Tetrahedron Letters No. 9, pp. 541-544, 1963. Pergamon Press Ltd.
Printed in Great Britain.

THE BIOSYNTHESIS OF NICOTINIC ACID BY MYCOBACTERIUM TUBERCULOSIS

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Herrn Prof. Dr. H.v. Euler-Chelpin zur 90. Wiederkehr seines Geburtstages in Verehrung gewidmet.

(Received 21 January 1963)

In many fungi like Neurospora crassa and in mammals it has been shown that nicotinic acid is formed from tryptophan.[1] This biosynthetic pathway from tryptophan to the pyridine ring system, has, however, not been realised in higher plants nor in many microorganisms.[2] In Xanthomonas pruni it is indicated that tryptophan is the precursor of nicotinic acid,[3] in many other bacteria, however, no intermediate products of the tryptophan metabolism can replace nicotinic acid as the essential growth factor.[4-7] Recent reports indicate that C_3-compounds like glycerol and C_4-compounds like succinic acid or aspartic acid are direct precursors in the biosynthesis of the pyridine ring.[2]

During the biosynthesis of nicotinic acid in a BCG-strain of Mycobacterium tuberculosis,+ it was observed that there is an increased secretion of nicotinic acid into the culture medium after addition of asparagine.[8] Therefore, it was assumed that there is a direct relation between the metabolism of asparagine and the formation of nicotinic acid - an observation recorded by other authors.[9]

It has been shown[10] that after feeding DL-aspartic acid-[4-^{14}C], radioactive nicotinic acid is formed in which the total radioactivity is in the carboxylic group. This means that the carboxylic group of the nicotinic acid originates from the C_4 of aspartic acid.

It has been shown during the incorporation of one molecule aspartic acid in nicotinic acid; that the C_1-carboxylic group is lost and that the

+ Herrn Prof. Dr. H. Knöll und Herrn Dipl. Biol. A. Zureck vom Institut für Mikrobiologie und experimentelle Therapie, Jena danken wir für die grosszügige Bereitstellung der verwendeten BCG-Kulturen.

pyridine nitrogen originates from the amino group of the aspartic acid.

In feeding experiments, 80 mg synthetic[11] DL-aspartic acid-[1.4-^{14}C,^{15}N] was added to 20 ml Sauton-culture medium and after 10 days incubation, 200 µg radioactive nicotinic acid was isolated by the method of Mothes.[8] This diluted with 25 mg inactive nicotinic acid, was crystallized for constant radioactivity. For decarboxylation, 5 mg of this radioactive nicotinic acid was diluted with 20 mg inactive nicotinic acid and mixed with 75 mg calcium oxide. The pyridine formed by heating this mixture was absorbed in a saturated alcoholic solution of picric acid. The pyridine picrate was crystallized for constant radioactivity, yield: 15 mg, m.p. 160°. All assays for ^{14}C were performed with a flow counter (Frieseke & Hoepfner, FH 49).

Table 1 Incorporation of DL-aspartic acid-[1.4-^{14}C,^{15}N] in nicotinic acid by Mycobacterium tuberculosis

compound	specific radioactivity	specific incorporation
DL-aspartic acid-[1.4-^{14}C,^{15}N]	3.35 $\cdot 10^8$ cpm/mMol	
nicotinic acid	1.6 $\cdot 10^8$ cpm/mMol	48%

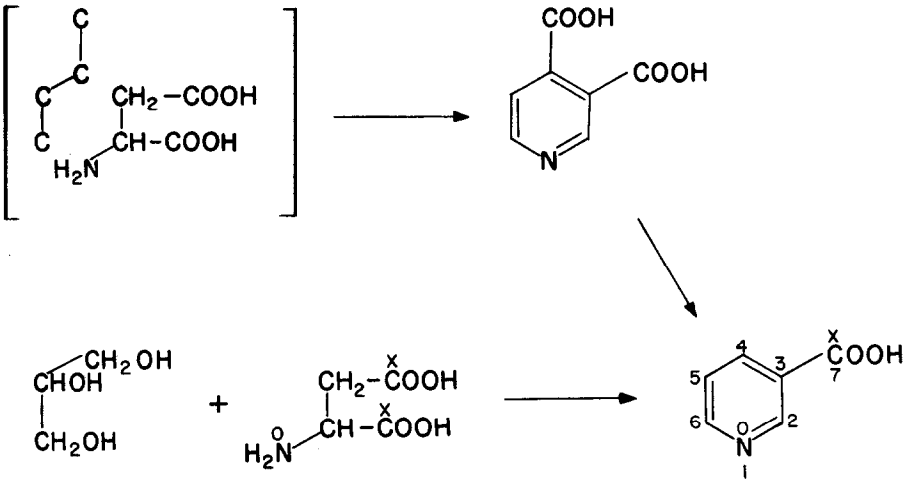
The decarboxylation of nicotinic acid yielded a pyridine picrate without radioactivity. Feeding of aspartic acid-[1.4-^{14}C,^{15}N] to Mycobacterium tuberculosis also yielded a nicotinic acid only labelled in the carboxylic group.

These two results indicate that since the C_1-carboxylic group of the aspartic acid is lost during the biosynthesis of nicotinic acid, the effective incorporation rate of the aspartic acid is 96% and not 48%. Further, the results show that the nicotinic acid is built up of only one molecule aspartic acid.

As cinchomeronic acid is a precursor of nicotinic acid in Lactobacillus arabinosus,[12] it is probable that the nicotinic acid originates from two molecules aspartic acid or a biochemical equivalent.

Experiments with Mycobacterium tuberculosis have proved this to be the case. It has been shown that BCG-bacteria are able to incorporate glycerol-

[1.3-^{14}C] into the nicotinic acid at a high specific rate. A chemical degradation proving the ^{14}C-distribution is in progress.



As aspartic acid and succinic acid are so closely related, it was necessary to determine which of these two compounds is the direct precursor and if the aspartic acid is incorporated inclusive of its nitrogen. Experiments with doubly labelled aspartic acid proved the latter to be the case.

Table 2 Isotopic Ratio $^{14}C/^{15}N$ after incorporation of aspartic acid-[1.4-^{14}C, ^{15}N] into nicotinic acid by Mycobacterium tuberculosis.

compound	Excess Atom % ^{15}N	specific radioactivity	Ratio specific radioactivity/ excess ^{15}N
DL-aspartic acid [1.4-^{14}C, ^{15}N]	33	$3.35 \cdot 10^{8}$ cpm/mMol	$1.01 \cdot 10^{7}$
nicotinic acid (diluted 1:125)	0.28	$1.28 \cdot 10^{6}$ cpm/mMol	$0.46 \cdot 10^{7}$

The ^{15}N-analyses were carried out by spectroscopic and mass spectrometric methods.

After incorporation of aspartic acid inclusive of its nitrogen and with only one carboxylic group in the nicotinic acid, theoretically only 50% of the $^{14}C/^{15}N$ ratio of the total aspartic acid can be expected, i.e. the isotopic ratio of $^{14}C/^{15}N$ in the nicotinic acid cannot be $1.01 \cdot 10^7$, but should be $0.505 \cdot 10^7$. The ratio found (0.46) is 91% of that theoretically possible.

These results prove that aspartic acid inclusive of its nitrogen is directly incorporated into the nicotinic acid molecule, and that it is of the origin atoms 2, 3 and 4. The other three C-atoms of the pyridine ring could come via glycerol or glycerolaldehyde.

Nicotinic acid is of great importance in the biosynthesis of many alkaloids, and proof that the amino group of an amino acid is incorporated into a heterocyclic system confirms the suggestion that the alkaloids are derived from proteinogenic amino acids.

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