ADECYPENOL, A UNIQUE ADENOSINE DEAMINASE INHIBITOR CONTAINING HOMOPURINE AND CYCLOPENTENE RINGS

TAXONOMY, PRODUCTION AND ENZYME INHIBITION

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Adecypenol, which exhibits potent inhibitory activity against calf intestinal adenosine deaminase (EC 3.5.4.4), was isolated from the cultured broth of *Streptomyces* sp. OM-3223. Adecypenol was classified as a semi-tight binding inhibitor. The *Ki* value against calf intestinal adenosine deaminase was 4.7×10^{-9} M. No acute toxicity of adecypenol was observed at 100 mg/kg in mice. Adecypenol exhibited no antimicrobial activity against various bacteria and fungi at the concentration of 1.0 mg/ml.

In the course of our screening work for new adenosine deaminase inhibitors from microorganisms, a new inhibitor containing cyclopentene ring, adecypenol (Fig. 1), was isolated from the cultured broth of *Streptomyces* sp. OM-3223 which was isolated from a soil sample collected at Inokashira Park, Musashino-shi, Tokyo. The isolation, physico-chemical properties and structure were reported in the preceding paper[1].

In the present paper, we describe the taxonomy of the producing strain, fermentative production and biological properties of adecypenol, and kinetics of its enzyme inhibition.

Taxonomy of the Producing Strain OM-3223

Morphology

The vegetative mycelia grow abundantly on both synthetic and complex agar media, and do not show fragmentation into coccoid or bacillary

Fig. 1. Structure of adecypenol.

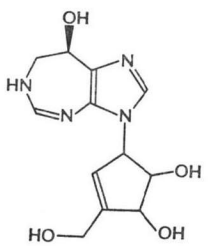


Plate 1. Scanning electron micrograph of aerial mycelia of strain OM-3223 grown on inorganic salts-starch agar.
Bar represents 1 μm.

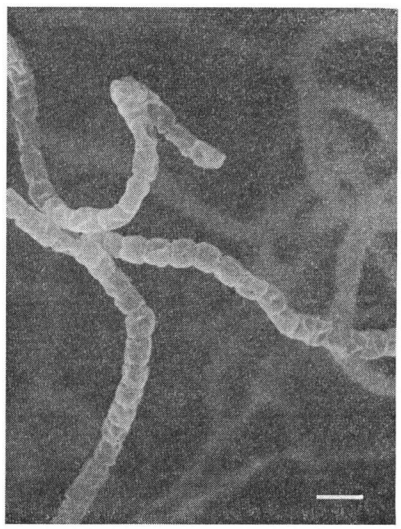


Table 1. Cultural characteristics of strain OM-3223.

Yeast extract - malt extract agar*	G:	Good, mustard gold (2pe)
	R:	Amber (3pe)
	AM:	Abundant, velvety, powder blue (13-ec) or pearl gray (13a)
	SP:	—
Oatmeal agar*	G:	Good, penetrant, light yellow (1-ea)
	R:	Light yellow (1-ea)
	AM:	Poor, shell pink (5ba)
	SP:	—
Inorganic salts - starch agar*	G:	Good, yellow maple (3ng)
	R:	Center, topaz (3ne); outside, light ivory (2ca)
	AM:	Abundant, velvety, dawn blue (15dc) or pearl (13ba)
	SP:	—
Glycerol - asparagine agar*	G:	Moderate, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	—
	SP:	—
Glucose - asparagine agar	G:	Moderate, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	Poor, white (a)
	SP:	—
Peptone - yeast extract - iron agar*	G:	Good, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	—
	SP:	—
Tyrosine agar*	G:	Good, bamboo (2qc)
	R:	Center, light ivory (2ca); outside, light beige (3ec)
	AM:	—
	SP:	—
Sucrose - nitrate agar*	G:	Moderate, pearl (3ba)
	R:	Pearl (3ba)
	AM:	—
	SP:	—
Glucose - nitrate agar**	G:	Poor, pearl (3ba)
	R:	Pearl (3ba)
	AM:	—
	SP:	—
Glycerol - calcium malate agar**	G:	Good, mustard gold (2ne)
	R:	Mustard gold (2ne)
	AM:	Poor, shell pink (5ba)
	SP:	—
Glucose - peptone agar**	G:	Moderate, pearl pink (3ca)
	R:	Pearl pink (3ca)
	AM:	Very poor, flesh pink (4ca)
	SP:	—
Nutrient agar**	G:	Good, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	—
	SP:	—

* Medium recommended by ISP.

** Medium recommended by S. A. WAKSMAN.

Abbreviations: G; Growth of vegetative mycelium, R; reverse, AM; aerial mycelium, SP; soluble pigment.

Table 2. Physiological properties of strain OM-3223.

Melanin formation	−
Tyrosinase reaction	−
H_2S production	−
Liquefaction of gelatin (21°C)	+
Peptonization of milk	+
Coagulation of milk	−
Cellulolytic activity	±
Hydrolysis of starch	+
Temperature range for growth	13~34°C

+; Active, ±; weakly active, −; not active.

Table 3. Utilization of carbon sources by strain OM-3223.

D-Glucose	+
D-Fructose	±
L-Rhamnose	−
D-Mannitol	−
L-Arabinose	±
i-Inositol	+
Raffinose	±
D-Xylose	+
Sucrose	±
Melibiose	−
Cellulose	±

+; Utilized, ±; weakly utilized, −; not utilized.

elements. The velvety aerial mycelia grow abundantly on yeast extract - malt extract agar and inorganic salts - starch agar. The sporophores are of the *Rectiflexibilis* type and have more than 20 spores per chain (Plate 1). The spores are cylindrical in shape, 0.5×0.8 μm in size and have a smooth surface (Plate 1). Sclerotic granules, sporangia and flagellated spores were not observed.

Type of Diaminopimelic Acid in Cell Wall

LL-2,4-Diaminopimelic acid (DAP) was detected in the cell wall of the strain by the method of LECHEVALIER and LECHEVALIER[2].

Cultural and Physiological Characteristics

The International Streptomyces Project (ISP) media recommended by SHIRLING and GOTTLIEB[3] and those recommended by WAKSMAN[4] were used. Cultures were observed after incubation at 27°C for two weeks. Color names and hue numbers indicated in Table 1 are those of Color Harmony Manual (4th Ed.) published by Container Corporation of America. The utilization of carbon sources was tested by growth on PRIDHAM and GOTTLIEB's medium containing 1% carbon source at 27°C. The cultural and physiological characteristics and the utilization of carbon sources of strain OM-3223 are shown in Tables 1, 2 and 3, respectively.

The strain exhibits the following properties. Sporophore, *Rectiflexibilis*; spores, cylindrical and smooth surface; color of vegetative mycelium, gold or ivory; color of aerial mycelium, bluish gray or pearl; soluble pigment, none; DAP in cell wall, LL-type. Based on the taxonomic properties described above, strain OM-3223 is considered to belong to the genus *Streptomyces*; and to be a strain of the gray series of the PRIDHAM and TRESNER's system[5]. The strain was deposited in Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the name *Streptomyces* sp. OM-3223 and the accession No. is FERM P-8447.

Production

A stock culture of strain OM-3223 was inoculated into 100 ml of a seed medium (pH 7.0) consisting of glucose 2.0%, peptone 0.5%, meat extract 0.5%, yeast extract 0.1%, NaCl 0.5% and $CaCO_3$ 0.3% in a 500-ml Erlenmeyer flask and incubated at 30°C for 3 days. The seed culture (600 ml) was transferred to 20 liters of a production medium (pH 8.0) containing glucose 2.0%, peptone 0.5%, meat extract 0.5%, yeast extract 0.1%, NaCl 0.5% and $CaCO_3$ 0.3% in a 30-liter jar fermentor and the aerobic fermentation was carried out at 30°C. Adecypenol concentration was monitored using its adenosine deaminase inhibiting activity assayed by the modified method[6] of MEIER and CONSCIENCE[7], and

Fig. 2. Time course of production of adenosine deaminase inhibitors.

Each simbol exhibits ● adecypenol; ○ coformycin; ▲ mycelial growth; ■ pH.

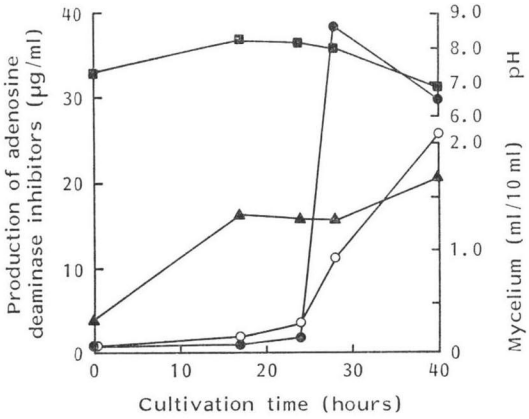
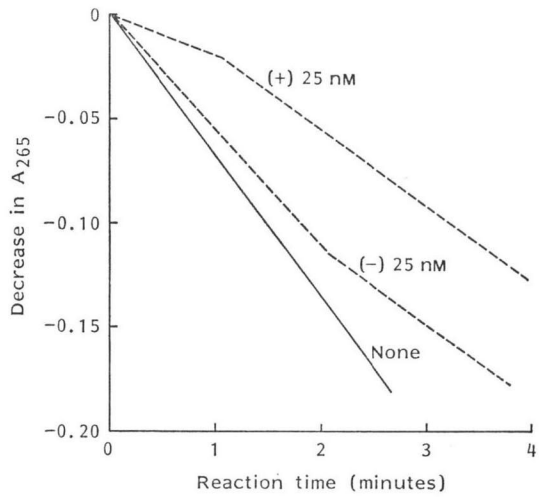


Fig. 3. The effect of preincubation on the adenosine deaminase inhibition by adecypenol.

Calf intestinal adenosine deaminase (EC 3.5.4.4 type VI; Sigma Chem. Co.) was used. +; Preincubated for 15 minutes, −; non-preincubated.



HPLC (ODS column, 0.1 M AcONa buffer (pH 5.75) - 5% CH_3CN, retention time 6.7 minutes).

As shown in Fig. 2, adecypenol production occurred at 24 hours after inoculation and then rapidly increased. The production reached maximum value (40 μg/ml) at 28 hours, then decreased gradually. The strain was found to coproduce coformycin, which occurred at 24 hours after inoculation, then reached 27 μg/ml at 40 hours.

Kinetics of Inhibition of Adenosine Deaminase by Adecypenol

Adenosine deaminase inhibiting activity was assayed by spectrophotometric tracing of the reaction mixture using calf intestinal adenosine deaminase (EC 3.5.4.4) as described by AGARWAL *et al.*[8]. Kinetics was also examined as described by them. They classified adenosine deaminase inhibitors into three types; readily reversible, semi-tight-binding and tight-binding inhibitors. As shown in Fig. 3, the inhibitory effect of adecypenol was enhanced by preincubation with the enzyme for 15 minutes. However, the inhibition was not complete and the enzyme reaction proceeded gradually. Thus, adecypenol was considered to be an inhibitor of semi-tight-binding type, like *erythro*-9-(2-hydroxyl-3-nonyl)adenosine (EHNA) reported by AGARWAL[8]. The *Ki* value of adecypenol was calculated according to the method of LINEWEAVER and BURK[9]. Fig. 4 shows the result of kinetic analysis for adecypenol. The velocity was determined after the attainment of a steady state in the binding between adecypenol and adenosine deaminase (after about 3 minutes). The double reciprocal plots yielded patterns consistent with classical competitive inhibition. The replot of the slope vs. adecypenol concentration was linear and yielded a *Ki* value of 4.7×10^{-9} M.

Biological Properties

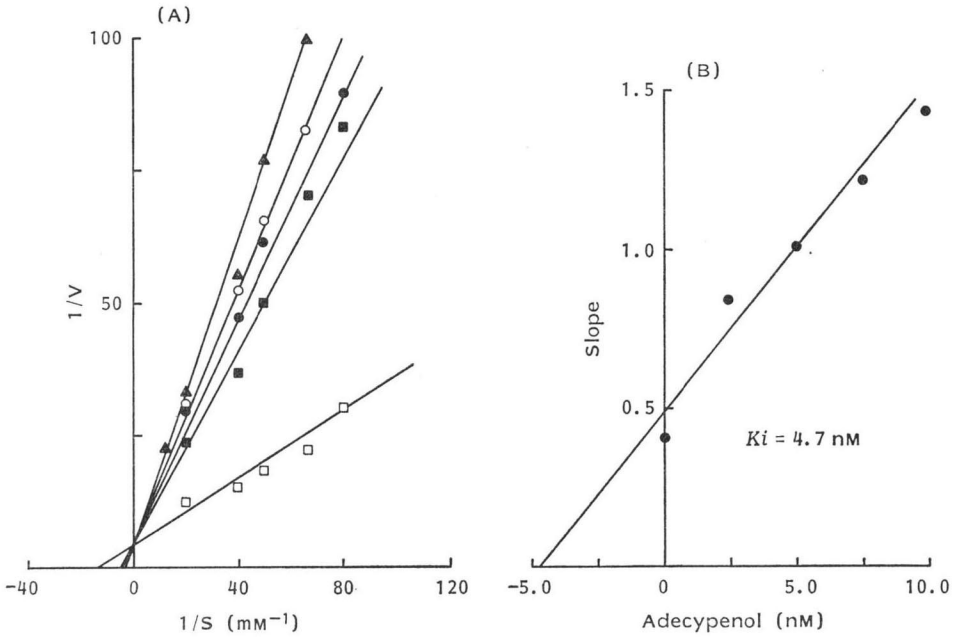
The adenosine deaminase inhibitor adecypenol did not exhibit antimicrobial activity against various bacteria and fungi tested at the concentration of 1.0 mg/ml by the paper-disc method.

When adecypenol was administered intravenously to mice at the dose of 100 mg/kg, no acute toxicity was observed.

Fig. 4.

A) Double reciprocal plot of adenosine deaminase reaction in the presence of adecypenol.
Adenosine deaminase (0.01 units) and adecypenol (concentration as indicated) were incubated at room temp for 10 minutes in a total volume of 1.8 ml containing 50 mM phosphate buffer, pH 7.5. The steady state velocities were taken 3 minutes after the start of the reaction. The reaction was started by the addition of 200 μl of varying concentrations of adenosine.
Adecypenol ▲; 10.0, ○; 7.5, ●; 5.0, ■; 2.5, □; 0 nM.

B) The replot of slope vs. adecypenol concentration.
The *Ki* value of adecypenol was estimated to be 4.7 nM.



Discussion

Three adenosine deaminase inhibitors, adechlorin, coformycin and deoxycoformycin, have been reported to be produced by actinomycetes. These inhibitors are classified as of the tight-binding type and possess potent inhibitory activity against the enzyme. This characteristic would be due to the fact that the inhibitors contain the unique chromophore homopurine, 8(*R*)-3,6,7,8-tetrahydroimidazo[4,5-*d*]-[1,3]diazepin-8-ol, which is a structural analog of an intermediate from adenosine to inosine[10].

On the other hand, the new adenosine deaminase inhibitor reported here, adecypenol, is of the semi-tight-binding type, although it contains the same chromophore as the three known inhibitors, the activity of which is equivalent to that of EHNA which contains adenine.

It is of interest to investigate the structure-adenosine deaminase inhibiting activity relationship of the analog containing the homopurine.

Acknowledgments

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