

**APPLICATIONS OF BIOCHEMICAL
SYSTEMS IN ORGANIC CHEMISTRY**

Part I

J. Bryan Jones, Charles J. Sih, &
D. Perlman

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APPLICATIONS OF BIOCHEMICAL SYSTEMS IN ORGANIC CHEMISTRY

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PART 1

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INTRODUCTION TO THE SERIES

Techniques of Chemistry is the successor to the Technique of Organic Chemistry Series and its companion—Technique of Inorganic Chemistry. Because many of the methods are employed in all branches of chemical science, the division into techniques for organic and inorganic chemistry has become increasingly artificial. Accordingly, the new series reflects the wider application of techniques, and the component volumes for the most part provide complete treatments of the methods covered. Volumes in which limited areas of application are discussed can be easily recognized by their titles.

Like its predecessors, the series is devoted to a comprehensive presentation of the respective techniques. The authors give the theoretical background for an understanding of the various methods and operations and describe the techniques and tools, their modifications, their merits and limitations, and their handling. It is hoped that the series will contribute to a better understanding and a more rational and effective application of the respective techniques.

Authors and editors hope that readers will find the volumes in this series useful and will communicate to them any criticisms and suggestions for improvements.

ARNOLD WEISSBERGER

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PREFACE

Despite the considerable literature that now exists documenting the value of applying biochemical methods to solve organic chemical problems, biologically oriented techniques are still largely ignored by most organic chemists. In the past, the reasons for this neglect have often been valid; however, this is no longer the case and the aim of this book is to promote a more widespread recognition of the potential of biochemical methods in structural and synthetic organic chemistry.

The task of organizing a book on a subject of this magnitude was undertaken only after considerable consultation with many organic and bioorganic chemists. It soon became evident that the range of problems that could benefit from applications of biological approaches was so broad that only a multiauthor format could cope satisfactorily with the variety of topics we thought should be covered.

We elected to interpret the term *biochemical systems* in a broad chemical sense in order to permit the inclusion of topics that, although not strictly biological, exploited biochemical principles in chemically useful ways. We felt this to be important because the interaction of the biological and chemical approaches will probably influence the development of the field to an increasing extent in the foreseeable future.

It was found that the material to be covered could be divided into two general areas, one dealing with well-established areas of biologically mediated transformations, and the one in which newer and more chemically based approaches could be grouped together. These divisions are reflected in Parts I and II of this book. The first chapter of Part I is an introduction to both volumes.

Part I focuses on microbiological and enzymic methods of synthesis and structure determination. The coverage of topics is deliberately uneven. For example, areas such as microbiological transformation which have been exhaustively reviewed in specialized books are afforded illustrative coverage only. On the other hand, applications of enzymes for asymmetric synthesis and configuration determination purposes are fairly comprehensively discussed because the data are not easily available in other single sources.

The chapters in Part II are devoted to biochemical areas that are growing rapidly in their relationships to organic chemistry, and also to strictly chemical topics that involve biochemically important principles. The authors of these

chapters were advised to write on their subject as they saw fit and were encouraged to speculate on the impact of their area on organic chemistry. Consequently, the chapters vary in their approach, degree of specialization, and depth of treatment. However, the flexibility that this arrangement affords should enable the readers to immerse themselves in the overall field at whatever level and pace they choose.

This book is intended to stimulate the interest of organic chemists and to provide them with information on how biochemical methods can be profitably used. In addition, many of the chapters will be of value to biochemists with interests at the molecular level. We have attempted to overcome the problems of language and philosophy that plagued the earlier books devoted to this task by inviting as contributors only organic chemists who have been using these biochemical methods or biochemists who have extensive chemical backgrounds. With the diversity of subjects it was inevitable that certain topics would be included by more than one author. Cross-references to the other chapters have been included where this has happened.

We realize that the application of biochemical systems in organic chemistry is already well established, and that no book of this size can hope to provide totally comprehensive coverage of what is known or what can be foreseen. Nevertheless, we hope that this treatise will prove of value to the growing circle of organic chemists who are interested in, or who wish to exploit, the principles and methods on which the life processes are dependent. We also hope that it will catalyze and stimulate further research in the field.

We are grateful to the many chemists and biochemists who have encouraged us during the preparation of this book, and also to Dr. Arnold Weissberger, Editor-in-Chief, for giving us this opportunity to put the volume together and for his unflagging enthusiasm for our objectives.

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Chapter I

BIOCHEMICAL SYSTEMS IN ORGANIC CHEMISTRY: CONCEPTS, PRINCIPLES, AND OPPORTUNITIES

J. Bryan Jones

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Chemists have been interested in biological problems since scientific studies on the origin of life were begun [1]. Following the studies carried out during the late eighteenth, nineteenth, and early twentieth centuries, the chemical nature of biochemical processes became firmly established, although not without considerable controversy [2]. During the remaining years of the first half of the twentieth century, the attention of almost all organic chemists was diverted from the immediate problems of biochemistry by the developments and challenges in structure determination, synthesis, and reaction mechanism

studies. In recent years, however, the interest of organic and physical-organic chemists in biological problems has been rekindled, with biosynthesis and mechanisms of enzyme action currently attracting the most attention.

Most of the investigators who have been drawn to the frontier area between chemistry and biochemistry have applied their expertise to examining chemical reactions catalyzed by various enzymes and to solving the stereochemical and mechanistic problems posed. In contrast, relatively little effort has been made to apply the knowledge gained in such studies to aid in the solution of synthetic, structural, stereochemical, and other problems of organic chemistry. The potential of biochemical systems in some of these areas has, of course, already been recognized. This is shown by the extensive applications of microbiological fermentation methods to structure modification [3-5] and to the production of chemicals [6], and by the use of hydrolytic enzymes in carbohydrate [7], protein [8], and nucleic acid [9] degradations. Synthetic uses of other enzymes in these specialized fields are also well documented [10]. Such examples of the practical utility of biochemical systems, of which the most dramatic so far is probably the enzymic synthesis of a gene [11], serve to illustrate the enormous potential that exists in this area; it is hoped that the subsequent chapters of this book will provide adequate documentation of the opportunities that exist.

Although an ever-increasing number of chemists are being attracted to the challenges of the chemistry-biochemistry frontier, many still hesitate to commit themselves to an area in which some of the principles and philosophies are not always immediately clear. This natural reluctance is often compounded by a lack of familiarity with biochemical terminology and methods [12]. Accordingly, the remainder of this introductory chapter is devoted to a brief, and in parts simplistic, review and discussion of the more important aspects of biochemical systems in the context of their practical relevance and potential to organic chemistry. For the most part the bibliography is restricted to illustrative and/or leading references only. More detailed treatments of most of the topics covered may be found through these references, in one of the excellent texts [13, 14] available, and in subsequent chapters of this book.

1 ENZYMES

A large proportion of the biochemical research carried out to date has been concerned with enzymic catalysis. Although major advances have been made in recent years in other areas of biology, the unique properties of enzymes and related model systems have had the greatest impact on organic chemistry, and it is this area that seems likely to dominate the interest of chemists in the foreseeable future. Many enzymes are now commercially available. Furthermore, if sufficient demand is created for a particular enzyme, the means to produce it

on a large scale can often be developed very rapidly. The speed with which large quantities of asparaginase became available once its anticancer potential was recognized illustrates this point. Furthermore, several agencies for the custom production of enzymes have been created.*

2 CLASSIFICATION OF ENZYMES

The protein nature of all enzymes is firmly established, [2, 13, 14] and almost 1800 different enzymes have now been identified. Of these, many have been fully or partly purified and more than 200 have been crystallized [15, 17, 18]. The molecular weight range encountered is very broad, varying from about 12,000 to more than 1,000,000.

In comparison with other catalysts used in organic chemistry, enzymes are exceptional in three main respects.

1. They are extremely versatile, and the spectrum of reactions catalyzed by them is very broad. Furthermore, the reactions take place under mild conditions, often at room temperature and close to neutral pH. These conditions minimize the problems, such as epimerization, racemization, and isomerization, that so often complicate the use of many organic reagents.
2. The enzymes are usually quite selective in terms of the type of reaction catalyzed and with respect to the structure and stereochemistry of the substrate and product.
3. The catalytic power of enzymes is very great. The rates of enzyme-mediated processes are faster than those of nonenzymic reactions by a factor of up to 10^9 to 10^{12} .

The types of enzyme-catalyzed reactions have been classified [18] into six main groups.

1. Oxidoreductases. Enzymes of this group catalyze oxidation-reduction reactions involving oxygenation or overall removal or addition of hydrogen atom equivalents.
2. Transferases. These enzymes mediate the transfer of one-carbon groups, such as aldehydic or ketonic, acyl, sugar, phosphoryl, and sulfur-containing ones, from one molecule to another.
3. Hydrolases. The range of functional groups hydrolyzed by such enzymes is very broad. It includes esters, anhydrides, peptide and other C-N-containing

*A list of enzyme suppliers and producers, together with the agencies from which microorganisms may be obtained, is provided in Appendix A at the end of Part I of this book. The properties of the enzymes available and information on how to handle them are often provided by the supplier. Comprehensive coverage of this basic information is available from treatises [15] or handbooks [16] devoted to this topic.

functions, glycosides, and others.

4. **Lyases.** The types of reactions catalyzed are additions to or formation of double bonds such as $C=C$, $C=O$, $C=N$.

5. **Isomerases.** A variety of isomerizations, including racemization, can be effected.

6. **Ligases.** These are often called synthetases, and catalyze the formation of C-O, C-S, C-N, and C-C bonds with accompanying adenosine triphosphate (ATP) or other nucleoside triphosphate cleavage.

Under the International Union of Biochemistry (IUB) enzyme nomenclature system [18], each of the above main groups is further divided into subclasses based on the nature of the transformation, coenzyme requirement, and other criteria, and a numerical classification derived from this approach is used to identify each individual enzyme. A four-number designation is used. For each enzyme the first number indicates the main class; the second and third indicate subclasses that designate the nature of the reaction quite specifically. The fourth number is simply the serial number of an enzyme within its own specific group. This classification permits each enzyme to be assigned a unique identification number. For example, the oxidoreductase yeast alcohol dehydrogenase is EC 1.1.1.1 and the hydrolase α -chymotrypsin is EC 3.4.21.1. The system is not an ideal one and contains several anomalies. One of the more serious disadvantages is that the separation of transferases, hydrolases, and ligases into different enzyme classes is not pedagogically sound. All enzymes of the main classes 2, 3, and 6 are in fact transferases that effect transfer of a group without changing the oxidation state of the reaction center. Despite its disadvantages use of the IUB classification method in papers or catalogs seems likely to continue for some time. The search for more satisfactory nomenclature systems continues [19].

A summary of the range of reactions catalyzed and their corresponding enzyme-type classifications is given in Appendix B at the end of Part I of this volume. The spectrum of possible functional group interconversions is extremely broad. Although at the present time many of the enzymes identified are too specific to be of much general utility to chemists, many of the reactions are encountered frequently in an organic laboratory. Unfortunately the enzymes studied in greatest detail do not always correspond to the processes of greatest current interest to organic chemists since, not surprisingly, most attention has been devoted to those enzymes that are most abundant and easily purified.

In contrast to the systematic classification developed for enzyme-catalyzed reaction types, development of a satisfactory nomenclature for the enzymes themselves is a much more difficult task, and the problem has not yet been solved. Naming on the basis of the chemical reaction catalyzed would seem at first sight to be the most satisfactory procedure, and many enzymes have been designated in this way by adding the suffix *-ase* to the name of the substrate

or reaction catalyzed. However many of the trivial names developed for the earliest discovered enzymes remain in current use. (Biochemistry is, of course, not the only scientific discipline that suffers the consequences of historical attachments to nonsystematic nomenclature.) This is particularly true for the proteolytic enzymes such as chymotrypsin, papain, rennin, and pepsin. In addition, complications often arise when an enzyme is named on the basis of a process that appears to be one reaction but is subsequently found to involve two or more distinct processes. An example of initial confusion of this type in an area of organic chemical interest is provided by the studies on the mechanism of the squalene to lanosterol oxidative cyclization process. It was originally thought that a single enzyme, formerly designated as squalene oxidocyclase EC 1.99.1.13, was involved. Subsequently, however, it was recognized that squalene oxide is a true intermediate and that squalene oxidocyclase is in fact two distinct enzymes [12, 20]. In view of these pitfalls, biochemists now usually rely on the functional classification (Appendix B) until the unique nature of an enzyme as a single protein has been unambiguously established. Even when the latter condition is met, a reaction-type classification remains preferable from the chemical viewpoint since pure enzymes can sometimes catalyze a spectrum of related or even different reactions. A number of enzymes exhibiting multiple specificities are known [12, 21].

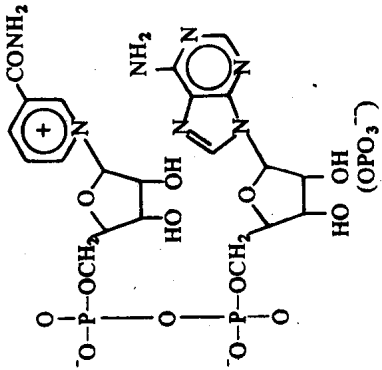
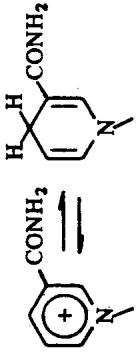
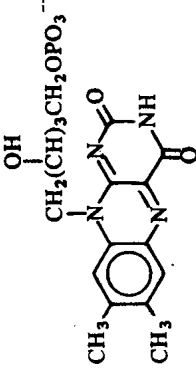
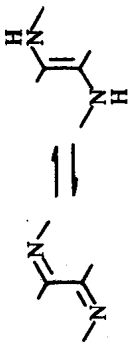
It should be noted that in some reactions by-products, such as ammonia, hydrogen peroxide, hydrogen sulfide, and hydrogen halides, are formed which may prove harmful to one or more of the components of the reaction mixture. Accordingly, whenever such systems are being applied for organic chemical purposes, precautions must be taken to counter any such effects. This can usually be done quite easily. For example, the concentration of any hydrogen peroxide produced can be reduced to a safe level by the addition of a peroxide-metabolizing enzyme such as catalase, and negative effects of acidic or basic species can be offset by maintaining the pH within a certain range.

3 COENZYMES

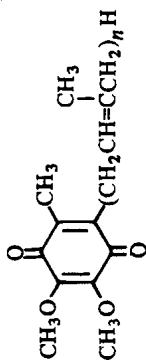
While catalytically active solutions of some enzymes are obtained by simply dissolving the protein in an aqueous buffer, many others require nonprotein cofactors. Such cofactors may be metal ions and/or relatively small organic moieties required as coenzymes. A coenzyme species that is tightly bound, covalently or otherwise, to the enzyme may be referred to as a prosthetic group. The structures of the most commonly encountered coenzymes and the range of reactions in which they are involved are summarized in Table 1.1.

A great deal is now known regarding the mechanisms of action of coenzymes, and this area continues to attract considerable interest [22-28]. Some coenzymes undergo a chemical transformation during the particular enzyme-catalyzed

Table 1.1 Some Common Coenzymes and Their Roles in Enzymic Transformations of Organic Chemical Interest

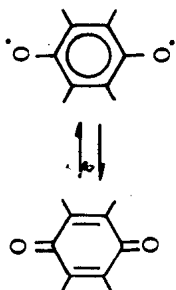
Coenzyme	Reaction types Involved	Key Coenzymic Reactions or Intermediates ^a
 <p>Nicotinamide adenine di- nucleotides (NAD⁺/NADH; NADP⁺/NADPH)</p>	Oxidoreductions	
	Oxidoreductions	

Flavin mononucleotide (FMN)
and related compounds.

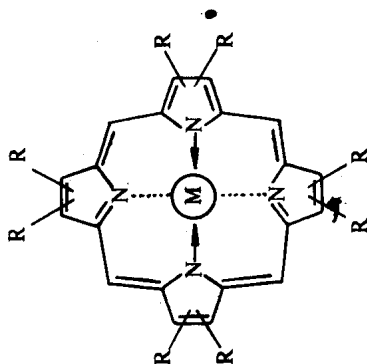
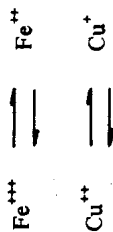


Ubiquinones (Coenzymes Q)

Oxidoreductive electron transfer

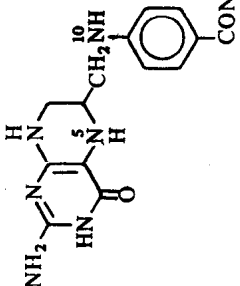
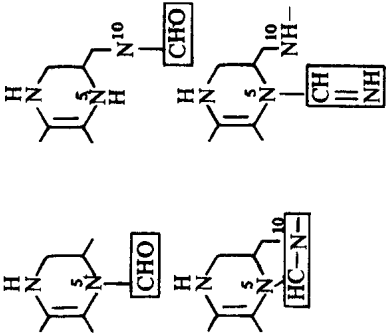
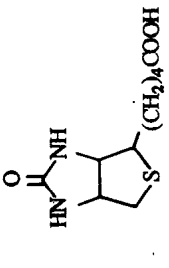


Oxidoreductive electron transfer



Cytochromes (M = Fe, Cu)

∞ Table 1.1 Some Common Coenzymes and Their Roles in Enzymic Transformations of Organic Chemical Interest (Cont.)

Coenzyme	Reaction types Involved	Key Coenzymic Reactions or Intermediates ^a
 Tetrahydrofolic acid	One-carbon group transfers	
 Biotin	Carboxylation and carboxyl transfer	