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NUCLEOSIDES XXXII. ON THE STRUCTURE OF BLASTICIDIN S, A NUCLEOSIDE ANTIBIOTIC (1)

Jack J. Fox and Kyoichi A. Watanabe

The Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York 21, N.Y.

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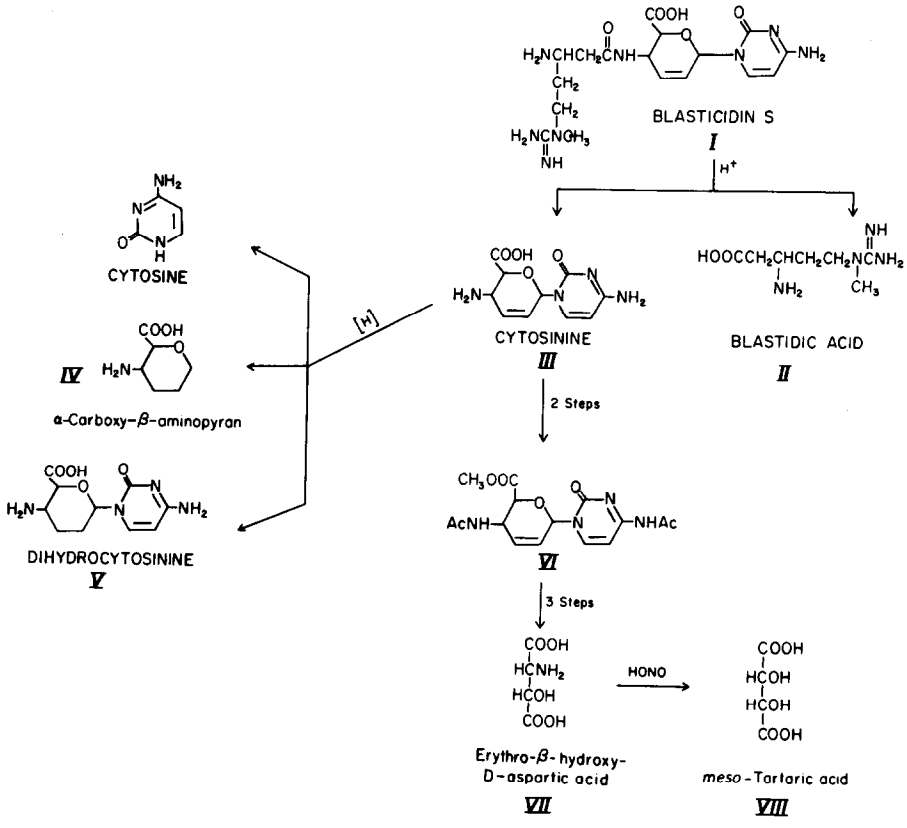
In the course of screening studies for antibiotics which would inhibit the virulent fungus, Piricularia oryzae, a serious cause of rice plant disease in Japan, a new antibiotic, blasticidin S, was isolated from culture broths of Streptomyces griseochromogenes by Takeuchi et al. (2). When rice plants infected with Piricularia oryzae were sprayed with solutions containin blasticidin S in concentrations as low as 5 p.p.m., the propagation of rice blast disease was markedly inhibited (3). The wide use of this antibiotic in Japan has stimulated a detailed investigation into its complex structure.

Early studies by Yonehara et al. (4) (Fig. 1) demonstrated that blasticidin S (I) was a 1-substituted cytosine nucleoside since it exhibited a cytidine-like ultraviolet absorption spectrum and also yielded cytosine after strong acid hydrolysis. Controlled acid hydrolysis of blasticidin S (I) yielded two fragments, blastidic acid (II) and cytosinine (III) (5). The structure of blastidic acid was investigated by Ōtake et al. (5) and was shown to be ε-N-Methyl-β-arginine (II).

The structure of cytosinine (III) was investigated by Ōtake, Takeuchi, Endo and Yonehara (6) who found that the ultraviolet absorption spectrum of III was similar to that for cytidine. They observed the presence of two

FIG. 1


BLASTICIDIN S
I
H+
CYTOSININE
III
BLASTIDIC ACID
II
[H]
CYTOSINE
IV
α-Carboxy-β-aminopyran
DIHYDROCYTOSININE
V
2 Steps
VI
3 Steps
HONO
Erythro-β-hydroxy-D-aspartic acid
VII
meso-Tartaric acid
VIII

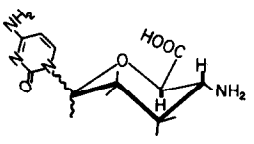
ethylenic groups in III by n.m.r. spectroscopy. Hydrogenation of III in acetic acid over platinum oxide catalyst afforded three products; cytosine, an α-carboxy-β-aminopyran (IV) and dihydrocytosinine (V). This facile hydrogenolysis of cytosinine indicated that the double bond in the sugar moiety was allylic to N-1 of the cytosine moiety. Confirmation of the 2',3'-ene structure of III was obtained later by oxidative and degradative studies.

Esterification of cytosinine followed by acetylation gave the di-N-acetate (VI) which was ozonolyzed, then oxidized with peroxide, and finally hydrolyzed with acid. A β-hydroxyaspartic acid (VII) was obtained which, after treatment with nitrous acid, afforded meso-tartaric acid (VIII). Since the latter reaction is known to proceed with retention of configuration (7), Ōtake et al. (6) concluded that VII was an erythro-β-hydroxyaspartic acid. From the optical rotation of VII (- 47°), they assigned the D configuration to this amino acid (rotation of L-isomer = + 51°) (7,8,9). It was concluded (7) that compound VII is erythro-β-hydroxy-D-aspartic acid.

Having thus established the configuration of the amino acid fragment as VII, they (7) concluded that the structure of the carbohydrate moiety in cytosinine (III) was of the (R) configuration (vide infra) at C-4' and C-5'. They depicted cytosinine as a nucleoside bearing an L-sugar (see structure IIIa, Fig. 2).

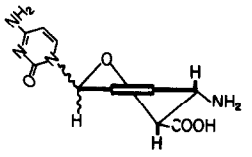
They gave the conformational structure of dihydrocytosinine as Va (Fig. 2) because the n.m.r. spectrum showed an axial-axial orientation of C-4' and C-5' protons (J value = 10.5 c.p.s.). The configuration at the glycosyl linkage (C-1') "remained uncertain but it was presumed to be beta, since nucleosides from natural sources were reported as beta." (6,10). Their proposed structure for blasticidin S is therefore Ia (Fig. 2).

FIG. 2



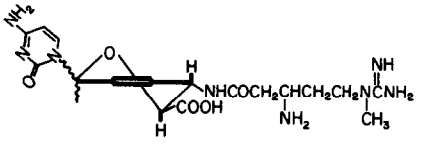
DIHYDROCYTOSININE

V a



CYTOSININE

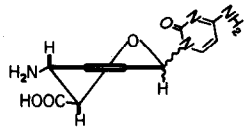
III a



Ōtake's structure of Blasticidin S

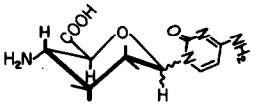
I a

FIG. 3



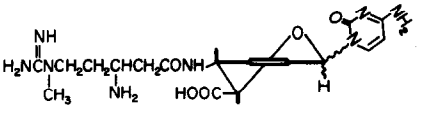
CYTOSININE

III b



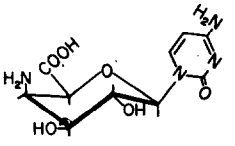
DIHYDROCYTOSININE

V b



BLASTICIDIN S

I b



IX

After careful scrutiny of their available data (5,6), we cannot accept their configurational assignment to the carbohydrate moiety. The C-4' (R), C-5' (R) configuration (11,12) given by Otake et al. (6) in their cytosinine IIIa or dihydrocytosinine Va structures cannot be reconciled with their erythro-β-hydroxy-D-aspartic acid structure VII.

Structure VII requires the D- rather than the L-configuration for the carbohydrate moiety in cytosinine and dihydrocytosinine [4'(S), 5'(S) in the Cahn-Ingold-Prelog system (12)]. We propose structures IIIb and Vb (Fig. 3) for cytosinine and its dihydro derivative, respectively. These structures are also consistent with the n.m.r. data (axial-axial C-4', C-5' protons) reported for dihydrocytosinine by the Japanese investigators. From these considerations, a revision of Ōtake's structure I and Ia (Fig. 1 and 2) for blasticidin S to the revised structure Ib (Fig. 3) is in order (13). It should be emphasized that our structural revisions are based solely on chemical data (5,6) presently available.

Since our cytosinine structure IIIb contains a 4'-amino-4'-deoxy-D-erythro-hex-2'-ene-uronic acid moiety, it should be possible to ascertain unequivocally the configuration at its glycosyl center. It will be recalled that C-substance (IX) derived from the nucleoside antibiotic, gougerotin, is of established configuration at C-1' and C-5' (β-D) (14,15). Oxidation of cytosinine to the 2',3'-glycol followed by periodate oxidation should give the same dialdehyde as would periodate oxidation of C-substance -- only if cytosinine (and, thereby, blasticidin S) is a β-nucleoside. If the same dialdehydes are not obtained from these oxidations, one would conclude that cytosinine is an α-nucleoside. Because of the importance of this antibiotic to the rice economy of Japan, such studies are most essential.

Establishment of the configuration at the anomeric center in blasticidin S would also be of great importance in any rationalization

of its biochemical behavior with structure.

It is of interest that from our revised blasticidin S structure (Ib), a further generalization on common structural features (15) of pyrimidine nucleoside antibiotics is now possible. Not only do these antibiotics contain a cytosine aglycon, at least one amino acid, and a 4-amino hexose; but also this aminohexose moiety is of the D-configuration.

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(9) The rotation of - 47° reported by Ōtake et al. (6) was taken in water. Actually, the rotations of erythro-β-hydroxy-L-aspartic acid reported by Sallach (7) are + 51° in N HCl and + 12° in water. However, the possibility of VII being a threo compound is easily excluded on the basis of Ōtake's n.m.r. assignment of the C-4', C-5' diaxial protons in dihydrocytosinine V (vide infra).

(10) From this statement on presumed beta configurations, it is difficult for the reviewers to understand why the Japanese investigators (6) portrayed cytosinine and dihydrocytosinine structurally as alpha nucleosides. To avoid confusion we have taken the liberty to depict their structures in the presumed beta form (Fig. 2) in order to conform with the text of their paper.

(11) The (R) configuration presumably derives from the Cahn-Ingold-Prelog system of nomenclature based on a 3-dimensional formula for asymmetric carbon atoms. A discussion of this system is given by Eliel (12). According to this nomenclature, the erythro-β-hydroxy-D-aspartic acid (VII) is a 2(R), 3(S) system.

(12) E. L. Eliel, "Stereochemistry of Carbon Compounds", McGraw Hill Book Co., Inc., New York, 1962, p. 92.

(13) It was stated (5) that X-ray analysis of blasticidin S monohydrobromide "... was parallelly investigated with the chemical work and reached

complete agreement ..." with their structure (I) (Fig. 1). In the absence of precise data, it is difficult to assess this statement. It should be noted that Ōtake's structures for cytosinine and dihydrocytosinine (Fig. 2) are mirror images of our revised structures (Fig. 3) for these nucleosides. Moreover, the configuration of the β-carbon of the blastidic acid residue in blasticidin S has not as yet been determined. More importantly, our revised structure for blasticidin S (Ib) (Fig. 3) was not taken into account in the X-ray analysis of the antibiotic. From all these considerations, the claim (5) of "complete agreement" between chemical studies and X-ray analysis on blasticidin S is certainly inconclusive.

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