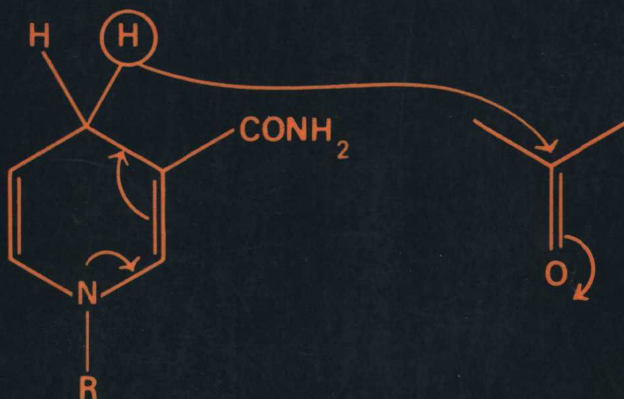


Biological chemistry

The molecular approach
to biological systems

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THE MOLECULAR APPROACH TO
BIOLOGICAL SYSTEMS

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FOREWORD

Living systems display an enormous and fascinating range of chemical processes; indeed, almost the entire development of organic chemistry over its first 100 years or so flowed from studies of natural materials. During this early period, there was particular emphasis on determination of the structures of isolated pure substances and gradually it was recognised by some of the pioneers that relationships existed among the structures which had been elucidated. So Nature did not produce a haphazard set of substances. There was order and it was correctly deduced that this must be a reflection of there being patterns in the biosynthetic processes. Though there was intense speculation at that time about possible biosynthetic pathways, the means (isotopes of carbon and hydrogen) which were used by organic chemists some 40 years later to discover biosynthetic routes were not then available.

However, vigorous development was occurring in other studies of living systems and the area of science we now know as biochemistry grew apace. Fundamentally though, these and other branches were growing from the same tree. Titles for the branches such as bio-, organic, bio-organic, bio-inorganic and so on tend to obscure the fact that scientists in all these areas have a basic common interest, namely, the chemistry of living systems. The danger is that one branch can stay rather isolated from another and if students reading biochemistry fail to grasp the basic principles of reaction mechanism and reactivity, they are hamstrung in their approach, for example, to enzymes and coenzymes. Similarly organic chemists who regard biochemistry as 'messy and non-crystalline' are missing exciting opportunities to enrich their studies.

This book aims, on the one hand, to help chemists see the beauty and order in the chemistry of living systems and to recognise that the processes are related to familiar reactions. On the other hand, it aims to help students of biochemistry to think in molecular and mechanistic terms. Such a weaving of these various threads is not an easy task and this book, written by two authors whose experience and interests span the various branches, is therefore particularly welcome. There will be few readers whether from chemical or biochemical backgrounds who do not benefit from it.

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A. R. Battersby

PREFACE

Many modern scientific and technological problems require contributions from scientists trained in several different disciplines. Nowhere is this more apparent than in the interaction of the life sciences with chemistry, where the chemist must understand the nature of the biological system that is under study and his biologist colleague must be conversant with the language and logic of chemistry. Biochemistry courses can easily underestimate the value of the molecular approach: similarly there is little room in chemistry courses to give students a feeling for the nature of the problems in the life sciences which require a contribution from them.

In this book we attempt to guide students of both chemistry and biochemistry through the common ground of their subject in what we believe is a novel way, so that they can appreciate fully the potential of the molecular approach in the life sciences. To achieve this we have concentrated more on communicating patterns of thought rather than on presenting a comprehensive account of modern biological chemistry. We have found this approach to be successful in teaching both chemistry students in Strathclyde and biochemistry students in Edinburgh and it also seems to be popular with students.

Our discussion develops from a principally chemical beginning to consideration of biological systems of increasing complexity, and we attempt to show the power of molecular thinking in all these systems. Thinking in molecular terms requires practice, and we have included problems designed to help the student acquire this desirable ability. Many have been tested in classes in Edinburgh and Strathclyde. Answers will be found at the end of the book or in the references cited in the problem.

We have included an outline of some central metabolic pathways in Appendix 1 to help the student not already familiar with these. Since mechanistic ideas are frequently expressed using the curly arrow formalism we have also included a note on their use (Appendix 2). Mrs Barbara Stewart and Miss Helen Scott made a valuable contribution to the typing of the manuscript and Professor D. T. Elmore and Mr Andrew Scott are to be thanked for their

critical reading of the earlier drafts. We would also like to thank the editorial staff of Cambridge University Press for their help in producing the volume which you now have before you.

Keith Suckling
Colin Suckling

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1 Chemistry and nature

1.1. Introduction: our aim

Chemistry is the fundamental science that deals with the properties and behaviour of molecules. It is a mature scientific discipline in that its axioms have been substantiated by experiment and can also be derived mathematically by rigorous theoretical methods. In the past, the chemist has practised his art upon relatively small molecules, and both theory and experiment with these systems have led to the establishment of a number of general chemical principles. For instance, up to 1950 molecules of molecular weights greater than several hundred were of little interest to the chemist; synthetic polymer chemistry was in its infancy and natural-product chemists were only concerned with molecules smaller than chlorophyll. Nevertheless, within this range chemistry developed into a more or less self-consistent body of experimental fact and related principles. If these principles are any good, they should also allow the chemistry of much larger molecules and molecular aggregates, such as are common in biochemistry, to be described.

We want to show you how a thorough understanding of the principles of chemistry derived from and applied to biochemistry allows us to say a good deal about what goes on in nature and to produce socially useful end results. If you are trained as a chemist, we shall try to demonstrate how to extend experience of simple reactions to understand complex biochemicals with many interactions both within and without the molecule. If you are a biochemist by background, we hope to convince you that disciplined chemical thought can help you in a more penetrating study of your subject.

We begin by tidying up our ideas about the elementary principles of organic chemistry and see in general terms how they relate to biologically important molecules.

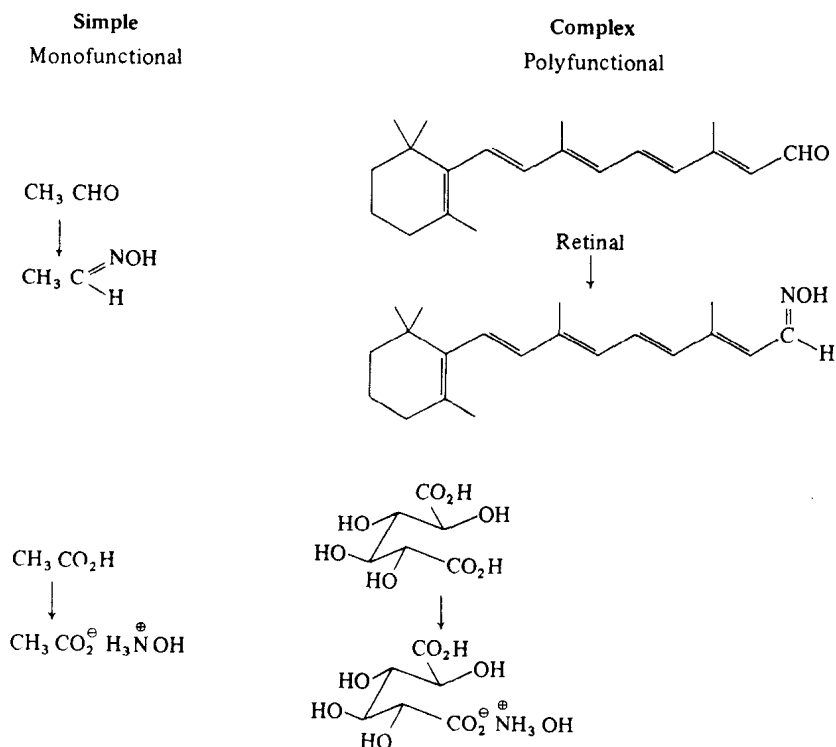
1.2. Systematic organic chemistry

One of the greatest difficulties for students of organic chemistry has always been the vast quantity of experimental information pertaining to the subject. To assimilate such material and to apply the knowledge, systematisation

of organic chemistry has been essential. Traditionally, since the early nineteenth century systematisation has been built around the recognition that the same **functional group** in one compound undergoes more or less the identical reactions to the same functional group in another compound; conversely, different functional groups behave differently under the same conditions. Thus hydroxylamine, for instance, forms an oxime with both the simple aldehyde acetaldehyde, and with the complex aldehyde retinal, but the carboxylic acids, acetic (simple) and glucaric (complex) form salts (Figure 1.1). We usually take it that a hydrocarbon chain is inert to most reactions (a paraffin derivative) and concentrate upon the behaviour of the functional group. What is 'functional' depends to some extent upon the reaction in question.

Having classified our compounds according to functional groups, we can say a good deal about the sort of chemistry that we would expect, provided that we know a few simple facts about representative members of each class of compounds. Acetic acid, for example, could be our prototype carboxylic acid and we can predict the behaviour of undecanoic acid, say, simply by *analogy* with acetic acid, at least as far as the carboxylic acid group is concerned. If you are not fluent in the typical reactions of functional groups, you

Figure 1.1.



should consolidate your knowledge by referring to a conventional organic textbook as you go along. Morrison & Boyd (1973) and Allinger *et al.* (1976) are recommended.

What can be said about cases like retinal, where in addition to the aldehyde there are also a number of carbon-carbon double bonds? How can we understand the chemistry of a polyfunctional compound such as this? Obviously it will show some of the typical reactions of each functional group independently, but it is also likely that some of the functional groups may interact with each other. The electronic theory of organic chemistry provides a rationalisation for the interaction of functional groups. It can describe in more or less pictorial terms why each functional group behaves as it does, and uses the same arguments to develop an understanding of how the functional groups affect each other (Suckling, Suckling & Suckling, 1978). The language used frequently refers to **effects**, inductive effects, resonance effects and steric effects, that change the properties of the functional group. These effects have been singled out chiefly as a result of experimental evidence, but they find some support in quantum-mechanical calculations. If you understand how to manipulate these effects, you can systematise much further information within them. For example, an electron-withdrawing effect exerts an acid-strengthening influence. Furthermore, you can make logical predictions about the behaviour of complex molecules or you can rationalise their observed behaviour. Such predictions are naive by the standards of modern computational chemistry but usually they are remarkably successful.

A third useful classification exists; reactions can be grouped together according to common features of **reaction mechanism**. These reaction types overlap with systematisation by functional groups and electronic theory because they employ concepts and methods from both. Thus, the above examples of aldehydes and carboxylic acids (Figure 1.1) illustrate a condensation reaction and the formation of a salt respectively. It is possible for condensation reactions to take place with esters, for example, and for salts to form with phosphoric acids as well as carboxylic acids. Further, we shall come across cases where we are not dealing with a simple recognisable functional group, although we can define the class of reaction occurring. Chapters 3 to 8 are built around relatively few single reaction types, but there is another equally important reason for this organisation. It is that we can bring the cohesive influence of reaction mechanism into our systematisation.

A reaction mechanism is best regarded as any description of the course of the reaction, however sophisticated or naive this description may be. It is a model of a reaction that guides us to make predictive leaps in the dark. Manipulating mechanism as an intuitive tool of chemists is very similar to manipulating electronic theory and we hope that you will develop a valuable facility in both as you proceed with this book.

1.3. Structural formulae and molecular models

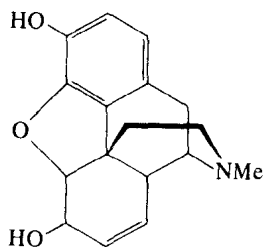
When we think of a functional group, we bring to mind not only its reactions but also its structure. The crux of chemical argument is to relate the structure of a compound to its chemical properties and vice versa. To communicate structures between chemists it is customary to employ drawings or structural formulae. These pictures can be considered as stylised models of the molecules in question: they are constructed within the limits of fairly strict conventions. Structures symbolise not only molecular shape, but also chemical behaviour.

Structural formulae have limitations. In particular, it is necessary to employ conventional projections to represent the three-dimensional structure of molecules on a flat page or blackboard. One way round this is to use molecular models that allow us to hold and manipulate a more readily acceptable representation of a three-dimensional system (Mislow, 1966). All students of chemistry should be familiar with such models. Living beings, too, are three-dimensional and stereochemistry, the understanding of which is greatly aided by molecular models, is an essential part of the chemistry of living things. Molecular models also have their limitations, but it will divert us too much from our aim to consider them at length now. Where the representation of a compound shows an important feature, we shall point it out. Let us meanwhile bear in mind the essence of communication through structures as distilled by Robinson who as early as 1917 wrote concerning arguments over the structure of the alkaloid morphine (Robinson, 1917*a*): 'This formula [Figure 1.2] has been adopted especially since the formulae which it is suggested should replace it cannot without hesitation be accepted as superior representations of the properties of the substance.'

1.4. How organic chemists became interested in biochemistry

For many reasons, some commercial, some scientific and some philosophical and pedagogic, molecular science has been tending to ignore interdisciplinary boundaries in the last two decades. It is now common to

Figure 1.2.



Morphine

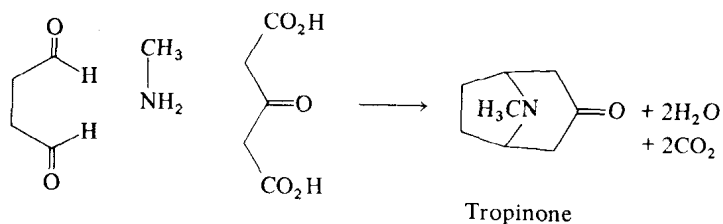
hear scientists described as hybrids of conventional disciplines, such as the bioinorganic chemist. Some hybrids have become an established and prolific strain, molecular biologists for example. But before about 1940, it was unusual for a scientist of one discipline to pay more than polite passing attention to a colleague's work in a different but related discipline. The eminent German organic chemist Hans Fischer, whose life's work was expended upon the chemical synthesis and structure determination of the important naturally occurring compounds porphyrins, typified the situation when he wrote in 1937 'So far, we can only speculate upon the physiological significance of porphyrins in the plant and animal kingdoms' (Fischer & Orth, 1937). However, some twelve years earlier, Keilin described the identification of the porphyrin-containing proteins, the cytochromes; Fischer appears to have been unaware of them.

In the nineteenth century, disciplines were not so conventionally defined and men of genius, like Pasteur, were able to embrace in their careers all fields of natural science from physics to microbiology, and to contribute to each. The whole of modern chemical and biochemical stereochemistry is foreshadowed in Pasteur's work on optical isomerism (Robinson, 1974), not so much in its scientific precision and elegance but principally in the vision with which he assesses the significance of his results.

The twentieth century too had its prophets. Robinson, an organic chemist, interpreted the probable biosynthetic origins of alkaloids from the structures that he had deduced for them chemically (Robinson, 1917a). He demonstrated that the alkaloid tropinone could be prepared in the laboratory from the probable biological reactants under mild conditions (Robinson 1917b); (Figure 1.3). These early achievements were not surpassed until the skill of chemists had developed further over thirty years. Eventually (1945), it became possible to test the ideas of Robinson and others concerning biosynthesis by the use of radioactive tracers. Also, chemists learned to synthesise many of the complex naturally occurring molecules and began to study their behaviour in terms of chemical mechanism both *in vivo* and *in vitro*.

Technique and methodology, therefore, in chemical and biological sciences steadily reached a maturity that was compatible and apt for marriage. At the same time, the intuitive and theoretical concepts of organic chemistry matured,

Figure 1.3. A simple biogenetic-type synthesis.



and chemists began to set themselves more severe tests posed by the complex molecules of nature. It was natural for organic chemists to be the first to be influenced by biochemists but, today, a chemist of any specialisation will readily apply his expertise to a biological problem. Complex problems in molecular science can now be approached with a formidable array of precedent, principle and perception that is remarkably self-consistent.

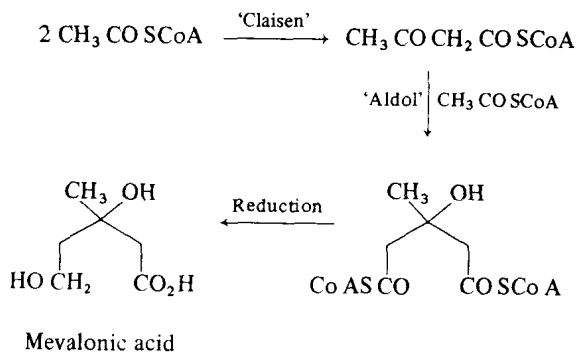
1.5. Chemistry assimilates larger and more complex systems

Our knowledge of the biosynthetic pathways of organisms is chiefly the result of the combined efforts of chemists and biochemists during the two decades up to 1965. This was the classical era of biosynthesis (Bu'lock, 1965; Hendrickson, 1965) and we shall draw on it for examples.

A chemist confronted with this large body of information will perceive that in reality there is less to learn than at first sight seemed the case. Most of the transformations that biosynthetic studies have discovered are readily absorbed into the existing body of systematic chemical fact. For example, mevalonic acid, the key intermediate of steroid and terpene biosynthesis, is built up in nature from acetic acid in a series of steps. The individual steps are analogues of the chemically familiar reactions, the Claisen and aldol condensations (Figure 1.4).

Relatively few organic reactions simply transform *A* into *B* without the intervention of a catalyst; Claisen ester condensations, for example, require an alkoxide ion as catalyst. Uncatalysed reactions occur in both chemistry and biochemistry, but are especially rare in the latter. They are often spontaneous decarboxylations or dehydrations, usually occurring at room temperature in the laboratory. Most other reactions require more extreme conditions easily obtainable in the laboratory but not available to living systems. Reactions in living organisms must, of course, be carried out under the mild ambient conditions prevailing in the organism. This means that catalysis is almost invariably essential, and it is provided by enzymes.

Figure 1.4.



Enzymes are proteins whose molecular weights range from 10^4 to 10^6 and they catalyse metabolic reactions very efficiently. Chemists have been greatly intrigued to discover how enzymes achieve their catalytic efficiency under such mild reaction conditions. Accordingly, an immense research effort has been expended upon chemical, biochemical and crystallographic methods aimed at determining both the chemical structures of enzymes and the relationship of their structures to the mechanism of action. Both are exceedingly complex problems which can be solved satisfactorily only by using a combination of techniques. When answers are available, the organic chemist wants to see whether they fit in with his model of chemical behaviour. By and large, enzyme-catalysed reactions can be understood using modern organic chemical logic, especially if attention is concentrated upon the **substrate** of the enzyme.

Much has been written (Gray, 1971; Bender & Brubacher, 1973), about the mechanism of action of enzymes, but attention has usually focused on the enzyme through a physical approach to the organic chemistry involved. This approach is necessary, although a great deal must be known about the basic chemistry of the system before the often involved arguments can be appreciated. The topic then becomes one only for advanced undergraduate and postgraduate study.

On the other hand, if we consider the substrates, we can discuss the new chemistry and the related biochemical consequences at the same time. It makes little difference whether the substrate is attacked by a hydrolysing enzyme or by hot caustic soda solution, since its chemical properties govern what reactions may occur. By use of the qualitative but powerful logic of chemistry, we introduce new classes of compound as developments of simple aliphatic and aromatic functional-group chemistry. We can then study the implications of the chemistry in the biological field as a direct consequence of the inherent chemical reactivity of the substrate.

1.6. A chemical approach: amide hydrolysis, chymotrypsin

When a chemist plans a reaction, he examines the structure of the starting material and assesses its inherent chemical reactivity. He then selects the reagents and the reaction conditions that are most likely to give the desired result. Many attempts may be necessary in which the conditions are successively modified to achieve improvements. Nature has had more experience than chemists in handling molecules, and has developed in the course of evolution enzymes that are very efficacious. So precise is the relationship of an enzyme's chemical function to its substrate that a study can reveal aspects of the chemistry of the substrate that may well have lain hidden even to a most thorough-minded chemist.

Consider the hydrolysis of an amide bond as a simple example of what we can find out. What can chemistry tell us? Experimentally, if we simply compare the conditions required to hydrolyse an ester and the corresponding amide,

we discover that the ester is more easily hydrolysed. An organic chemist would rationalise this by explaining that the contribution of the canonical form **B** (Figure 1.5a) to the resonance hybrid is much greater in the case of the amide because nitrogen is less electronegative than oxygen and therefore more readily donates its electrons. This fact is also apparent in the infrared stretching frequencies of the carbonyl groups. Typically an amide absorbs at about 1680 cm^{-1} and an ester close to 1740 cm^{-1} , this reflects the weaker carbon-oxygen double bond in the amide, again a consequence of the large contribution of canonical form **B**.

Hydrolysis occurs by attack of a nucleophile (water or hydroxide ion) upon the carbon of the carbonyl group of the ester or amide. The resonance effect in the amide means that the attacking nucleophile senses a much weaker partial positive charge on the amide carbonyl group than the ester and, accordingly, more energy is required to form the intermediate adduct (Figure 1.6). Hydrolysis of amides and esters thus are examples of nucleophilic addition to carbonyl groups.

There is one other important consequence of the resonance interaction in amides. The canonical form **B** represents the donation of the lone pair of the nitrogen atom to interact with the p-orbital of sp^2 -hybridised carbon in the

Figure 1.5. Bonding in amides and esters.

