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STRUCTURE OF CYTOSININE, A STRUCTURAL COMPONENT OF BLASTICIDIN S

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In previous papers[1,2], we have reported the isolation and characterization of a new nucleoside designated cytosinine from the hydrolysis products of blasticidin S, a useful antibiotic against rice blast desease. We wish to present the details leading to the assignment of structure I to cytosinine.

Cytosinine (I), $C_{10}H_{12}O_4N_4$, m.p. 244-245°, $[\alpha]_D^{18}$ -20°(c, 1 in H_2O), pKa 2.4, 4.6 and 8.0, $\lambda_{max}^{0.1NHCl}$ 274 mμ (ε 13,800), $\lambda_{max}^{0.1N.NaOH}$ 267 mμ (ε 7,500), showed the existence of two ethylenic groups (NMR (in D_2O) ppm: 7.72 and 6.14 (2H d, AB type, J = 7.5 cps) and 6.45-6.00 (2H broad)), two amino groups (Van Slyke determination) and one carboxylic group. By the action of methanol containing 3% hydrogen chloride, I gave corresponding methyl ester dihydrochloride, $C_{10}H_9O_3N_4(OCH_3)\cdot 2HCl$,

m.p. 220-223°(dec.), IR (Nujol) 1730 and 1230 cm^{-1}, pKa 4.6 and 8.0, which was acetylated with acetic anhydride and triethylamine to afford N, N'-diacethyl methylester (III), $C_{10}H_9O_3N_4\cdot(OCH_3)\cdot(CH_3CO)_2$, m.p. 263-266°.

UV spectral evidence indicated probable presence of cytosine nucleus, on which a substituent attached at N-1 position.

Acid hydrolysis of I gave one mole each of cytosine, levulinic acid, ammonia and carbon dioxide as characterizable products, whereas further attempt to cleave the glycosidic linkage of I was unsuccessful without extensive decomposition.

In an effort to gain information regarding the sugar portion*1, reduction and oxidative cleavage of ethylenic function in I were undertaken. On reduction with PtO_2 in acetic acid, I gave a mixture of the dihydrocytosinine (IV), $C_{10}H_{14}O_4N_4$, m.p. 227-229°(dec.), NMR (D_2O) ppm: 7.25 and 5.45 (2H d, J = 7.5 cps), 5.10 (1H m), 3.50 (1H d, J = 10.5 cps), 2.70 (1H m) and 1.40 (6H m), hydrogenolysis product (V), $C_6H_{11}O_3N$, m.p. 275-278°, and cytosine. The facile hydrogenolysis of I to cytosine and V indicated that an ethylenic function in C_6 moiety located at allylic position to the glycosidic C-N bond.

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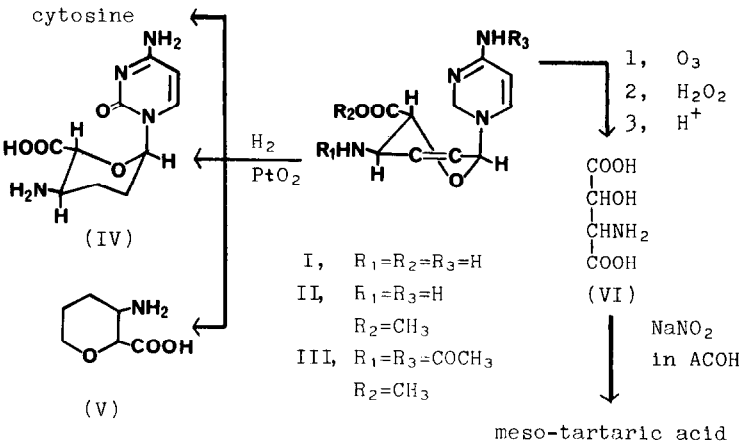
*1 The presence of sugar moiety was presumed, since I gave levulinic acid on acid hydrolysis.

V characterized as monobasic monoacidic compound of pKa 9.2 and 2.4 gave corresponding N-acethyl methylester, $C_6H_9O_2N\cdot(OCH_3)\cdot(CH_3CO)$, m.p. 130°, IR (Nujol) 1740, 1670 and 1230 cm^{-1}. The IR spectra of V and deuterated V exhibiting characteristic bands of tetrahydropyran[3] at 1100 and 1060 cm^{-1} (C-O-C stretching vibration) established the presence of tetrahydropyran ring.

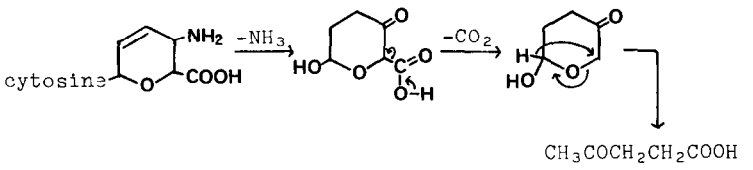
Ozonolysis of III in acetic acid followed by oxidation with hydrogen peroxide and acid hydrolysis furnished β-hydroxy aspartic acid (VI), $C_4H_7O_5N$, m.p. 287-288°(dec.), $[\alpha]_D^{20}$ -47° (c, 1 in H_2O). On diazotization, VI afforded meso-tartaric acid, therefore, the erythro-D-configuration[4] was established. VI was a substantially important structural fragment of the sugar portion in I and was also obtained from blasticidin S itself by a similar ozonolysis.



These results established the structure of V as 3-amino-tetrahydropyran-2-carboxylic acid. On the basis of these accumulated evidence the structure of cytosinine was elucidated as formula I.

Stereochemical feature of I was next to be considered, e.g. the configuration of erythro-D-β-hydroxy aspartic acid provided evidence for the (R)-configuration at C_4' and C_5' respectively. The NMR spectrum of IV showing a distinct doublet at 3.50 ppm (1H, J = 10.5 cps) assigned for C_5' proton established the C_4'-C_5' protons to be diaxial. The configuration of glycosidic linkage remained uncertain, but it was presumed to be β form, since nucleosides from natural source were reported as β.

It was necessary to consider the mechanism of levulinic acid formation from I. Transformation of hexoses into levulinic acid by the action of acids was well established[5]. Similarily, the formation of levulinic acid, ammonia and carbon dioxide from I could be explained as follows:



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