ESSENTIALS OF BIOLOGICAL CHEMISTRY

Fairley and Kilgour

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Biological Chemistry

Library of Congress Catalog Card Number 63-13546
Printed in the United States of America

Preface

This book has been written specifically to serve as a text for the introductory course in biochemistry at the undergraduate level. With suitable supplementation it will also fill the needs of survey courses offered for graduate students whose areas of specialization require a basic familiarity with the principles of biochemistry, rather than a working knowledge of the field.

We were prompted to write this text by a definite need and our strong desire for a book which would present the fundamental concepts of biochemistry in a manner suited to the broad spectrum of students in the undergraduate classes at Michigan State University. These students come to us with widely varying preparations in chemistry and in biology and with major interests ranging from home economics through agriculture, veterinary medicine, microbiology, and other biological sciences to chemistry. The composition of these classes reflects in major degree the great impact that the unusually rapid growth of biochemical information has had upon all areas of biology. The resulting interest in biochemistry is gratifying to the biochemist, but it poses major problems for the instructors of introductory courses in the selection of material. On one hand, the students are being derived from an ever-widening pool of scientific interests, and on the other, the mass of biochemical information in any single area is rapidly becoming increasingly detailed and complex.

We feel that the logical solution of these problems requires major emphasis upon the basic concepts and fundamental information of general biochemistry rather than detailed information on a specific organism such as the human, or a superficial treatment which includes mention of every possible peripheral aspect of the field. Accordingly, our primary goal in this book is the presentation of a coherent picture of the major metabolic activities of living cells, a picture which places special attention on the relationship of chemical structure to biological function. This we firmly believe to be the core of the study of biochemistry. It is this material which a student must master as an approach to the understanding of the phenomena of life in chemical terms.

We have treated the material we feel to be basic in considerable depth, sufficiently, we believe, so as to permit real understanding. At the same time much material traditionally included in introductory courses has been omitted. This includes a certain amount of information on the purely chemical aspects of the compounds found in biological systems. We have discussed only those details most necessary for the comprehension of the processes of metabolism and of the research methods of the biochemist. Similarly, we have deleted many data related specifically to the understanding of human physiology and nutrition and to the clinical detection of disease or metabolic deficiencies. Much of this subject matter will currently be covered in courses in the student's own field and no longer need be considered as necessary in a general biochemistry course.

Suggestions for "Additional Reading" have been included at the end of each chapter in order to lead the way to the current literature for those who are so inclined. Where possible, we have chosen articles at several levels in order to provide a range between general background and precise detail.

The material on the chemical constitution of biological materials has been put into the first few chapters of the book in order to permit maximum flexibility in adapting the text to different classes having somewhat different backgrounds, particularly in organic chemistry. Students with a good chemistry background will be able to proceed with relative rapidity through the first few chapters and spend more time on the later material. Other classes may require more time for the first five chapters, and may cover the remaining material in less detail.

In Chapter 12, we have considered some of the current information regarding the chemical mechanisms by which metabolic activities are controlled within cells. This is an area of paramount importance for the maintenance of normal cellular structure and function. At the present time we have only a few glimpses of how the controls function, but they are so important that we felt justified in inserting some of this information even though it is only fragmentary.

In over-all approach, we have tried to proceed on what we feel to be a logical progression of complexity. Thus we begin with a consideration of the organic chemistry of cellular constituents, then go on to consider the most basic chemical feature of all life—the enzymatic catalysis of chemical reactions. The next step is to look at the chemical nature of an individual cell with emphasis on its energy-transfer mechanisms and the general means by which it maintains the living state. This leads readily into a rather extensive examination of many of the basic metabolic reaction sequences within cells, with consideration of their interactions and integration into a functional whole. The discussion of special problems peculiar to multicellular organisms or tissues has been left until the basic cellular groundwork has been laid.

We would like to express our thanks to the many colleagues and students who have read part or all of the manuscript during its preparation and have given us the benefit of their comments and suggestions. Our thanks also go to Mary Ann Kiesler, who prepared most of the figures for publication, and to the staff members of the Reinhold Publishing Corporation, who have been of so much assistance to us in proceeding from thoughts to print.

James L. Fairley
Gordon L. Kilgour

Michigan State University
East Lansing, Michigan
April, 1963

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1

Some Pertinent Aspects of Organic Chemistry

Certain aspects of organic chemistry are particularly important to a clear understanding of the structures, functions, and reactions of various compounds of biochemical importance. Some of these aspects have been selected for very brief review and re-emphasis at this point. More detailed information on any of the material presented in this chapter may be found in any standard text or reference book of organic chemistry.

FUNCTIONAL GROUPS AND THEIR REACTIONS

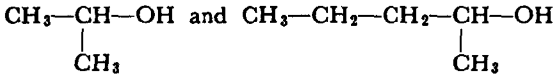
Alcohols R—OH

The alcohols can be classified into three groups on the basis of their structures:

Primary (those having at most one other carbon attached directly to the hydroxylated carbon), e.g.:

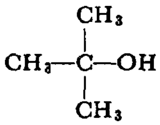
$$CH_3—OH, \quad \text{and} \quad CH_3—CH_2—OH$$

Secondary (those having two other carbons attached directly to the hydroxylated carbon), e.g.:



$$CH_3—\underset{\displaystyle CH_3}{\underset{|}{CH}}—OH \quad \text{and} \quad CH_3—CH_2—CH_2—\underset{\displaystyle CH_3}{\underset{|}{CH}}—OH$$

Tertiary (those having three other carbons attached directly to the hydroxylated carbon), e.g.:

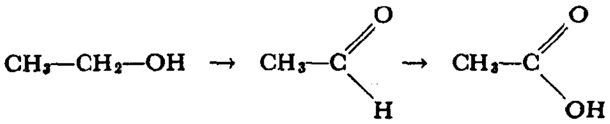


$$CH_3—\overset{\displaystyle CH_3}{\overset{|}{\underset{\displaystyle CH_3}{\underset{|}{C}}}}—OH$$

The differences in structure among the three groups are also reflected in differences in reactivities. Thus, for example, tertiary alcohols are most easily dehydrated, followed by the secondary and then by the primary alcohols. On the other hand, the reverse order of reactivity holds for ester formation.

REACTIONS

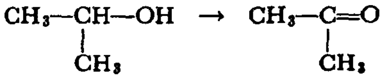
1. *Oxidation:* A *primary alcohol* is oxidized to the corresponding aldehyde, which in turn may be oxidized to a carboxylic acid:



$$CH_3—CH_2—OH \rightarrow CH_3—C\begin{matrix}\nearrow\!\!\!\!\nearrow O\\ \searrow H\end{matrix} \rightarrow CH_3—C\begin{matrix}\nearrow\!\!\!\!\nearrow O\\ \searrow OH\end{matrix}$$

aldehyde acid

Secondary alcohols are oxidized to ketones:

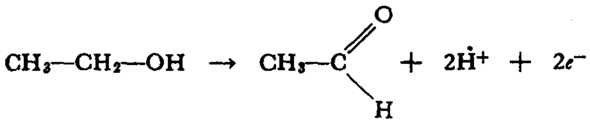


$$CH_3—\underset{\displaystyle CH_3}{\underset{|}{CH}}—OH \rightarrow CH_3—\underset{\displaystyle CH_3}{\underset{|}{C}}=O$$

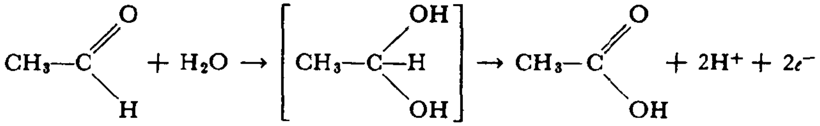
ketone

Tertiary alcohols cannot be oxidized except under conditions which bring about rupture of the carbon chain.

The oxidation of an alcohol to an aldehyde or of an aldehyde to an acid actually involves the removal of two hydrogens with their electrons:



$$CH_3—CH_2—OH \rightarrow CH_3—C\begin{matrix}\nearrow\!\!\!\!\nearrow O\\ \searrow H\end{matrix} + 2\dot{H}^+ + 2e^-$$

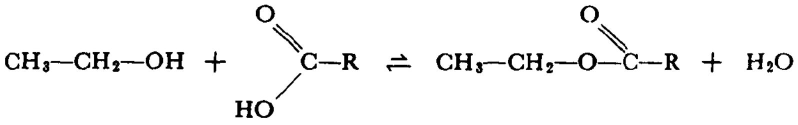


It is easier to see that the oxidation of the aldehyde to the acid does occur by removal of two hydrogens, when the aldehyde is written in the hydrated form. In fact, this form predominates in water solutions of simple aldehydes.

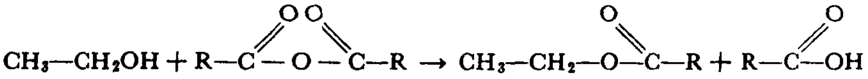
2. *Dehydration:* Under proper conditions, alcohols can be dehydrated to yield the corresponding unsaturated compound:

$$CH_3-CH_2-CH_2-OH \rightarrow CH_3-CH=CH_2 + H_2O$$

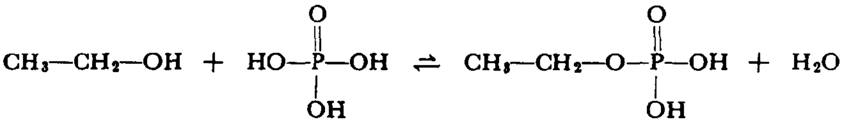
3. *Ester Formation:* When an alcohol and an acid are mixed together, they will react to produce some of the corresponding ester and an equivalent amount of water.



Chemically, this equilibrium reaction is usually avoided by use of the anhydride of the acid and removal of the free acid that is formed during the reaction by the addition of excess base.

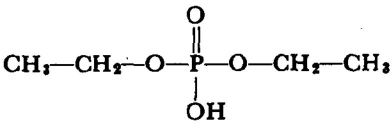


Note also that it is especially important for biochemical purposes to remember that the acid involved in ester formation need not be an organic acid, but can equally well be an inorganic acid such as phosphoric.



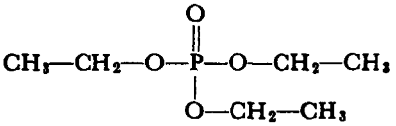
$$CH_3-CH_2-OH + HO-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-OH \rightleftharpoons CH_3-CH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-OH + H_2O$$

This product is called a *monoester* of phosphoric acid since only one of the three acidic groups is involved in the ester bond. A second group can be esterified to give a *phosphodiester;* this can react further to yield a *phosphotriester*. For example:



$$CH_3-CH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-O-CH_2-CH_3$$

a phosphodiester



$$CH_3-CH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O-CH_2-CH_3}{|}}{P}}-O-CH_2-CH_3$$

a phosphotriester

Thioalcohols R—SH

The sulfur analogs of the alcohols in which a sulfur atom replaces an oxygen atom are called mercaptans or thioalcohols. The —SH group of a thioalcohol is referred to as a *thiol* or as a *sulfhydryl* group.

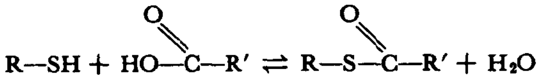
The thiols undergo many of the reactions of alcohols but have several outstanding differences:

REACTIONS

1. *Oxidation:* Thiols can be reversibly oxidized to form *disulfides:*

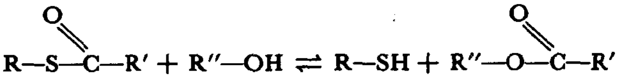
$$2\ CH_3-CH_2-SH \rightleftharpoons CH_3-CH_2-S-S-CH_2-CH_3 + 2H^+ + 2e^-$$

2. *Thioester Formation:* Reaction of a thiol with an acid (or acid anhydride) generally yields a *thioester:*



$$R-SH + HO-\overset{\overset{\displaystyle O}{\|}}{C}-R' \rightleftharpoons R-S-\overset{\overset{\displaystyle O}{\|}}{C}-R' + H_2O$$

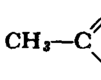
Since thioalcohols are stronger acids than are alcohols, thioesters have a stronger tendency to act as anhydrides than do true esters. Thus, they are much more easily hydrolyzed, and in some cases may transfer the acid portion of the molecule to an alcohol; e.g.:



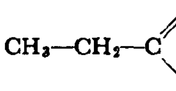
$$R-S-\overset{\overset{\displaystyle O}{\|}}{C}-R' + R''-OH \rightleftharpoons R-SH + R''-O-\overset{\overset{\displaystyle O}{\|}}{C}-R'$$

Aldehydes and Ketones $R-\overset{\overset{\displaystyle O}{\|}}{C}-H$ and $R-\overset{\overset{\displaystyle O}{\|}}{C}-R$

The aldehydes are generally named for the acid which is formed on their oxidation; e.g.:

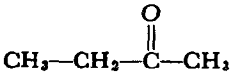


acetaldehyde



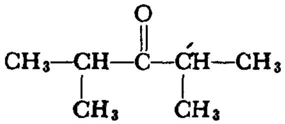
propionaldehyde

The ketones are named for the two groups which are attached to the carbon carrying the oxygen. (This C=O group as found in the aldehydes and ketones is referred to as a *carbonyl group.*) For example:



$$\mathrm{CH_3{-}CH_2{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}CH_3}$$

methyl ethyl ketone

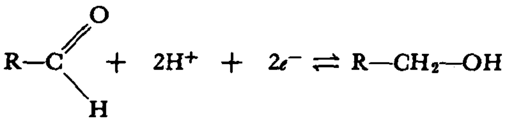


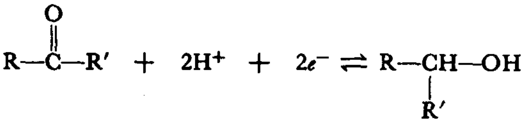
$$\mathrm{CH_3{-}\underset{\underset{\displaystyle CH_3}{|}}{CH}{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}\underset{\underset{\displaystyle CH_3}{|}}{CH}{-}CH_3}$$

diisopropyl ketone

REACTIONS

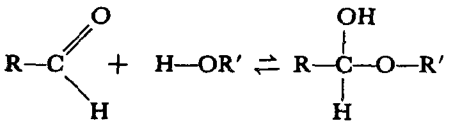
1. *Reduction:*





$$\mathrm{R{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}R'} + 2H^+ + 2e^- \rightleftharpoons \mathrm{R{-}\underset{\underset{\displaystyle R'}{|}}{CH}{-}OH}$$

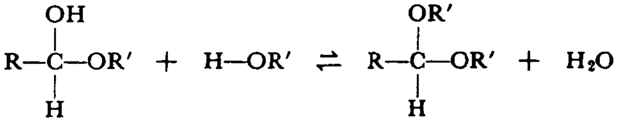
2. *Addition to Carbon-Oxygen Double Bonds:* In each of the following cases, the hydrogen from the compound being added across the double bond goes to the oxygen; the remainder of the compound becomes attached to the carbon. One important example is the addition of an alcohol to an aldehyde to yield a *hemi-acetal.*



hemi-acetal

The formation of a hemi-acetal (or hemi-ketal if formed from a ketone) is a true addition reaction. That is, two molecules are joined together to form a new molecule without loss or gain of atoms. Hemi-acetals are constantly in equilibrium with the free aldehyde and alcohol. This equilibrium usually lies far to the left. Exceptions to this are certain ring structures, common among the sugars, in which the equilibrium is very much in favor of the hemi-acetal.

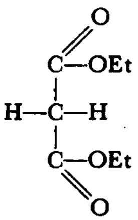
Hemi-acetals react with alcohols to yield *acetals*. This reaction is a dehydration involving the hemi-acetal and alcohol hydroxyl groups and is *not* an addition reaction. The corresponding derivative of a ketone is usually called a *ketal*.



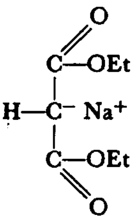
$$R\text{—}\underset{H}{\overset{OH}{C}}\text{—}OR' + H\text{—}OR' \rightleftharpoons R\text{—}\underset{H}{\overset{OR'}{C}}\text{—}OR' + H_2O$$

A second type of addition reaction, involving *active methylene groups*, is also common in biological systems. When a compound has one or more electron-withdrawing groups adjacent to a methylene (or sometimes a methyl) group, the methylene group is more acidic than usual. Accordingly this type of methylene group is more reactive in carbonyl-group addition reactions. We often refer to such methylene groups as "active methylene groups." Electron-withdrawing groups include phenyl groups, esters, acids, carbonyl groups, and, under some circumstances, double bonds.

A compound with an exceptionally active methylene group is malonic acid (or its esters). Here the two carboxyl or ester groups are so effective in decreasing the electron density at the central carbon that the ester becomes sufficiently acidic to form salts in such solvents as ethyl alcohol:



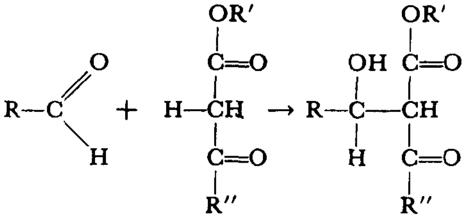
diethyl malonate



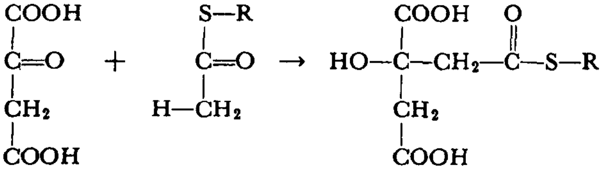
sodium diethyl malonate

Examples of typical additions of active methylene groups to carbonyl double bonds are:

(a)

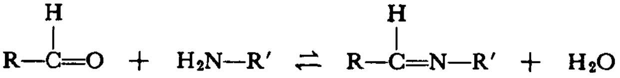


(b)



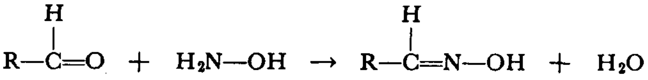
$$\mathrm{HOOC{-}CO{-}CH_2{-}COOH} + \mathrm{R{-}S{-}CO{-}CH_2{-}H} \rightarrow \mathrm{HO{-}C(COOH)(CH_2COOH){-}CH_2{-}C({=}O){-}S{-}R}$$

A third type of addition to carbonyl double bonds involves compounds of the general type $H_2N—X$, where the X represents a variety of groups ranging from a hydroxyl group to substituted ring systems. A typical example is the reaction of an aldehyde with an amine:



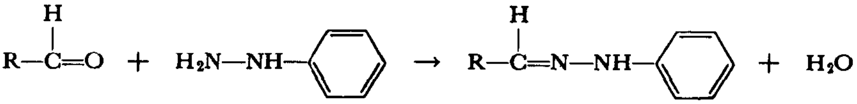
$$\mathrm{R{-}\overset{H}{\overset{|}{C}}{=}O} + \mathrm{H_2N{-}R'} \rightleftharpoons \mathrm{R{-}\overset{H}{\overset{|}{C}}{=}N{-}R'} + \mathrm{H_2O}$$

The product in this case is called a "Schiff base" and is very unstable in water solution. However, stable products are formed in reactions of aldehydes with compounds such as hydroxylamine or phenylhydrazine. The products, oximes and phenylhydrazones, respectively, are readily crystallized and are often prepared to assist in the identification of unknown aldehydes or ketones.



$$\mathrm{R{-}\overset{H}{\overset{|}{C}}{=}O} + \underset{\text{hydroxylamine}}{\mathrm{H_2N{-}OH}} \rightarrow \underset{\text{an oxime}}{\mathrm{R{-}\overset{H}{\overset{|}{C}}{=}N{-}OH}} + \mathrm{H_2O}$$

hydroxylamine an oxime



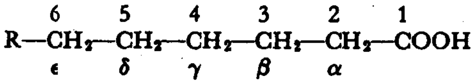
phenylhydrazine a phenylhydrazone

Acids R—COOH

The following is a list of common unbranched-chain monocarboxylic acids. The common name is shown first, followed by the more systematic IUPAC nomenclature in parentheses:

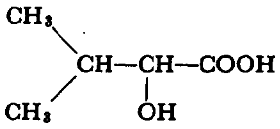
$H—COOH$	formic (methanoic) acid
$CH_3—COOH$	acetic (ethanoic) acid
$CH_3CH_2—COOH$	propionic (propanoic) acid
$CH_3CH_2CH_2—COOH$	butyric (butanoic) acid
$CH_3CH_2CH_2CH_2—COOH$	valeric (pentanoic) acid
$CH_3CH_2CH_2CH_2CH_2—COOH$	caproic (hexanoic) acid
$CH_3—(CH_2)_{10}—COOH$	lauric (dodecanoic) acid
$CH_3—(CH_2)_{12}—COOH$	myristic (tetradecanoic) acid
$CH_3—(CH_2)_{14}—COOH$	palmitic (hexadecanoic) acid
$CH_3—(CH_2)_{16}—COOH$	stearic (octadecanoic) acid

It should be noted that for those acids having four or more carbon atoms, a variety of isomers is possible, depending upon the arrangement of the carbons in the side chain; for example, *n*-butyric (butanoic) and *iso*-butyric (2-methylpropanoic) acids. The positions of substituents on the carbon chain of the acids may be indicated either by numbering of the carbons, or by the use of Greek letters as shown below:



$$R—\underset{\epsilon}{\overset{6}{CH_2}}—\underset{\delta}{\overset{5}{CH_2}}—\underset{\gamma}{\overset{4}{CH_2}}—\underset{\beta}{\overset{3}{CH_2}}—\underset{\alpha}{\overset{2}{CH_2}}—\overset{1}{COOH}$$

Generally, the numbers are used with the IUPAC system of nomenclature and the Greek letters when the common names are employed. For example:



α-hydroxy-*iso*-valeric acid
or
2-hydroxy-3-methylbutanoic acid

Another important group of acids contains two carboxylic groups per molecule and is referred to as *dicarboxylic acids*. The more important ones for our purposes are: