

STUDIES ON THE BIOSYNTHESIS OF
BLASTICIDIN S. VII¹⁾ ISOLATION OF
DEMETHYLBlasticidin S

Sir:

During a course of the investigation on the biosynthesis of blasticidin S, we have isolated a blasticidin S related metabolite from the strongly basic fraction of the filtered broth of *Streptomyces griseochromogenes*²⁾, a blasticidin S producing organism. We wish to report the isolation, structure and biological activity of this compound, demethylblasticidin S.

S. griseochromogenes was cultivated using Medium-1 as described previously³⁾ and the filtered broth was adsorbed on Amberlite IRC-50 (Na⁺) and after washing with distilled water, the resin was eluted with 0.5 N HCl. Fractions showing biological activity against *Bacillus cereus* were pooled and concentrated *in vacuo* to a small volume. Storage at 4°C overnight gave crystals of blasticidin S, which were removed by filtration. The filtrate was adjusted to pH 8 and adsorbed on active carbon. Elution with 60% acetone gave UV absorbing fractions, which were, after removal of acetone *in vacuo*, adsorbed on Dowex 50W (H⁺). The resin was first washed with 5% pyridine and then successively with 0.5 N NH₄OH. The fraction eluted with ammonia was concentrated *in vacuo* to dryness and the residue thus obtained was subjected to resin chromatography on Dowex 50W, X-2, (3 × 88 cm). Development with pyridine-acetate buffer, pH 5.0, 1.0 M gave demethylblasticidin S (I) in fractions 336~380 (17 ml each), preceded by blasticidin S⁴⁾ (II) in fractions 255~330. The fractions containing I were combined and evaporated to dryness. The residue was dissolved in distilled water and passed through a column of Dowex 50W (H⁺). Elution with 0.5 N NH₄OH followed by concentration under reduced pressure afforded crystals of I. Recrystallization from CO₂ free hot water gave an analytically pure sample. [m.p. 244~248°C (dec.) C₁₆H₂₄O₅N₈·H₂O. Found, C: 45.16, H: 6.27, O: 22.66, N: 25.87. Calcd., C: 45.06, H: 6.15, O: 22.51, N: 26.27. pK_a 2.4, 4.3, 8.5 and >12.5, titration equivalent 486. [α]_D²⁵ +59° (c 1.0, 0.1 N HCl), λ_{max}^{H+} 274 nm (ε 12900) and λ_{min} 240 (2600), λ_{max}^{OH-} 267 (9200) and λ_{min} 248 (7200). Positive color reactions; ninhydrin, alkaline ferricyanide-nitroprusside and SAKAGUCHI. Paper chromatography (Rf value) *n*-BuOH - MeOH -

conc. NH₄OH - H₂O = 10: 4: 3: 3, 0.22 (II, 0.27), *n*-BuOH - AcOH - H₂O = 2: 2: 1, 0.53 (II, 0.57)]. On alkaline treatment⁴⁾, I changed with a loss of one molecule of ammonia to demethylcytomyacin, III, (obtained as a monohydrochloride. m.p. browning at ~187°C. C₁₆H₂₁O₅N₇·HCl·H₂O. Found, C: 43.19, H: 5.55, O: 21.72, N: 21.78, Cl: 7.64. Calcd., C: 43.10, H: 5.43, O: 21.53, N: 21.99, Cl: 7.95. Negative to ninhydrin and SAKAGUCHI reactions).

These physicochemical properties of I are very similar to those of II and the only differences between the two antibiotics, *i.e.* the molecular formula (I contains one less carbon and two less hydrogens than II does) and positive SAKAGUCHI reaction (II is negative to this color reaction), might be reasonably explained by the lack of the N-methyl group in I (Fig. 2).

The comparison of ¹H-NMR spectra* of I and II gave conclusive evidence on the structure of I. As expected, the N-methyl singlet at 3.10 in II is lost in the ¹H-NMR spectrum of I (Fig. 1). The upfield shift of the C-5 methylene (3.55 in II and 3.42 in I) is also explained by the removal of the N-methyl group. The similar upfield shift of a methylene adjacent to a guanidino group [HOOC·CH₂·N(CH₃)·C(=NH)·NH₂ 4.33, HOOC·CH₂·NH·C(=NH)·NH₂ 4.20] supports the above conclusion.

Acid hydrolysis of I gave the nucleoside, cytosinine⁵⁾ and a new amino acid, demethylblasticidic acid (β -arginine) which is apparently different from blasticidic acid⁴⁾ on paper chromatogram (Rf value, *n*-BuOH - MeOH - NH₄OH - H₂O = 10: 4: 3: 3, demethylblasticidic acid 0.33, blasticidic acid 0.41). Since demethylblasticidic acid could not be obtained as pure crystals, it was characterized after conversion to demethylpseudoblastidone (m.p. gradually softened at ~230°C, C₆H₁₁O₂N₈, Found C: 45.72, H: 7.09, N: 26.82. Calcd., C: 45.82, H: 7.05, N: 26.74) which was also obtained by acid hydrolysis of III (see Fig. 2). The ¹H-NMR spectrum of demethylpseudoblastidone was very similar to that of pseudoblastidone⁴⁾ except for the absence of the N-methyl singlet (H₂; 2.75 (2H), d, J = 7 Hz, H₃; ~3.95 (1H), complex multiplet, H₄; ~1.85 (1H) and ~2.05 (1H) complex multiplet and H₅; 3.40; (2H), t, J = 7.5]. Thus, the structure of I is established as demethylblasticidin S as shown

* ¹H-NMR spectra were obtained in conc. DCl at 100 MHz using DSS as internal standard.

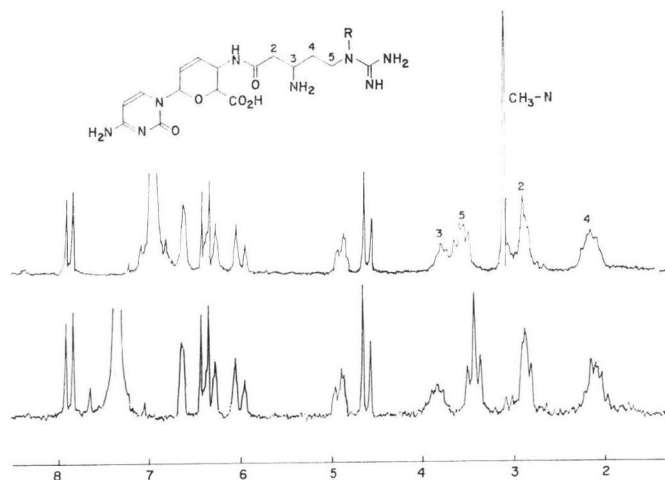
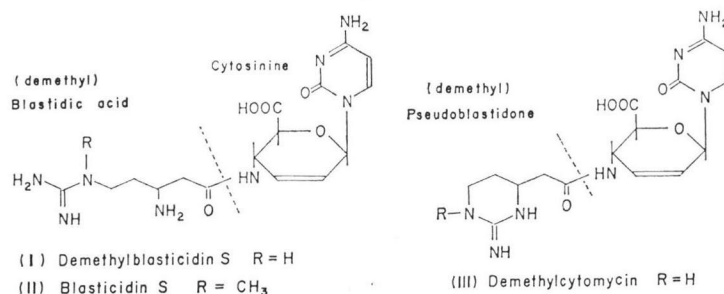
Fig. 1. $^1\text{H-NMR}$ spectra of blasticidin S (upper trace, $\text{R}=\text{CH}_3$) and demethylblasticidin S (lower trace $\text{R}=\text{H}$).

Fig. 2.



in Fig. 2.

With the structure of **I** having been established, we then investigated the effect of ethionine, a strong inhibitor of transmethylation, on the production of **I**. Addition of the amino acid to the fermentation broth (15 mg/ml) at 48 hours after inoculation increased the yield of **I** without considerably affecting the production of **II**. (At 72 hours, **I**: 43 $\mu\text{g/ml}$, **II**: 83 $\mu\text{g/ml}$. control **I**: 19 $\mu\text{g/ml}$, **II**: 93 $\mu\text{g/ml}$. At 96 hours, **I**: 196 $\mu\text{g/ml}$, **II**: 410 $\mu\text{g/ml}$. control **I**: 95 $\mu\text{g/ml}$, **II**: 331 $\mu\text{g/ml}$). Thus, ethionine may be a useful compound to attain the increased production of **I**.

Finally we compared the biological activity of **I** with that reported for **II**³⁾. As shown in Table 1, **I** is almost as active as **II** against the pathogen of the rice blast disease, *Piricularia oryzae*. However, the MIC values of **I** for other microorganisms seem to be somewhat larger than those of **II**, due probably to slight differences in

the experimental conditions.

Green house experiments (Table 2) showed that **I** might be as useful an antibiotic as **II** for protection against the rice blast disease in the field.

The LD_{50} values (oral administration) of **I** and **II** were 35 mg and 38 mg/kg to mice, respectively.

Acknowledgement

We thank Dr. K. SAGA of Kaken Chemical Co. for determining the toxicity of demethylblasticidin S.

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(Received August 29, 1977)

Table 1. Antimicrobial spectrum of demethyl-blasticidin S.

Test organisms	Minimum inhibitory concentration ^{a)}	
	Demethyl-blasticidin S	Blasticidin S ^{b)}
<i>Micrococcus luteus</i>	20	
<i>Bacillus subtilis</i>	> 100	50
<i>Staphylococcus aureus</i>	100	
<i>Klebsiella pneumoniae</i>	> 100	
<i>Mycobacterium smegmatis</i>	100	
<i>Shigella dysenteriae</i>	100	
<i>Pseudomonas aeruginosa</i>	> 100	
<i>Escherichia coli</i>	> 100	50
<i>Trichophyton mentagrophytes</i>	> 100	
<i>Trichophyton rubrum</i>	> 100	
<i>Cryptococcus neoformans</i>	100	
<i>Candida albicans</i>	> 100	> 100
<i>Aspergillus fumigatus</i>	> 100	
<i>Piricularia oryzae</i>	10	5~10
<i>Pellicularia filamentosa</i>	10	
<i>Alternaria kikuchiana</i>	50	50
<i>Ophiobolus miyabeanus</i>	> 100	> 100
<i>Deapolte citri</i>	50	
<i>Fusarium oxysporum</i>	> 100	> 100

a) Determined by agar dilution method.

b) See ref. 2 in the text.

References

- 1) For part VI see, SETO, H. & H. YONEHARA:

Table 2. Effect of demethyl-blasticidin S on the rice blast disease^{a)}

Materials	Concentration (mcg/ml)	Number of lesions per leaf	Protective value
Demethyl-blasticidin S	5	0.2	99.4
	10	0.1	99.7
	20	0	100.0
Blasticidin S	5	0.4	98.8
	10	0.2	99.6
Control	—	34.2	

a) Determined by pot test. The antibiotics were sprayed on the same day of the inoculation of the pathogen.

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