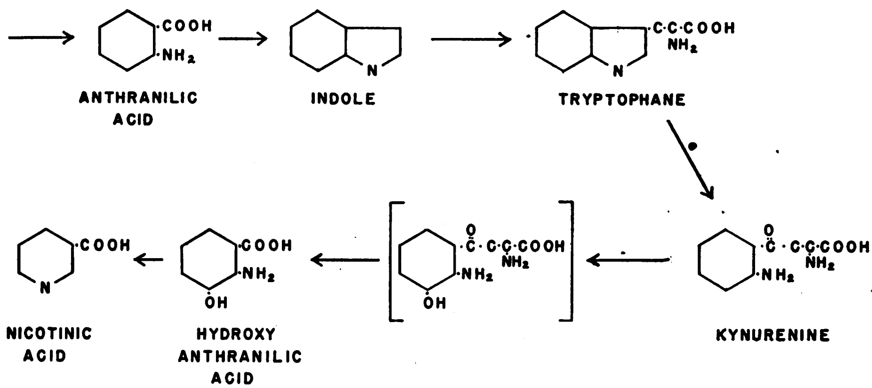

FIGURE 2

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.

* These investigations were supported by funds from the Rockefeller Foundation and the Williams-Waterman Fund for the Combat of Dietary Diseases.

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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 requiring nicotinic acid, nicotinamide, or related compounds for growth.[1, 2] Genetic investigation of these strains indicates at least three genetic types[1] which in accord with the usual interpretation[3] suggests at least three separate steps in the biosynthesis of nicotinic acid. Since in theory different biosynthetic steps are blocked in the various mutant strains requiring nicotinic acid for growth, culture filtrates were tested for the accumulation of intermediates. Strain #4540 when grown in limiting amounts of nicotinamide was found to accumulate a substance possessing nicotinic acid activity for a strain of a second genetic type (#39401).[1] Fractionation of culture filtrates yielded a small amount of a crystalline compound as active as nicotinic acid for growth of strain #39401.[1]

Elementary analysis of the isolated material establishes the probable empirical formula $C_7H_7O_3N$. Due to the difficulty encountered in preparing sufficient amounts of pure substance, however, analysis of material of unquestionable purity has not been carried out.

	% C	% H	% N
Calculated for $C_7H_7O_3N$	54.8	4.6	9.2
Found	54.6	4.8	10.1

Determination of the molecular weight suggests either a C-6 or C-7 structure. Since the physical properties of the isolated material resembled those of the pyridone carboxylic acids, several pyridones were prepared. The 6-oxy-nicotinic acid, 4-oxy-nicotinic acid, 2-oxy-nicotinic acid, 4 amino-nicotinic acid, and 2,3-dicarboxy pyridine were prepared, tested and found inactive. In addition samples of N-methyl-6-oxy-nicotinic acid, and N-methyl-6-oxy-nicotinamide† were also tested and found inactive. Following a different approach in their investigation of the biosynthesis of nicotinic acid Mitchell and Nyc[4] prepared 3-hydroxy-anthranilic acid (2-amino, 3-hydroxy benzoic acid) and found it active as a precursor of nicotinic acid for strain #39401. Comparison of the physical and biological properties of the precursor isolated from Neurospora culture filtrates with a sample of 3-hydroxy-anthranilic acid, generously supplied by Drs. H. K. Mitchell and J. F. Nyc, California Institute of Technology, indicates identity of these two compounds. Table 1 lists the melting point and sublimation behavior of the two compounds, and figure 1 shows a comparison of the absorption spectra of the two compounds at a concentration of 10 γ/cc. 1MHCl from 320 mμ to 230 mμ.

TABLE 1

A COMPARISON OF THE PHYSICAL PROPERTIES OF THE PRECURSOR ISOLATED FROM NEUROSPORA FILTRATES, AND OF 3-HYDROXY-ANTHRANILIC ACID

	ISOLATED	SYNTHETIC
Melting point	255 °C.-d-vig. gas evolution	255 °C.-d-vig. gas evolution
Mixed melting point	255 °C.-d-vig. gas evolution	
Sublimation *in vacuo*	170–180 °C.	170–180 °C.
Absorption maxima	297 and 235 mμ	297 and 235 mμ

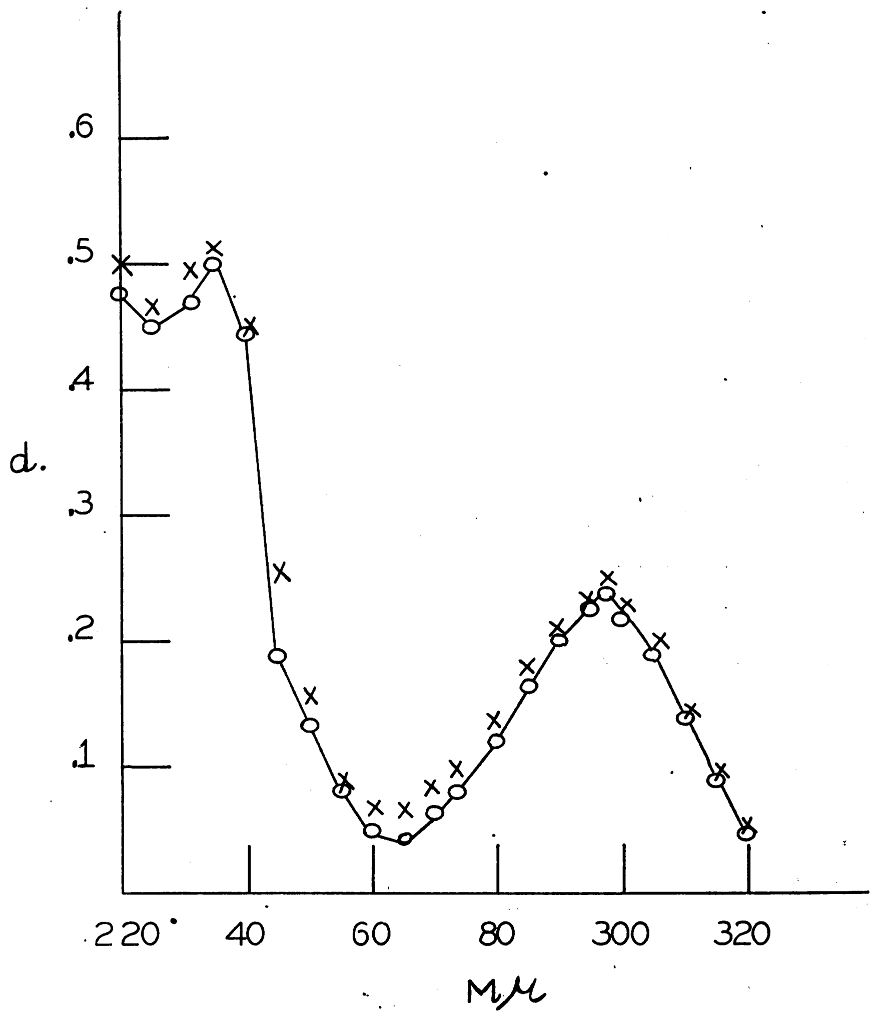

FIGURE 1

Comparison of the absorption spectra of the isolated Neurospora precursor and of 3-hydroxy-anthranilic acid. Concentration 10γ/ml. 1MHCl. ⊙–⊙, 3-hydroxy-anthranilic acid. x–x, isolated Neurospora precursor.

The physiological activity of both preparations is identical as shown in table 2, and both compounds are as active as nicotinic acid for each mutant strain tested. The strains listed in table 2 include those strains which can use indole, tryptophane, kynurenine or the Neurospora precursor, and in addition, a mutant strain recently isolated from material treated with a nitrogen mustard, which cannot utilize indole, tryptophane or kynurenine in place of nicotinic acid, but which can utilize the isolated precursor. The activity of 3-hydroxy-anthranilic acid is the same as that of the isolated precursor for the growth of this mutant strain.

TABLE 2

GROWTH OF VARIOUS MUTANT STRAINS OF NEUROSPORA ON NICOTINIC ACID AND RELATED COMPOUNDS

STRAIN NO.	ANTHRANILIC ACID	TRYPTOPHANE	KYNURENINE	ISOLATED PRECURSOR	3-HYDROXY-ANTHRANILIC ACID	NICOTINIC ACID
39401	−	+	+	+	+	+
65001	−	+	+	+	+	+
Y31881	−	−	−	+	+	+
4540	−	−	−	−	−	+
3416	−	−	−	−	−	+
75001	+	+	+	−	−	−
10575	−	+	−	−	−	−

The physical properties and biological activity of the precursor prepared from Neurospora filtrates appear, therefore, to be identical with those of 3-hydroxy-anthranilic acid. On the basis of these comparisons, the isolated Neurospora precursor is assigned the structure, 3-hydroxy-anthranilic acid (2-amino 3-hydroxy-benzoic acid).

Discussion.—With the identification of the Neurospora precursor as 3-hydroxy-anthranilic acid, the scheme of biosynthesis of nicotinic acid previously proposed would appear as:

39401
65001 Y-31881 4540 3416
A ⟶ B ⟶ 3-hydroxy-anthranilic acid ⟶ C ⟶ nicotinic acid

From work of Beadle, *et al.*,[2] kynurenine, tryptophane and the tryptophane precursor indole are known to replace nicotinic acid for strains 39401 and 65001. No strain has been found to date which can utilize anthranilic acid in place of nicotinic acid. This might suggest the sequence:

75001 10575 39401
65001
⟶ anthranilic acid ⟶ ⟶ indole ⟶ tryptophane ⟶

Y-31881 4540 3416
kynurenine ⟶ 3-hydroxy-anthranilic acid ⟶ ⟶ nicotinic acid

Such a scheme has been suggested by Beadle, *et al.*,[2] and by Mitchell and Nyc.[4] There are, however, certain discrepancies which are difficult to reconcile with such an interpretation. Mutant strains are known which accumulate anthranilic acid[5] yet which give no growth response to nicotinic acid, and there are also mutant strains known which can use tryptophane, indole, anthranilic acid or kynurenine which cannot utilize hydroxy-anthranilic acid or nicotinic acid for growth[5] (see table 2). Using appropriate genetic stocks it has also been impossible to detect the conversion of tryptophane or kynurenine to 3-hydroxy-anthranilic acid. The inactivity of kynurenine in replacing tryptophane or nicotinic acid as a precursor of N'-methyl nicotinamide in the rat has been reported by Rosen, *et al.*[6] Both kynurenine and 3-hydroxy-anthranilic acid have been found inactive in replacing tryptophane and nicotinic acid in preliminary growth experiments with rats.[7] It should be pointed out, therefore, that while these compounds are related to nicotinic acid synthesis, the specific rôle of each compound cannot as yet be definitely assigned.

Summary.—On the basis of the similarity in the physical and biological properties of a natural precursor of nicotinic acid isolated from Neurospora filtrates, and of 3-hydroxy-anthranilic acid, it is concluded that these two compounds are identical.

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† Generously supplied by Dr. W. Eugene Knox, College of Physicians and Surgeons, Columbia University.

ON LINE CONGRUENCES

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1. This paper summarizes without proofs some results on the following problem in projective three-dimensional space.

Let a two-parameter family of curves γ be given such that through every point of some region R of three-space there passes one and only one curve γ. Moreover consider a p-parameter family of surfaces Σ, such that in R every curve γ and every surface Σ have precisely one point in common. Then the family of curves γ determines in R a one-to-one correspondence between any two of the surfaces Σ. Now, assuming that for each pair of surfaces Σ this correspondence is *asymptotic*, i.e., maps the asymptotic lines of the one surface onto those of the other, we ask for the maximal value of p and furthermore we want to determine those congruences of