MINIMYCIN, A NEW ANTIBIOTIC

YOHKO KUSAKABE, JUNSAKU NAGATSU, MITSUO SHIBUYA,
OSAMU KAWAGUCHI, CHIEKO HIROSE
and SHIROH SHIRATO

Research Division of the Kaken Chemical Co., Ltd., Tokyo, Japan

(Received for publication September 11, 1971)

A new crystalline antibiotic, minimycin[1], $C_9H_{11}NO_7$, was isolated from the culture broth of *Streptomyces hygroscopicus*. Isolation and properties of the new antibiotic are described. Minimycin is active against both Gram-positive and Gram-negative bacteria, and also possesses significant antitumor activity against transplantable tumors.

During the course of our screening program for new antibiotics, *Streptomyces* sp. 80432 isolated from a soil sample was found to produce a water-soluble antibiotic. This antibiotic, named minimycin, showed not only inhibitory activity against Gram-positive and Gram-negative bacteria, but also antitumor activity. Isolation and purification by a chromatography gave a colorless crystalline substance as needles or prisms. The physical, chemical and biological properties of minimycin were examined, and it was found to be a new antibiotic. This paper describes the fermentation production, isolation procedures, and characterization of minimycin together with its antimicrobial and antitumor activities.

Antibiotic Production

Streptomyces sp. 80432 was isolated from a soil sample collected at Nyugawa, Ehime Prefecture, Japan, and identified as *Streptomyces hygroscopicus*. The antibiotic minimycin was produced in a medium composed of glucose 4.0 %, soy bean meal 2.0 %, wheat embryo 1.0 %, brewer's yeast 0.4 %, sodium chloride 0.2 %, potassium chloride 0.2 % and calcium carbonate 0.2 %, at pH 6.8 before sterilization. Fermentation was carried out in a stirred-jar fermenter for 48~60 hours at 27°C.

Isolation and Purification

The antibiotic was isolated from the culture filtrate; no activity was demonstrated in the mycelium. A procedure for preparation of minimycin is as follows: The cultured broth filtrate (100 liters) was acidified to pH 2.0 with 10 % hydrochloric acid, treated with active carbon (2.0 g per 100 ml), and the mixture was stirred for 30 minutes. The carbon cake was filtered, washed with water, and eluted with 50 % aqueous acetone. The elution was repeated three times with the same solvent, and the combined eluate was concentrated under reduced pressure to a small volume. The concentrate was then chromatographed on a carbon column, and developed with 10 % aqueous acetone after washing with water. The active fraction, eluted with 20 %

aqueous acetone, was collected, and concentrated *in vacuo* to a small volume.

Crude minimycin (4 g) was purified by Sephadex G-15 chromatography in water. The active fraction detected by assay using *Staphylococcus aureus* FDA 209 P was collected, and concentrated under reduced pressure. This active concentrate was again applied to a column of Sephadex G-10, developing with water. The active fraction was concentrated under reduced pressure and finally freeze-dried.

The colorless powder thus obtained was recrystallized either from water or from water-miscible organic solvents (*e.g.* ethanol, methanol, acetone *etc.*). Recrystallization from water gave needles melting at 166°C, and from the other solvents prisms melting at 161°C were obtained. The total yield was 2 g.

Physical and Chemical Properties

Physical and chemical properties of minimycin are summarized in Table 1. Fig. 1 shows the infrared absorption in KBr of minimycin in the needle form and Fig. 2 the spectrum of minimycin in the prism form. The nuclear magnetic resonance spectrum of minimycin in deuterium oxide is shown in Fig. 3.

The above properties of minimycin are similar to those of showdomycin[2,3]. However, minimycin differs from showdomycin with respect to molecular formula, ultraviolet absorption spectrum and infrared absorption spectrum. Thus, the antibiotic minimycin differs from all known antibiotics, and is a new antibiotic. The structural investigation will be reported in near future.

Biological Activity

Table 2 shows the antimicrobial spectrum of minimycin using diffusion techniques on bouillon and glycerine bouillon agar. Minimycin is active against Gram-positive and Gram-negative bacteria, but has no activity against yeasts and fungi.

Table 1. Physical and chemical properties of minimycin.

Appearance	Colorless needles or prisms
Melting point (dec.)	161°C (prisms) 166°C (needles)
Solubility	Soluble in water, methanol Slightly soluble in ethanol, propanol, acetone Insoluble in benzene, hexane, ether
Optical rotation	$[\alpha]_D^{25}+18°$ (c 1, water)
Ultraviolet absorption	Inflection at 230 mμ ($E_{1cm}^{1\%}$ 188.0) in 0.01 N HCl
Color reaction	Positive : hydroxamic acid-$FeCl_3$, BALJET, oxyvanadium-salt, $KMnO_4$ (decolorized) Negative : ninhydrin, ELSON-MORGAN, biuret, tetrazolium chloride, ferric chloride
Molecular weight	245 (vapor pressure osmometry)
Elemental analysis	Found : C 44.15, H 4.62, N 5.84, O 45.39 % Calcd. for $C_9H_{11}NO_7$: C 44.08, H 4.57, N 5.71, O 45.68 %
Rf values on silica gel TLC	wet BuOH 0.42 BuOH - AcOH - H_2O (4 : 1 : 2) 0.57 EtOH-H_2O (4 : 1) 0.75 $CHCl_3$-MeOH-17 % NH_4OH (2 : 1 : 1) upper 0.88 PrOH-Pyr-AcOH-H_2O (15 : 10 : 3 : 12) 0.85
Stability	Stable in acidic and neutral media Unstable in alkaline medium

Fig. 1. Infrared absorption spectrum of minimycin in needle form (KBr).

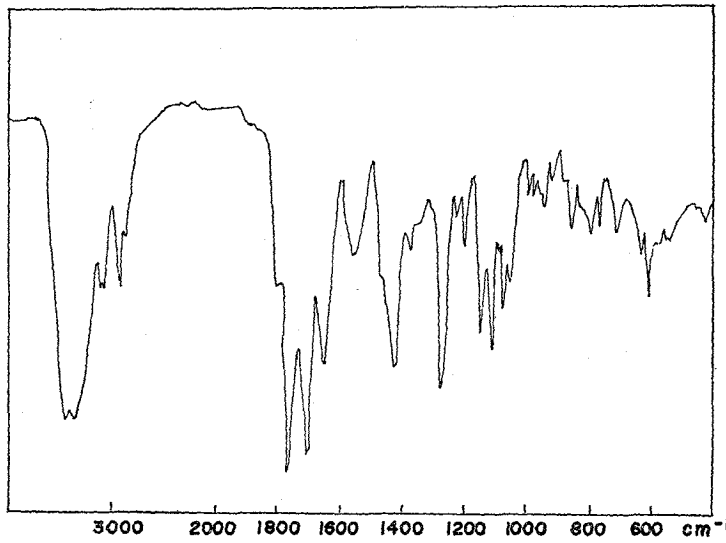

Fig. 2. Infrared absorption spectrum of minimycin in prism form (KBr).

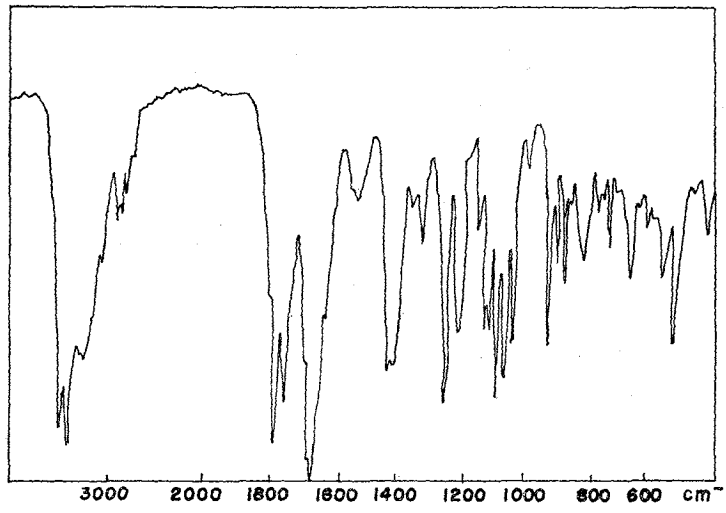

Fig. 3. Nuclear magnetic resonance spectrum of minimycin (D_2O).

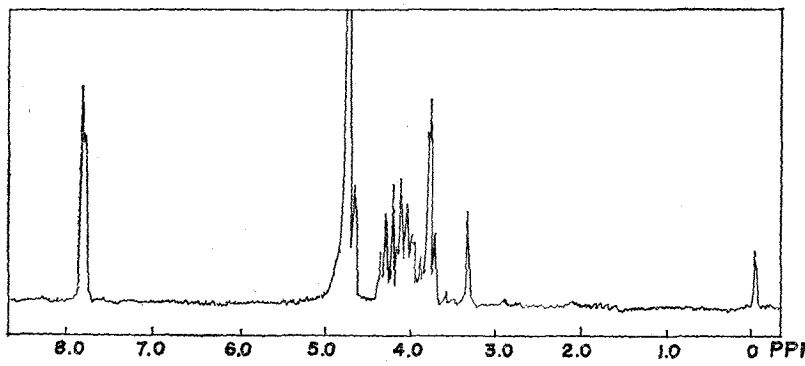

Table 2. Antimicrobial spectrum of minimycin.

Test organisms	Inhibition zone diameter (mm)		
	100 mcg/ml	50 mcg/ml	25 mcg/ml
Bacillus subtilis	27.0	25.0	23.0
Staphylococcus aureus (sensitive)	24.0	20.5	17.5
(resistant)*	24.5	21.5	18.5
Escherichia coli (sensitive)	19.6	17.5	15.5
(resistant)**	17.0	15.0	13.0
Mycobacterium smegmatis	0	0	0

* Resistant to penicillin, chloramphenicol, tetracycline, erythromycin, kanamycin, streptomycin and sulfonamide.
** Resistant to chloramphenicol, tetracycline, kanamycin, streptomycin and sulfonamide.

The acute toxicity of minimycin in mice was examined by intravenous, intraperitoneal and subcutaneous routes of administration. The LD_{50} for mice was 30 mg/kg intraperitoneally, 20 mg/kg subcutaneously and 80 mg/kg intravenously.

The antitumor activity of minimycin was examined against EHRLICH ascites carcinoma, sarcoma 180 (ascites) and sarcoma 180 (solid form) in mice. In the case of the ascites form of EHRLICH carcinoma, inhibition of the tumor growth was observed by intraperitoneal injection at a daily dose of 2 mg/kg for 10 days. The effects on the ascites form of sarcoma 180 and sarcoma 180 (solid form) were similar to those observed in the case of EHRLICH carcinoma.

References

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