

MECHANISMS OF
ORGANIC AND
ENZYMIC
REACTIONS

S. G. WALEY

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BY

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FOREWORD

As the author mentions in his preface, this book is intended to appeal both to biochemists and chemists, and it is from the viewpoint of the latter that I introduce it. The study of the mechanism of action of enzymes is one of the most interesting and fruitful fields of biochemistry at the present time. This development is long overdue. For many years the study of enzyme-catalysed reactions was still faintly obscured by the last remnant of the tradition of vitalism, which manifested itself in the feeling that enzymic reactions are in some mysterious way different from 'chemical' reactions.

This obscurantist attitude has finally been dispelled. The isolation of crystalline urease by Sumner, with the demonstration that enzymes are proteins and can be purified and characterized as such, was a great stride forward in this direction. The classical work of Warburg then laid the foundations for the understanding in chemical terms of the function of coenzymes, and of the relationship between vitamins and coenzymes. These advances opened the way to a study of enzyme reactions in terms of the chemistry of the coenzymes, but the role of the enzyme protein itself remained quite obscure.

A new level of understanding of enzyme chemistry was achieved when it was shown that the enzyme protein itself may react stoichiometrically with a substrate, with the formation of stable bonds. In an investigation of the enzyme sucrose phosphorylase, Barker, Hassid, and Doudoroff obtained evidence of the formation of glucosyl-enzyme intermediate, implicating the enzyme itself as a reactant rather than a 'noble' catalyst. To take another example, several esterases have been thoroughly studied with the aid of organo-phosphorus poisons (such as di-isopropyl phosphorofluoridate) which react specifically with them. This has led not only to a much more satisfactory knowledge of the mechanism of the enzymic hydrolysis of esters, but also has yielded valuable information on the actual sequence of amino acids near the reactive centres of such enzymes.

Parallel to these developments in enzymology, there has been a rapid growth in the knowledge of the mechanism of organic reactions. It has become imperative for the biochemist (and especially the enzymologist) to master at least the fundamentals of this relatively new area of organic chemistry, since the application of this information to biological systems

will be of growing importance in the years to come. In the present volume, Dr. Stephen G. Waley presents in non-mathematical terms an up-to-date account of the physical-chemical theory of organic reactions. In addition, he considers the mechanism of certain enzyme-catalysed reactions in the light of such theory. The book is thus admirably designed to bridge the gap between the two fields. For the biochemist who is not a specialist in organic chemistry, or for the organic chemist who is interested in learning about enzymic reactions, this volume should be of special interest and value.

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PREFACE

MUCH is now known about the detailed course of many organic reactions. Indeed, the results of the studies of organic reactions, taken as a whole, form an impressive and coherent body of knowledge, which gives much insight into the reactivities of organic compounds. An understanding of mechanism has thus become a necessary part of the education of every student of chemistry and of biochemistry. Ordinarily, books on organic reactions do not discuss enzyme-catalysed reactions, interesting though these are. Symposia on the mechanism of enzyme action cannot give the background knowledge necessary to appreciate the remarkable advances made recently. I hope, therefore, that both biochemists and chemists will find it useful to have a book in which the mechanisms of organic reactions, and of enzyme-catalysed reactions, are discussed together. Throughout this book, enzymic reactions are discussed shortly after their non-enzymic counterparts.

The selection of topics in a large and rapidly growing subject is necessarily difficult, but I have tried to describe sufficient examples in enough detail for the reader to be able to master the principles. Practice in applying the ideas of theoretical organic chemistry is necessary; familiarity comes gradually. The treatment has to be fairly detailed, because if one only gives conclusions about mechanisms, with very little discussion of how the conclusions are reached, then the reader makes no real progress towards being able to judge for himself how well based the conclusions are.

The first two chapters are introductory and deal, in outline, with the structure of organic molecules, and with the general nature and classification of organic reactions. Nucleophilic substitutions are the first reactions described, as several important concepts can be introduced here. Olefine formation often competes with nucleophilic substitution, so this reaction, and its reverse, are then described.

The next three chapters form a (loosely connected) group. Carbonyl addition reactions are especially important in biochemistry, as they seem to be the main way in which carbon-carbon bonds are formed *in vivo*. After aldehydes and ketones, the discussion continues with other compounds containing the carbonyl group, such as carboxylic esters and acids, and thence to other esters and acids, such as the phosphoric esters. These chapters have provided examples of substitutions, additions,

and eliminations; rearrangements form the fourth main class of reactions and some examples are discussed in the next chapter.

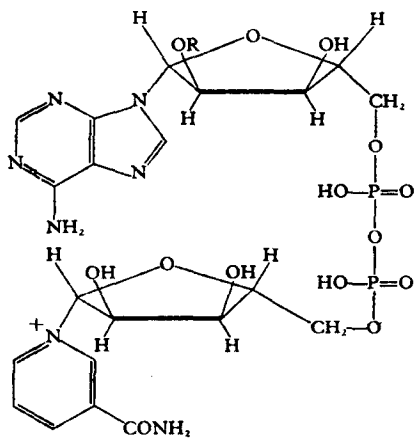
The chemistry of aromatic compounds has always been a corner-stone of theoretical organic chemistry, and many individual aromatic compounds play notable roles in cells; some aromatic substitutions are described in the penultimate chapter. Finally, the importance of macromolecules in biology has led to the inclusion of a chapter describing some polymerizations.

My indebtedness to many is manifest. I have tended to cite books and reviews, and to give the most recent references, as these refer back to earlier papers. I can only hope that this practice will not cause any researcher to feel slighted. I have been most fortunate in the kindness of the following, who have read all or part of the manuscript, and made many helpful comments: Mr. R. P. Bell, F.R.S., Dr. R. O. C. Norman, Dr. A. G. Ogston, F.R.S., and Dr. G. T. Young. I am grateful to Professor Eugene Kennedy for writing the Foreword, to my colleagues at this laboratory for their encouragement, and to Miss Hutchinson for preparation of the typescript.

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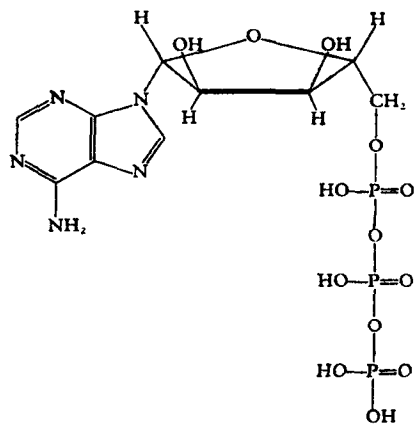
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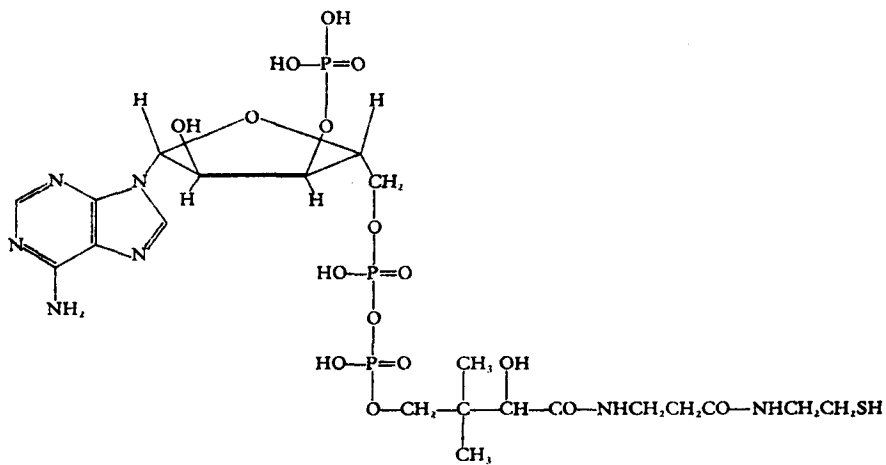


R=H: Diphosphopyridine nucleotide (DPN)
(Coenzyme I)

R=PO₃H₂: Triphosphopyridine nucleotide (TPN)
(Coenzyme II)



Adenosine triphosphate (ATP)



Coenzyme A

CONTENTS

Formulae for Coenzyme I (DPN), Coenzyme II (TPN), ATP, and
Coenzyme A

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ILLUSTRATIONS

Plate I is facing page 12 and Plate II is facing page 16

I

MOLECULAR STRUCTURE

1. Atoms and molecules

(a) *Introduction.* Accounts of the mechanisms of organic reactions are couched in terms of the relative dispositions of the atomic nuclei and electrons of the reacting species. Such ideas cannot be grasped without some familiarity with modern concepts of molecular structure, so an introduction to these concepts is presented here: the next four sections (§ 1 b to § 1 e) are of an elementary nature.

(b) *Nuclei and electrons.* Atoms consist of positively charged nuclei and negatively charged electrons. The positively charged nuclei are small; their diameter is of the order of 10^{-12} cm, whereas the diameter of the atom is of the order of 10^{-8} cm, i.e. 1 Å. However, virtually all the mass of an atom is due to the nucleus. Thus the hydrogen atom consists of the nucleus (called the proton), and one electron which is some 2,000 times lighter than the whole atom. The unit of charge that we shall use is the charge on the electron (-1 , as the electron is negatively charged). The charge on the proton is $+1$.

The number of electrons in the atom of an element is equal to the atomic number of the element in the Periodic Table. It is the electrons which affect chemical properties, and so the isotopes of a given element (which differ in nuclear mass but have the same number of electrons) have qualitatively the same chemical properties. Thus hydrogen, deuterium, and tritium have nuclei of masses of approximately one, two, and three (in atomic mass units) respectively, but in each case there is one electron in the atom.

(c) *The quantum theory.* We now consider the simplest atom, the hydrogen atom, in rather more detail. As the nucleus is positively charged and the electron is negatively charged there is an attractive force between the two particles. This tends to pull the electron into the nucleus and so, for the atom to be stable, the electron must be supposed to be rotating round the nucleus. But, according to classical ideas, the electron would then emit radiation continuously, gradually lose energy by so doing, and thus move nearer and nearer to the nucleus. This model of the atom, then, cannot account for its stability. A way

out of this difficulty was seen by Bohr, who applied the quantum theory to the hydrogen atom.

The quantum theory, put forward by Planck in 1900, was concerned with the radiation emitted by a heated body. The problem was how to account for the way the energy of the heated body is related to the wavelength of the emitted radiation. A familiar example is that a white-hot poker is at a higher temperature than a red-hot one. Planck showed that the experimental results could be explained if the energy of the body could only change by integral multiples of a definite unit of energy. In rather the same way, the second-hand on some clocks jumps from one second-mark to the next, so that time, on these clocks, is measured with a quantum of one second. Planck's idea was applied by Einstein to the relation between the energy of electrons emitted from an irradiated metal plate and the frequency of the radiation used. The value of the quantum of energy (E) is proportional to the frequency (ν) of the radiation:

$$E = h\nu,$$

where h is a constant, called Planck's constant ($h = 6.62 \times 10^{-27}$ erg sec).

These ideas were then applied to the hydrogen atom by Bohr, as follows. On the basis of two assumptions he accounted for the stability of the atom, and (quantitatively) for the wavelengths of the lines in the spectrum of the hydrogen atom. The first assumption was that the electron could only move in definite orbits, and when it was moving in these orbits radiation was neither emitted nor absorbed. The second assumption was that when the electron moved from one orbit to another nearer the nucleus, radiation was emitted of frequency ν , which was related to the energy difference ($E_1 - E_2$) between the orbits by the equation

$$E_1 - E_2 = nh\nu,$$

where n is a whole number. The frequency (ν) and wavelength (λ) are related by $\nu = c/\lambda$, where c is the velocity of light, so that

$$E_1 - E_2 = nhc/\lambda. \quad [1]$$

When a discharge is passed through a tube containing hydrogen, an electron may acquire enough energy to 'jump' to a relatively distant orbit; as this 'excited' electron drops back to an orbit nearer to the nucleus it emits radiation. Thus the origin of spectral lines on the Bohr model of the hydrogen atom may be depicted as in Fig. 1, in which lines of wavelengths 972.5, 1,026, and 1,216 Å (observed by Lyman) and the electronic transitions responsible for them are shown.

(d) *Energy levels.* By the use of equation [1], the energy differences

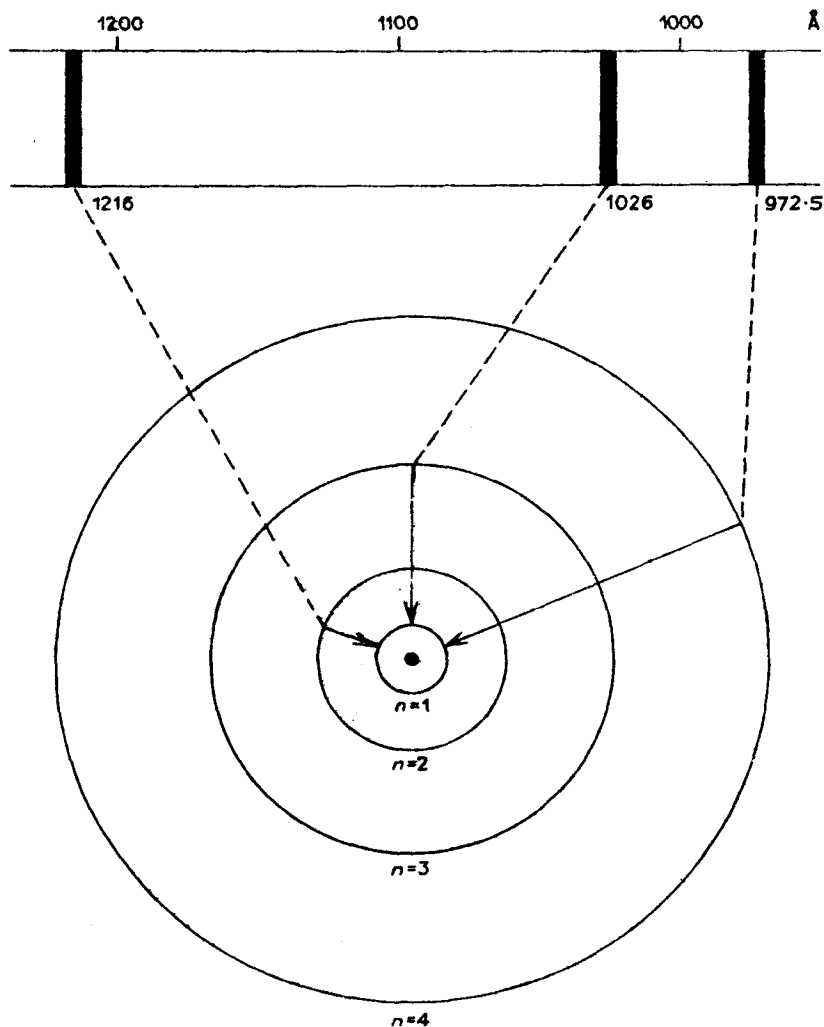


FIG. 1. The origin of spectral lines on the Bohr model of the hydrogen atom. The orbits are shown as circles, and the nucleus is the central dot. The radiation emitted when the electron moves from one orbit to another that is nearer to the nucleus is represented by the spectrum at the top of the figure.

between the orbits are obtained from the wavelength of the radiation emitted. If sufficient energy is absorbed by the hydrogen atom, the electron is effectively removed from the field of force of the nucleus; this is ionization, and this state is taken as the zero in Fig. 2, which shows the energy of the hydrogen atom when the electron is in the various possible orbits. When the electron is in, say, the $n = 2$ orbit,

the energy level is given as -78 kcal/mol in Fig. 2; this means that a hydrogen atom with the electron in this ($n = 2$) orbit is more stable than a bare proton and an electron at a distance by 78 kcal/mol. These energy levels are especially important as they apply (approximately) to elements other than hydrogen.

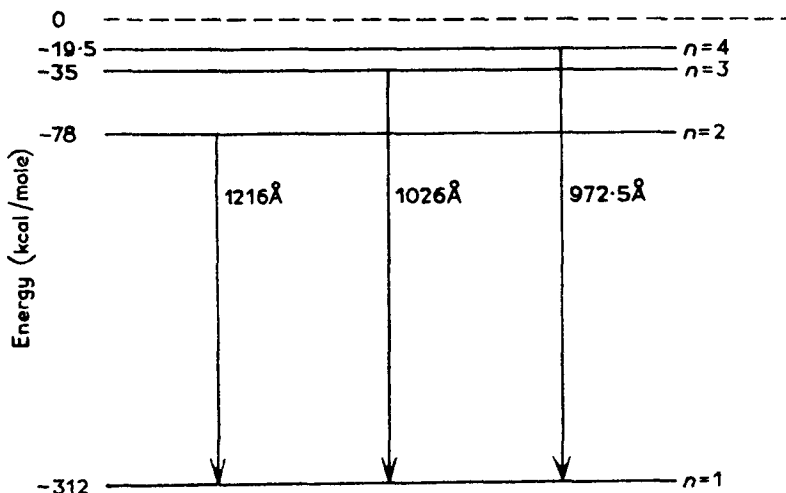


FIG. 2. Some energy levels in the hydrogen atom. The same electronic transitions as in Fig. 1 are also shown here.

(e) *Electronic configuration of atoms.* When the electron is in the orbit nearest to the nucleus, the hydrogen atom is in its state of lowest energy (ground state); this orbit is characterized by the value $n = 1$ in equation [1], and is the lowest energy level (Fig. 2). The energies of successive orbits depend mainly on the value of n , and n is called the *principal quantum number*.

We now consider other atoms, in outline. As in the hydrogen atom, the electrons will be assigned to orbits. We shall be guided by three principles:

- (i) The energy levels of the orbits are similar to those shown in Fig. 2.
- (ii) An orbit with principal quantum number n can accommodate up to $2n^2$ electrons.
- (iii) A completely filled orbit is chemically inert.

The application of these principles to the first three periods of the Periodic Table is shown in Fig. 3. The next element after hydrogen in the Periodic Table is helium. It has two electrons in the most stable, innermost, orbit, the orbit with principal quantum number one. This