# Organic and Bio-organic Mechanisms

## 有机和生物有机机理

Michael Page & Andrew Williams

Addison Wesley longman
世界图书出版公司

# Organic and Bio-organic Mechanisms

**Michael Page**
The University of Huddersfield
and
**Andrew Williams**
The University of Kent

世界图书出版公司
北京·广州·上海·西安

书　　名: Organic & Bio－Organic Mechanisms
作　　者: Michael Page & Andrew Williams
中 译 名: 有机和生物有机机理
出 版 者: 世界图书出版公司北京公司
印 刷 者: 北京中西印刷厂
发　　行: 世界图书出版公司北京公司(北京朝内大街137号　100010)
开　　本: 大32　　印张: 9.75
版　　次: 1997年9月第1版　　1997年9月第1次印刷
书　　号: ISBN 7－5062－3388－6/O・205
版权登记: 图字01－97－1168
定　　价: 44.00元

**Addison Wesley Longman**
Addison Wesley Longman Limited,
Edinburgh Gate, Harlow,
Essex CM20 2JE, England
*and associated companies throughout the world*



First published 1997

**British Library Cataloguing in Publication Data**

A catalogue entry for this title is available from the British Library

ISBN 0-582-07484-3

**Library of Congress Cataloging-in-Publication Data**

A catalog entry for this title is available from the Library of Congress

This edition of **Organic & Bio-Organic Mechanisms, First edition** is published by arrangement with Addison Wesley Longman Limited, London

# Preface

The fascinating chemical logic of the sequence of the reactions which occur in living systems is matched by the fascination of the reaction mechanisms of the individual steps involved. An understanding of how bonds are made and broken is essential to the understanding of life. There have been enormous advances in the application of instrumentation to elucidating chemical structures—from the smallest molecule to the largest biopolymer. These static structures have made an invaluable contribution to chemistry and biology. However, it is the knowledge of the dynamic interconversion of these structures which remains an intriguing challenge. How are the bonds between atoms rearranged? What sort of structural changes take place to cause bond fission and formation? How do catalysts lower the activation energies of reactions?

To some extent chemistry and biology are still dominated by the consideration of static structures. The three-dimensional structures of enzymes and the identification of active sites, although necessary to understand mechanisms, are too often used as the only vehicle on which to base mechanistic speculation. An appreciation of the dynamic processes involved and a deeper understanding of the *assumptions* involved in many models and descriptions will advance our understanding of the processes involved.

It is now clear that an understanding of reaction mechanisms is essential for the application of enzymes to organic synthesis and their use as biosensors and as targets for drug design. The pharmaceutical industry has been extremely successful in realizing that the development of drugs as enzyme inhibitors is strongly dependent upon an understanding of reaction mechanisms. The chemical industry, interested in clean technology, high product yield and purity, wants to know how bonds are made and broken and uses this information to show how unwanted side reactions can be prevented.

The assumptions involved in elucidating standard reaction mechanisms, and indeed the basis of the models which are used in defining the structure of states along a reaction path, are often forgotten in advanced chemical and biochemical studies. The nature of a 'state' itself is often not made clear, but such omissions are natural considering the time between the student's learning the fundamentals and entering advanced work. This text aims to redress this failing and is an introduction to the diagnosis of mechanism particularly in its application to bio-organic chemistry; we

hope it will provide a handbook for the student starting research into mechanisms and reactivity. Continued advancement and development in biochemistry and chemistry require an understanding of chemical reaction mechanisms and how they are elucidated.

We discuss bonding in terms of the line formalism which remains the best working model for most chemists and biochemists; although it has limitations the model has both the 'feel' of chemical intuition and graphic utility. The book is intended to help the specialist and the non-specialist come to some meaningful conclusions about mechanism and its elucidation. There are chapters on the fundamental assumptions involved in describing reactions and structures, and there are descriptions of the methods used to elucidate mechanisms as well as examples of biologically important reactions, catalysis and enzymes. Suggestions for reading 'in depth' are given at the end of most chapters; and readers can judge for themselves from the titles which references are useful for general reading.

M. I. Page and A. Williams. October 1995
Huddersfield and Kent

# Contents

# 1 The transition state

## 1.1 Mechanism as a progression of states

The mechanism of a reaction is often described as the structure and energy of a molecule through its progress from reactants to products. Such a definition is obviously suspect because the properties of a single, static molecule cannot be measured and because structure itself requires a definition. Energy is required to transform reactants to products and the energy barrier in a single-step reaction arises from a transitional structure which has an existence of only $10^{-13}$ to $10^{-14}$ s. The assembly of transitional structures 'in passage' from reactant to product states is known as the *transition state*. The collection of reactant molecules is converted into the collection of product molecules through a transition state which embraces a collection of transient species which effectively has a 'normal' thermodynamic distribution of energies even though these structures/energies are not interconvertible within their lifetimes. Thus the mechanism of a reaction could be defined as the structures of states on progression from reactant through transition states to product. A definition of mechanism which can be fulfilled experimentally, at least in principle, is a description of any intermediates and all the transition-state structures connecting these intermediates, reactants and products.

Theory and gas-phase work have provided information on the energy of single entities as they go through to products for a limited number of reactions. A high-level definition of mechanism is the energy surface of such a progression as a function of all degrees of freedom. This definition is not attainable for reactions in solution.

## 1.2 Structure and its interpretation

In order to discuss the mechanism of any reaction and transition-state structures it is necessary to know precisely what the term 'structure' means. The chemist visualizes a pure compound as an assembly of molecules, each atom of which has identical topology relative to its neighbour. The relative position of each atom varies with time and the *average* positions of the atoms are measured. Even the most explicit method of structure

determination, namely X-ray crystallography, cites the atomic coordinates with a degree of uncertainty, partly because the method depends on X-rays being diffracted by the electrons and not the nuclei. Moreover, analytical methods provide results for assemblies rather than for an individual molecule. A single crystal used for an X-ray structure determination is likely to contain up to $10^{20}$ molecules.

It is important to remember that, when chemists write structures with bonds represented by lines (Lewis bonds), these are hypothetical *models*. These structures (which are commonly called Kekulé structures) are often taken for reality but they are simply representations of hypotheses which fit experimental knowledge of compounds. There are many ways available to represent molecules, each with its own advantages. Most have the disadvantage that they refer to a single molecule and assume that the constituent atoms have time-stable spatial coordinates relative to each other. There is nothing superior to the line bonding model, which readily graphs an assembly with a facility that is readily comprehensible to all chemists. Most reactions are carried out in solution and there is a great, but unfulfilled, need for a simple, graphical, model of an assembly of molecules in solution.

Descriptions of mechanisms in this book are couched in a language devised for structural studies and can therefore be misleading if the assumptions are forgotten. For convenience, and following precedent, solvent is often omitted from descriptions of state in this text; moreover the term 'bond' is invariably used to mean the summation of electronic bonds (in its 'Lewis' sense) and solvation.

Structure requires a description of the relative positions of nuclei and the electron density distribution, and how these vary with energy. Even at absolute zero, the exact positions of the atoms in a molecule are uncertain, as reflected in zero-point energies. As the temperature is increased, higher quantum states are occupied for each degree of freedom so that fluctuations around the mean positions of the atoms increase. Most vibrational motions are decoupled from each other so that the apparently static pictures which are drawn of the relative positions of atoms in a molecule can occasionally be very misleading. For example, at room temperature, most covalent C–C bond lengths are 1.54 Å but individual ones fluctuate with time by ±0.05 Å; bond angles at saturated carbon are, on average, 111° but vary between 106° and 116°. The mean square amplitude of vibration is inversely proportional to the reduced mass and force constant, so that either a small mass or a low force constant gives rise to a large vibrational amplitude. For example, the classical turning points for the bending mode of water are at HOH angles of 83° and 127°. Non-covalently bonded atoms move even more with respect to each other than normal vibrational motions; this means that O—H···O bond lengths and angles may be ±0.15 Å and ±25°, respectively.

Intrinsic uncertainty in nuclear positions is inversely matched by an equivalent ambiguity about electron density. The distribution of electrons

is important because nuclear motion is much slower than that of the lighter electrons. It is often considered that nuclei can be imagined to move within a constant force field generated by the electrons. Crudely speaking, electron transfer occurs when a suitable nuclear configuration has been achieved. This simple fact explains the chemist's pre-occupation with electrons and 'curved arrows'. Most tools used to elucidate transition-state structures give a measure of the apparent electron density/charge around an atom and the geometrical arrangement of the atoms. However, the relationship between charge and structure is not straightforward, even in stable systems. For example, the resonance structures of amides include negative and positive charges on oxygen and nitrogen respectively. However, even if the absolute partial charges on these atoms were known, they would not necessarily be informative about the relative single/double bond character and bond lengths of the amide. Furthermore, the charge will vary on transfer of an amide hydrogen-bonded in water to a 'free-molecule' in a non-polar solvent.

Knowledge only of the structure of ground, intermediate and product states does not enable us to calculate the appropriate rate constants for an enzyme-catalysed reaction, or any other reaction. This problem is neatly summarized in an amusing analogy, attributed to Jeremy Knowles: *knowing the structure of a reactant such as an enzyme and possessing the picture of a horse tells us neither about the catalytic activity of the enzyme nor about the Derby winning propensities of the horse!*

The development of the idea of mechanism since the first studies at the beginning of the 20th century (Lapworth, 1903, 1904, 1907) went hand in hand with progress in methods for its determination. The concepts of mechanism sought by Lapworth were not very different from the descriptions pursued today in that they were couched in Lewis-type language. The most important advance since Lapworth's era is the development of the concept of the transition state. Our improved understanding of molecular structure demands an ability to think in terms of multidimensional space if this is extended to descriptions of mechanism.

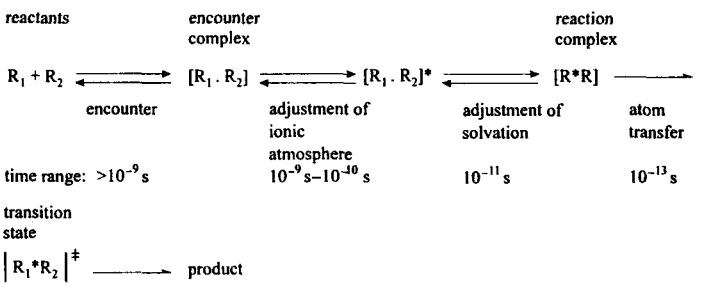
The maximum of the potential energy along the reaction coordinate between reactants and products corresponds to the *transition-state structure*, or activated complex. The *transition state* is a quasi-thermodynamic *state* and is at the maximum of the Gibbs' free energy along the reaction coordinate. This free energy represents the pseudo-collection of molecules of the transition state distributed among the available quantum states of the various degrees of freedom as reflected in the entropy. The maximum in the potential energy along the reaction coordinate is temperature-independent, whereas the transition-state structure may be temperature-dependent because of entropy effects. 'Structure' usually refers to potential energy and strictly we should always refer to the transition structure or 'transition-state structure' but common usage abbreviates this simply to the 'transition-state'. In this text we adopt this rather casual approach but it is important to remember that it is a simplification of phraseology.

## 1.3 Interconversion of states—reaction and encounter complexes

A bimolecular reaction in solution occurs via the following series of events. Two reactant molecules diffuse through the assembly of solute and solvent molecules and collide to form an *encounter complex* within the same solvent cage. If the molecules are charged, then the ionic atmosphere adjusts to any changes in the combined charge. Reaction may still not be possible until any necessary changes in solvation occur (such as desolvation of lone pairs) to form a *reaction complex*, in which bonding changes take place. The encounter complex remains essentially intact for the time period of several collisions because of the protecting effect of the solvent surrounding molecules once they have collided. The products of the subsequent reaction could either be converted back to reactants or diffuse into the bulk solvent.

A similar description applies to a unimolecular reaction except that the transition state, formed from a single reactant molecule, is initiated by energy accumulation in the solvated reactant by collision between reactant and solvent molecules.

Scheme 1.1 gives typical half-lives for reactant molecules *destined* to react. Many encounters do not lead to reaction and only a small fraction of the complexes will have the appropriate transition-state solvation in place for reaction to take place.



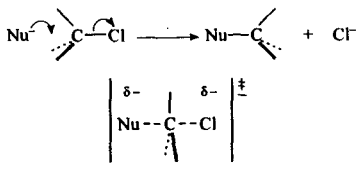

**Scheme 1.1** Bimolecular mechanism in solution.

Reaction complexes in enzyme-catalysed reactions are more ordered than those in reactions of simple molecules and often constitute relatively thermodynamically favourable species. The enzyme active site provides a special microsolvation for the reaction compared with that for the uncatalysed reaction in bulk solution; time must elapse after the first encounter of substrate with enzyme molecule before the active site is occupied.

## 1.4 Methods of representing reaction mechanisms

The mechanism of nucleophilic aliphatic substitution was the first to be studied in depth. It is exemplified by reactions of alkyl halides (Eqn [1.1]

and structure **1**). The reaction type is the paradigm for many bio-organic reactions, including biological methylation and the transfer of the glycosyl moiety between nucleophiles.




[1.1]

**1**

Equation [1.1] illustrates a 'mechanism' as represented in most studies of organic reactions. A knowledge of reactant and product structure indicates that only two bond changes are involved. The nucleophile donates electrons and the leaving group attracts electrons; the passage of pairs of electrons is represented by a sequence of curved arrows. This description does not indicate the structure of the transition state although some structure similar to the trigonal bipyramidal arrangement (**1**) must be traversed in the reaction.

The transition state is effectively an assembly of molecular structures which exists for less than $10^{-13}$ s; the measurement of its properties by conventional means is not possible because the measuring devices have relaxation times larger than this. Recent gas-phase work employing femtosecond ($10^{-15}$ s) light pulses as probes can glimpse the molecule at various stages along the reaction coordinate (Pilling & Smith, 1987; Zewail, 1988; Baggott, 1989; Smith, 1990; Polanyi & Zewail, 1995). Since the velocity of separation of atoms constituting a bond is about 0.01 ångstroms per femtosecond (1 Å per $10^{-13}$ s) the time-scale for separation is a few hundred femtoseconds and resolution is therefore possible with light pulses of a few femtoseconds duration. Such studies are limited by the *uncertainty principle*, especially as it cannot be assumed that the interaction of the light pulses with the molecule is 'innocent'. It is only possible to study simple gas-phase reactions by this technique; nevertheless the results are very useful as models for more complicated systems.

In general it is not possible to determine the structure of species in a transition state in the same way that we can measure structure for a regular assembly of molecules. The structural information that we require involves the positions of the atoms and bond order (in particular that for the bonds undergoing major changes) and knowledge of the electronic structure such as the electronic charge at atoms.

The description represented by structure **1** is commonly called a mechanism, but the real reaction is between *assemblies* of molecules and in bio-organic chemistry the molecules are in solution. For example, in aqueous solution there will be a dramatic change in the solvation around the chlorine as it is converted from a relatively neutral entity to an anion. The solvent

contributes to the activation energy and is fundamental in determining the relative charge and atom distribution drawn to represent the transition-state structure. It is the general convention to neglect the effect of the solvent molecules in the description as shown, because of the difficulties of graphical representation.

The only measurable property of the transition state is its energy relative to reactant or product states and this is obtained by kinetics, or indirectly, by product distribution studies. *All* experimental knowledge of transition-state structures for solution reactions comes from such measurements and includes stereochemical, trapping, isotopic labelling and product isolation techniques. These techniques will be discussed in Chapters 2, 3 and 4.

## 1.5 General considerations concerning reaction mechanisms

### *Single- and double-electron transfer*

Ingold (1953) divided mechanisms broadly into those proceeding by bond fission through two-electron transfer (heterolytic) and those by single-electron transfer (homolytic) (Scheme 1.2).

| | |
|---|---|
| Electron pair transfer | |
| Heterolysis | $A{:}B \longrightarrow A^+ + B{:}^-$ |
| Single electron transfer | |
| Homolysis A | $A{:}B \longrightarrow A^{\cdot} + B^{\cdot}$ |

**Scheme 1.2** Types of mechanism.

These divisions are still relevant except that there is now considerable overlap between the two types, and radical cations and radical anions can also be involved in many solution reactions. For example, nucleophilic substitution at an aromatic centre can involve a radical anion in an $S_{RN}$ process (Kim & Bunnett, 1970). Reactions in polar solution are often heterolytic because of the massive solvation stability afforded to ions, whereas gas-phase reactions are often homolytic. The distinction between electron-pair and single-electron transfer (SET) may not be clear-cut if the apparent heterolytic reactions involve 'inner-sphere' single-electron transfers which do not express themselves as free radicals or even radicals caged in encounter complexes (Pross, 1985; Shaik, 1990; Savéant, 1990, 1993).

The observation of free radicals or radicals caged in encounter complexes by use of CIDNP (chemically induced dynamic nuclear polarization) experiments in NMR is incontrovertible evidence for SET processes; however, the absence of evidence for radicals is not sufficient to disprove the existence of 'inner-sphere' SET mechanisms (Ashby & Pham, 1987; Newcomb & Curran, 1988). 'Inner-sphere' and 'outer-sphere' single-electron transfer mechanisms for nucleophilic displacement (Rossi *et al.*, 1989) are illustrated in Scheme 1.3.

*Inner sphere:*

$$R{-}X + Nu{:}^- \longrightarrow [Nu \cdot\cdot R{-}X]^- \longrightarrow [Nu{-}R \cdot\cdot X]^- \longrightarrow Nu{-}R + X{:}^-$$

*Outer sphere:*

$$R{-}X + Nu{:}^- \longrightarrow R{-}X^{\cdot -} + Nu^{\cdot}$$
$$R{-}X^{\cdot -} \longrightarrow R^{\cdot} + X{:}^-$$
$$R^{\cdot} + Nu{:}^- \longrightarrow R{-}Nu^{\cdot -}$$

or

$$R^{\cdot} + Nu^{\cdot} \longrightarrow R{-}Nu$$
$$R{-}Nu^{\cdot -} + R{-}X \longrightarrow R{-}Nu + R{-}X^{\cdot -}$$

Sum:

$$R{-}X + Nu{:}^- \longrightarrow R{-}Nu + X{:}^-$$

**Scheme 1.3** Inner- and outer-sphere single-electron transfer (SET) mechanisms.

The distinction between electron-pair and single-electron transfer involves identifying processes in which electron transfer, bond breaking and bond making are stepwise, and processes where they are concerted. Although the 'outer sphere'/'inner sphere' terminology was used originally for electron-transfer reactions involving metal complexes it can be applied to organic reactions (Lexa *et al.*, 1987). Bond making and bond breaking in outer-sphere reactions occur in separate steps distinct from electron transfer. If all the steps are concerted the reaction occurs by inner-sphere electron transfer mechanisms (Scheme 1.3) which is difficult to distinguish from a classical $S_N2$ mechanism involving electron-pair transfer.

The transition state of a single-electron transfer from $Nu:^-$ to X can be represented by the resonance hybrids represented by structure **2**.

$$\left| Nu{:}^- \quad R \quad X \longleftrightarrow Nu \quad R \quad {}^-{:}X \right|^{\ddagger -}$$

**2**

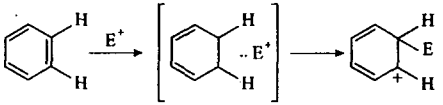
The extent of bond breaking and the extent of electron transfer cannot readily be separated (Perrin, 1984). The length of the R–X bond is itself an essential coordinate in controlling the occurrence of electron transfer, subject to Franck–Condon restrictions, in the concerted electron transfer–bond breaking pathway and in the outer-sphere electron transfer in the framework of the Born–Oppenheimer approximation (see Section 1.9). The electronic 'reshuffle' from reactant to product configurations takes place 'instantaneously' when the nuclei, which move more slowly than electrons, adopt the appropriate intermediate configuration between that of reactants and that of products. The occurrence of electron transfer depends on solvent reorganization and vibrational modes other than R–X stretching, but it does not seem appropriate, in general, to regard electron transfer and bond breaking as two independent phenomena.

It is difficult to define whether two, one or a non-integral quantity of electrons transfer in a regular $S_N2$ reaction. The conventional hypothesis,

until recently, has been that two electrons always 'go together'. Since the mid-1980s there has been considerable discussion (Shaik, 1985; Bordwell, 1987) indicating that electrons may move one at a time (Scheme 1.3), particularly with nucleophiles which are members of one-electron reversible redox couples. The factors governing whether radical reactions occur (i.e. whether the radical escapes from the reaction complex) are those which govern the coupling of the spin paired electrons following the electron shift. This enables us to understand why reactions sometimes involve radicals and sometimes involve straightforward heterolytic processes.

In the above discussion we have exemplified the problem of electron transfer with nucleophilic aliphatic substitution. Proton transfer between bases could also be considered as an SET process (Eqn [1.2]), as can electrophilic substitution in benzene (Eqn [1.3]).

$$B:^- + H\text{–}A \longrightarrow [B{:}H \cdot\cdot A]^- \longrightarrow [B \cdot\cdot H{:}A]^- \longrightarrow B\text{–}H + A:^- \qquad [1.2]$$




[1.3]

### *Classification of reactions*

So far we have discussed the way in which individual bond changes can occur. Most reactions involve at least two major bonding changes and Ingold classified reactions into four main types—substitution, addition, elimination and rearrangement (Scheme 1.4). The classification is based on the stoichiometry and *not* on the mechanism.

| | |
|---|---|
| Substitution (S) | $Y + B\text{–}X \longrightarrow B\text{–}Y + X$ |
| Addition (Ad) | $A + B \longrightarrow A\text{–}B$ |
| Elimination (E) | $A\text{–}B \longrightarrow A + B$ |
| Rearrangement (not designated) | $X\text{–}A\text{–}B\text{–}Y \longrightarrow Y\text{–}A\text{–}B\text{–}X$ |

**Scheme 1.4** Ingold's classification of reactions.

It is important to recall that this classification records observations about the structure of *reactants* and *products*. Since mechanism is strongly connected with classification, the symbols that Ingold and later workers used to denote reaction types have come to be used as symbols for mechanistic types. A IUPAC group has proposed a new symbolism to refer to *mechanisms*; its application is hotly disputed so we gather the most important current symbols together with a brief description of the IUPAC scheme in Appendix A.1 (Guthrie & Jencks, 1989).

When mechanisms are studied it is surprising how the basic types described above suffice, often in combination, to describe the overall reaction. For example, the mechanism for the reaction of hydroxide ion with