SERINOMYCIN, A NEW ANTIBIOTIC

Sir:

A new antifungal antibiotic serinomycin was isolated from the culture filtrate and mycelia of a *Streptomyces* sp. No. 87-55, which was isolated from a soil sample collected at Nagatsuta, Yokohama city. Serinomycin is active against some species of phytopathogenic fungi.

Fermentation was carried out using 600 liters tank at 27°C. The composition of the medium was 2% glucose, 1% starch, 2.5% soybean meal, 0.4% dried yeast, 0.1% beef extract, 0.2% sodium chloride and 0.005% dipotassium phosphate. The culture filtrate of 480 liters was adjusted to pH 8 and extracted with 200 liters of butyl acetate. The antibiotic was then back-extracted into 23 liters of water acidified to pH 2 with hydrochloric acid. The aqueous layer was separated and allowed to stand in a refrigerator to give a precipitate of 7.1 g of crude serinomycin hydrochloride. The precipitate was filtered and recrystallized from 1.5 liters of hot water, yielding 1.84 g of crystalline serinomycin hydrochloride.

The filtrate was concentrated to 8 liters *in vacuo*, and extracted with 4 liters of ethyl acetate at pH 9.0. Serinomycin was back-extracted from the solvent layer into one liter of water acidified to pH 2 with sulfuric acid. A precipitate of 40.5 g of crude sulfate was obtained in the manner described above, and 11.43 g of crystalline serinomycin sulfate was obtained by recrystallization.

Fig. 1. Ultraviolet spectra of serinomycin sulfate.

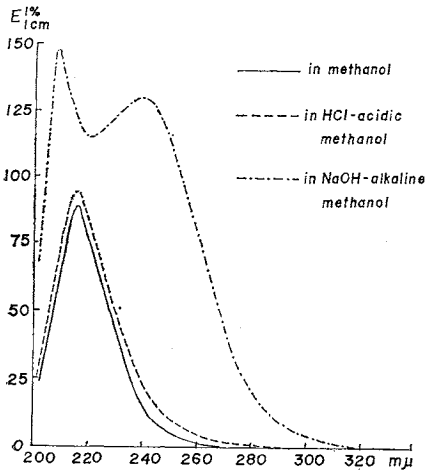
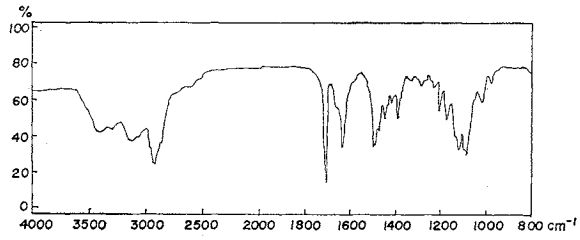


Fig. 2. Infrared absorption spectrum of serinomycin sulfate (in KBr tablet).



Serinomycin sulfate and hydrochloride are colorless needles. They are soluble in pyridine, dimethylformamide, dimethylsulfoxide and diethylene glycol, slightly soluble in water, methanol, *n*-butanol, methylcellosolve and acetic acid, and insoluble in ethanol, acetone, ethyl acetate, dioxane, chloroform, benzene, *n*-hexane and petroleum ether.

Serinomycin gave positive FOLIN (blue), PAULY (orange), ferric chloride (red), and ninhydrin tests on silica gel plate (yellow), but negative FEHLING, anthrone, MOLISCH,

Table 1. Antimicrobial activity of serinomycin sulfate

Test organism	M.I.C. (μg/ml)
Alternaria kikuchiana	0.8
Sclerotinia cingulata	0.4
Glomerella cinerea	1.6
Diaporthe citri	0.8
Elsinoe fawcetti	50
Piricularia oryzae	50
Ophiobolus miyabeanus	0.8
Pellicularia filamentosa	0.4
Fusarium oxysporum	25
Colletotrichum coffeanum	6
Aspergillus oryzae	12.5
Aspergillus niger	>100
Penicillium chrysogenum	0.1
Rhizopus oryzae	12.5
Candida albicans	50
Saccharomyces cerevisiae	25
Rhodotorula glutinis	6
Bacillus agri	>100
Staphylococcus aureus	>100
Escherichia coli	>100
Micrococcus luteus	>100
Xanthomonas oryzae	>100

MILLON, DRAGENDORFF, TOLLENS, EHRLICH, SAKAGUCHI, biuret and ninhydrin tests.

The melting point of the hydrochloride is 158~161°C (dec.), and that of the sulfate is 158~160°C (dec.). The optical rotation of the sulfate in pyridine is $[\alpha]_D^{25}$ $-6° \pm 1°$ (c 0.685). The ultraviolet absorption spectra in methanol is shown in Fig. 1. The sulfate shows a maximum at 214 mμ ($E_{1cm}^{1\%}$ 89), being shifted to 215 mμ ($E_{1cm}^{1\%}$ 95) in HCl acidic solution, and to 209 mμ ($E_{1cm}^{1\%}$ 148) and 238 mμ ($E_{1cm}^{1\%}$ 130) in NaOH alkaline solution. The infrared absorption spectrum of the sulfate in KBr shows 3400, 3300, 3130, 2930, 1710, 1635, 1490, 1445, 1390, 1290, 1205, 1173, 1120, 1085 and 1020 cm^{-1} (Fig. 2). Elementary analysis is as follows: Hydrochloride: C 53.44, 54.48; H 8.98, 8.96; N 6.21, 6.40; Cl 11.06, 10.98 %. Sulfate: C 54.09, 54.10; H 8.98, 8.92; N 6.46, 6.34; S 3.93, 3.78 %. Any attempt to measure the molecular weight has been unsuccessful.

Silica gel thin-layer chromatography gave Rf values: 0.78 with n-butanol – acetic acid – water (4 : 1 : 2), and 0.42 with benzene – n-butanol – ethanol – water (10 : 7 : 3 : 1).

Serinomycin sulfate yielded about 20 % of L-serine after hydrolysis with 1 N NH_4OH at room temperature.

The antimicrobial spectrum of serinomycin sulfate by the agar dilution method is shown in Table 1. The LD_{50} for mice was found to be 100 mg/kg intraperitoneally.

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