

Purine Metabolism and Alkaloid Biosynthesis in Leaves of *Coffea arabica* L.

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SUMMARY

The occurrence of the classical pathway for the *de novo* biosynthesis of purine nucleotides was suggested by demonstration of the incorporation of several [¹⁴C]precursors into IMP and Σ Ade and by the inhibition of this incorporation by azaserine, a known inhibitor of *de novo* purine biosynthesis in other organisms.

Evidence demonstrating purine degradation via the classical pathway was provided by incorporation of the radiolabeled purine ribosides inosine and adenosine and the radiolabeled bases adenine and hypoxanthine into xanthine. In addition, allopurinol, a known inhibitor of xanthine oxidase and xanthine dehydrogenase, in all cases inhibited the incorporation of radiolabeled precursors into xanthine. Coffee leaves degraded 316 nmoles of [8-¹⁴]xanthine to urea per g fresh weight in 3-h confirming that urea is a product of degradation.

Incorporation of radiolabeled bicarbonate into IMP and Σ Ade was inhibited by the addition of adenosine to excised roots of *C. pepo* and leaves of *C. arabica* suggesting that the purine *de novo* pathway was regulated by end-product inhibition in both species. Regulation by end-product inhibition in *C. pepo*, a species that does not synthesize caffeine, was different from regulation in *C. arabica*. Although we were unable to identify the specific site of the *de novo* purine pathway sensitive to end-product inhibition in coffee, results suggest that an early step in the pathway prior to the synthesis of PRAICA is the site of physiological importance in the regulation of the pathway. In contrast, the site of physiological importance in the regulation of the *de novo* purine pathway in squash was at one or both of the enzymatic reactions converting PRAICA to IMP.

The pathway for the biosynthesis of theobromine, theophylline and caffeine was also investigated. Theobromine, theophylline and paraxanthine are logical precursors of caffeine. However, our results provided strong evidence that theobromine, theophylline and caffeine are the products of different pathways. The results suggested that theobromine is synthesized from adenine nucleotides, theophylline from guanine nucleotides and caffeine from the degradation of purines via hypoxanthine to xanthine with paraxanthine the immediate precursor of caffeine.