STUDIES ON THE BIOSYNTHESIS OF BLASTICIDIN S. VI[1] THE ISOLATION AND STRUCTURE OF BLASTICIDIN H

Sir:

Blasticidin S has been produced in a large scale as an effective fungicide for the rice blast disease in Japan.

As part of biosynthetic studies of this antibiotic, we have looked for its biosynthetic intermediates in a mother liquor* of blasticidin S crystallization obtained at a factory during blasticidin S production. As a result of screening for compounds showing the UV absorption characteristic to 1-substituted cytosine, we have obtained as described herein a metabolite named blasticidin H (H means hydrated.) which seems to be an intermediate of blasticidin S biosynthesis.

The mother liquor* was adsorbed on active carbon and eluted with 60% aqueous acetone. After concentration *in vacuo*, the eluate was subjected to resin chromatography on Dowex 50 X-2, (3×88 cm) equilibrated with pyridine-acetate buffer, pH 5.0, 1.0 M. Elution with the same buffer gave blasticidin H in fractions 175~205 (17 ml each), blasticidin S being present in fractions 255~330.

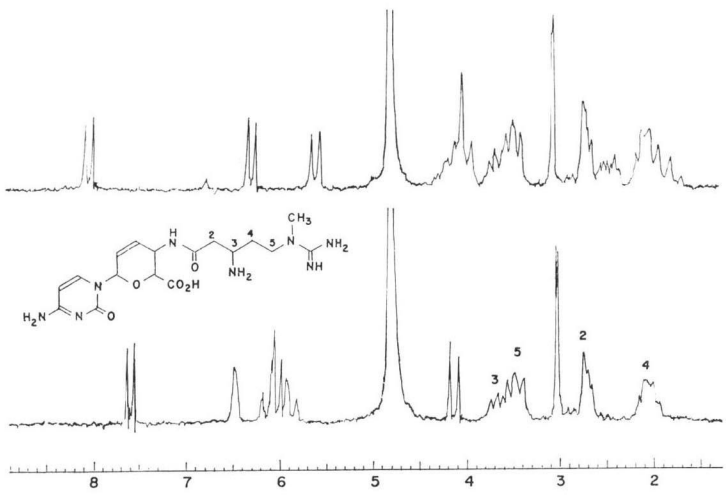
The blasticidin H containing fractions were combined and evaporated to dryness. The residue was dissolved in distilled water and passed through a column of Sephadex G-15. Fractions showing UV absorption were combined and concentrated to give crystals of blasticidin H (**I**). **I** was purified as dihydrochloride by recrystallizing from a mixture of dilute hydrochloric acid and ethanol.

The physicochemical properties of **I**·2HCl are as follows: m.p. 230~235°C (dec.), $C_{17}H_{28}O_6N_8 \cdot 2HCl \cdot H_2O$, Found C: 38.77, H: 6.27, O: 21.39, N: 20.92, Cl: 13.20. Calcd. C: 38.42, H: 6.07, O: 21.07, N: 21.09, Cl: 13.34. pKa′ 2.8 (carboxylic acid), 4.2 (amino group of cytosine), 8.2 (amine) and >12.5 (guanidine). $\lambda_{max}^{H^+}$ 277 nm (ε 13500) and λ_{min} 241 (2500), $\lambda_{max}^{OH^-}$ 270 (9600) and λ_{min} 250 (7500), positive to ninhydrin.

These properties suggest the close structural relationship between blasticidin S (**IV**) and **I**, and the molecular formula indicates that **I** would be a monohydrated derivative of **IV**.

The comparison of ^{1}H-NMR spectra** (Fig. 1) of **I** and **IV** revealed that the amino acid moiety (blastidic acid[2]) is also present in **I** with its amino function being unsubstituted, and that two olefinic protons in **IV** disappeared in **I**. This structural difference was confirmed by acid degradation (6 N HCl, 95°C, 8 hours). The acid hydrolysate was concentrated *in vacuo* to dryness, and the residue was dissolved in distilled water and

Fig. 1. ^{1}H-NMR spectra of blasticidin S (lower trace) and blasticidin H (upper trace).



* This fraction was obtained at Kaken Chemical Co. by adsorbing the filtered broth of *Streptomyces griseochromogenes* on Amberlite IRC-50, and by eluting the resin with hydrochloric acid solution. Concentration of the eluate followed by chilling had removed most of blasticidin S as crystals from this fraction.

** ^{1}H-NMR spectra were taken in D_2O at 100 MHz using DSS as internal standard.

passed through a column of Amberlite IRA-410 (OH-type). From this effluent was isolated blastidic acid as described previously[2] and its identity with an authentic sample was confirmed. Elution of the column with 0.5 N HCl, followed by concentration gave a ninhydrin-positive novel nucleoside named pentopyranamine D (**II**) as a dihydrochloride which was recrystallized from conc.hydrochloric acid (m.p. browning at ~235 °C, $C_{10}H_{14}O_5N_4 \cdot 2HCl$, found C: 34.70, H. 5.07, O: 23.36, N: 15.96, Cl: 20.84. Calcd. C: 34.99, H: 4.70, O: 23.31, N: 16.33, Cl: 20.66. $\lambda_{max}^{H^+}$ 277 nm (ε 12200) and λ_{min} 241 (3100), $\lambda_{max}^{OH^-}$ 270 (8200) and λ_{min} 250 (6500).

Potentiometric titration proved the presence of a carboxylic acid (pKa′ 2.0), amino group of cytosine (4.1) and amine (7.8).

The ^{1}H-NMR spectrum of **II** together with spin decoupling experiments (Fig. 2) revealed the following structural sequence; H_1 (5.75 ppm, $J_{1,2}=10.0$ Hz), H_2 (4.13, $J_{1,2}=10.0$, $J_{2,3a}=10.2$, $J_{2,3e}=4.5$), H_{3a} (2.03 $J_{2,3a}=10.2$, $J_{3a,3e}=10.2$, $J_{3a,4}=10.2$), H_{3e} (2.74, $J_{2,3e}=4.5$, $J_{3e,3a}=10.2$, $J_{3e,4}=4.5$), H_4 (3.77, $J_{3e,4}=4.5$, $J_{3a,4}=10.2$, $J_{4,5}=10.1$) and H_5 (4.57, $J_{4,5}=10.1$).

The magnitude of the coupling constants of these protons implies that the sugar moiety takes a pyranose form and that all the protons but for H_{3e} are in axial orientation as shown in Fig. 3.

The remaining problem with regard to the positions of a hydroxy group and an amino group which must be located at C-2 or C-4 was settled as follows: On alkaline treatment, **I** changed with a loss of one molecule of ammonia to a ninhydrin-negative compound named cytomycin H (**III**) (obtained as a dihydrochloride. m.p. 217~218°C, $C_{17}H_{25}O_6N_7 \cdot 2HCl \cdot H_2O$, Found C: 39.98, H: 5.99, O: 21.94, N: 18.93, Cl: 13.97. Calcd. C: 39.69; H; 5.68, O: 21.77, N: 19.06, Cl: 13.79. pKa′ 2.3, 4.2 and >12.5), just as did **IV** to cytomycin[2]. Since this reaction (Fig. 3, A→B) can take place only when the amino group of the blastidic acid part is free, and since only one primary amino group (except for the amino group of cytosine) is present in **I** (see above), the primary amino group of **II** must be blocked in **I**.

Acetylation of **III** gave an N,O-diacetate (m.p.

Fig. 2. ^{1}H-NMR spectrum of pentopyranamine D.

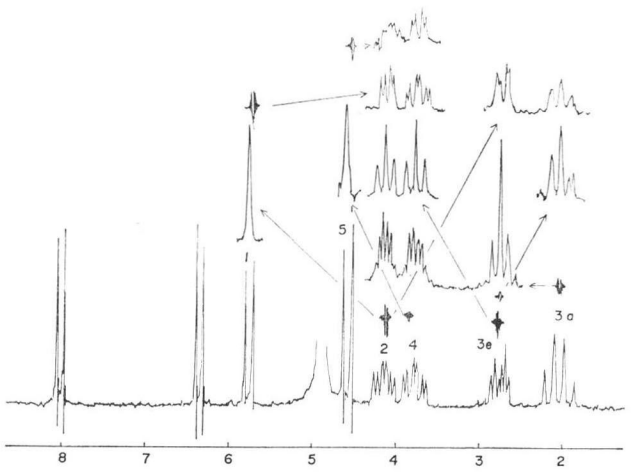
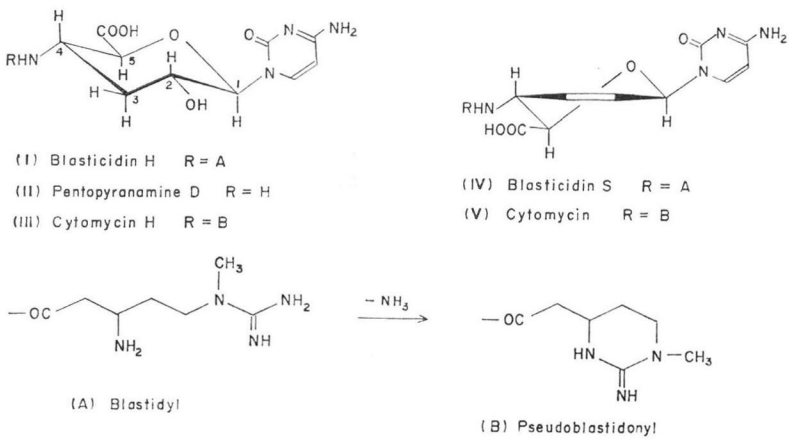


Fig. 3.



browning at ~270°C, $C_{17}H_{23}O_6N_7$ $(CH_3CO)_2 \cdot \frac{1}{2}H_2O$. Found C: 48.88, H: 5.87, O: 26.47, N: 19.10. Calcd. C: 48.82, H: 5.85, O: 26.33, N: 18.98). Its IR spectrum (1740 cm^{-1}, ester carbonyl) and UV spectral change [$\lambda_{max}^{H_2O}$ 249 nm (ε 13700), 297 (6700) and λ_{min} 226 (4650), 275 (4400)] showed that a hydroxy group and the amino group of cytosine were acetylated. In the ^{1}H-NMR spectrum of the diacetate, not only H_1 but also H_2 proton suffered a considerable down field shift [H_1 5.65 and H_2 4.1, $J_{1,2}=10.0$ Hz in **III**, H_1 5.95 and H_2 5.20, $J_{1,2}=9.5$ Hz in the diacetate of **III**]. Thus, it follows that the hydroxy function should be present at C-2 but not at C-4.

The similar CD spectral pattern (Fig. 4) of **II** and C-substance[3] [a 3-hydroxy isomer of **II**] established the absolute structure of **II** as 1-(4-amino-3,4-dideoxy-β-D-*ribo*hexopyranosyluronic acid)-cytosine as shown in Fig. 3. This structure has recently been confirmed by chemical synthesis[4].

Since the amino group in the sugar moiety of **II** is not free in **I**, it must be connected with blastidic acid through an amide bond to give the structure of **I** as 1-(4-blastidylamino-3,4-dideoxy - β - D - *ribo*hexopyranosyluronic acid) - cytosine.

This structural feature strongly implies that **I** is the direct precursor of **IV**, only removal of water from **I** being formally necessary for the formation of **IV***.

I is slightly active against *Piricularia oryzae*, the pathogen of the rice blast disease, at higher concentration. This means that the double bond in the sugar part of **IV** is strongly associated with its biological activity.

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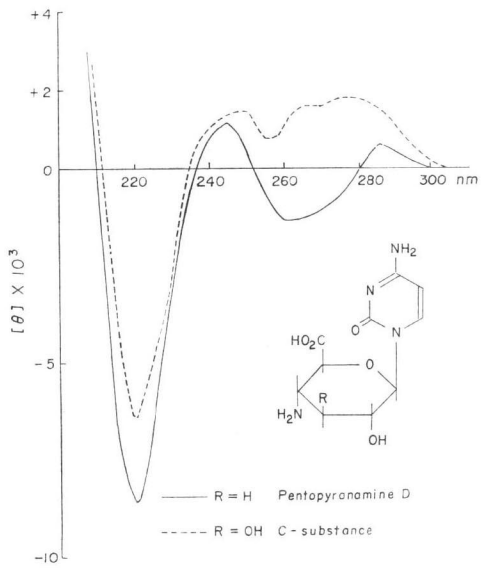
Fig. 4. CD-spectra of pentopyranamine D and C-substance in H_2O.



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* Since leucylblasticidin S has been known to be involved in the biosynthesis of **IV**[5], **I** may be either a precursor or a shunt pathway product of a hypothetical intermediate, leucylblasticidin H which has not yet been isolated.