Advances in Physical Organic Chemistry

Volume 11

Edited by

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England

1975

Academic Press
London · New York · San Francisco

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS INC. (LONDON) LTD
24–28 Oval Road, London NW1

U.S. Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue,
New York, New York 10003, San Francisco

Library of Congress Catalog Card Number: 62–22125
ISBN 0–12–033511–5

PRINTED IN GREAT BRITAIN BY
WILLIAM CLOWES & SONS LIMITED
LONDON, COLCHESTER AND BECCLES

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Preface

With the appearance of Volume 11, *Advances in Physical Organic Chemistry* enters the second decade of its life. Many exciting developments of the subject have taken place since the publication of the first volume. It is a matter of some pride to be able to state that the contents of the first ten volumes have not only reflected these advances but, in many cases, have pointed the way and had a seminal influence on later work.

Over the same period, Physical Organic Chemistry has become "respectable", as is shown by the titles of several books and by the institution of professorships and other academic appointments in the subject. The value of physical organic methods has become widely appreciated in industry. Biennial International Conferences on Physical Organic Chemistry have been started under the auspices of the International Union of Pure and Applied Chemistry, who have also appointed a Commission on Physical Organic Chemistry.

No doubt, these tokens of recognition give some satisfaction to many scientists who have found the search for glimpses of a quantitative understanding of organic chemistry the most fascinating field of study. Yet this very respectability has its dangers. What made Physical Organic Chemistry a dynamic movement in science was that some outstanding men refused to be type-cast as physical or organic chemists and instead pursued the investigation of absorbing problems irrespective of the nature of the techniques required. Physical Organic Chemistry as an institutionalized "discipline" runs the risk of losing this spirit. It will be a continuing objective of our series not to allow this to happen, by encouraging the publication of contributions which do not as yet conform to established notions of the scope of the field.

It is natural that a living subject should at times produce quite extreme divergences of opinion. This series will continue to include

contributions which may present a topic from an unfashionable point of view. At other times, complementary accounts of controversial issues may be published, with the dual aim of pinpointing problems and resolving confusion. Thus, the present volume contains a reappraisal of the structure of the norbornyl cation by George Kramer, and in a subsequent one the problem of the norbornyl cation and of its significance in organic chemistry will be discussed in an article by George Olah, Eric Nordlander and Paul Schleyer.

Beginning with the present volume, the series will have the benefit of the specialist knowledge of Dr. Donald Bethell who is joining me as Associate Editor. At the same time, some changes have been made in the production of volumes, so as to improve lay-out and speed up publication.

It is a pleasure to acknowledge the service to the subject provided by the publishers, Academic Press, and especially the indefatigable assistance given to the Editor over the years by Messrs R. S. Lawrence and R. Adams. My main thanks go to the contributors who have so readily come forward with ideas or responded to suggestions for timely reviews. Dr. Bethell and I hope that we shall continue to receive such cooperation, and will always welcome expressions of views on the series and its contents.

V. Gold

London, November 1974.

Contents

Physical Organic Model Systems and the Problem of Enzymatic Catalysis

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1. INTRODUCTION

Enzymes are the best catalysts known. They catalyse chemical reactions in aqueous solution near neutral pH and at mild temperatures with attainment of great velocities. Although suitable standards

of comparison are often lacking, still it can be calculated (Bruice and Benkovic, 1966; Jencks, 1969; Bender, 1971) that certain enzymes will give rate enhancements by factors of the order of 10^{10}–10^{14} compared with similar non-enzymatic reactions. Therefore, knowledge of the mechanism of action of enzymes is of great importance, not only because enzymes are of biological interest, but also for the greater chemical understanding of catalysis in aqueous solution that would result.

A complete description of the mechanism of action of an enzyme requires knowledge of a number of factors, among which are:

(a) The structure of the active site and of enzyme-substrate complexes.
(b) The specificity of substrates and their ability to bind to the enzyme.
(c) The rate constants for the various steps in the reaction and the chemical mechanism of each step, i.e., a description of all of the intermediates and transition states along the reaction coordinate.
(d) An explanation for the magnitude of the rate constants.

Hence, in approaching the problem of enzymatic catalysis, information must be obtained from a number of experimental techniques.

Being proteins, enzymes are extremely complicated molecules, and the mechanistic interpretation of kinetic data is generally difficult. It is this complexity that in large part explains why, although kinetic studies have been conducted with many enzymes since the end of the nineteenth century, there is still no enzyme for which a mechanism has been definitely established. Extensive effort has been expended in recent years on mechanistic investigations of model reactions, chemical reactions similar to a post-binding enzymatic reaction, but where the number of variables is limited and strong evidence can be obtained pertaining to mechanism. Model work can begin only after enzymologists have provided information about the structure of the enzyme and the identity of groups in the active site. Once this basic information is available, the next step is to attempt to gain understanding of the chemistry of the process. In some cases it has not been possible to begin with sophisticated models, but rather, chemical work has been necessary to determine whether mechanisms suggested for an enzyme were even chemically feasible. Work on acetal hydrolysis (Fife, 1972) related to the mechanism of action of lysozyme is an illustration of this point.

In addition to determining the mechanism of action of an enzyme, it is important to be able to explain, in quantitative terms, the rapid rate of the enzyme reaction. That is, if factors A, B, and C are important, we would like to analyse the observed rate of the reaction in terms of these factors according to equation 1 and give numerical values to the coefficients. To date this has not been possible for most

$$\log k_{obsd} = aA + bB + cC + \ldots nN \qquad (1)$$

enzymes and possible only to an approximation with α-chymotrypsin (Bender *et al.*, 1964). It is apparent that this type of information can best come from detailed chemical studies where the various factors are examined in isolation. Chemical models then fulfil a twofold purpose: first, in providing reasonable mechanisms for the enzyme and casting doubt on others, thereby suggesting experimental approaches having a heightened chance of being fruitful; and second, in leading to explanations of the observed rates in terms of structure and mechanism [items (c) and (d)].

The number of different functional groups that an enzyme can use in the catalytic process is quite limited. Among them are the imidazole ring, aliphatic and phenolic hydroxyl, carboxyl, sulphhydryl, and amino groups. A reasonable speculation is that enzymes which catalyse similar reactions will have mechanistic features in common. This supposition is supported by the fact that several different esteratic enzymes having serine at the active site have an identical sequence of amino-acids around the active serine (Bruice and Benkovic, 1966; Bender, 1971). The amino-acid sequences for chymotrypsin and subtilisin (Wright *et al.*, 1969) are dissimilar, but the catalytically important groups are the same and are arranged in nearly the same geometrical relationship, implying identical mechanisms of action. Also, it seems reasonable that common mechanistic features may extend to enzymes which catalyse different reactions if the same catalytic group can function. For example, if an aliphatic hydroxyl group acts as a nucleophile in two different reactions, at least some common factors will probably be important in promoting catalysis. Consequently, from studies of catalysis by relatively few functional groups in carefully selected reactions it may be possible to formulate general principles for catalysis in aqueous solution including enzyme catalysis.

The purpose of the present review is to summarize the pertinent data concerning several well-studied reactions, to draw reasonable conclusions, and to point out some of the questions remaining to be

answered. To restrict the discussion to a manageable level, concern will be mainly with hydrolysis reactions relevant to the research interests of the author. Many important mechanism studies must therefore be omitted. For the most part only those reactions will be considered in detail which bear direct analogy to corresponding enzyme reactions and can therefore be considered true models. The enzymes themselves will not be discussed except to give necessary background information pertaining to postulated mechanisms. Chemical model investigations will be described which relate to three hydrolytic enzymes, α-chymotrypsin, carboxypeptidase, and lysozyme. Numerous excellent review articles have appeared concerning these enzymes, and their reactions (see for example, Boyer, 1970) and the mechanisms of the enzyme-catalysed reactions (Westheimer, 1962; Bruice and Dunn, 1973; Kaiser and Kaiser, 1972). These enzymes serve as perhaps the best examples of the model approach because detailed structural information is available. In each case the complete amino-acid sequence of the enzyme is known, and the three-dimensional structure has been determined by x-ray crystallographic analysis at 2 Å resolution (for three dimensional structural drawings, see Dickerson and Geis, 1969). Also, the basic chemistry of the types of reactions catalysed has been extensively studied over a long period of time, and there is a wealth of background chemical information (Bender, 1960; Bruice and Benkovic, 1966; Johnson, 1967; Jencks, 1969; Cordes, 1967; Fife, 1972). The enzymes have the aliphatic hydroxyl group of serine, the imidazole ring of histidine, and carboxyl groups as catalytically important functional groups at the active sites. The problem resolves itself into determining how such functional groups can participate in the hydrolytic reactions and how the rates of the enzymatic reactions can be accounted for in mechanistic terms.

Model studies have made a major contribution towards an understanding of the mechanistic possibilities which lysozyme might employ. Indeed, the model approach is perhaps the method with the greatest chance of success in attempts to comprehend the complex reactions of enzymes such as lysozyme where the natural substrates are not suitable for detailed kinetic work. α-Chymotrypsin can be studied with a variety of ester and amide substrates of low molecular weight, and much mechanistic information is available from direct work on the enzyme. Chemical models have been chiefly useful in promoting understanding of the chemistry of the reactions and in providing a basis for comprehension of the magnitude of the rate

constants in terms of individual mechanistic factors. At the other extreme, crucial model systems have not yet been investigated in relation to carboxypeptidase, but that enzyme illustrates types of questions that well-designed chemical models might answer.

Information is currently available which allows more definite conclusions than have previously been possible. Rate enhancements have been obtained in several simple chemical reactions that are of similar magnitude to those observed in analogous enzyme-catalysed reactions, and the goal of analysing the individual factors that can give such large rate accelerations is now perhaps within reach. The recent work will be stressed in this review.

Deeper insight into complex enzyme reactions has resulted from the study of simpler chemical models; but also, fundamental and novel observations concerning catalysis in aqueous solution have been made. The chemical work is therefore of interest in its own right, apart from any applicability to enzymatic catalysis. It is the author's opinion that this has not been stressed to a sufficient extent in previous discussions of enzymatic catalysis. Apart from the practical advantages that might result from understanding how enzymes function, the primary scientific importance would appear to be greatly increased knowledge of chemical catalysis. From this inverted viewpoint it is the chemistry that is elucidated that will prove to have ultimate scientific value, whether or not it directly applies to enzymes.

2. ENZYMATIC CATALYSIS: GENERAL PRINCIPLES

Intramolecular Catalysis

Several fundamental aspects of enzymatic catalysis must be considered in any discussion of the chemistry of enzymatic reactions. First, an enzyme-catalysed reaction proceeds with formation of an

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P \qquad (2)$$

enzyme-substrate complex which then breaks down to product and free enzyme [equation (2)]. The evidence for this is in most cases only suggestive (saturation kinetics), but an ES complex has been observed in some reactions (Chance, 1943, 1947), and it is generally assumed that such a complex is formed in most, if not all,

enzyme-catalysed reactions. Second, it is held that reaction takes place in a limited, specific area of the protein surface (the active site) with functional groups of amino-acid residues participating in the reaction.

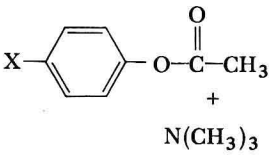
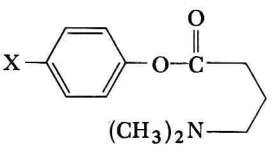
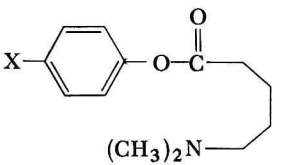
These ideas have been highly advantageous in regard to the development of chemical catalysis in aqueous solution. If the above concepts are correct, then an enzymatic reaction proceeding through an enzyme-substrate complex with the substrate bound close to appropriate functional groups is quite analogous to a chemical intramolecular reaction. Substantial effort has therefore been expended on the study of such reactions in attempts better to comprehend enzyme catalysis (Bruice, 1970; Kirby and Fersht, 1971).

Intramolecular reactions usually occur rapidly in comparison with similar bimolecular reactions, and a number of explanations have been put forth concerning the efficiency of intramolecular catalysis. Westheimer (1962) pointed out that intramolecular reactions should have a more favourable entropy of activation than their bimolecular counterparts because translational entropy of the catalyst is not lost as it is in the bimolecular reaction. To determine the efficiency of an intramolecular reaction, the first-order rate constant is often compared with the second-order rate constant of the corresponding bimolecular reaction proceeding by the same mechanism. This ratio has units of molarity ($s^{-1}/M^{-1}s^{-1}$) and is taken to be the "effective molarity" of the neighbouring group, i.e. the concentration of bimolecular catalyst required to give a pseudo-first order rate constant of the magnitude observed in the intramolecular reaction. An intramolecular dimethylamino-group has an effective molarity of 1000–5000 M for attack at the ester carbonyl (Bruice and Benkovic, 1963) and there is a more favourable $\Delta S^{\ddagger}$ value than in the intermolecular reaction between trimethylamine and aryl acetates (see the data in Table 1). Page and Jencks (1971) have calculated that favourable changes in $\Delta S^{\ddagger}$ in intramolecular reactions could be large enough to explain the rapid rates of some enzymatic reactions, and effective concentrations of the order of 10^8 M may be accounted for without introducing new chemical concepts.

Intramolecular nucleophilic reactions could also be facilitated over their intermolecular counterparts if the reaction centre and the nucleophile are compressed in the ground state. Part of the van der Waals repulsion energy could thereby be overcome in the ground state, resulting in a more favourable $\Delta H^{\ddagger}$ value. Solvation

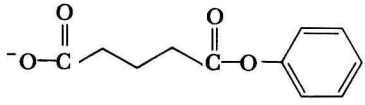
TABLE 1

Activation Parameters (kcal mole^{-1}) for Nucleophilic Displacement by the Dimethylamino-Group at 25° (Bruice and Benkovic, 1963)

X Substituent						
	$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$	$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$	$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$
p-NO_2	12·3	−6·3	11·9	−1·9	11·5	−2·6
m-NO_2	12·1	−8·0	11·5	−4·3	11·8	−4·4
p-Cl	12·5	−9·1	15·9	−2·2	13·8	−4·1
H	12·9	−9·4	12·5	−5·7	12·3	−6·4
p-CH_3			13·7	−5·1	14·4	−5·5

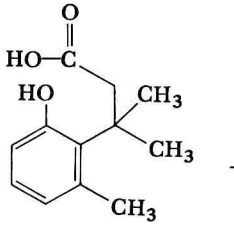
factors might also be of great importance. Before a nucleophile can attack it must be desolvated, requiring expenditure of substantial energy. However, an intramolecular nucleophile would be less heavily solvated in the ground state than an intermolecular nucleophile in dilute solution if the neighbouring group and the reaction centre are immediately adjacent in the same molecule so that water molecules cannot fit between.

As an intramolecular nucleophile is more rigidly held with respect to the reaction centre, the rate of the reaction increases as illustrated in Table 2. Bruice and Pandit (1960b) concluded that the rate increases were due to restriction of unfavourable rotamer distribution. The most energetically favourable ground state conformation would have the carboxyl group extended into the solvent, *viz.*

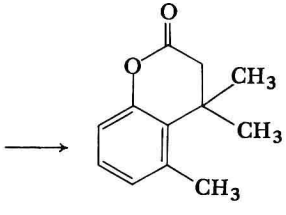


where it could not attack the carbonyl. Removal of rotational degrees of freedom would therefore greatly enhance the rate of reaction. As seen in Table 3, similar rate enhancements have been observed in lactonization reactions as the reacting groups are held in proximity to each other (Storm and Koshland, 1970, 1972a). A rate enhancement of 10^{15} M is observed in lactonization of [1] with respect to the bimolecular esterification in equation (4) (Milstien and Cohen, 1972). The methyl group in the 3-position of [1] fits between the geminal methyl groups in the side chain and locks the system.

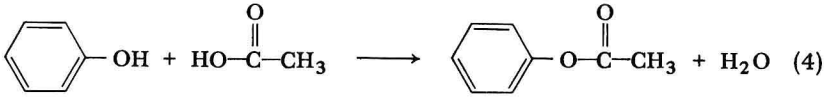
One function of an enzyme is to bring the substrate by binding at the active site into proximity with functional groups of the enzyme.



[1]



(3)



(4)