

COMPREHENSIVE BIOCHEMISTRY

EDITED BY
MARCEL FLORKIN
AND
ELMER H. STOTZ

VOLUME 5

CARBOHYDRATES



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GENERAL PREFACE

The Editors are keenly aware that the literature of Biochemistry is already very large, in fact so widespread that it is increasingly difficult to assemble the most pertinent material in a given area. Beyond the ordinary textbook the subject matter of the rapidly expanding knowledge of biochemistry is spread among innumerable journals, monographs, and series of reviews. The Editors believe that there is a real place for an advanced treatise in biochemistry which assembles the principal areas of the subject in a single set of books.

It would be ideal if an individual or small group of biochemists could produce such an advanced treatise, and within the time to keep reasonably abreast of rapid advances, but this is at least difficult if not impossible. Instead, the Editors with the advice of the Advisory Board, have assembled what they consider the best possible sequence of chapters written by competent authors; they must take the responsibility for inevitable gaps of subject matter and duplication which may result from this procedure.

Most evident to the modern biochemist, apart from the body of knowledge of the chemistry and metabolism of biological substances, is the extent to which he must draw from recent concepts of physical and organic chemistry, and in turn project into the vast field of biology. Thus in the organization of *Comprehensive Biochemistry*, the middle three sections, Chemistry of Biological Compounds, Biochemical Reaction Mechanisms, and Metabolism may be considered classical biochemistry, while the first and last sections provide selected material on the origins and projections of the subject.

It is hoped that sub-division of the sections into bound volumes will not only be convenient, but will find favour among students concerned with specialized areas, and will permit easier future revisions of the individual volumes. Toward the latter end particularly, the Editors will welcome all comments in their effort to produce a useful and efficient source of biochemical knowledge.

Liège/Rochester
July 1962

M. FLORKIN
E. H. STOTZ

PREFACE TO SECTION II

(VOLUMES 5-II)

Section II on the Chemistry of Biological Compounds deals with the organic and physical chemistry of the major organic constituents of living material. A general understanding of organic and physical chemistry is presumed, but the reader will find the special topics in Section I of value in the fuller understanding of several parts of Section II. The Editors have made special effort to include a sound treatment of the important biological high polymers, including sections on their shape and physical properties. A number of substances peculiar to plants, certain isoprenoids, flavonoids, tannins, lignins, and plant hormones, often omitted from textbooks of biochemistry, are included. Nevertheless, it is inevitable that some omissions, hopefully minor ones, have occurred. The only intentional omission is the chemistry of the coenzymes and certain components of biological oxidation, which will be covered in connection with their function in Section III.

The previous policy of dividing the section into smaller volumes has been continued, resulting in seven volumes for Section II. Two of the volumes each contain a complete area, namely Carbohydrates (Volume 5) and Sterols, Bile Acids and Steroids (Volume 10). Comments from readers will be appreciated by the Editors and be most helpful for possible future revisions.

Liège/Rochester
December 1962

M. FLORKIN
E. H. STOTZ

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Chapter I

The Monosaccharides

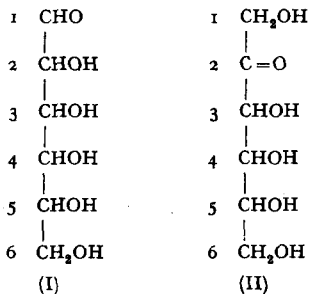
ELIZABETH PERCIVAL

Chemistry Department, University of Edinburgh (Great Britain)

1. General structure of monosaccharides¹

Monosaccharides or simple sugars are aliphatic carbon compounds concerned in many of the metabolic processes of both plants and animals. From them macromolecules, polysaccharides, are built up. They normally consist of chains of carbon atoms of varying length and are classified according to the number of these atoms in the molecule; the simplest, tetroses*, containing four, pentoses five, hexoses six and heptoses seven carbon atoms and at least one octose has been found in Nature. The majority of monosaccharides are unbranched and may be defined as straight-chain polyhydroxy aldehydes (aldoses) or ketones (ketoses); the former having the carbonyl group at carbon atom number one (C-1) (I) and the latter at C-2 (II); the remaining carbon atoms being hydroxylated.

The monosaccharides are water-soluble solids which are not easy to crystallise, and are very liable to form syrupy supersaturated solutions. They possess varying degrees of sweetness to the taste, give optically active



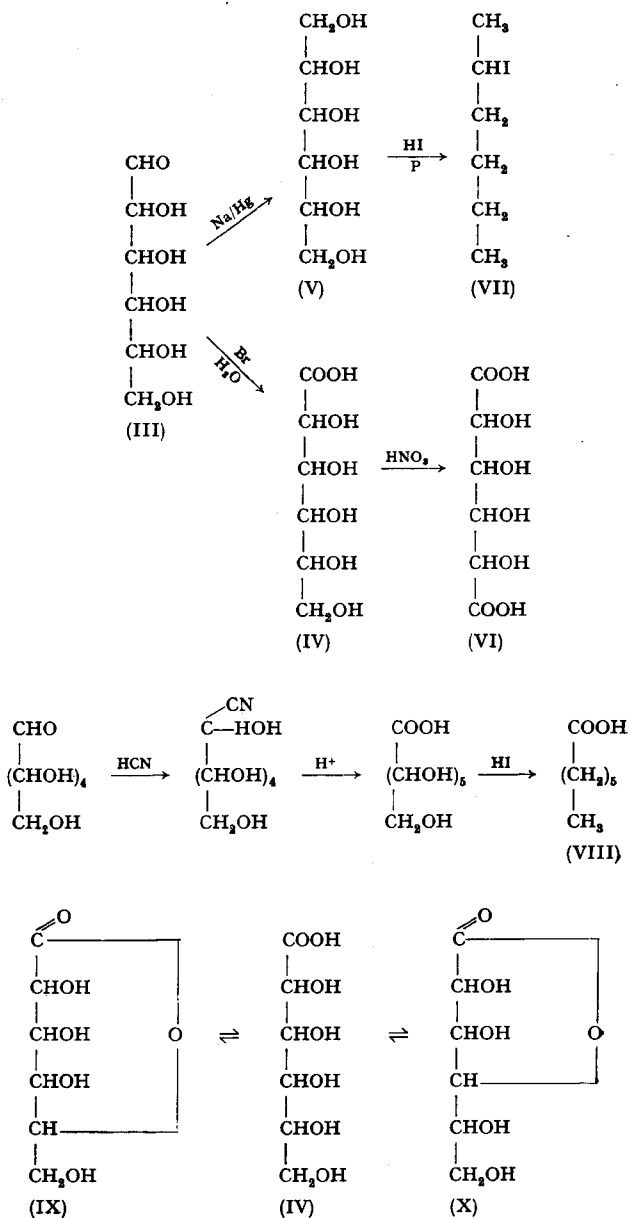
* The systematic nomenclature recommended jointly by American² and British³ authorities will be used throughout this chapter.

solutions and are readily oxidised, especially in alkaline solutions, *e.g.*, the cuprosodium ion in Fehling's solution becomes reduced to cuprous oxide. Although these simple carbohydrates normally exist in six (pyranose) or five (furanose) membered ring forms, many of their properties are consistent with the open-chain structure. In the first instance, the characteristic properties of the two types of functional groups, the carbonyl and the hydroxyl, and the configurations of the different monosaccharides will be described in the acyclic form. Indeed all these facts were established when the monosaccharides were still regarded as being nothing more than open-chain compounds. Furthermore, it is important to remember that a very small proportion of the acyclic form is always present in solution in equilibrium with the various cyclic structures.

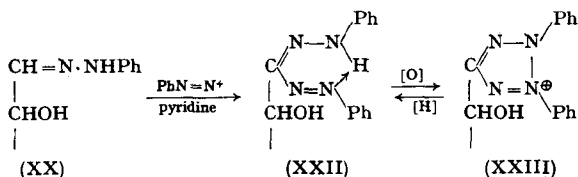
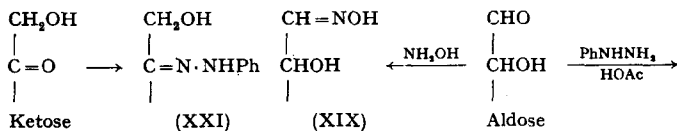
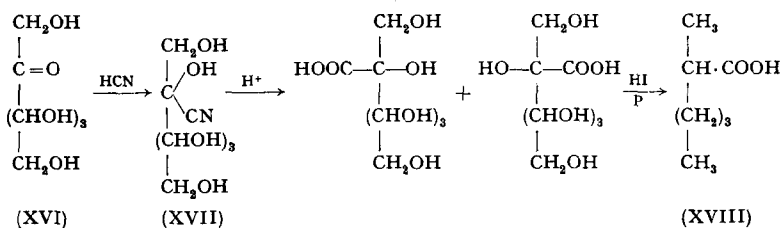
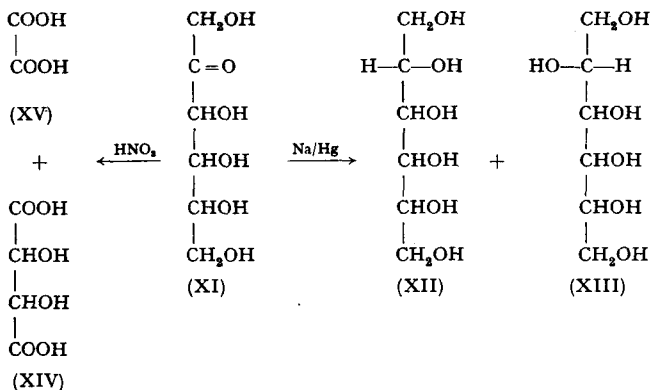
(a) Properties of the aldehydic and ketonic groups

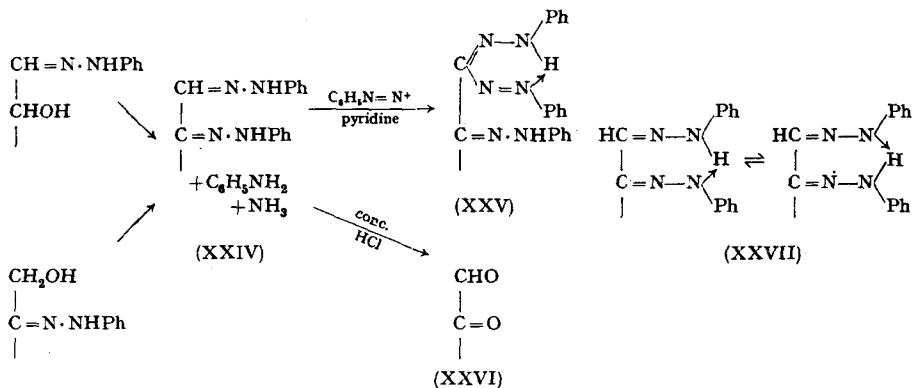
A typical aldohexose (the suffix *-ose* denotes all aldose sugars) has the general formula (III). The aldehydic group of the sugar can be oxidised with bromine water or reduced with sodium amalgam and water to yield, respectively, an aldonic acid (IV) or a hexitol (alditol) (V). Careful oxidation of (IV) with, *e.g.*, nitric acid yields a tetrahydroxy dibasic acid (an aldaric or saccharic acid) (VI) (p. 43). Further drastic reduction of the hexitol with hydriodic acid gives 2-iodohexane (VII) clearly showing that no branching occurs in the hexose chain. Additional evidence for this is derived from the formation of *n*-heptylic (*n*-heptanoic) acid (VIII) from a hexose after addition of hydrogen cyanide⁴ followed by hydrolysis and reduction (p. 37). The aldonic acids (IV) on storage in aqueous solution form equilibrium mixtures with their 1,5- (δ -) (IX) and 1,4- (γ -) (X) lactones (p. 42). Both types of lactone can be prepared as crystalline compounds. They are readily hydrolysed and can be titrated with alkali; the 1,4-lactones, being the more stable, are cleaved more slowly than the 1,5-lactones.

Ketose sugars, denoted by the suffix *-ulose*, have similar properties to the aldoses, but modified by their being ketonic. Reduction of a hexulose (XI) yields two hexitols (alditols) (XII, XIII). Oxidation of ketoses by nitric acid yields tartaric acid (XIV), and oxalic acid (XV), and must take place by cleavages of the carbon chain. The formation of tartaric acid is in accordance with a ketone group at C-2. Proof of the straight chain in ketose sugars is derived from the formation of 2-methylcaproic acid (2-methylhexanoic) (XVIII) after combination of a hexulose (XVI) with hydrocyanic acid and hydrolysis of the resulting two cyanhydrins and reduction. Further evidence of the presence of an aldehydic or ketonic group in monosaccharides is the formation of typical carbonyl derivatives such as oximes (XIX) or phenylhydrazones⁶ (XX, XXI) by the action of hydroxylamine



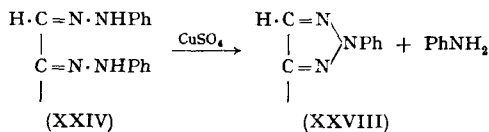
and phenylhydrazine respectively. Diazonium compounds in pyridine or alkaline ethanolic solution couple with acyclic aldose hydrazones derived from phenylhydrazine itself to yield bright red crystalline formazans (XXII).





The structure present in (XX) is necessary for the reaction; it therefore takes place neither with hydrazones derived from *N*-substituted phenylhydrazines, for example, methyl phenylhydrazine, nor with any hydrazones derived from ketoses, nor with cyclic aldose phenylhydrazones. The reaction has proved of value in distinguishing these different forms of hydrazone⁷. The reversible oxidation of the coloured formazans into colourless tetrazolium derivatives (XXIII) has proved of value as an indicator in biological systems.

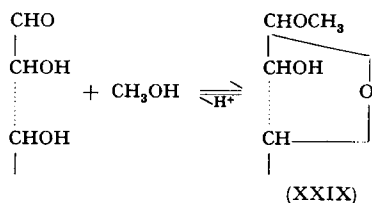
Prolonged action by at least three molecules of phenylhydrazine, in the hot, attacks C-2 in the aldose and C-1 in the ketose by an intermolecular oxidation-reduction yielding a yellow crystalline osazone or bis-hydrazone (XXIV). Various ring structures have been advanced for D-glucose phenylosazone but this substance gives a positive formazan reaction (XXV) due to the presence of $-\text{CH}=\text{N}-\text{NHPH}$ structure on C-1, and must therefore have the acyclic structure (XXIV) which is probably stabilised by a chelate ring structure⁸ (XXVII). Removal of the phenylhydrazine residues either by hydrolysis with concentrated hydrochloric acid or exchange with a competing aldehyde yields a dicarbonyl compound known as an osone⁹ (XXVI).



In the early studies on monosaccharides the osazones proved to be valuable crystalline derivatives in the identification and characterisation of sugars, but the more easily purified osatriazoles (XXVIII) prepared by Hudson¹⁰ by oxidation of the osazones (XXIV) with copper sulphate are to be

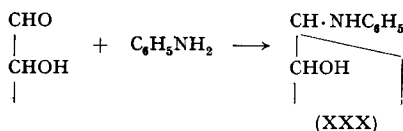
preferred since they are very stable, have characteristic melting points, and their optical rotations can easily be measured as they are colourless in solution.

Treatment of an aldo- or keto-pentose or hexose with alcohols such as methanol containing hydrogen chloride instead of yielding a diacetal or diketal typical of an ordinary aldehyde or ketone results in reaction with only one molecule of the alcohol, the elimination of a molecule of water, and the formation of a crystalline hemiacetal or methyl glycoside (XXIX). These derivatives exist as cyclic compounds; the size of the ring will be discussed later (p. 21). In contrast to the free sugars the alkyl glycosides are non-reducing, and stable to alkali, but are readily hydrolysed by dilute



acids to the parent sugar and the alcohol. The carbohydrate moiety of the glycoside is known as the glycosyl residue and the non-carbohydrate portion is generally called the *aglycone*. In the above glycosides, methanol is the aglycone and the glycosyl link is through oxygen. A vast number of natural glycosides derived from phenols and alicyclic alcohols have been isolated from natural environments and studied (p. 28).

Sugars also react readily, under mild conditions, with compounds containing the primary amino group, such as amino acids¹¹ and with a wide variety of amines¹². With secondary amines the reaction is less facile, but similar products are derived. A typical reaction is between a hexose and



aniline to give *N*-phenylhexopyranosylamine (XXX). These derivatives are crystalline with characteristic melting points, but some may decompose on keeping at room temperature, the stability of *N*-aryl-D-glucosylamines increasing in the order *N*-phenyl, *N*-*p*-tolyl, *N*-*m*-tolyl, *N*-*o*-tolyl. In aqueous and alcoholic solution the majority mutarotate to an equilibrium involving