LYSOCELLIN, A NEW POLYETHER ANTIBIOTIC

I. ISOLATION, PURIFICATION, PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES

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A new antibiotic, lysocellin (K-5610), was isolated from *Streptomyces cacaoi* var. *asoensis* K-9 Met⁻. Lysocellin was obtained as a colorless crystalline needles from both the cultural filtrate and the mycelium of the organism. The antibiotic melted at 158~160°C and had a molecular formula $C_{34}H_{59}O_{10}Na \cdot \frac{1}{2} H_2O$. It had antimicrobial activity against gram-positive bacteria, antibiotic-resistant *Staphylococcus aureus* and some fungi, but not against gram-negative bacteria. Based on its physico-chemical and biological properties lysocellin was identified as a new polyether antibiotic.

Lysocellin was isolated from a mutant of the polyoxin-producing strain *Streptomyces cacaoi* var. *asoensis*[1]. Unlike polyoxins which are antifungal substances, lysocellin is antibacterial. The mutant strain also produced polyoxin. The present paper describes the physico-chemical and biological properties on lysocellin.

Material and Methods

Streptomyces cacaoi var. *asoensis* K-9 Met⁻ was used in these studies. It is one of a number of methionine auxotrophic strains derived after treatment of the organism with N-methyl-N'-nitro-N-nitrosoguanidine and produced an antibacterial substance. We designated this mutant strain as *Streptomyces cacaoi* var. *asoensis* K-9 Met⁻ and the antibiotic as K-5610 respectively. Later, K-5610 was given the name "lysocellin".

Production of the antibiotic

A few grains of rice cultured spores of *S. cacaoi* var. *asoensis* K-9 Met⁻ were placed into 500-ml conical flasks containing 40 ml of seed medium which consists of 2 % glycerol, 1 % beef extract, 1 % peptone, and 0.3 % K_2HPO_4, and grown on a rotary shaker at 33°C for 40 hours. Two ml of seed cultured mycelium were transferred to 500-ml conical flasks containing 40 ml of the second seed medium which consists of 0.5 % sucrose, 0.5 % soybean oil, 2 % soybean meal, 0.2 % dry yeast and 0.3 % K_2HPO_4 and grown as described above for 24 hours. Seven ml of the second seed-cultured mycelium were transferred to 1.0-litre conical flasks contianing 150 ml of the main culture medium which consists of 1 % sucrose, 1.5 % soybean oil, 4 % soybean meal, 0.2 % dry yeast and 0.3 % K_2HPO_4 and grown in the same manner for one week. Antibacterial activity was found in both the cultural filtrate and the mycelium.

Antibiotic production was assayed on petri dishes by the paper disc method. *Bacillus subtilis* PCI-219 was used as a test organism. Maximum antibacterial activity was obtained in 120-hour culture.

Purification Procedure

Since the antibiotic was precipitated at acidic pH, the brew was adjusted to pH 2.0 with 1 N HCl, centrifuged, and the supernatant was discarded. The precipitated material was immediately suspended in acetone and the pH was adjusted to 8.0 with 1 N NaOH and kept

at room temperature overnight. The suspension was filtered on a Buchner funnel; the filtrate contained the antibiotic.

The acetone solution was concentrated *in vacuo*, and the concentrated material was extracted by *n*-butanol. The *n*-butanol extract was concentrated *in vacuo* which resulted in a brownish syrup. This syrup was dissolved in acetone and was filtered or centrifuged to separate the insoluble matter. The resulting supernatant was concentrated under reduced pressure, and the residue was dissolved in methanol. Lysocellin was crystallized from the concentrated methanol solution after storage at room temperature. Recrystallization was also from methanol.

When the parent strain *S. cacaoi* var. *asoensis* was used in a similar fermentation, no lysocellin production was detected in the culture broth.

Results

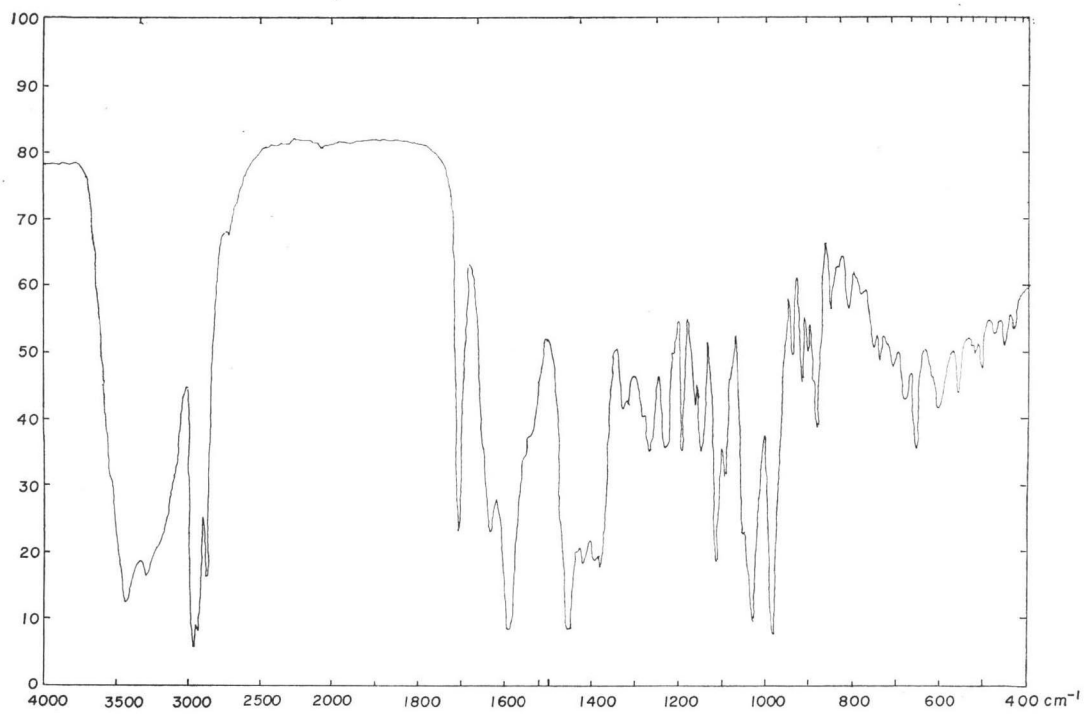
Physico-Chemical Properties

The sodium salt of lysocellin formed colorless needles and melted at 158~160°C. The optical rotation is $[\alpha]_D^{25}+11.5°$ (*c* 1, methanol) and the compound exhibits an absorption maximum of low intensity at 292 nm ($E_{1cm}^{1\%}$ 0.68) in ethanol.

The infrared spectrum in a KBr tablet is shown in Fig. 1. Characteristic bands were observed at 3450~3300 cm^{-1} corresponding to hydroxyl group; 2960 and 2930 cm^{-1} correspounding to methyl and methylene groups; 1710 and 1590 cm^{-1} to carbonyl groups. Elementary analysis and measurement of the molecular weight (656) by the vapor pressure equilibrium method indicated a molecular formula $C_{34}H_{59}O_{10}Na \cdot \frac{1}{2} H_2O$ (molecular weight 659) for sodium salt of lysocellin.

Calcd. for $C_{34}H_{59}O_{10}Na \cdot \frac{1}{2} H_2O$: C 61.88, H 9.18, O 25.46, Na 3.48
Found : C 61.58, H 9.10, O 25.05, Na 3.37

Fig. 1. Infrared spectrum of lysocellin (KBr)



Lysocellin is a monocarboxylic acid with pKa′ of 6.6 when titrated in 66 % dimethylformamide. The sodium salt of lysocellin was soluble in methanol, ethanol, *n*-butanol, ethyl acetate, acetone, benzene and chloroform, and insoluble in water. Lysocellin was stable in neutral solution but labile in acidic solution.

It produced a positive reaction to 2,4-dinitrophenylhydrazine and turned brown with concentrated sulfuric acid. The compound gave negative reactions to potassium permanganate, ferric chloride and MOLISCH reactions. On thin-layer chromatograms of silica gel, the following Rf values were observed: 0.54 with ethyl acetate, 0.30 with benzene-methanol (9 : 1), 0.63 with chloroform-methanol (9 : 1).

Biological Properties

Antimicrobial activities of lysocellin are listed in Table 1. Lysocellin is active against gram-positive bacteria, antibiotic resistant *Staphylococcus aureus*, and is also active against some fungi, but it is not active against gram-negative bacteria. When *Bacillus subtilis* was grown on liquid medium, lysocellin caused bacterial lysis.

Lysocellin was inactive against *Staph. aureus* infection in mice when given by intraperitoneal administration. The LD_{50} of this antibiotic is about 70 mg/kg, given intraperitoneally in mice.

Table 1. Antimicrobial spectra of lysocellin

Microorganims	Minimum inhibitory concentration (μg/ml)
Staphylococcus aureus FDA 209P	10
Antibiotic resistant *S. aureus*[1]	4
Sarcina lutea	10
Bacillus subtilis	10
Mycobacterium smegmatis	20
Escherichia coli	>100
Antibiotic resistant *E. coli*[2]	>100
Shigella dysenteriae	>100
Salmonella typhimurium	>100
Pseudomonas aeruginosa	>100
Botrytis cinerea	50
Helminthosporium oryzae	50
Fusarium oxysporum	100
Alternaria kikuchiana	100

1) Resistant to streptomycin, erythromycin, chloramphenicol and penicillin.
2) Resistant to streptomycin, chloramphenicol, kanamycin, tetracycline and sulfonamide.

Discussion

Lysocellin is in the polyether group of antibiotics. To this group also belong the monensins[2], nigericin[3], dianemycin[4], X-206[5], X-537A (lasalocid A)[5], grisorixin[6], A204A[7] and salinomycin[8]. These compounds can be distinguished from the new antibiotic, lysocellin, on the basis of their physico-chemical properties.

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References

1) ISONO, K.; J. NAGATSU, Y. KAWASHIMA & S. SUZUKI: Studies on polyoxins, antifungal antibiotics. I. Isolation and characterization of polyoxins A and B. Agr. Biol. Chem. 29: 848~854, 1965
2) HANEY, M. E., Jr. & M. M. HOEHN: Monensin, a new biologically active compound. I. Discovery and isolation. Antimicr. Agents & Chemoth. -1967: 349~352, 1968
3) HARNED, R. L.; P. H, KIDY, C. J. CORUM & K. L. JONES: Nigericin, a new crystalline antibiotic from an unidentified *Streptomyces*. Antibiot. & Chemoth. 1: 594~596, 1951
4) HAMILL, R. L., M. M. HOEHN, G. E. PITTENGER, J. CHAMBERLIN & M. GORMAN: Dianemycin, an antibiotic of the group affecting ion transport. J. Antibiotics, Ser. A 22: 161~164, 1969
5) BEGER, J.; A. I. RACHLIN, W. E. SCOTT, L. H. STERNBACH & M. W. GOLDBERG: The isolation of three new crystalline antibiotics from *Streptomyces*. J. Am. Chem. Soc. 73: 5295~5298, 1951.
6) GACHON, P.; A. KERGOMARD, H. VESCHAMBRE, C. ESTEVE & T. STARON: Grisorixin, a new antibiotic related to nigericin. Chem. Commun. 1970: 1421~1422, 1970
7) JONES, N. D.; M. O. CHANEY, J. W. CHAMBERLIN, R. L. HAMILL & SUE CHEN: Structure of A 204A, a new polyether antibiotic. J. Am. Chem. Soc. 95: 3399~3400, 1973
8) MIYAZAKI, Y.; M. SHIBUYA, H. SUGAWARA, O. KAWAGUCHI, C. HIROSE, J. NAGATSU & S. ESUMI: Salinomycin, a new polyether antibiotic. J. Antibiotics 27: 814~821. 1974