DEISOVALERYLBLASTMYCIN PRODUCED BY *STREPTOMYCES* SP.*

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A new antifungal antibiotic was isolated from the fermentation broth of *Streptomyces* sp. 5140-A_1. Degradation studies of the crystalline antibiotic, m.p. 186~188°C, $C_{21}H_{28}O_8N_2$, suggested its structure to be deisovaleryl-blastmycin. It exhibited antimicrobial activity against *Piricularia oryzae* and less toxicity against killifish than antimycin A—blastmycin antibiotics.

During screening of new antibiotics, a strain of *Streptomyces* sp. 5140-A_1, isolated from a soil sample collected at Moiwa Forest, Sapporo-city, Japan, was found to produce a new antifungal antibiotic, which was closely related to the antimycin A—blastmycin group of antibiotics[1~5]. Its structure was finally determined as deisovaleryl-blastmycin (**II**). In this report, fermentation, purification procedures, and chemical and biological properties are discussed.

Fermentation

Streptomyces sp. 5140-A_1 was cultivated in the medium containing starch 2.5%, soybean meal 1.5%, dry yeast 0.2%, ammonium sulfate 0.2%, sodium chloride 0.5% and calcium carbonate 0.4% at pH 6.2 (before sterilization).

Antibiotic production rose to a maximum 24~28 hours after inoculation, and differential bioassay using *Piricularia oryzae* as a test organism showed 10~20 mcg/ml of new antibiotic accumulated together with blastmycin in the fermentation broth.

Isolation Procedure

Cultured broth of the streptomyces was harvested after 24 hours fermentation and the mycelial cake was extracted with 60% aqueous acetone. After removal of the acetone, the aqueous phase was extracted with ethyl acetate. The organic layer, combined with the ethyl acetate extract of the filtered beer, was washed with water, dried over sodium sulfate and concentrated *in vacuo* to a brownish solution, which was passed through a silica gel column.

The colorless active filtrate was concentrated and applied to a silicic acid column, which was washed with benzene, then developed carefully with benzene - ethyl acetate, whereby two active fractions were separated. From the first fraction was isolated crystalline blastmycin. Later fractions were concentrated and diluted with hexane to give colorless crystals, which were recrystallized from ethyl acetate - hexane or aqueous methanol.

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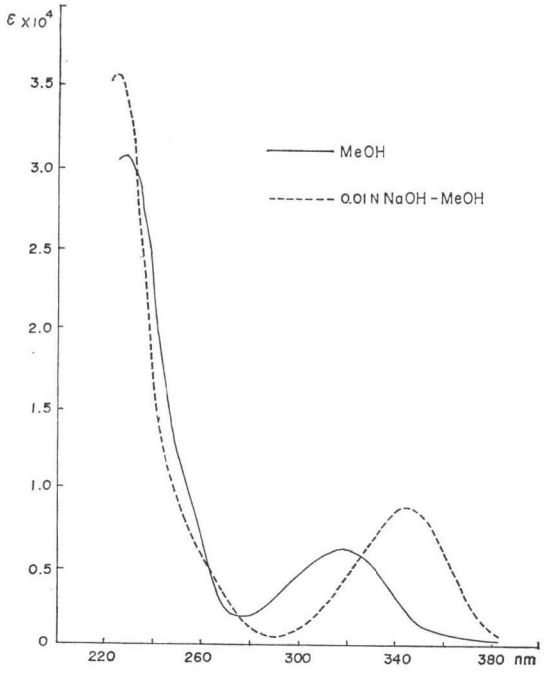
In another procedure, blastmycin was removed with benzene from the fermentation beer or mycelial extract, then the deacyl compound was extracted from the residual liquor with butanol, and purified by silicic acid column chromatography. This method was applied for differential assay of fermentation products.

Fig. 1. UV spectra of deisovaleryl-blastmycin



Physicochemical Properties

The colorless crystalline compound had m.p. 186~188°C and $[\alpha]_D^{24}$ +36° (*c* 1.0, methanol). It gave positive color reactions with ferric chloride and LEMIEUX's reagent, but negative with ninhydrin, FEHLING and silver nitrate reagents. It is soluble in alcohols, ethyl acetate and acetone, slightly soluble in benzene, ether and chloroform, and almost insoluble in petroleum ether, hexane and water. Elemental analysis and molecular weight determination by mass spectrometry suggested $C_{21}H_{28}O_8N_2$ for its molecular formula.

Calcd. for $C_{21}H_{28}O_8N_2$;
C 57.79, H 6.47, O 29.33, N 6.42
MW 436.45

Found; C 57.99, H 6.63, O 28.88, N 6.42
MW 436 (M^+)

Fig. 2. IR spectrum of deisovaleryl-blastmycin ($CHCl_3$)

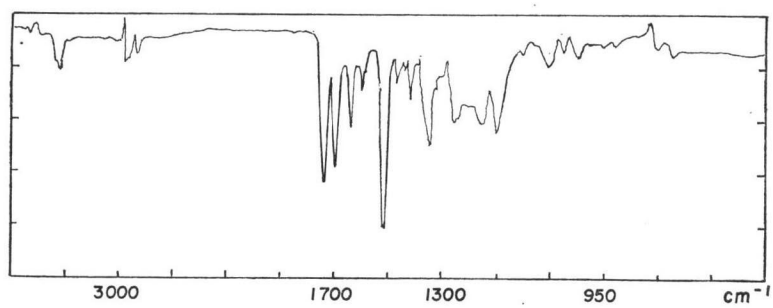
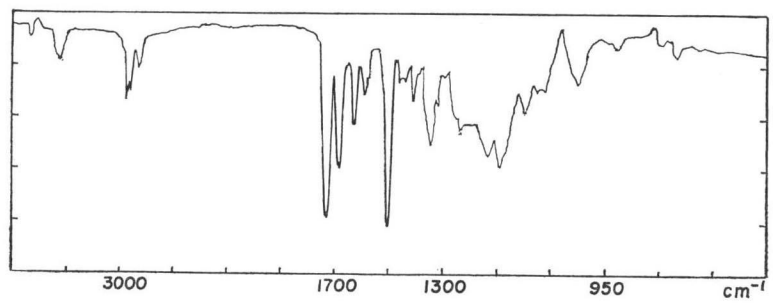


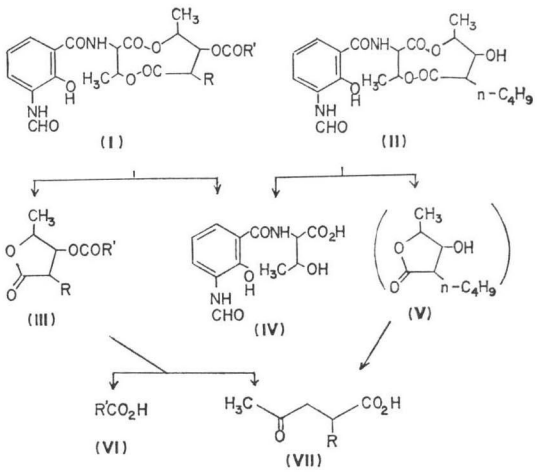
Fig. 3. IR spectrum of blastmycin ($CHCl_3$)



The UV spectra of the crystalline compound (Fig. 1) show maxima at λ_{max} 226 nm (ε 30,400), 320 (6,000) in methanol and N/100 HCl-MeOH and at λ_{max} 225 nm (ε 35,300), 345 (8,500) in N/100 NaOH-MeOH.

These maxima are essentially the same as those of antimycin A or blastmycin, except for their molecular extinction coefficients. The differences between the crystalline antibiotic and these antibiotics are shown in their IR spectra (Figs. 2 and 3) and in their thin-layer chromatograms. The crystalline antibiotic had Rf values of 0.25 and 0.16 in benzene - ethyl acetate (3 : 2) and chloroform - methanol (20: 1) system respectively, whereas blastmycin had Rf 0.70 and 0.90.

Fig. 4. Degradation scheme of blastmycin and de-isovaleryl-blastmycin



Chemical Structure

The new antibiotic (**II**) was considered to be a compound similar to antimycin A or blastmycin (**I**) from the data mentioned above. Similarity of UV spectra, and the detection of L-threonine from the hydrolysate of **II** (5.7 N HCl, 120°C, 12 hours) showed the same amino acid composition as **I**. Formation of α-butyllevulinic acid (**VII**) by vigorous alkaline hydrolysis (3 N NaOH, 100°C, 1 hour), but no volatile acid (**VI**) suggested the absence of the acyl group at R′ position, as in formula (**II**).

When **I** and **II** were acylated in isovaleric anhydride-pyridine solution, acyl-**I** and acyl-**II** showed the same mobility on TLC.

Mild alkaline hydrolysis of antimycin A or blastmycin (**I**) (1 N NaOH, room temperature, few minutes) gave blastmycic acid (**IV**) and antimycinone or blastmycinone (**III**), which are mixtures of alkyl

Fig. 5. Gas chromatograms of blastmycinones.

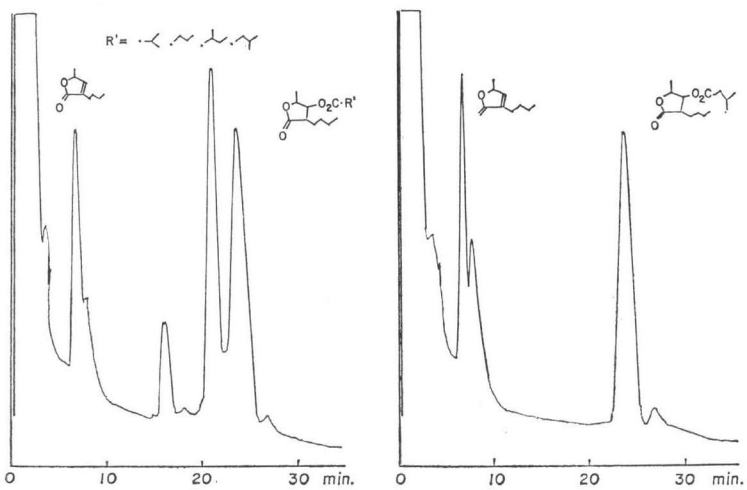


Table 1. Antifungal activity of deisovaleryl-blastmycin.

Fungi	M. I. C. (mcg/ml)
Alternaria kikuchiana IAM-5005	25
Aspergillus oryzae	>100
Botrytis cinerea IAM-5127	>100
B. fabae IAM-5125	>100
Cladosporium fulvum NIAS	50
Fusarium lini NIAS	>100
Gibberella fujikuroi NIAS	>100
G. saubinetti NIAS	50
Gloeosporium kaki NIAS	50
Glomerella cingulata IAM-8050	>100
G. lagenarium NIAS	>100
Helminthosporium sesamum NIAS	12.5
H. sigmoideum	100
Macrosporium bataticola IAM-5014	>100
Mucor ramannianus IAM-6128	>100
Ophiobolus miyabeanus NIAS	25
Penicillium chrysogenum Q 176	25
Piricularia oryzae NIAS	0.8
Trichophyton mentagraphytes NIHJ-640	25
Candida albicans IAM-4905	>100
C. utilis IAM-4215	3.125
Cryptococcus neoformans IAM-4514	>100
Rhodotorula glutinis IAM-4757	50
Saccharomyces cerevisiae NIHJ-F-130	>100

Table 2. Preventive effect of deisovaleryl-blastmycin against rice-blast disease.

DV-BM (ppm)	Blasticidin S (ppm)	Spots/ leaf	Effectivity (%)	Phyto-toxicity
400	0	4.0	86.1	—
200	0	17.5	39.2	—
100	0	16.7	42.0	—
0	10	1.1	96.2	—
0	0	28.8	0.0	

residues at R and R′ and which were separated on gas chromatograms (20% DEGS, Fig. 5)[6]. Under these conditions, the blastmycin mixture was observed to be a mixture of R′=$(CH_3)_2$-CH–, *n*-C_3H_7–, $C_2H_5(CH_3)$CH– and $(CH_3)_2$CH-CH_2– with R = *n*-C_4H_9–, and our antimycin mixture so far determined was a mixture of R = *n*-C_4H_9–, *n*-C_6H_{13}– and R′ = $(CH_3)_2$CH–, *n*-C_3H_7–, $C_2H_5(CH_3)$CH–, $(CH_3)_2CHCH_2$–.

Isovalerylated **II**, on mild alkaline treatment, gave only a peak corresponding to R = *n*-C_4H_9– and R′ = $(CH_3)_2CHCH_2$– moiety on gas chromatography. For these reasons, structure **II** was assigned to the original antibiotic, named deisovalerylblastmycin.

As antimycin A components, more than twenty compounds have been detected with combinations of R (C_2~C_8) and R′ (C_1~C_7) side chains[6~11] and the present antibiotic is characterized by no acyl group at R′ position.

Biological Properties

The antimicrobial activity of deisovaleryl-blastmycin is shown in Table 1. It is primarily active against *Piricularia oryzae* and *Candida utilis*, and weakly active against some fungi, but not active against species of bacteria. The LD_{50} of deisovaleryl-blastmycin was 15 mg/kg (i.v., dd mice) and 25 mg/kg (i. p.).

Toxicity against fish was 0.5 mcg/ml (killifish), 1/1,000 as toxic as antimycin A or blastmycin mixtures.

The preventive effect of deisovaleryl-blastmycin against rice-blast disease was determined on pot test in a green house, and was 86.1% effective at 400 ppm concentration.

Discussion

Deisovaleryl-blastmycin (**II**) was isolated from the fermentation broth of *Streptomyces* sp. 5140-A_1 in an early stage of the fermentation.

Recently, SINGH *et al.*[12] reported the enzymatic preparation of deacylantimycin A by hog kidney deacylase. In the present case, the deacyl compound was isloated from the same *Streptomyces* which produces blastmycin (**I**, R = *n*-C_4H_9– and R′ = $(CH_3)_2CHCH_2$–) and with regard to the fermentation time of this strain, the deacyl compound may be a biosynthetic precursor of blastmycin.

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