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Preface

Interest in the biological origins of natural products is nearly as old as the study of natural products themselves. In the early 1950s, the ready availability of radioisotopes of carbon and hydrogen opened up the classical era of experimental biosynthetic investigations. The results of numerous *in vivo* incorporation experiments with intact plants and microorganisms have largely confirmed and amplified the early biogenetic speculations of J. N. Collie, R. Robinson, L. Ruzicka and other pioneers. The application of high field nuclear magnetic resonance techniques in combination with stable isotopes brought about a further dramatic increase in the pace and sophistication of biosynthetic investigations by eliminating the necessity of lengthy chemical degradation procedures to determine the distribution of isotopes in biosynthetically labeled metabolites. The introduction of stable isotope NMR methods alone, however, did not change the basic experimental or philosophical approach to the design and interpretation of precursor – product experiments with intact organisms and the field of natural products biosynthesis, for all its conceptual and methodological elegance, remained largely an indirect science.

As an alternative to incorporation experiments with intact organisms, biosynthetic investigators have turned with increasing frequency over the last 15-20 years to the study of the enzymes responsible for natural product biosynthesis, at first using crude cell-free extracts and more recently highly purified, homogeneous proteins. This shift in emphasis has allowed the first detailed insights into the *mechanism* of individual biosynthetic reactions, the determination of cofactor requirements and the elucidation of the structure and sequence of reactive intermediates. At the same time it has also become possible for the first time to consider the structure and properties of the protein catalysts themselves, from the measurement of physical properties to the determination of the active site residues. The study of multistep enzyme-catalyzed reactions has presented particularly interesting challenges and brought the study of natural products biosynthesis to the frontiers of modern mechanistic enzymology.

The low titres of many biosynthetic enzymes and the attendant difficulties in obtaining sufficient quantities of protein for structural and mechanistic analysis have stimulated increasing interest in the application of modern recombinant DNA techniques for the isolation, molecular cloning, sequencing, and overexpression of the relevant structural genes for biosynthetically important enzymes. Indeed, in some cases the application of molecular genetics has leaped far ahead of the complementary biochemical studies. Thus several laboratories have recently succeeded in isolating and sequencing entire gene clusters responsible for the formation of polyketide antibiotics for which none of the relevant enzymes have yet to be characterized even in crude form. Investigations in the field of molecular genetics are also opening up new areas of inquiry, providing new information about the molecular regulation and control of secondary metabolic pathways while promising new insights into the genetic relationships among enzymes from different species performing similar biosynthetic transformations.

In the pages which follow there is a representative sampling of work being carried out by a variety of groups at the enzymological and genetic frontiers of natural products biosynthesis. Poulter, Scott, and Croteau have each contributed papers on the enzymes of the isoprenoid biosynthetic pathway. Zenk has described the cloning and heterologous expression of cDNA for two enzymes of alkaloid biosynthesis while Saito and Kawaguchi report their studies of the use of transgenic plant teratomas to study the metabolism of solanaceous alkaloids. Sankawa has investigated the role of P-450 dependent enzymes in plant isoflavonoid biosynthesis and Gould has studied the detailed mechanism of a bacterial isochorismate synthase. The use of recombinant DNA techniques to study two enzymes of longstanding interest, isopenicillin N synthase and uroporphyrinogen III synthase (cosynthetase) is reported by Baldwin and by Abell, respectively. Several papers deal with advances in polyketide enzymology and molecular genetics. Jordan has used chirally labeled malonates in combination with purified 6-methylsalicylic acid synthase and fatty acid synthase to elucidate the stereochemical course of polyketide chain elongation reactions. Sherman and his collaborators have investigated a proposed bifunctional cyclase/dehydrase gene from the actinorhodin gene cluster which is thought to be involved in controlling the regiochemistry of aromatic polyketide cyclization. Further investigations of the *act* gene cluster are described by Floss and Strohl along with a description of related work on thiopeptide antibiotics, while Omura has used *act* I and *act* III as probes to isolate the gene cluster for the benzoisochromane antibiotic kalafungin. Finally, Parry reports the use of a crude cell-free extract of *Streptomyces griseolus* to investigate the origin of the adenylyl moiety of the nucleoside antibiotic sinefungin.

The contributions to this Symposium-in-Print are only a small example of the wide range of activity and broad scope of this emerging area. Over the next several years, we should expect to see the most powerful tools of modern chemistry, enzymology, and molecular biology brought to bear on these complex and intriguing problems at the interface of chemistry and biology.

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