approximately 4 : 6 as shown in Fig. 1. From this experiment, we concluded that the antibiotic is a tautomeric mixture as in the case of tautomycin,[1] which we reported previously.

Tautomycetin is acidic yellowish gum possessing the following physico-chemical pro-

Fig. 2. UV spectrum of tautomycetin.
—— MeOH, ---- 0.1 N HCl - MeOH (1 : 1), —·— 0.1 N NaOH - MeOH (1 : 1).



perties. It is optically active, $[\alpha]_D^{20}$ +19.4° (*c* 0.83, $CHCl_3$) and soluble in MeOH, Me_2CO, EtOAc, $CHCl_3$ and benzene, but hardly soluble in hexane and water. It has a characteristic absorption in UV region: λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 268 (298) (Fig. 2). Main absorption bands in the IR spectrum appeared at the following wavelength: ν_{max}^{neat} cm^{-1} 3400, 2950, 1760, 1700, 1580, 1450, 1370, 1250, 900, 730 (Fig. 3). The absorption at 1760 cm^{-1} suggested the presence of lactone. The molecular formula was established to be $C_{33}H_{50}O_{12}$ from elemental analysis and secondary ion mass spectrometry (SI-MS): *m/z* 661 $(M+Na)^+$, 677 $(M+K)^+$. Calcd for $C_{33}H_{50}O_{12}$: C 62.07, H 7.84. Found: C 62.23, H 8.12. The signals in the ^{13}C NMR spectrum taken in $CDCl_3$ accounted for 33 carbons (Fig. 4). Properties of the carbon atoms were determined by INEPT experiment as follows: $CH_3\times7$, $CH_2\times8$, $CH\times8$, $=CH_2\times1$, $=CH\times4$, $=C<\times1$, $C=O\times4$. ^1H NMR spectrum is shown in Fig. 5. It gave a positive reaction to anisaldehyde - H_2SO_4, but was negative to 2,4-dinitrophenylhydrazine, ferric chloride, and ninhydrin tests. It is an acidic compound. On high voltage paper electrophoresis, it migrated to an anode at pH 7.8 and 4.0. Its *pKa'* value was determined to be 5.2 by potentiometric titration in 50% EtOH.

Tautomycetin showed inhibitory activity against various fungi (Table 1) and as low as 25 μg/ml of the antibiotic showed a good preventive effect to cucumber gray mold in pot test. It showed no inhibitory activity against Gram-positive and Gram-negative bacteria tested. The antibiotic induced bleb-formation on human erythroid leukemia cell K562[2] at the concentration of 3~100 μg/ml. Interestingly, 100 μg/ml of tautomycetin reversed the bleb-formation induced by phorbol 12,13-dibutyrate (1 μg/ml). LD_{50} to mice was approximately 35 mg/kg when administered orally.

Comparing with lipophilic antifungal antibiotics have no nitrogen in the molecule, *e.g.*, tautomycin,[1] oligomycin,[3] venturicidin B,[4]

Table 1. Antimicrobial activity of tautomycetin.

Test organism	MIC (μg/ml)
Botrytis cinerea IFO 5365	12.5
Alternaria mali IFO 8984	50
Pyricularia oryzae IFO 5994	50
Colletotrichum lagenarium IFO 7513	25
Glomerella cingulata IFO 9767	50
Cochliobolus miyabeanus	25
Rhizoctonia solani IFO 6258	200
Fusarium oxysporum	200
Aspergillus niger	50
Penicillium chrysogenum	50

The conventional agar dilution method was used. Medium: Potato - sucrose agar.

Fig. 3. IR spectrum of tautomycetin (film).

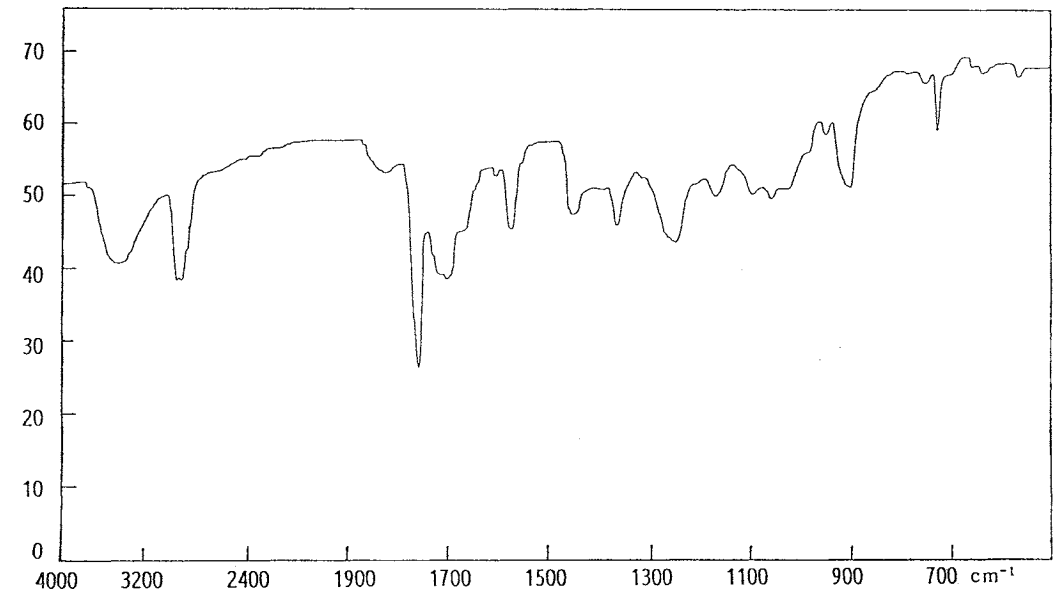


Fig. 4. ^{13}C NMR spectrum of tautomycetin (25 MHz, $CDCl_3$).

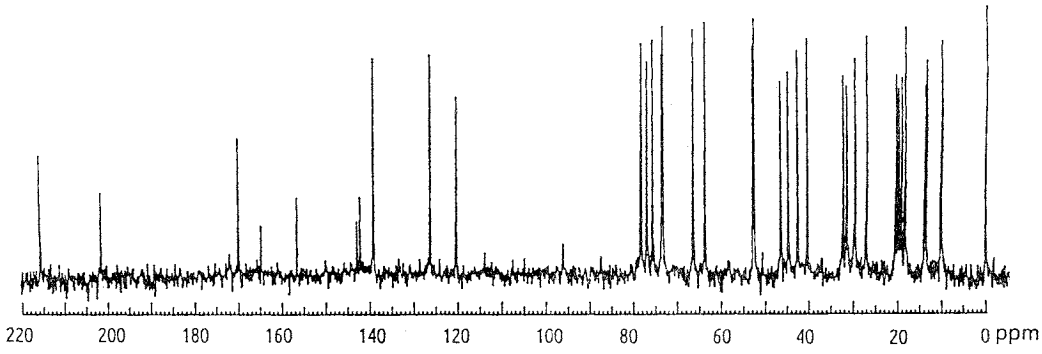
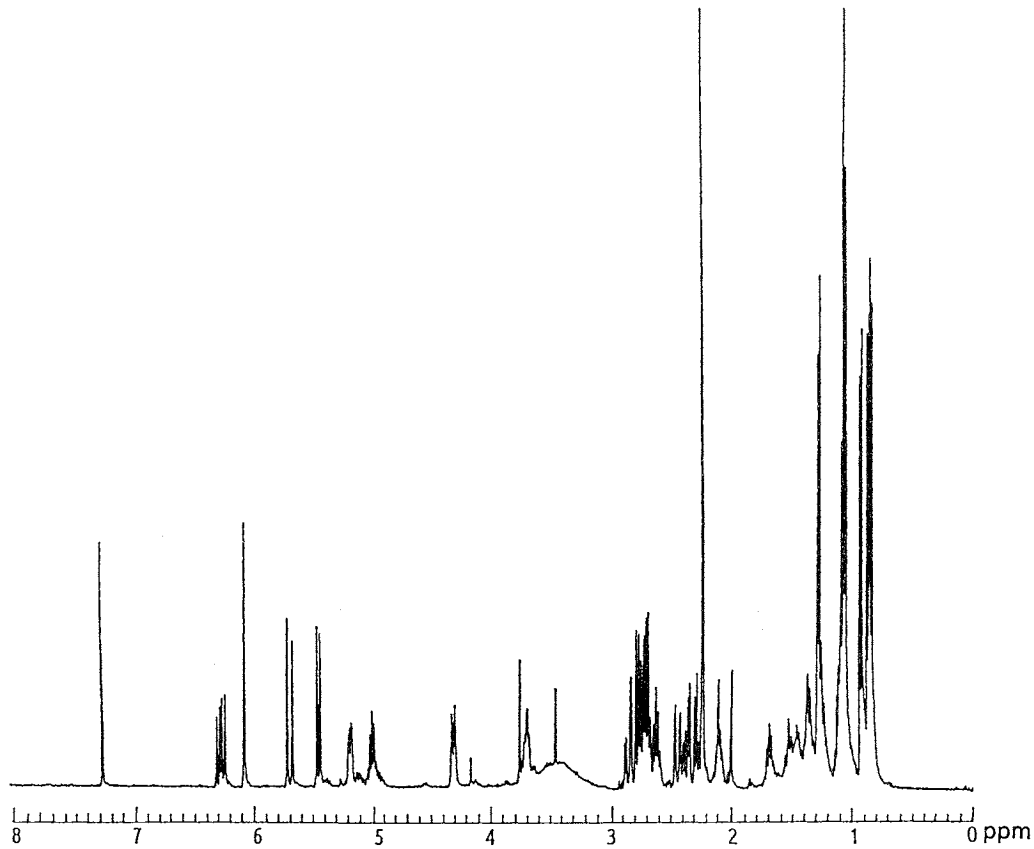


Fig. 5. ^{1}H NMR spectrum of tautomycetin (500 MHz, $CDCl_3$).



cytovaricin[5] *etc.*, tautomycetin is most similar to tautomycin in its tautomeric property and also in the biological activity. However, there are distinct difference in UV spectrum, optical rotation and molecular formula between them, justifying novelty of the compound.

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