DEXTROCHRYSIN, A NEW ANTIBIOTIC

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Dextrochrysin is produced in culture broths of *Streptomyces calvus* var. *dextrochrysus*, and can be isolated in crystalline form by solvent extraction and silica gel column chromatography. It is obtained as neutral yellowish brown platelets, m. p. 250~255°C and shows strong dextrorotation. It has antimicrobial activity against Gram-positive and Gram-negative bacteria, a *Mycobacterium* and some bacteriophages, but is highly toxic to mice. In *in vivo* anti-tumor test with EHRLICH carcinoma and YOSHIDA sarcoma cells it did not show a favorable effect.

In the course of our anti-phage antibiotic screening program[1], a *Streptomyces* initially designated strain 1615 was found to produce an antibiotic with strong activity against some bacteriophages, Gram-positive and Gram-negative bacteria. From its unique physicochemical properties, it was judged to be a new antibiotic and named dextrochrysin.

In this paper, the characteristics of strain 1615, fermentation and isolation procedures, and chemical and biological properties of dextrochrysin are described.

Characters of Strain 1615

Streptomyces No. 1615 was isolated from a soil sample collected at Hattori, Toyonaka city; Osaka Prefecture. The cultural characteristics, morphological and physiological properties of strain 1615 are listed in Table 1. The media used for taxonomic studies were inoculated and incubated for 13~15 days at 30°C, except the gelatin stab. The growth on the gelatin stab was examined after 20 days at room temperature.

The distinctive characters of 1615 strain are as follows:

(1) The color of well matured aerial mycelium is gray and that of substrate growth white or yellowish brown. Some greenish yellow or yellowish brown soluble pigment is produced on nutrient or synthetic medium.
(2) This strain belongs to the so-called non-chromogenic group. However, it has a somewhat chromogenic character as seen on nutrient agar or on milk.
(3) Aerial mycelium is short and branched, tips of hyphae form loops or spirals.
(4) Proteolytic and diastatic activities are strong.
(5) The type of carbon utilization is characteristic. Pentoses are hardly utilized, whereas sugar alcohols are readily utilized.

The taxonomic keys of WAKSMAN's "The Actinomycetes" Vol. 2[2] were used to compare the culture with recognized species of the genus *Streptomyces*. Strain 1615

Table 1. Cultural characteristics of strain 1615

Medium	Growth	Aerial mycelium	Soluble pigment	Remarks
CZAPEK's agar	Pale grayish white Poor growth	Pale gray Poor growth	None	
Starch-ammonium agar	Yellowish gray-white	Dark gray powdery	None	Diastatic action rather strong
Glucose asparagine agar	White pale cream	White powdery	None	
Calcium malate agar	Pale yellowish	Thin, white powdery	Pale yellowish	
Tyrosin agar	Colorless	White powdery Poor growth	None	
Bouillon agar	Yellowish brown	White Thick cottony	Pale yellowish brown	
BENNETT's agar	Yellow	Dark gray powdery	Pale greenish yellow	Growth at 37°C weak
Glucose bouillon	Thick cream ring growth	White powdery	None	pH unchanged
Glucose CZAPEK's solution	Thin colorless membranous	None	Pale yellowish	Nitrite negative
Milk	Brownish cream Ring growth	None	Pale brownish	Peptonization strong Coagulation negative
Gelatin	Creamy growth	None	None	Liquefaction strong
Potato plug	Brown flat growth	White powdery	Brown	
Cellulose	No growth			
Morphology	Aerial hyphae	Short branched with much curvature		
Carbon utilization	Utilized	Glucose, fructose, trehalose, mannit, inosit		
	Doubtful	Arabinose, mannose, sucrose, lactose, raffinose		
	Not utilized	Xylose, rhamnose, salicin		

showed some characteristics in common with *Streptomyces albogriseolus*, *S. cacaoi*, *S. calvus*, *S. chibaensis*, *S. diastaticus*, *S. flaveolus* and *S. griseoluteus*, but seemed to be identical with *S. calvus* in the main criteria used for identification of *Streptmyces*. Strain 1615 differed from *S. calvus* in the following characters: it produced some soluble pigment, did not coagulate milk, showed strong peptonization and produced a new antibiotic dextrochrysin and not nucleosidin which is known to be a product of *S. calvus*[3]. From these considerations, it seems to be most reasonable to identify strain 1615 as a variant of *S. calvus* and the name of *Streptomyces calvus* var. *dextrochrysus* is proposed.

Fermentation

S. calvus var. *dextrochrysus* grows well at 25~30°C in agitated and aerated submerged culture. A typical fermentation medium suitable for production of

dextrochrysin contains the following ingredients: glucose 2%, gluten meal (Nihon Shokuhin) 2%, corn steep liquor 2% and calcium carbonate 0.3%. Tap water was used to prepare the medium and the pH was adjusted to 6.2~6.5 by adding NaOH solution.

Dextrochrysin was produced by a 2-day fermentation under suitable submerged culture conditions and the activity was found mainly in the broth filtrate. Excessive aeration seemed to be harmful for dextrochrysin production.

Antibiotic potency was determined by an agar diffusion method with *Staphylococcus aureus* 209P as the test organism on nutrient agar at pH 7.0. Crystalline dextrochrysin was used as a standard.

Isolation Procedure

Approximately 90 liters of whole broth, with 0.5% of 'Filter Cel', was filtered. The filtrate was extracted with three 20-liter portions of *n*-butanol. The extracts were combined and concentrated *in vacuo* to about 1 liter. The concentrate was redissolved in 2 liters of *n*-butanol and extracted with three 2-liter portions of very dilute hydrochloric water (pH 3.0). Some impurities were removed by this acidic water extraction. The butanol layer was concentrated *in vacuo* at 40°C to 300 ml of syrup, to which 1 liter of *n*-hexane was added. The resulting precipitate was filtered and dried. About 30 g of brown powder was obtained. This powder was resolved in methanol and subjected to chromatography through a 50 mm×450 mm column containing 350 g of Merck Silica Gel. After impurities were washed out with ethyl acetate, antimicrobially active fractions were eluted with an ethyl acetate-methanol mixture (9:1). Active fractions were collected and evaporated *in vacuo* to yield about 300 mg of product. The dried powder was dissolved in 2 ml of dimethylformamide and the solution diluted with 20 ml of methanol. When this was kept in cold overnight about 200 mg of dextrochrysin was obtained as yellowish brown platelets.

Chemical Properties

Dextrochrysin is a neutral substance, m.p. 250~255°C (dp). Elementary analysis gave the following data: C 61.47, H 6.82, N 10.25, O (difference) 21.46. The molecular weight of this antibiotic was difficult to determine because of its low solubility in suitable solvents. The ultraviolet absorption spectrum (Fig. 1) in methanol exhibits maxima at 230~240 mμ (shoulder) and 330~338 mμ (broad band). The infrared and NMR spectra of dextrochrysin are shown in Fig. 2 and Fig. 3, respectively. Dextrochrysin

Fig. 1. Ultraviolet absorption spectrum of dextrochrysin in methanol.

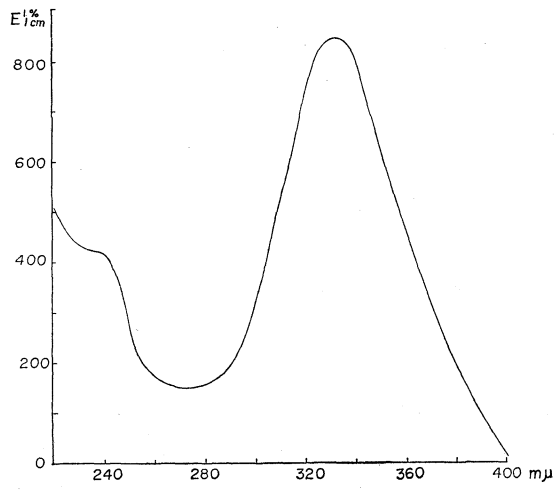


Fig. 2. Infrared spectrum of dextrochrysin (in nujol).

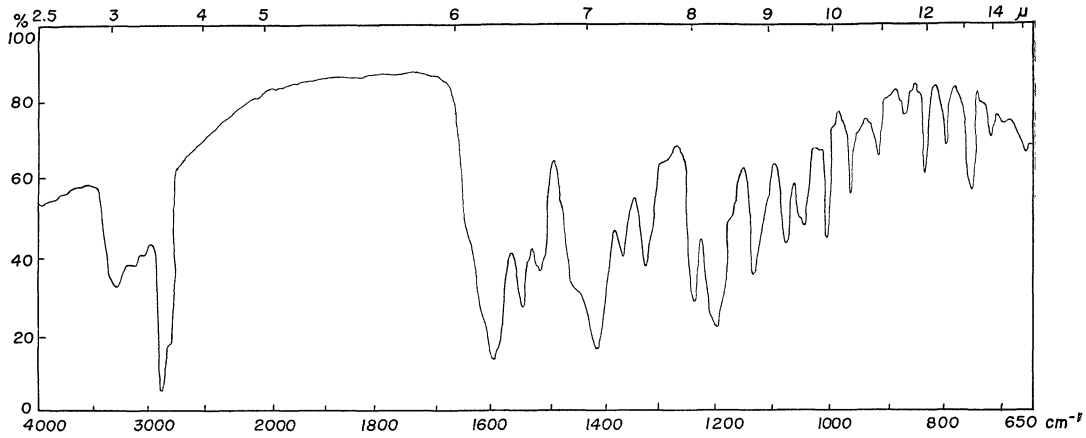
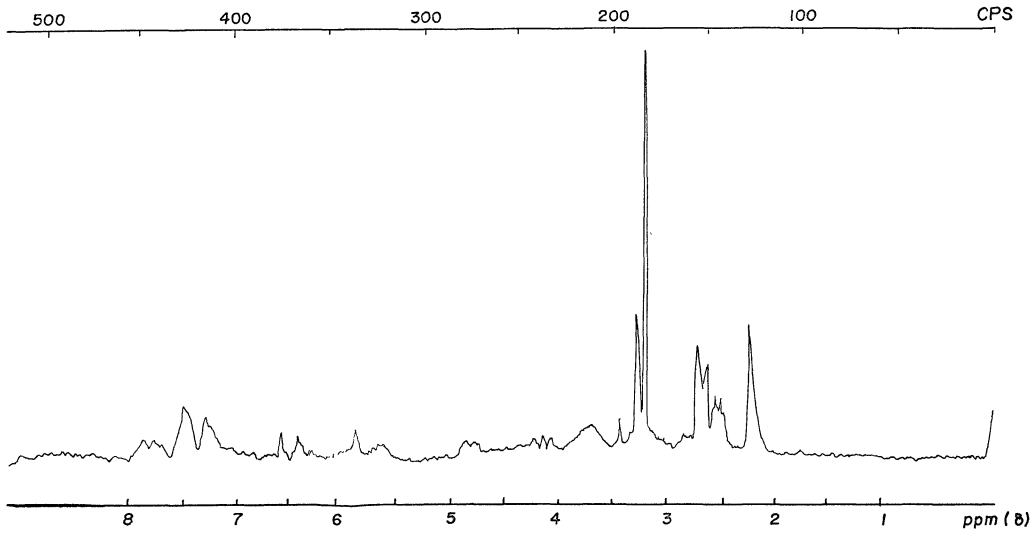


Fig. 3. NMR spectrum of dextrochrysin in dimethyl sulfoxide.



is optically active and shows high dextrorotation of $[\alpha]_D^{24}$ +837.9° (1 %, dimethyl formamide). Dextrochrysin is easily soluble in dimethylformamide and dimethylsulfoxide, fairly soluble in methanol, ethanol and *n*-butanol, and slightly soluble in ethyl acetate.

This antibiotic decolorizes potassium permanganate solution, gives positive EHRLICH and ferric chloride reactions, and negative ninhydrin and DRAGENDORFF tests.

Biological Characteristics

The antimicrobial spectrum of dextrochrysin by the agar dilution method is shown in Table 2. The antibiotic has activity against Gram-positive and Gram-negative bacteria (except *Pseudomonas*) and *Mycobacterium* sp. 607.

Dextrochrysin is highly active against some bacteriophages. Using AOKI's anti-phage-screening method[1], agar plates containing 10^4 phages and 10^7 host cells were

prepared and pulp discs of 6 mm diameter which adsorbed 100 mcg of dextrochrysin were placed on the plates, which was then incubated at 37°C overnight. An inhibition zone of 34 mm was observed on SP 10 cl phage mixed with *B. subtilis* W 23 and 30 mm on phage mixed with *E. coli* K-12.

The anti-tumor tests were carried out by Dr. NISHIDA of our research laboratory. The results are shown in Table 3. In spite of strong activity against bacteriophage *in vitro*, no *in vivo* effect against EHRLICH ascitic carcinoma or YOSHIDA sarcoma was observed.

Dextrochrysin is highly toxic to animals. The LD_{50} of dextrochrysin is 0.75 mg/kg when administered intravenously to mice.

Table 2. Antimicrobial spectrum of dextrochrysin

Test organisms	Minimum inhibitory concentration (mcg/ml)
Bacillus subtilis PCI 219	1.25
Bacillus megaterium	1.25
Staphylococcus aureus 209P	0.63
// penicillin resistant	0.63
// kanamycin resistant	0.63
// streptomycin resistant	0.63
Sarcina lutea PCI 1001	2.5
Escherichia coli NIH	5.0
Corynebacterium xerosis	2.5
Proteus vulgaris	1.25
Pseudomonas aeruginosa	160
Pseudomonas solanacerum	80
Erwinia aroidae	20
Mycobacterium sp. 607	1.25
Saccharomyces cerevisiae	10
Candida utilis	10
Candida albicans	>160
Penicillium chrysogenum	10
Aspergillus niger	40

Table 3. Anti-tumor activity of dextrochrysin

(1) On EHRLICH ascitic carcinoma

Days after inoculation	Daily dose mcg/kg	Total dose mcg/kg	No. of mice	Average survival days	Average survival time ratio $\frac{\text{Treated gr.}}{\text{Control gr.}} \times 100$ (%)
1	200	200	7	18	100
1	100	400	7	16	89
1	50	350	7	17	95
1	20	140	7	18	100
Control 1	—	—	7	18	

2×10^6 cells/mouse were inoculated.
Dextrochrysin was dissolved in dimethylformamide, diluted with phosphate buffer and injected intraperitoneally.

(2) On YOSHIDA sarcoma

Hours after inoculation	Daily dose mcg/kg	Total dose mcg/kg	No. of rat	Average survival days	Average survival time ratio
1	50	50	4	8.8	140
1	100	100	4	9.0	143
1	200	200	4	4.8	76
1	400	400	4	1.5	24
Control	—	—	4	6.3	100
2	25	75	4	5.5	71
2	50	150	4	11.0	141
2	100	300	4	11.0	141
Control	—	—	4	7.8	100
2	12.5	62.5	4	9.8	110
2	25	125	4	9.3	104
2	50	250	4	5	56
Control	—	—	4	8.9	100

1×10^8 cells/rat were inoculated intraperitoneally.

Discussion

Dextrochrysin seems to be related to anthramycin[4] in its physicochemical and biological properties. However, some clear differences were found, as seen in Table 4. It was concluded that dextrothrysin is a new antibiotic. Its name reflects its strong dextrorotatory activity and the yellowish color of its crystalline powder.

Table 4. Comparison of dextrochrysin and anthramycin

	Dextrochrysin	Anthramycin
MP	250~255°C (dp)	188~194°C (dp)
$[\alpha]_D^{25}$	+837.9° (*c* 1, dimethyl formamide)	+930° (*c* 1, dimethyl formamide)
Analysis	C 61.47	C 61.17
	H 6.82	H 5.56
	N 10.25	N 13.26
Rf. (Silica gel / EtOAc : MeOH 4 : 1)	0.46	0.50

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References

1) AOKI, H. & H. SAKAI: Antiphage property as a screening test for antitumor agents. J. Antibiotics, Ser. A 20 : 87~92, 1967.
2) WAKSMAN, S. A.: The Actinomycetes, Vol. 2. The Williams and Wilkins Co., 1961.
3) BACKUS, E. J.; H. D. TRESNER & T. H. CAMPBELL: The nucleocidin and alazopeptin producing organisms: Two new species of *Streptomyces*. Antibiot. & Chemoth. 7 : 532~541, 1957.
4) LEIMGRUBER, W.; V. STEFANOVIC, F. SCHENKER, A. KARR & J. BERGER: Isolation and characterization of anthramycin, a new anti-tumor antibiotic. J. Am. Chem. Soc. 87 : 5791~5793, 1965.