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*HYDROXYANTHRANILIC ACID AS A PRECURSOR OF NICOTINIC ACID IN NEUROSPORA**

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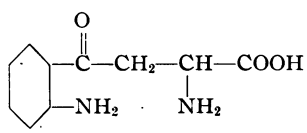
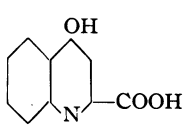
Recent investigations in this laboratory[1] have provided evidence that the biosynthesis of nicotinic acid in *Neurspora* proceeds from tryptophane through the intermediate kynurenine I.

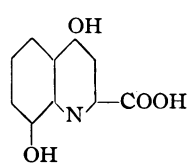


Kynurenine I

Further studies suggested that the pyridine ring of nicotinic acid might arise by ring closure of the α keto acid corresponding to kynurenine to give the naturally occurring compound kynurenic acid II[2] or, if preceded by oxidation, xanthurenic acid III.[3]



Kynurenic acid II

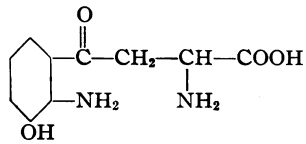


Xanthurenic acid III

In addition to these two compounds a series of nicotinic acid derivatives was synthesized and tested for growth promoting or growth inhibiting properties on *Neurospora* mutant 65001.[1] These compounds included: 3-carboxy-4-hydroxy pyridine, 3-carboxy-4-amino pyridine, 2-hydroxy-3-carboxy pyridine, 3-carboxy-6-hydroxy pyridine, 2,3-dicarboxy pyridine, 3,4-dicarboxy pyridine, 2,6-dimethyl-3,4-dicarboxy pyridine, 2,3,4-tricarboxy-6-methyl pyridine, 3-carboxy-4-chloro pyridine and 2,6-dimethyl-3-carboxy-4-chloro pyridine. In high concentrations, the compound 3-

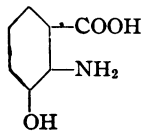
carboxy-4-amino pyridine promoted a small amount of growth, but the remaining compounds possessed no stimulatory or inhibitory action under the conditions utilized.

From the above facts it was concluded that the pyridine ring of nicotinic acid does not arise from kynurenine through kynurenic acid II or xanthurenic acid III followed by oxidation of the benzene ring. It also appeared evident that the oxidation in position 8 of xanthurenic acid III precedes formation of the pyridine ring, a possible intermediate being 3-hydroxykynurenine IV.



Hydroxykynurenine IV

A consideration of this hypothetical compound suggested the possibility of biological oxidation to give 3-hydroxyanthranilic acid (2-amino-3-hydroxybenzoic acid) V instead of xanthurenic acid.



Hydroxyanthranilic acid V

A trimethyl derivative of this compound V is indeed found in nature as the alkaloid damascanine (2-methyl-amino-3-methoxy-methyl benzoate).[4,5] The alkaloid has been isolated from the seeds of two species of *Nigella* (common name of flowers, Love in a Mist).

It is the purpose of the experimental part of the present paper to present evidence that hydroxyanthranilic acid is an intermediate in the biological synthesis of nicotinic acid from tryptophane in *Neurospora*.

Hydroxyanthranilic acid has been synthesized in this laboratory by two independent methods. These methods and the proof of structure of the active compound will be presented elsewhere.

Experimental.—Media and conditions for growth of mutant 65001 have been previously described.[1,6] Growth curves for this mutant in the presence of nicotinamide and hydroxyanthranilic acid (filter sterilized) are presented in figure 1. For these experiments the pH of the medium was adjusted to 4.1 since hydroxyanthranilic acid, like nicotinic acid, is less active at a higher pH where dissociation is greater. In four days at a pH of 5.6 the compound is 50 to 70% as effective as nicotinamide in promoting growth. It is thus more effective than nicotinic acid at pH 5.6.[7]

The growth-promoting activity of hydroxyanthranilic acid on several

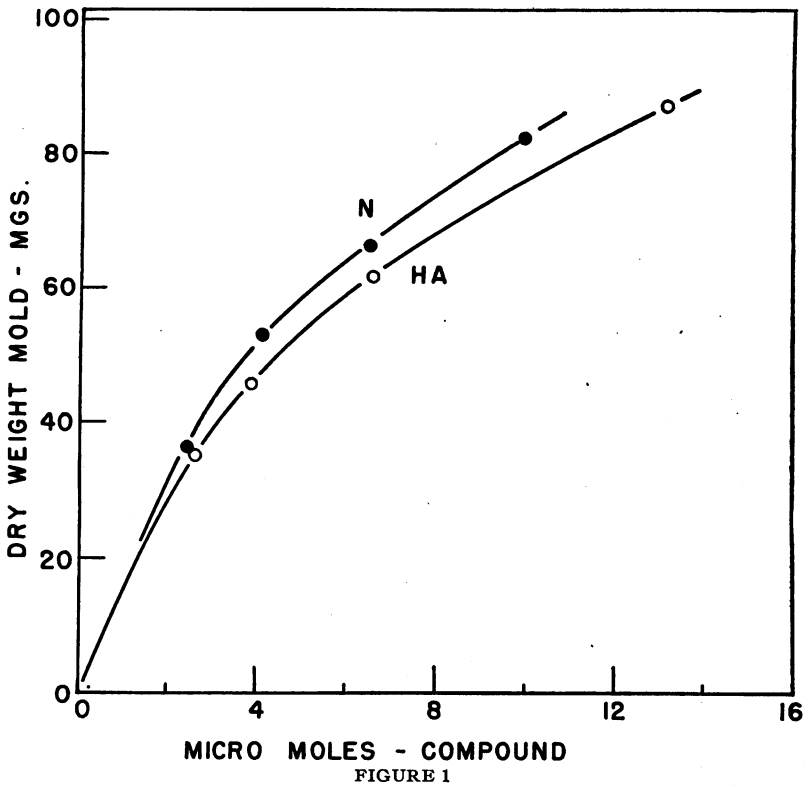


FIGURE 1

Growth curves of mutant 65001 ($6^1/_2$ days) in the presence of nicotinamide (curve *N*) and hydroxyanthranilic acid (curve *HA*).

genetically different mutants of *Neurospora* was compared to that of anthranilic acid, indole, tryptophane, kynurenine and nicotinamide, and qualitative data is presented in table 1.

TABLE 1

ACTIVITY OF HYDROXYANTHRANILIC ACID COMPARED TO ANTHRANILIC ACID, INDOLE, TRYPTOPHANE, KYNURENINE AND NICOTINAMIDE ON *Neurospora* MUTANTS

MUTANT STRAIN	ANTHRANILIC ACID	INDOLE	TRYPTOPHANE	KYNURENINE	HYDROXYANTHRANILIC ACID	NICOTINAMIDE
44008	−	+	+	+	+	+
65001	−	+	+	+	+	+
39401	−	+	+	+	+	+
4540	−	−	−	−	−	+
3416	−	−	−	−	−	+

It was previously shown[1] that an excess of a compound with nicotinamide activity is produced by mutant 65001 when it is grown in the presence of an

excess of kynurenine. Similar experiments with hydroxyanthranilic acid are summarized in table 2. Nicotinic amide activity was determined by use of strain 3416 which does not utilize hydroxyanthranilic acid. Determinations were made on culture fluids of six-day-old cultures of 65001 and 44008 grown in the presence of various quantities of hydroxyanthranilic acid.

TABLE 2

PRODUCTION OF NICOTINAMIDE ACTIVITY FROM HYDROXYANTHRANILIC ACID BY MUTANTS 65001 AND 44008

HYDROXYANTHRANILIC ACID, μG. PER 20 ML.	DRY WEIGHT		NICOTINAMIDE ACTIVITY, μG. PER 20 ML. OF CULTURE FLUID	
	65001	44008	65001	44008
0	2	0	0	0
20	84	97	0	0
50	97	95	0	0
100	108	94	10	12
200	103	85	12	12

Discussion.—It is evident from the experimental data presented, that for certain *Neurospora* mutants, hydroxyanthranilic acid possesses growth-promoting activity that is quite comparable to the activity of nicotinamide. In addition it has been demonstrated that in the presence of an excess of hydroxyanthranilic acid two of the *Neurospora* mutants produce an excess of a substance with the biological activity of nicotinic acid or nicotinamide. This was determined by use of a mutant that utilizes either of the latter two compounds but does not utilize hydroxyanthranilic acid.

Thus it appears probable that this substance is a natural intermediate in the biological synthesis of nicotinic acid by the mold *Neurospora*. It is of interest to note the complex series of reactions that are required by the mold to convert anthranilic acid to hydroxyanthranilic acid. These reactions are illustrated schematically in figure 2.

No comparison has been made, in this laboratory, between the properties of hydroxyanthranilic acid and those of the nicotinic acid precursor from *Neurospora* described by Bonner and Beadle.[7] From the published data it can be seen that the molecular formula is similar. The isolated precursor, however, is reported to be a pyridine derivative. As such it would be expected to be further along in the series of reactions leading to nicotinic acid synthesis. In this connection it may be suggested that hydroxyanthranilic acid can be converted to nicotinic acid by oxidation and loss of carbon three of the compound, followed by ring closure or rearrangement in the six carbon amino acid residue. If this occurs in animals and in *Neurospora* the 3-carboxy-6-pyridone isolated by Knox and Grossman[8] may well be a by-product of the reaction. Similarly, the occurrence of damascanine in *Nigella* may be accounted for as resulting from a side reaction in the biosynthesis of nicotinic acid in the organism.

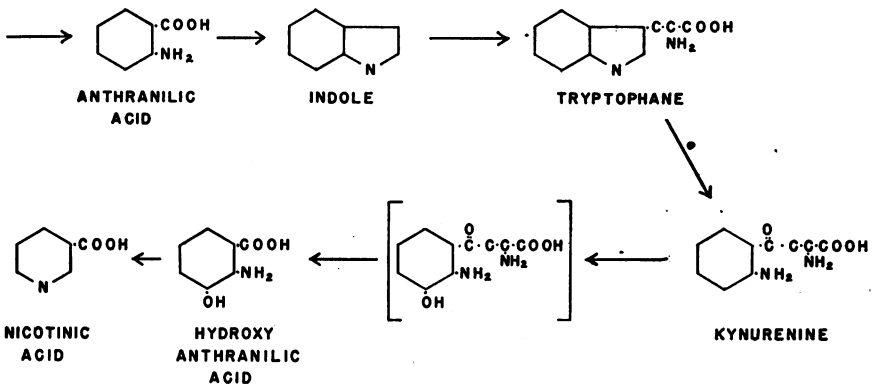


FIGURE 2

A schematic representation of a series of reactions leading to the biosynthesis of nicotinic acid in *Neurospora*.

Summary.—1. Evidence is presented to show that hydroxyanthranilic acid (2-amino-3-hydroxy benzoic acid) is an intermediate in the biosynthesis of nicotinic acid in *Neurospora*.

2. Several nicotinic acid derivatives and other related compounds are shown to lack significant biological activity.

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[1] Beadle, G. W., Mitchell, H. K., and Nyc, J. F., *Proc. Nat. Acad. Sci.*, **33**, 155 (1947).
[2] Ellinger, A., *Ber.*, **37**, 1801 (1904).
[3] Lepkovsky, S., Roboz, E., and Haagen-Smit, A. J., *J. Biol. Chem.*, **149**, 195 (1943).
[4] Pommerehne, F., *Archiv. Pharm.*, **238**, 531 (1900).
[5] Keller, O., *Ibid.*, **246**, 1 (1908).
[6] Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **32**, 678 (1945).
[7] Bonner, D. M., and Beadle, G. W., *Archiv. Biochem.*, **11**, 319 (1946).
[8] Knox, W. E., and Grossman, W. I., *J. Biol. Chem.*, **166**, 391 (1946).

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*THE IDENTIFICATION OF A NATURAL PRECURSOR OF NICOTINIC ACID**

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In a previous paper[1] the production and isolation of a natural precursor of nicotinic acid was described. The present paper deals with the identification of this precursor.

Several mutant strains of *Neurospora crassa* have been characterized as