A NEW ANTIBIOTIC, GOUGEROXYMYCIN

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A new antifungal substance, gougeroxymycin (m. p. 103°~105°C, λ_{max} 234 ~235 mμ in methanol) has been isolated from a *Streptomyces*. Production, isolation, physico-chemical, and biological properties of the antibiotic are described.

In the course of screening for antibiotics active against *Trichophyton mentagrophytes* and having no polyene character, a strain MA 428-Cl resembling *Streptomyces gougeroti* was found to produce a new antibiotic named gougeroxymycin. Production, isolation and properties of the antibiotic are presented in this paper.

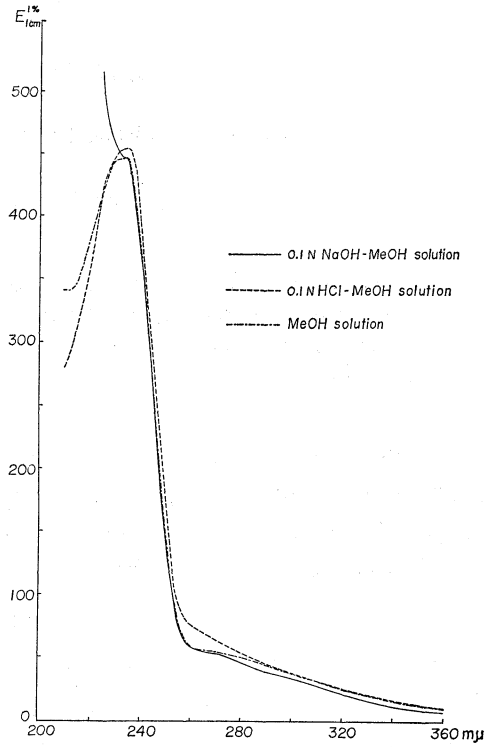
1. Production and Isolation

The strain MA 428-Cl was shaken-cultured (120 strokes/min., 8 cm amplitude) in a medium (pH 7.0) containing 2% glucose, 1.5% soybean meal, 0.3% NaCl, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.0007% $CuSO_4 \cdot 5H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0008% $MnCl_2 \cdot 4H_2O$, 0.0002% $ZnSO_4 \cdot 7H_2O$ at 27°C for 5 days. The amount of gougeroxymycin was determined by a cylinder plate method described by one of the authors[1]. About 70% of the gougeroxymycin in the cultured broth was in the mycelium mass; the rest was in the liquid. The antibiotic in the liquid was extracted with *n*-butanol, and that in the mycelium cake with methanol. For example, 14 liters of cultured broth containing 14.6 mcg/ml of gougeroxymycin was filtered and the filtrate extracted twice with 3.5 liters of *n*-butanol. The mycelium cake was extracted five times with 1 liter of methanol, and the methanol extracts were combined, cencentrated *in vacuo*, and the concentrated solution extracted with *n*-butanol. The *n*-butanol extracts obtained from filtrate and mycelium were combined and concentrated under reduced pressure to 200~300 ml. To the concentrate 100 ml of distilled water were added. The pH was adjusted 9.0 with N/10 NaOH, and the antibiotic was extracted with 200 ml of *n*-butanol by a countercurrent method using 5 separatory funnels. Butanol layers were combined (700 ml), and concentrated under reduced pressure to dryness. The residue was washed with 200~300 ml of petroleum ether, yielding 2.89 g of yellowish brown powder. This powder was extracted with 53 ml of ethanol and after concentrating to 8~10 ml, was filtered to remove a white inactive powder (0.5 g). The antibiotic in the filtrate was purified by a silica-gel (70 g Mallinckrodt Chemical Works, U. S. A.) column (2.3 cm diameter) chromatography. The antibiotic was eluted with ethanol (0.35 ml/min.), and the eluate collected in 7 g fractions. The antibiotic appeared in fractions 9~91 in a yeild of 90.6%. The rest of the activity was eluted with

methanol. The active ethanol fractions were concentrated to a small volume, and subjected to silica-gel column chromatography as described above, and the same elution pattern was obtained. Identity of the active compounds in ethanol and in methanol were confirmed by thin-layer chromatography on silica-gel (Wakogel B-O) using methanol-water-2.5 % acetic acid (3:1:0.5), and methanol-1 N NH_4OH (2:1), by paper chromatography using methanol-water-2.5 % acetic acid (3:1:0.5), and water-saturated *n*-butanol, and by their infrared spectra (Fig. 2) as KBr pellet.

The ethanol eluate in fractions 9~30 contained a brown impurity and was further purified by the same silica-gel chromatography. The colorless fractions 31~91 were concentrated under reduced pressure to dryness, and washed with ethyl ether to yield pure gougeroxymycin 23 mg. The overall yield was about 29.6 %.

Fig. 1. Ultraviolet absorption spectrum of gougeroxymycin



2. Physical and Chemical Properties

Gougeroxymycin is a white powder, m. p. 103°~105°C, $[\alpha]_D^{26}$ +20° (*c* 1, methanol), soluble in methanol, ethanol, *n*-butanol, chloroform and acetone, sparingly soluble in water and insoluble in petroleum ether, ethyl ether and hexane. It is stable in aqueous solution of pH 7.0 and pH 9.0 at 60°C for 30 minutes, but slightly unstable at pH 2.0. The UV spectrum shows maxima at 234~235 mμ ($E^{1\%}_{1cm}$ 452.5) in methanol, 234 mμ

Fig. 2. Infrared absorption spectrum of gougeroxymycin

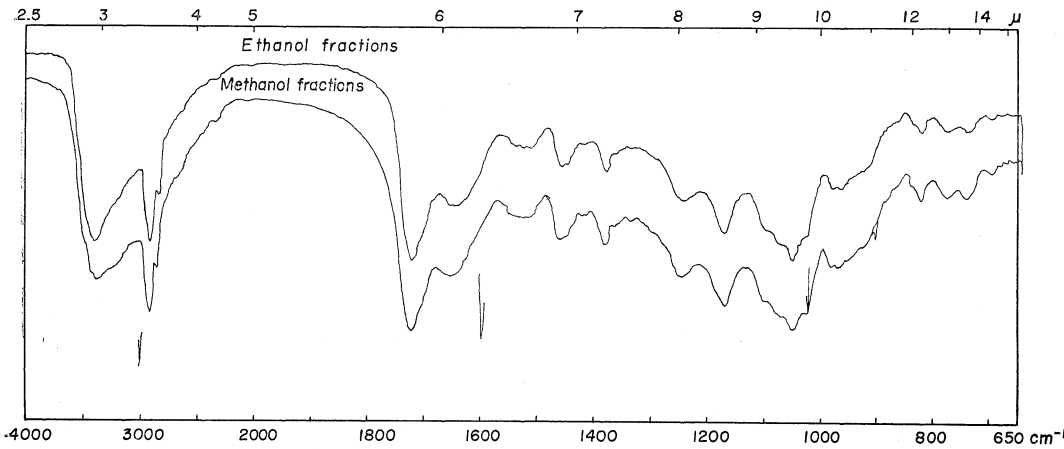
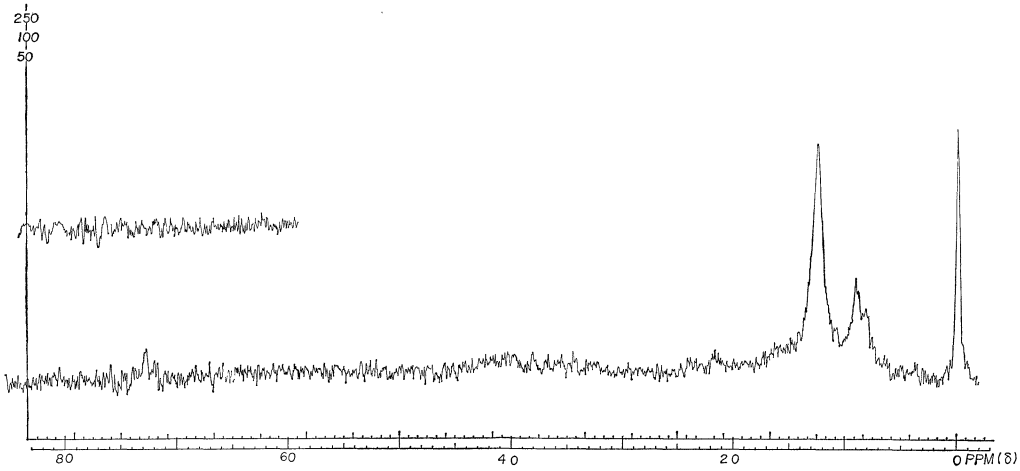


Fig. 3. NMR spectrum of gougeroxymycin with $CDCl_3$



($E^{1\%}_{1cm}$ 445) in 0.1 N HCl methanol, and 235 mμ ($E^{1\%}_{1cm}$ 442.1, shoulder) in 0.1 N NaOH methanol, as shown in Fig. 1. The IR spectrum in potassium bromide is shown in Fig. 2 and the NMR spectrum with $CDCl_3$ in Fig. 3.

The elemental analysis:

Anal: Calcd. for $C_{11}H_{19}O_5N$:	C 53.86, H 7.81, O 32.62, N 5.71.
Found:	C 53.40, H 7.32, O 33.97, N 5.31.

It decolorizes potassium permanganate solution and gives a positive EHRLICH reaction. Negative tests were obtained with hydroxamic acid-ferric salt, ninhydrin, ferric chloride, BEILSTEIN, TOLLENS, SAKAGUCHI and BENEDICT reagents.

On paper chromatography at room temperature using Toyo filter paper No. 51, gougeroxymycin gives a spot at Rf 0.72 with methanol - water - 2.5 % acetic acid (3 : 1 : 0.5), Rf 0.54 with water-saturated *n*-butanol, Rf 0.49 with methanol, Rf 0.76 with 50 % acetone, Rf 0.73 with phosphate buffer pH 9.0 saturated with *n*-butanol, Rf 0 with water, and tailing spot with ethanol. On the thin-layer chromatography at room temperature using silica-gel (Wakogel B-O) gougeroxymycin gives an iodine-positive and biologically active spot at Rf 0.4 with methanol - water - 2.5 % acetic acid (3 : 1 : 0.5), Rf 0.37 with methanol - 1 N NH_4OH solution (2 : 1), tailing spot with methanol, Rf 0 with ethanol, chloroform, water-saturated *n*-butanol - methylcellsolve (1 : 1), and methanol - dioxane (1 : 1), and at Rf 0.55 on cellulose (Microcrystalline cellulose spot film) with methanol - water - 2.5 % acetic acid (3 : 1 : 0.5) and tailing spot with ethanol.

On high voltage paper electrophoresis (3,500 V/42 cm, 65 mA/20 cm, 15 min.) using a buffer of formic acid - acetic acid - water (25 : 75 : 900 by volume) gougeroxymycin stays at the origin.

3. Biological Properties

In 1 % glucose nutrient agar gougeroxymycin inhibits *Trichophyton mentagrophytes* (No. 589) and *Penicillium chrysogenum* (No. a-176) at 3.12 mcg/ml, *Aspergillus niger*

and *Trichophyton asteroides* at 1.56 mcg/ml, *Candida pseudotropicalis* and *Candida albicans* (No. 3147) at 6.25 mcg/ml, *Candida* sp. Yu 1200, *Candida krusei* and *Saccharomyces cerevisiae* at 25 mcg/ml. But *Candida tropicalis* (NI 7495), *Torula utilis*, *Cryptococcus neoformans* (No. 7496), *Pseudomonas fluorescens*, *Pseudomonas tabaci* and *Xanthomonas oryzae* are not inhibited at 100 mcg/ml. It shows no inhibition against the following Gram-positive and negative bacteria on a nutrient agar at 100 mcg/ml: *Staphylococcus aureus* (FDA 209P, Smith, Terajima), *Sarcina lutea* (PCI 1001), *Micrococcus flavus* (FDA 16), *Bacillus cereus* (No. ATCC 10702), *Bacillus anthracis*, *Bacillus subtilis* (PCI 219, NRRL B-558), *Escherichia coli* (NIHJ, K 12), *Proteus vulgaris* OX 19, *Shigella flexneri* 1a (Ew 8), *Salmonella enteritidis*, *Klebsiella pneumoniae* (PCI 602), *Mycobacterium smegmatis* (ATCC 607).

The intravenous injection of 25 mg/kg causes no death of mice.

Among known antibiotics, hygroscopins A and B[2,3], Antibiotic 5901[4], melanosporin[5] and copiamycin[6,7], are most closely related to gougeroxymycin having similar UV spectra and antifungal activity. However, the latter can be differentiated by its physico-chemical and biological activities.

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