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# THE STRUCTURE OF BLASTICIDIN S

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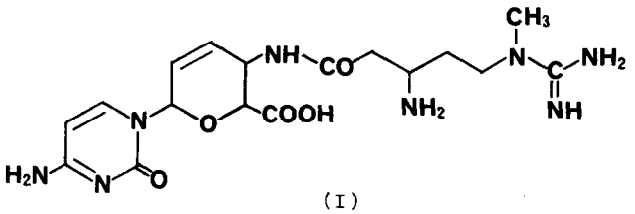
Blasticidin S, an antibiotic effective against rice blast disease, was isolated from the culture broth of Streptomyces griseochromogenes by the authors[1,2]. We wish to present the evidences which led us to the conclusion of structure I for blasticidin S.




(I)

Blasticidin S (I) $C_{17}H_{26}O_5N_8$*1, m.p. 252-253°(dec.),

*1 The formula $C_{18}H_{24}O_5N_8 \cdot H_2O$ previously reported should be revised to the formula $C_{17}H_{26}O_5N_8$ which was most likely to accommodate the experimental results.

$[\alpha]_D^{11}$ = + 108.4°(c, 1.0 in $H_2O$), $\lambda_{max}^{0.1N\,HCl}$ 274 mμ (ε 13,400); $\lambda_{max}^{0.1N\,NaOH}$ 266 mμ (ε 8,850), pKa 2.4, 4.6, 8.0 and above 12.5*2, gave corresponding monohydrochloride, $C_{17}H_{26}O_5N_8 \cdot HCl$, m.p. 229°(dec.), dihydrochloride, $C_{17}H_{26}O_5N_8 \cdot 2HCl$, m.p. 195° (dec.) and monomethyl ester*3 trihydrochloride, $C_{17}H_{25}O_4N_8(OCH_3) \cdot 3HCl$, m.p. 206-208.5°(dec.). Therefore, I was characterized as monoacidic tribasic compound. Functional group analysis demonstrated the presence of one N-$CH_3$ group, NMR (in $D_2O$) ppm: 2.92 (3H s), two ethylenic groups, ppm: 7.73, 6.15 (2H d, J = 7.5 cps AB type) and ppm: 6.45-6.00 (2H broad), and two $NH_2$ groups (Van Slyke determination).

Under a milder alkaline hydrolysis, liberation of one mole of ammonia took place to afford a crystalline compound designated cytomycin (II) $C_{17}H_{23}O_5N_7$, m.p. 237-239°(dec.), $\lambda_{max}^{0.1N\,HCl}$ 274 mμ (ε 12,000), $\lambda_{max}^{0.1N\,NaOH}$ 266 mμ (ε 6,930), pKa 2.4, 4.6 and above 12.5*4. Further alkaline hydrolysis

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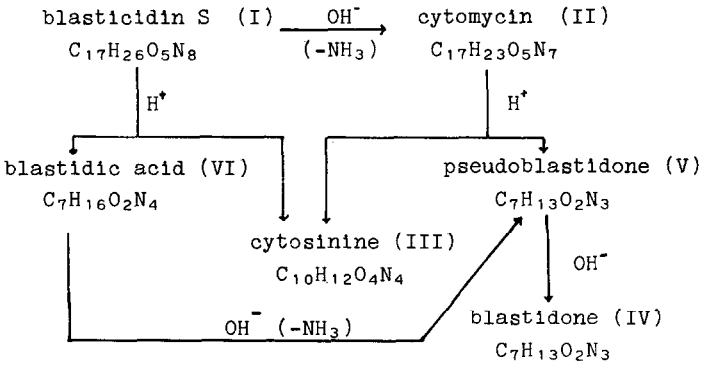
*2 Usual potentiometric titration cannot precise the pKa value above 12.5, whereas blasticidin S methyl ester trihydrochloride was titrated to be dibasic, therefore the presence of one more strong basic function dissociating above 12.5 was presumed.

*3 When I was treated with methanol containing 3% hydrogen chloride, blasticidin S methyl ester trihydrochloride was obtained as well defined crystals. NMR (in $D_2O$) ppm:3.60 (3H singlet), IR(Nujol)1730 $cm^{-1}$ and 1230 $cm^{-1}$. The parent antibiotic was recovered completely when the methyl ester trihydrochloride was passed through the column of IRA-410.

*4 The basic center of pKa 8.0 in I disappeared in II; nevertheless, II gave corresponding monomethyl ester dihydrochloride with methanolic hydrogen chloride.

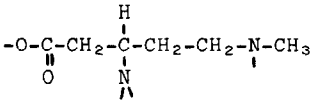
resulted in cleavage of II into cytosinine (III), $C_{10}H_{12}O_4N_4$, whose structure was elucidated in the preceding paper[3], and blastidone (IV) $C_7H_{13}O_2N_3$, m.p. 209-210°(dec.). The structure of IV was elucidated previously as 4-ureido-N-methyl-2-piperidone by synthesis[4]. It is noteworthy that the interpretation on the origin of piperidone ring, whether it is natively contained in I, or arise during the degradation, becomes a key problem in this work.

On acid hydrolysis, II gave III and pseudoblastidone (V)*5, $C_7H_{13}O_2N_3$, m.p. 282-283°(dec.), $\lambda_{max}^{H_2O}$ only end absorption, IR (Nujol) 1720 (shoulder), 1640 and 1610 $cm^{-1}$, NMR (in $D_2O$) ppm: 1.8 (2H m), 2.50 (2H d, J = 7.5 cps), 3.05 (3H s, N-$\underline{CH_3}$), 3.40 (2H t) and 3.65 (1H m), which was characterized as monoacidic monobasic compound of pKa 3.75 and above 12.5.




*5 V could be transformed in good yield into IV by the action of dilute alkali, but reversed reaction could not be taken place[2].

I could be cleaved with acid under selected condition to afford III and a new amino acid designated blastidic acid (VI) as dihydrochloride, $C_7H_{16}O_2N_4 \cdot 2HCl$, m.p. 192-192.5° (dec.), $[\alpha]_D^{15}$ + 25.0° (c, 1.0 in $H_2O$), pKa 3.2, 8.0 and above 12.5, containing one amino group (Van Slyke determination). VI showed NMR peaks (in $D_2O$ + DCl) at ppm: 1.8 (2H m), 2.50 (2H d, J = 7.5 cps), 2.95 (3H s, N-$\underline{CH_3}$), 3.15 (2H t) and 3.50 (1H m). Spin decoupling data established relationship between ten protons and provided proof for the partial structure (VII) in VI.



(VII)

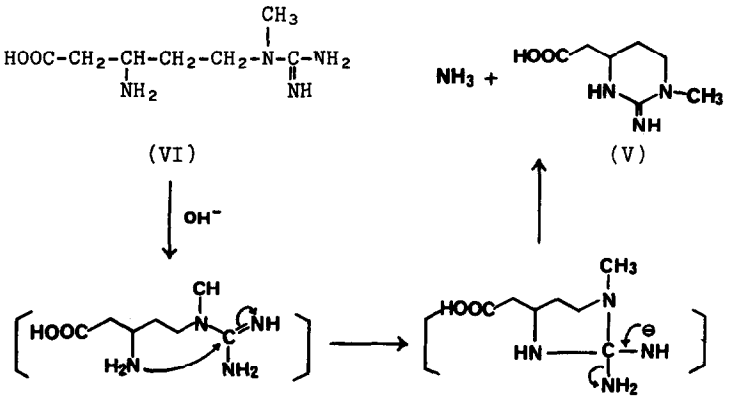
VI was transformed almost quantitatively to V with liberation of one mole of ammonia by the action of dilute alkali just like I furnished II.

Potassium permanganate oxidation of VI afforded N-methylguanidine, characterized as picrate, m.p. 200-201°, which was identified unequivocally by comparison of IR spectra and mixed melting point with the authentic sample.

These evidences allowed to the assignment of structure VI for blastidic acid[*6], and the transformation of VI to V

[*6] VI being called, ε-N-methyl-β-arginine, is a new amino acid, which is found for the first time in the natural source. The configuration of VI will be reported later.

could be reasonably explained as follows:

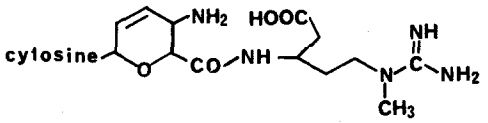


Indeed, V gave N-methylguanidine characterized as picrate by the oxidation with potassium permanganate. This result in addition to NMR data, supported the assignment of structure V for pseudoblastidone.

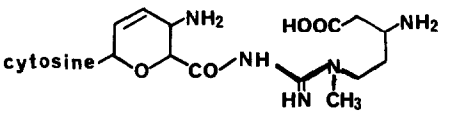
Approach to the complete elucidation of the structure I was provided by the spectral evidence, namely, spin decoupling experiment of I showed intact presence of partial structure VII in the antibiotic.

Taking account of the information in so far as had been obtained, the following structures I, VIII and IX were possible, but the data of dissociation constant excluded structure IX.

The following experiments gave unequivocal support to structure I. I or II were hydrogenolysed with $PtO_2$ in



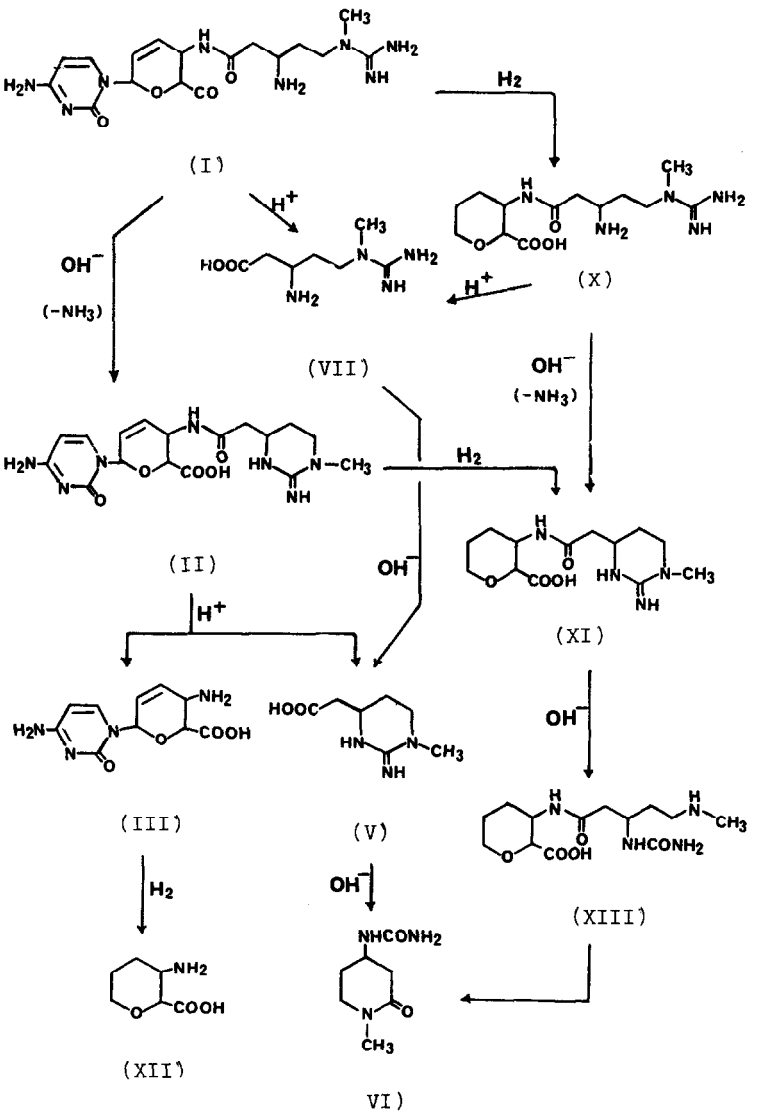

(VIII)




(IX)

acetic acid to afford the product designated $SC_{13}$ compound (X), $C_{13}H_{25}O_4N_5$, m.p. 208-209°(dec.), pKa 3.2, 8.0 and above 12.5, or $C_{13}$ compound (XI), $C_{13}H_{22}O_4N_4$, m.p. above 320°, pKa 3.2 and above 12.5, respectively. Acid hydrolysis of X, 3-amino-tetrahydropyran-2-carboxylic acid[3] (XII) and VI were obtained, similarily, XI gave XII and V.

Under the selected condition, XI was partially hydrolized by alkali to a compound designated PH-$C_{13}$ (XIII) $C_{13}H_{24}O_5N_4$[5], m.p. 177-177.5°, pKa 3.0 and 10.8, IR (Nujol) 1595, 1650 and 2760 $cm^{-1}$, NMR ($D_2O$) ppm: 2.0-1.4 (6H m), 2.29 (2H d, J = 6.5 cps), 2.54 (3H s, N-$\underline{CH_3}$), 2.90 (2H t), 3.49 (1H d, J = 8.5 cps) and 3.7 (3H m). XIII gave corresponding crystalline ethyl ester hydrochloride, $C_{13}H_{23}O_3N_4(OC_2H_5)\cdot HCl$, m.p. 222-223°(dec.), IR (Nujol) 1740, 1650 and 1250 $cm^{-1}$, and also gave mono dinitrobenzene derivative, $C_{13}H_{21}O_4N_4(C_6H_3O_4N_2)$, m.p. 188.5-190°, which on drastic acid hydrolysis afforded


(I)
(X)
(VII)
(II)
(XI)
(III)
(V)
(XIII)
(XII)
VI)

N-methylaniline and XII as characterizable products. These data demonstrated the presence of one free N-methylamino group and one free carboxyl group in XIII. Two amino groups in XIII could be detected slowly by Van Slyke determination, nevertheless, they did not show basicity, therefore, they were assigned to ureido function. Indeed, XIII exhibited characteristic color reaction of diphenylcarbohydrazide for urea[6].

These results permited to the assignment of structure XIII for $PHC_{13}$ compound. Clearly, the piperidone ring was formed by the recyclization, since either by acid or alkaline hydrolysis, XIII gave rise to IV and XII. Simultaneous formation of terminal N-methyl and ureido groups could be explained by the rupture of the guanidino ring (XI⟶XIII).

On the basis of these accumulated evidences, the structures could be assigned unequivocally, XI to $C_{13}$ compound, X to $SC_{13}$ compound, II to cytomycin and I to blasticidin S respectively.

The structural elucidation by X-ray analysis by Saito and his collaborators[7] using blasticidin S monohydrobromide was parallely investigated with the chemical work, and reached a complete agreement with the structure I.

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