BIOSYNTHESIS OF THE PYRIDINE RING OF NICOTINE FROM SUCCINATE AND ACETATE

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Received January 17, 1963

The pyridine moiety of several compounds of plant origin is labeled with C^{14} from two, three, and four carbon metabolites (Griffith et al., 1962; Ortega and Brown, 1959; Waller and Henderson, 1961). Acetate-2-C^{14}, propionate-2-C^{14} and aspartate-3-C^{14} fed to tobacco plants through the roots were shown to be relatively specific precursors of the pyridine ring since in each case nearly one-half the C^{14} was incorporated in carbon 3. On the basis of these experiments, it was suggested that carbon from positions 2 and 3 was derived from the methylene carbons of succinic acid or a closely related compound (pathway a, Fig. 1; Griffith et al., 1962). Carbons 2 and 3 of the pyridine ring should be labeled by this pathway. Dawson and Christman (1961) have expressed a different hypothesis concerning the origin of these carbon atoms; they suggest that carbons 2, 3 and 4 of the pyridine ring of nicotine are derived from the internal carbons of citric acid (pathway b, Fig. 1).

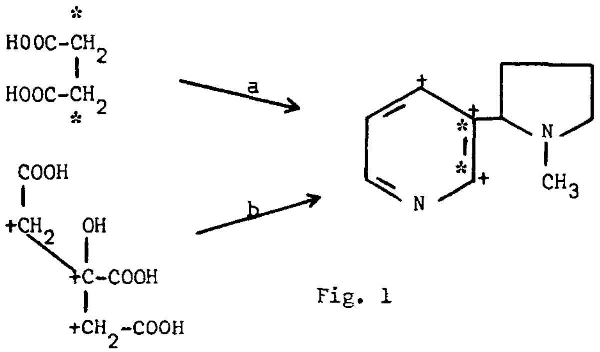

Fig. 1

This study presents results from specific degradations of nicotine from plants fed acetate-2-C^{14} and succinate-2,3-C^{14}. Carbons 2, 3, and 6 of the pyridi ring were isolated and assayed for C^{14}.

The results of measurements of C^{14} in nicotine and its degradation products following the feeding of C^{14} labeled metabolites are shown in Table 1. The distribution of C^{14} within the molecule is similar when either acetate-2-C^{14} or succinate-2,3-C^{14} was the precursor. The pyridine moiety contained essentiall the same percentage of C^{14} in each experiment. No data are presented concerning the distribution of C^{14} within the N-methylpyrrolidine ring of nicotine, but the specific activity of this ring would be the difference between the nicotine dipicrate and the pyridine picrate.

Carbon 3 of the pyridine ring had 34% (line 3) and benzoic acid derived from 2-phenylnicotine contained 38% of the C^{14} of the pyridine moiety (line 4). Thus, after feeding acetate-2-C^{14}, 72% of the C^{14} of the pyridine ring was associated with carbons 2 and 3 and was about equally divided between these two positions. Eleven percent of the C^{14} in the pyridine ring was found to be located at position 6 (line 5) and thus, 17% would be located in carbons 4 and/or 5. Results from the succinate-2,3-C^{14} experiment were nearly identical to those of the acetate-2-C^{14} studies. Therefore, as with acetate-2-C^{14}, when succinate-2,3-C^{14} was fed, a near equal quantity of C^{14} was associated with carbons 2 and 3 of the pyridine ring. Carbon 6 contained 7% of the C^{14} and the C^{14} not accounted for (15%) was located in carbons 4 and/or 5.

These data give strong support to the hypothesis that carbons 2 and 3 of the pyridine ring are derived from the methylene carbons of succinate or from equivalent carbons of a closely related acid. Acetate-2-C^{14} would yield succinate-2,3-C^{14} through the reactions of the tricarboxylic acid cycle. Succinate also has been shown to be an effective precursor of ricinine in the castor plant (Waller and Henderson, 1961) and nicotinic acid in *E. coli* (Ortega and Brown, 1959). The carbon of the pyridone ring of ricinine is very likely derived from the same intermediates as the pyridine ring of other plant compounds.

Table 1

DISTRIBUTION OF C^{14} IN THE PYRIDINE RING OF NICOTINE
FROM PLANTS FED ACETATE-2-C^{14} AND SUCCINATE-2,3-C^{14}

Compound	Acetate-2-C^{14}		Succinate-2,3-C^{14}	
	Specific Activity	Percent	Specific Activity	Percent
	(c.p.m./mmole x 10^{-3})		(c.p.m./mmole x 10^{-3})	
1. Nicotine dipicrate	19.1#		20.7#	
2. Pyridine picrate	11.1	100	12.2	100
3. Barium carbonate from carboxyl carbon of hygrinic acid (carbon 3 of pyridine ring of nicotine)	3.8	34	4.7	39
4. Benzoic acid from oxidation of 2 phenyl-nicotine (carbon 2 of pyridine ring of nicotine)	4.2	38	4.8	39
5. Benzoic acid from oxidation of 6 phenyl-nicotine (carbon 6 of pyridine ring of nicotine)	1.2	11	0.8	7

\# Specific activity of nicotine dipicrate after mixing nonradioactive nicotine with isolated radioactive nicotine. Nicotine dipicrate isolated from plants fed succinate-2,3-C^{14} was twice as radioactive as nicotine dipicrate from plants fed acetate-2-C^{14} when the two compounds were fed under essentially identical conditions.

* Percent C^{14} in pyridine moiety of nicotine.

METHODS

Tobacco plants (N. rustica) were grown and prepared for hydroponic feeding of the radiochemicals as reported previously (Henderson et al., 1959). Six hours after feeding radioactive metabolites, the plants were harvested and radioactive nicotine was isolated as the dipicrate salt. It was observed that the total incorporation of isotopes into nicotine from succinate-2,3-C^{14} was almost twice that of acetate-2-C^{14} (Pumplin and Byerrum, unpublished) when the isotopic compounds were fed to plants using standard procedures (Griffith et al., 1960).

Different quantities of non-radioactive nicotine were added to the radioactive samples in the two experiments to provide a sufficient amount of material to perform the degradations.

The procedures for isolation of carbon 3 of the pyridine ring and for the preparation of pyridine from nicotine have been reported earlier (Griffith et al., 1960; Lamberts and Byerrum, 1958). Carbons 2 and 6 of the pyridine ring were isolated by first preparing the 2- and 6-phenyl derivatives of nicotine (Abramovitch et al., 1960). The phenyl derivatives were separated by vapor phase chromatography using essentially the same conditions reported by Abramovitch, et al., (1960). Samples of the phenylnicotines were methylated with methyl iodide to form dimethiodides (Pictet and Genequand, 1897). These compounds were oxidized successively with alkaline potassium ferricyanide and chromic acid. Benzoic acid produced during these oxidations was extracted from the chromic acid oxidation media with ethyl ether and purified by sublimation and recrystallization from water. Generally, the yield of benzoic acid was about 10% of the theoretical.

All compounds to be analyzed for C^{14} were subjected to combustion and the carbon dioxide isolated as barium carbonate. Assays for C^{14} were performed with a Nuclear-Chicago proportional flow counter.

Since the carboxyl carbons of succinate or acetate are not utilized to a significant extent for synthesis of the pyridine ring the relatively small quantity of C^{14} associated with carbons 4, 5, and 6 indicated that this part of the molecule probably is not formed from a compound closely related to acetate or succinate.

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