MUTALOMYCIN, A NEW POLYETHER ANTIBIOTIC

TAXONOMY, FERMENTATION, ISOLATION AND CHARACTERIZATION

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Mutalomycin is a new metal-complexing polyether antibiotic produced by a strain of *Streptomyces mutabilis* NRRL 8088. The metabolite, a monocarboxylic acid, was isolated as the sodium salt $C_{41}H_{69}NaO_{12}$. The structure of this polyether was established by X-ray analysis of its potassium salt $C_{41}H_{69}KO_{12}$. Mutalomycin contains six heterocyclic rings and is structurally related to nigericin. The metabolite is active against gram-positive bacteria and *Eimeria tenella* (chicken coccidiosis).

In the course of our search for new antibiotics from soil actinomycetes a strain (S 11743/A) of *Streptomyces mutabilis* was isolated which produced a metabolite effective against gram-positive bacteria and chicken coccidiosis. The active compound S 11743/A-1 designated as mutalomycin is a new member of the polyether antibiotic group. This report presents the taxonomy of the producing strain, as well as the fermentation, isolation, characterization and biological activities of mutalomycin.

Taxonomy of the Producing Strain

The mutalomycin-producing strain (NRRL 8088) was isolated from a soil sample collected in the Central African Republic in 1965. The microorganism was identified as a strain of *Streptomyces mutabilis*[1]. It has the fundamental characteristics of the organism, namely, the sporophores have loose open spirals with one to three coils, but generally fitting the description Retinaculum-Apertum (Fig. 1). The silver or lead-gray spores are oval to elliptical to cylindrical, 0.7 ~ 0.9 nm by 1.3 ~ 1.5 nm; the spore surfaces are smooth as determined from the electron micrograph (Fig. 2). However, in contrast to the type strain[1], our strain NRRL 8088 did not utilize sucrose.

Fermentation

Fermentation was performed on a 10-liter scale in New Brunswick glass fermenters. Ten milliliters of a dense spore suspension of the culture NRRL 8088 was used to inoculate one liter of the vegetative growth medium, consisting of 3.0% soluble potato starch, 2.0% cerelose (glucose technical grade), 1.0% soybean meal (Nurupan Inc., Düsseldorf, Germany), 1.0% corn steep powder (Nurupan, Inc., Düsseldorf, Germany), 0.5% Polypeptone (Kobe, Japan), 0.3% NaCl and 0.5% $CaCO_3$ with a pH 7.0. Incubation of the inoculated vegetative flasks was made at 27°C for 3 days on a rotary shaker machine (200 rpm), then the vegetative stage was used to inoculate 10 liters of the fermentation medium composed of 2.0% sucrose, 0.2% peptone (Cudahy Labs., Omaha, Nebraska), 0.2% malt extract, 0.2% yeast extract (NG & SF, Delft, Holland), 0.2% KH_2PO_4 and 0.2% $MgSO_4 \cdot 7H_2O$; pH 7.2. Media were sterilized at 120°C for 20 minutes. The inoculated fermentation tank was incubated for 5 days at 27°C, stirring at 200 rpm and aerating with 1 liter air per minute per liter of medium.

Fig. 1. Photomicrograph of *Streptomyces mutabilis*, strain NRRL 8088 (Malt-yeast extract agar ×960)

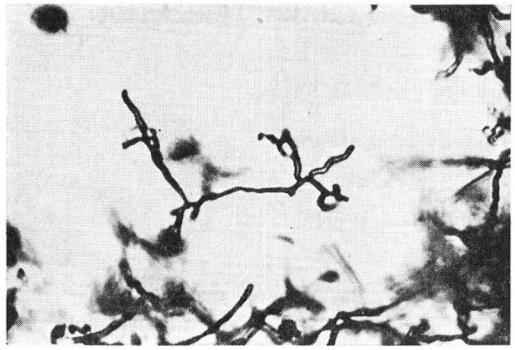
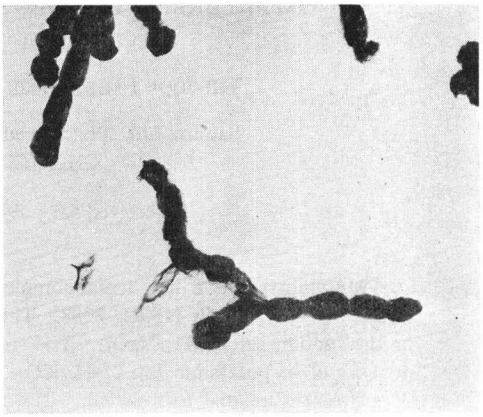


Fig. 2. Electronmicrograph of spores of *Streptomyces mutabilis*, strain NRRL 8088 (Malt-yeast extract agar ×10,500)



Isolation

The fermentation broth (100 liters) was centrifuged, the mycelial cake (6 kg) separated and the supernatant (90 liters) was brought to pH 9 by addition of 1 N NaOH. After filtration the filtrate was extracted three times with 90 liters of ethylacetate, the organic layers washed with water and evaporated under reduced pressure at 20~40°C giving 16 g crude extract.

The mycelial cake was extracted with 12 liters of methanol and twice with 12 liters of methanol-water (9: 1) using an Ultra-Turrax homogenizer. Water (5 liters) was added to the combined filtrates, the resulting solution concentrated under reduced pressure at 40°C to a volume of 3 liters and brought to pH 9 with 1 N NaOH. Extraction of this solution with ethylacetate in an analogus manner as described above furnished an additional 9.4 g crude extract.

The combined extracts were dissolved in 500 ml of toluene, washed with water, the organic phase dried with sodium sulfate and evaporated *in vacuo* at 40°C. The yellow oily residue (11 g) was chromatographed on 550 g silica gel (Merck, 0.06~0.2 mm) using as eluants toluene-acetone (4: 1 and 1: 1), each eluant containing 1% triethylamine. The fractions containing the main activity against *Staphylococcus aureus* were pooled and the solvents removed *in vacuo*.

The resulting solid (7.07 g) was further purified by chromatography on silica gel using chloroform and chloroform with increasing amounts of methanol as solvents. Chloroform-methanol (98: 2) eluted the main portion of mutalomycin. Each fraction was checked by bioassay and by tlc on silica gel plates (silica gel G Merck) in the system toluene-acetone (4: 1) with 1% triethylamine. Detection was made by spraying with a solution of 20% perchloric acid followed by heating to approximately 150°C. Active and pure fractions were combined and the solvents removed *in vacuo*. The residue was dissolved in chloroform and treated with 1 N NaOH, the organic layer washed with water, dried with sodium sulfate and evaporated to dryness. The residue (2.3 g) was then crystallized from acetone (3.5 ml) to yield 0.55 g sodium salt of mutalomycin as white crystals.

Physical and Chemical Properties

Mutalomycin sodium salt crystallizes with 1 mole acetone and melts at 159~163°C (decomp.). A solvent-free preparation was made by crystallization from methanol, m.p. 163~171°C (decomp.),

$[\alpha]_D^{20} + 83.5°$ (*c* 1.0, methanol) and $[\alpha]_D^{20} + 106.2°$ (*c* 1, chloroform). Mutalomycin sodium salt is practically insoluble in water, but dissolves readily in benzene, chloroform, acetone, methanol, ethylacetate and ether.

The proposed formula $C_{41}H_{69}NaO_{12}$ is supported by elemental analysis and determination of the molecular weight by mass spectrometry (M.W. 776).

Calcd. for $C_{41}H_{69}NaO_{12}$ (M.W. 776): C, 63.4; H, 9.0; Na, 2.9; O, 24.7
Found: C, 63.5; H, 9.1; Na, 3.2; O, 24.7

Fig. 3. IR spectrum of mutalomycin sodium salt in CH_2Cl_2

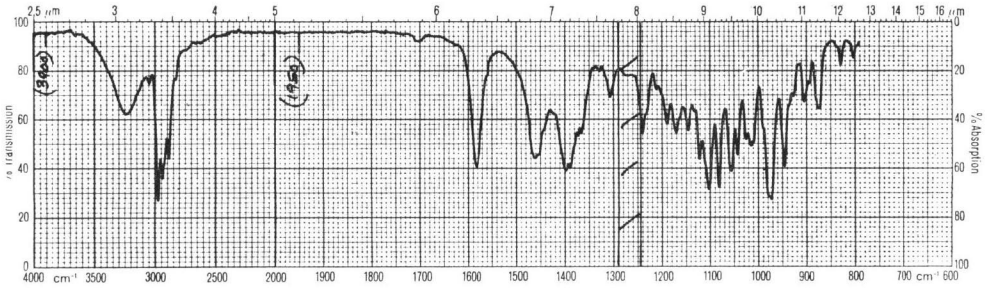
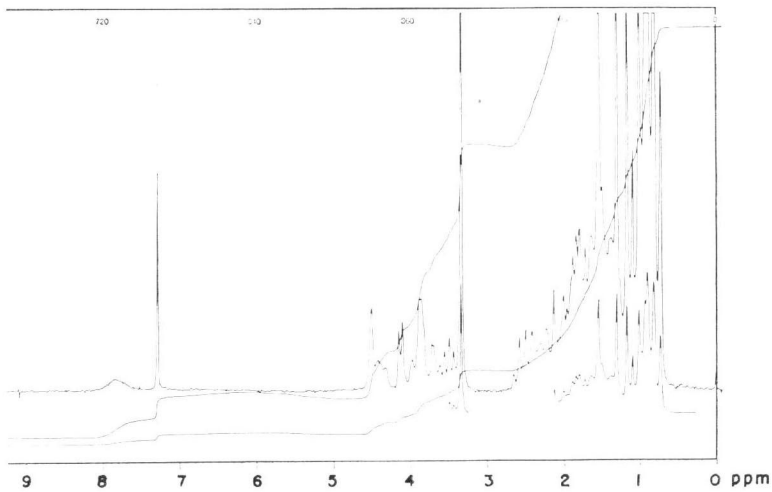


Fig. 4 90 MHz NMR spectrum of mutalomycin sodium salt in $CDCl_3$ (TMS as internal standard)



Mutalomycin sodium salt has no characteristic UV maximum in methanol except end absorption. The IR spectrum (Fig. 3) shows typical bands at 1583 cm^{-1} accounting for carboxylate ion and at 1050/1150 cm^{-1} indicating the ethereal character of several oxygen atoms. In the NMR spectrum (Fig. 4) peaks in the range of δ 0.7~1.6 indicate the presence 10~11 C-methyl groups and a signal at δ 3.3 corresponds to 1 methoxy group.

Comparative thin-layer chromatography of mutalomycin sodium salt and the ionophores nigericin and X-206 is shown in Table 1.

On the basis of the physico-chemical data and the biological properties, mutalomycin was considered to represent a member of the polyether antibiotic group.

Table 1. Rf-values of mutalomycin sodium salt, nigericin[2] and X-206 salt[8] in different solvent systems on silica gel G plates

	Rf-values in solvent systems		
	1	2	3
Mutalomycin	0.57	0.80	0.65
Nigericin	0.11	0.13	0.30
X-206	0.25	0.44	0.49

Solvent systems:
1) Toluene - acetone (4: 1) + 1% triethylamine
2) Chloroform - ethylacetate (1: 1)
3) Chloroform - methanol (95: 5)

Table 2. Antimicrobial spectrum of mutalomycin sodium salt

Organism	MIC (μg/ml)
Staphylococcus aureus	0.3
Streptococcus faecalis	0.01
Sarcina lutea	0.3
Micrococcus lysodeikticus	0.1
Clostridium pasteurianum	0.01
Neisseria pharyngis	0.3
Mycoplasma laidlawii B	0.01

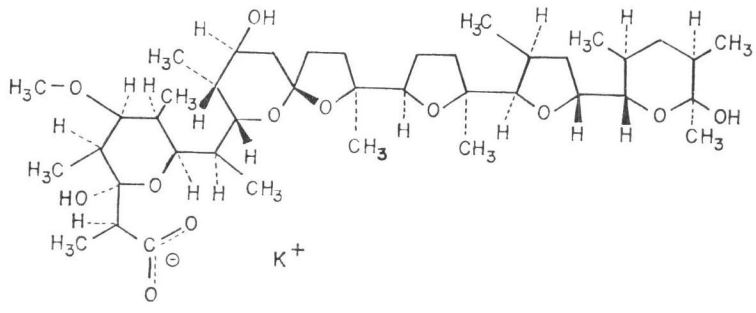
Structure of Mutalomycin

To establish the structure of mutalomycin by X-ray crystallographic studies we synthesized the *p*-bromophenacyl ester as described for septamycin[4], however the compound did not crystallize. We then prepared the potassium salt of mutalomycin which was, as was the potassium salt of alborixin[5], suitable for X-ray analysis. A solution of mutalomycin sodium salt (280 mg) in 40 ml of chloroform was treated twice with 40 ml each of ice cold 2 N HCl and with water to furnish the free acid. This solution immediately was washed twice with 40 ml each of 2 N KOH then with water. After drying the organic layer with K_2CO_3 the solvent was removed *in vacuo*. The residue was dissolved in 2 ml of methanol, filtered and crystallized (146 mg). Recrystallization from 2 ml of boiling methanol furnished 97 mg colourless crystals, m.p. 158~160°C, $[\alpha]_D^{20} + 89.1°$ (*c* 0.98, methanol) and $[\alpha]_D^{20} + 102°$ (*c* 1.1, chloroform).

Calcd. for $C_{41}H_{69}KO_{12}$ (M.W. 793): C, 62.1; H, 8.8; K, 4.9.
Found: C, 61.9; H, 8.9; K, 4.8.

Recrystallization from methanol gave colourless monoclinic prisms of mutalomycin potassium salt. X-ray analysis[6] revealed the structure of this salt (Fig. 5), and its absolute configuration was postulated on its close structural relationship to nigericin[7].

Fig. 5. Structural formula of mutalomycin potassium salt



Biological Properties

Mutalomycin possesses antibiotic activity against gram-positive bacteria. The antimicrobial spectrum *in vitro*, obtained by broth-dilution assay, is shown in Table 2.

Mutalomycin also exhibits an anticoccidial activity in chickens similar to other polyether antibiotics. It is effective in reducing mortality and increasing average body weight of chickens infected with *Eimeria tenella* and other *Eimeria* species.

Acknowledgement

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References

1) BERGEY's Manual of Determinative Bacteriology, 8th Ed., pp. 749, 772 and 777. Williams & Wilkins Co., 1974
2) STEINRAUF, L. K.; M. PINKERTON & J. W. CHAMBERLIN: The structure of nigericin. Biochem. Biophys. Res. Comm. 33: 29~31, 1968
3) BLOUNT, J. F. & J. W. WESTLEY: Crystal and molecular structure of the free acid form of antibiotic X-206 hydrate. J. Chem. Soc. Chem. Comm. 1975: 533, 1975
4) KELLER-JUSLÉN, C.; H. D. KING, Z. L. KIS & A. VON WARTBURG: Septamycin, a polyether antibiotic. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 28: 854~859, 1975
5) ALLEAUME, M.; B. BUSETTA, C. FARGES, P. GACHON, A. KERGOMARD & T. STARTON: X-Ray structure of alborixin, a new antibiotic ionophore. J. Chem. Soc. Chem. Comm. 1975: 411~412, 1975
6) PETCHER, T. J. & H. P. WEBER: X-Ray crystal structure and absolute configuration of mutalomycin potassium salt. Helv. Chim. Acta, in preparation
7) WESTLEY, J. W.: A proposed numbering system for polyether antibiotics. J. Antibiotics 29: 584~586, 1976