Meetings

Aromatic Biosynthesis and Metabolism

Recent research on the biosynthesis and metabolism of aromatic compounds was reviewed and discussed at a symposium held at the University of Saskatchewan, 16–17 May.

In the opening address D. B. Sprinson (Columbia University) described the biosynthesis of aromatic amino acids by microorganisms. He summarized the work of Davis, who applied the penicillin method to the isolation of several auxotrophs of *Escherichia coli* which required for growth phenylalanine, tyrosine, tryptophan, and *p*-aminobenzoic acid. Shikimic acid could replace the aromatic amino acids in some strains. The pathway from glucose to shikimic acid was established by tracing the conversion of glucose, specifically labeled with carbon-14, to shikimic acid and by the isolation of the enzymes involved. The shikimic acid is converted to shikimic acid-5-phosphate which, in turn, yields 3-enolpyruvyl-5-phospho-shikimic acid after condensation with phosphoenolpyruvate. Elimination of phosphate and a shift of the existing double bond gives chorismic acid. This acid, recently isolated by F. Gibson, is the compound where the pathways to the various amino acids diverge.

Chorismic acid, in the presence of glutamine and an enzyme preparation, is converted to *p*-aminobenzoate and anthranilate; the latter is the precursor of tryptophan. If glutamine is absent, then *p*-hydroxybenzoate and prephenate are obtained; the latter is the precursor of phenyalanine and tyrosine.

This pathway is the only known route for the synthesis of aromatic amino acids in *E. coli*. There is increasing evidence that shikimate is apparently an intermediate for the synthesis of the phenylpropane unit in lignin, flavonoids, and anthocyanins in plants and for the aromatic amino acids in some microorganisms.

The metabolism of phenylpropionic, cinnamic, and phenylacetic acids by a *Pseudomonas* bacterium isolated from soil was discussed by E. R. Blakley (National Research Council, Saskatoon). The C_6-C_3 acids were metabolized to a 2,3-dihydroxypropionic acid through a metahydroxy-C_6-C_3 intermediate, while phenylacetic acid was metabolized through a 3,4-dihydroxyphenylacetic intermediate.

J. C. MacDonald (National Research Council) described a series of experiments concerned with the biosynthesis of fungal metabolites containing the pyrazine-N-oxide ring. Aspergillic acid is synthesized by *Aspergillus flavus* from leucine and isoleucine, and is the precursor of hydroxyaspergillic acid. A new compound 3,6-diisobutyl-2-hydroxypyrazine-1-oxide is formed by *Aspergillus sclerotiorium* from either leucine or 3,6-diisobutyl-2-hydroxypyrazine, and is the precursor of neohydroxyaspergillic acid. Pulcherriminic acid is synthesized by *Candida pulcherrima* from leucine.

T. L. Sourkes (Allan Memorial Institute, McGill University) opened the session on catecholamine metabolism with a paper on clinical and experimental studies of aromatic amino acids and amines. He discussed the effects of certain drugs, such as α-methyldihydroxyphenylalanine and some hydrazine derivatives, on the intermediary metabolism of aromatic amino acids or of their derived products. Studies on the metabolism of tyrosine, tryptophan, and 3,4-dihydroxyphenylalanine show the methods of mechanisms affected by toxic substances and drugs.

In a study on the chemical basis of catecholamine assays, R. A. Heacock (University Hospital, Saskatoon) showed how the extracted catecholamines are oxidized by iodine to their corresponding 7-iodoaminochromes, which in turn, are converted to suitable fluorescent derivatives. Use of iodine for aminochrome formation could give high values for adrenaline and noradrenaline at neutral and mildly alkaline *p*H. In one procedure described, the aminochromes are rearranged to fluorescent 5,6-dihydroxyindoxyls with a solution of ascorbic acid in concentrated sodium hydroxide. The possible decomposition of ascorbic acid under the reaction conditions and the nature of the necessary protective action were considered. Most investigators reported that the fluorophore obtained from noradrenaline was less fluorescent than that from adrenaline. Studies on pure noradrenolutin have now indicated the converse was true and that pure noradrenolutin was about twice as fluorescent as adrenolutin (on a weight basis).

A similar procedure has been described for the assay of dopamine. However, rearrangement of the aminochrome obtained from dopamine is carried out with alkaline sodium sulphite and then followed by acidification of the reaction mixture. It was suggested that the relatively stable fluorophore was, in this case, the sodium bisulphite addition product of 5,6-dihydroxyindole.

A paper on the influence of the thyroid gland on the metabolism of catecholamines by A. D'Iorio (University of Ottawa) explained the potentiation of the effects of catecholamines by thyroxine on a biochemical basis. The pretreatment of animals with thyroxine generally leads to a decrease of monoamine oxidase. This enzyme, which is partially responsible for the inactivation of catecholamines, can also be inhibited by a number of specific compounds without presenting the same potentiation as with thyroxine. Evidence indicates that pretreatment of rats with thyroxine leads to a decreased activity in catechol-O-methyltransferase, the most important inactivating enzyme of catecholamines. However, thyroxine does not in vitro inhibit O-methyltransferase while triiodo- and diiodothyroxine inhibit noncompetitively. The tetraiodo-, triiodo-, and diiodothyroformic acetic and propionic acid derivatives all behave as noncompetetitive inhibitors toward a semi-purified enzyme preparation. General iodophenolic acids also inhibit catechol-O-methyltransferase. The potentiation of catecholamines by thyroxine could be partly explained by the effect of the latter hormone on the two inactivating enzymes, monoamine oxidase and catechol-O-methyltransferase.

In the session on the biosynthesis of aromatic compounds in plants, E. E. Conn (University of Califronia, Davis)

discussed some of the enzymes which in plants catalyze the reactions in which phenylalanine is converted to its various derivatives. He mentioned, in particular, the series of reactions in which phenylalanine is converted to coumarin through the intermediates *o*-coumaric acid, *o*-coumaric acid glucoside, β-glucoside of coumarinic acid, and coumarinic acid. Conn noted that little is known about the initial hydroxylation of the benzenoid nucleus although subsequent hydroxylation is catalyzed by a phenolase complex. The biosynthesis of dhurrin, a cyanogenetic glucoside, has also been studied by Conn's group.

A. C. Neish (National Research Council, Halifax) spoke generally on aromatic biosynthesis in plants, and emphasized particularly some of its evolutionary aspects. He said the work of Birch and his group showed that benzenoid nuclei in many fungal metabolites were synthesized by the "head to tail" condensation of acetate units. These metabolites could be excreted into the growth medium of the fungus. However, in higher plants, which have developed from aquatic plants, numerous mutations have led to a highly developed phenylpropanoid metabolism in the plants. Hence, with vascular plants, lignin and tannins represent the end products of the metabolism of phenylpropanoid compounds and are not excreted but stored in the plant tissue.

Coumarins—lactones of *cis-o*-hydroxycinnamic acids—are formed by numerous higher plants and by some microorganisms. Studies with C^{14} have shown that the plant coumarins are formed by means of the shikimic acid pathway, phenylalanine, and cinnamic acid (S. A. Brown, National Research Council, Saskatoon). Plants which elaborate coumarin contain both *cis* and *trans* isomers of *o*-hydroxycinnamic acid glucoside. The *cis* glucoside, coumarinyl glucoside, is a "bound coumarin" which liberates free coumarin on hydrolysis with an endogenous β-glucosidase upon disruption of the cells. It is the major form in which coumarin exists in the plant, and the *trans* glucoside, *o*-coumaryl glucoside, is an intermediate in its formation. Other coumarins whose biosynthesis has been studied are herniarin, scopoletin, and aesculetin. All of them are simple hydroxylated or methoxylated coumarins and appear to be formed from phenylpropanoid carboxylic acids. Herniarin (7-methoxycoumarin), like coumarin, exists in lavender almost exclusively as the glucoside of the corresponding *cis*-cinnamic acid. It is formed from cinnamic and *p*-coumaric acids, probably by means of *p*-methoxycinnamic and 2-hydroxy-4-methoxycinnamic acids and the *trans* glucoside of the latter acid. The coumarin nucleus of the antibiotic novobiocin seems to be a special case from a biosynthetic standpoint; the lactone ring is formed through an oxidative cyclization from tyrosine. Existing evidence points to *ortho* hydroxylation as the mechanism of the lactone ring formation in plant coumarins.

In discussing some aspects of alkaloid biosynthesis, Leo Marion (National Research Council, Ottawa), described some of his recent work on the biosynthesis of pyridine-ring alkaloids, particularly ricinine. He outlined a scheme for the complete carbon skeleton degradation of ricinine. Marion then discussed the significance of the data obtained from carbon-14 experiments in which sodium acetate-1-C^{14} and -2-C^{14} succinic acid-2,3-C^{14}, glycerol-1-C^{14} and -2-C^{14}, and DL-lysine-2-C^{14}, were used as tracers which gave radioactive ricinine labeled in various positions. The results of these experiments and those from studies on the biosynthesis of nicotine have shown that for these two alkaloids, the pyridine ring system had not been incorporated, as such, into the alkaloid but rather had been formed of smaller units. Marion noted that tryptophan served as the precursor of pyridine rings in some microorganisms, in contrast to the results observed here.

Over a period of years, a number of hypotheses regarding biosynthetic pathways have been advanced but few have been substantiated experimentally (I. D. Spenser, McMaster University). Spenser outlined arguments concerning the amino acid and carbohydrate hypotheses on the formation of the isoquinoline alkaloids. He indicated that neither morphine nor papaverine is suitable for a study to distinguish between the two routes. However, the alkaloid hydrastine would allow the biosynthetic route to be elucidated because its synthesis requires the addition of a one-carbon fragment. The experiments showed that the biosynthesis of hydrastine occurred by the classical amino acid route. Similar data were obtained for the biosynthesis of berberine.

E. Leete (University of Minnesota) concluded the symposium with a paper entitled "Recent work on the biosynthesis of indole and related alkaloids." It was established with the aid of radioactive tracers that ajmaline, reserpine, serpentine, ibogaine, and gramine are all derived from tryptophan. The non-tryptophan-derived portion of ajmaline and related alkaloids has been the subject of much speculation. It had been suggested that this part of these alkaloids was derived from a hydroxylated phenylalanine or from mevalonic acid or shikimic acid. Tracer experiments did not substantiate any of these hypotheses. However, it was discovered that acetate and malonic acid were incorporated, and a new hypothesis involving these molecules has been developed. Quinine, a quinoline alkaloid, has also been shown to be derived from tryptophan.

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