The Pyridine Ring and the Problem of its Biosynthesis

THE relationships of anthranilic acid and tryptophane to nicotinic acid are well established in a number of organisms[1] and it has been suggested that in the higher plants the pyridine ring may be formed in a like manner[2]. It has been shown, however, that tryptophane-3-^{14}C is not a precursor of damascenine in *Nigella damascena* L., nor did it give rise to radioactive trigonelline when fed to pea seedlings[3]. It is possible that in the two plants studied the enzyme system necessary for the initial oxidation of tryptophane to kynurenine is lacking and that the hydroxyanthranilic acid in one case (damascenine) and the pyridine ring in the other (trigonelline) are synthesized from different precursors and by a different pathway.

These two cases, however, do not exclude the possibility that in some other plants tryptophane might be the precursor of the pyridine ring. It has been claimed that *Nicotiana tabacum* does not utilize tryptophane in the biogenesis of the pyridine ring of nicotine[4], but the location of the carbon-14 in the labelled tryptophane used was such as to make it very unlikely that it would appear in the nicotine. Since the present work has been completed, however, Leete[5] has shown that tryptophane labelled in the benzene ring at the position to which the nitrogen is attached is not utilized by *N. tabacum* in the formation of nicotine. Further, 3-hydroxyanthranilic acid, a metabolite of tryptophane, does not serve as a precursor of trigonelline when fed to excised soybean leaves[6].

It was of interest to determine whether a plant that cannot utilize tryptophane in its synthesis of the pyridine ring might be able to convert anthranilic acid (also a metabolite of tryptophane in certain moulds) into the pyridine ring since the initial oxidation of the tryptophane would be by-passed. Chemically, there is no reason why anthranilic acid as well as kynurenine could not be hydroxylated. We have now tested this point with regard to nicotine formation in *Nicotiana tabacum* var. White Mammouth fed with anthranilic acid-1-^{14}C. The carbon-14 label, however, was not transferred to the nicotine isolated from these plants.

The starting material used for the synthesis of anthranilic acid was toluene-1-^{14}C (purchased from Tracerlab Inc., Boston, Mass.). Nitration followed by oxidation of the resulting nitrotoluenes with permanganate afforded a mixture of nitrobenzoic acids from which most of the *p*-isomer was separated as its sparingly soluble copper salt[7]. Catalytic reduction of the recovered crude *o*-nitrobenzoic acid yielded anthranilic acid which was precipitated from neutral solution as its insoluble cobalt complex[8]. Treatment of the complex with hydrogen sulphide liberated very pure anthranilic acid which was isolated with ether, sublimed and crystallized from water from which it separated as needles, m.p. 144–145°.

The tobacco plants were grown from seed in soil for two months and then transferred to a nutrient solution prepared as described by Leete[9], but modified by substituting ferric tartrate as the source of iron. The nutrient solution was aerated continually and changed weekly. It was necessary to add further quantities of ferric tartrate (4·0 mgm. per l.) twice a week to suppress signs of iron deficiency in the plants. After three weeks, five plants were each suspended in a quantity of the nutrient solution and anthranilic acid-1-^{14}C (300 mgm.) of specific activity $1{\cdot}1 \times 10^7$ disint. per min. per m.mol. was divided equally between the five solutions. After 10 days of contact the nutrient solutions (total activity $1{\cdot}65 \times 10^6$ disint. per min.) were discarded and replaced by fresh solution (without labelled material). Eighteen days after feeding the anthranilic acid the plants were harvested, extracted with dilute hydrochloric acid and the nicotine separated from this extract (total activity $4{\cdot}8 \times 10^6$ disint. per min.) by distillation from alkaline solution[10] followed by precipitation as the dipicrate. Nicotine dipicrate crystallized from 30 per cent dimethylformamide as yellow needles, m.p. 223–224°, unaltered by mixture with an authentic sample. A quantity of the dipicrate was converted to the diperchlorate separating from the ethanol as needles, m.p. 207–208° either alone or in admixture with an authentic sample. Paper chromatography[11] showed nicotine to be the only pyridine base detectable in the crude plant extract. The nicotine salts showed no significant radioactivity.

From Leete's work[9] there is no doubt that the tobacco plant synthesizes nicotine at the stage of growth at which the present experiments were performed. Hence anthranilic acid does not function as a precursor of the pyridine ring of nicotine. Surprisingly, lysine does not give rise to the pyridine ring of nicotine either[9], nor is it the precursor of the α-pyridone ring of ricinine (Reist, E. J., and Marion, L., unpublished results). A body of evidence is, therefore, accumulating according to which it seems improbable that in the higher plants the pyridine ring would have its source in either tryptophane or lysine, which appeared to be the two most plausible precursors. It is true that according to Dawson *et al.*[12] the preformed pyridine ring (nicotinic acid) can be utilized by the plant in the synthesis of nicotine; but this does not seem to have much bearing on the initial formation of the pyridine structure. It now appears likely that the pyridine ring might be built directly from smaller units arising from alanine and glycine, or possibly from non-nitrogenous precursors, and ammonia. In micro-organisms where tryptophane functions as the precursor of nicotinic acid, the pyridine ring must arise by ring closure of an oxidative intermediate compound, which in the higher plants might be built up at a much faster rate from smaller units than it can be formed from the metabolites of tryptophane. The fact that nicotinic acid takes part in the biogenesis of nicotine[12], whereas tryptophane does not[5], lends support to this possibility, which is being investigated.

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[1] Mehler, A. H., in "A Symposium on Amino Acid Metabolism", edit. by McElroy, W. D., and Glass, H. B. (The Johns Hopkins Press, Baltimore, 1955).
[2] Mortimer, P. I., *Nature*, **172**, 74 (1953).
[3] Leete, E., Marion, L., and Spenser, I. D., *Can. J. Chem.*, **33**, 405 (1955).
[4] Bowden, K., *Nature*, **172**, 768 (1953).
[5] Leete, E., *Chem. and Indust.*, 1270 (1957).
[6] Aronoff, S., *Plant Physiol.*, **31**, 355 (1956).
[7] Schall, C., *Z. Electrochem.*, **21**, 70 (1915); **19**, 832 (1913).
[8] Morgan, G. T., and Moss, J. E., *J. Chem. Soc.*, **121**, 2866 (1922).
[9] Leete, E., *J. Amer. Chem. Soc.*, **78**, 3520 (1956).
[10] Smith, H. H., and Smith, C. R., *J. Agric. Res.*, **65**, 347 (1942.)
[11] Kuffner, F., Schlick, K., and Bühn, H., *Monatsh.*, **87**, 749 (1956).
[12] Dawson, R. F., Christman, D. R., Anderson, R. C., Solt, M., D'Adamo, A. F., and Weiss, U., *J. Amer. Chem. Soc.*, **78**, 2645 (1956).