Polysaccharides of Micro-Organisms

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POLYSACCHARIDES OF MICRO-ORGANISMS

BY

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PREFACE

THE giant sugar macromolecules known as the polysaccharides are synthesized at some stage in the growth of every living cell. They may act as energy reserves, as structural units, as colloids for ion and food transfer, as defensive barriers for the cell, etc. During the life-cycle of micro-organisms the polysaccharides may be produced both by intracellular and extracellular enzymes and they are often made manifest by the striking appearance of large quantities of slime material. The carbohydrate nature of microbial slime has been recognized from the time of Pasteur.

Attention was sharply focused on bacterial polysaccharides by the discovery in 1923 by Avery and Heidelberger that the soluble specific substances of the *Pneumococcus* were polysaccharides. They were shown to be responsible for type specificity and this discovery stimulated researches into the relationship between chemical structure and serological specificity and focused attention on the role of polysaccharides in the whole field of immunochemistry. Modern chemical and physical techniques have now placed within our reach a real prospect of determining the fine structural chemistry of even the most complex polysaccharide macromolecules. Since we are at the beginning of a new era in microbial polysaccharide chemistry especially in relation to immunological specificity, virus studies, nitrogen-containing polysaccharides, and medical application of polysaccharides, etc., it seemed appropriate to review the very widely scattered chemical knowledge in the whole field of the polysaccharides of micro-organisms.

In order to appeal to the non-specialist the first five chapters deal with the modern techniques used for the isolation, purification, and complete structural determination of complex polysaccharides such as occur in micro-organisms. The remaining seven chapters are devoted to summarizing the present state of knowledge regarding the polysaccharides of Gram-positive and Gram-negative bacteria, viruses, rickettsia, moulds, yeasts

yeast-like fungi, and protozoa. The book lays the foundation for a correlation of the chemical structure of microbial polysaccharides and the bacteriological relationship between the microorganisms which elaborate them. We hope that it will be of interest not only to the carbohydrate chemist but to all those interested in the biology of macromolecules.

We are pleased to acknowledge the assistance of Mrs. M. Patrick in typing this manuscript, Mr. E. T. J. Chelton in proof reading, and Mr. Wheeler in the preparation of the photographs.

M. S.
S. A. B.

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I

CARBOHYDRATE NOMENCLATURE

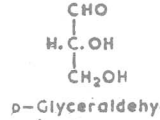
1. Introduction

IT is now widely recognized that polysaccharides, together with nucleic acids, proteins, and lipids, are the most vital macromolecules present in micro-organisms. Although in many cases polysaccharides occur as separate entities in bacteria and fungi they are also known to exist as complexes such as lipopolysaccharides, which are notable for their pyrogenic activity, or as the antigenic complexes, which are composed of firmly bound polysaccharide, protein, and lipid. It appears that the existence of the polysaccharide in the form of a complex is particularly important where biological activity is involved since in neither of the two cases cited is the polysaccharide itself either pyrogenic or a complete immunizing antigen. Our account, however, will deal only with the polysaccharide structures since so little is known about the complexes.

2. Monosaccharides

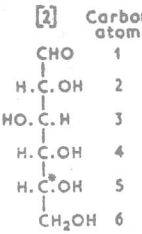
In order to explain some of the nomenclature involved in the study of polysaccharides we must first consider the 'building bricks' or monomeric repeating units of which they are composed, namely the monosaccharides. These are classified according to the number of carbon atoms they contain, thus: triose (3), tetrose (4), pentose (5), hexose (6), and heptose (7). In the case of the hexoses there are eight D-stereoisomers together with an equal number of L-stereoisomers. The configurational relationships denoted by the prefix D- or L- are used as follows: where the configuration of the highest numbered asymmetric carbon atom is the same as that of D (dextro)-glyceraldehyde (or D-glycerose [1]) the monosaccharide (e.g. [2]) will belong to the D-series, monosaccharides having the opposite configuration (e.g. [3]) will belong to the L-series.

[1]



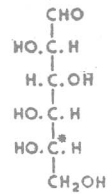
D-Glyceraldehyde
(D-Glycerose)

[2]



D-Glucose

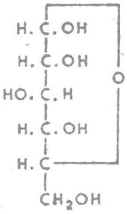
[3]



L-Glucose

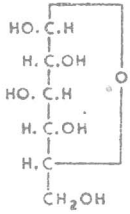
With the realization that monosaccharides existed predominantly in cyclic forms rather than in the open-chain forms depicted above came the necessity of describing the configuration at carbon atom no. 1. Irrespective of whether the ring was pyranose (6-membered—see [4] and [5]) or furanose (5-membered—see [6] and [7]) a new asymmetric carbon atom was created at C_1. These stereoisomers, termed 'anomers', were designated by the prefixes α- and β-

[4]



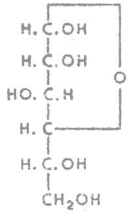
α-D-Gluco-pyranose

[5]



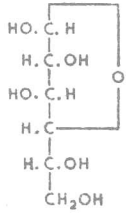
β-D-Gluco-pyranose

[6]



α-D-Gluco-furanose

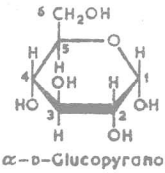
[7]



β-D-Gluco-furanose

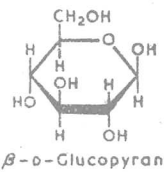
The formulae [4]–[7] are so-called Fischer projection formulae. The obvious disadvantages of depicting a ring structure in such a way prompted Haworth, after a series of classical researches, to propose the perspective representation illustrated in [8]–[11].

[8]



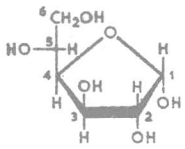
α-D-Glucopyranose

[9]



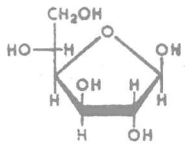
β-D-Glucopyranose

[10]



α-D-Glucofuranose

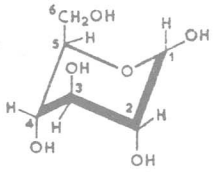
[11]



β-D-Glucofuranose

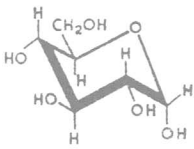
These Haworth type formulae, although the now generally adopted method of depicting sugars, are still only approximate representations of the true molecular structure. The most important discrepancy in them is that they represent the ring as coplanar. X-ray structures have proved that this is not so for in the case of each pyranose sugar there are at least eight types of possible stereoisomeric structures—two of them are the so-called 'chair' forms depicted in [12] and [13] and the remainder are various 'boat' forms. The 'chair' and 'boat' designations describe the shape of the ring.

[12]



1C-α-D-Glucopyranose

[13]



C1-α-D-Glucopyranose

3. Conformation of sugars

It is now recognized that the actual shape of the ring determines the reactivity of any particular hydroxyl group (Aspinall and Zweifel, 1957), the optical activity (Whiffen, 1956), and various other physical properties. The factors which govern the preferred form or conformation of a pyranose sugar are mainly as follows:

(1) The preferred conformation will be that where the non-bonded interactions between atoms are a minimum.

(2) In general the pyranose ring will assume a chair form in preference to a boat form.

(3) Any substituent (other than hydrogen) prefers to adopt a position lying in the plane of the ring (designated 'equatorial' or 'e' position) rather than one perpendicular to the plane of the ring (designated 'axial' or 'a' position). The preferred conformation will therefore be that which has the least number of bulky axial substituents.

(4) A particular element of instability is introduced where bulky axial substituents are on the same side of the ring and attached to carbon atoms β to each other.

(5) Intramolecular hydrogen bonding between hydroxyl groups and the ring oxygen may also be a factor in determining a preferred conformation.

The dominating feature with most hexoses or heptoses that exist in the pyranose form are the bulky $-CH_2OH$ or $-CH(OH)-CH_2OH$ substituents. In most cases the preferred conformation will be that chair form where this substituent is in the equatorial position. Table 1 shows those carbon atoms in the pyranose sugar ring which would carry bulky substituents in axial positions in each of the two chair forms.

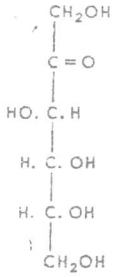
TABLE 1

Hexose	C1 *Chair form*	1C *Chair form*
β-D-Glucose	none	1, 2, 3, 4, 5
α-D-Glucose	1	2, 3, 4, 5
β-D-Galactose	4	1, 2, 3, 5
α-D-Galactose	1, 4	2, 3, 5
β-D-Mannose	2,	1, 3, 4, 5
α-D-Mannose	1, 2,	3, 4, 5
β-D-Allose	3,	1, 2, 4, 5
α-D-Allose	1, 3,	2, 4, 5
β-D-Altrose	2, 3,	1, 4, 5
α-D-Altrose	1, 2, 3,	4, 5
β-D-Gulose	3, 4	1, 2, 5
α-D-Gulose	1, 3, 4	2, 5
β-D-Talose	2, 4	1, 3, 5
α-D-Talose	1, 2, 4	3, 5
β-D-Idose	2, 3, 4	1, 5
α-D-Idose	1, 2, 3, 4	5

It is evident from Table 1 that glucose, galactose, and mannose (the most abundant hexoses in nature) have the least numbers

of bulky substituents in both their α- and β-forms. The next candidates would be allose and idose (rare or non-existent in nature) but here both sugars in one of their anomeric forms would possess the unstable arrangement of bulky axial substituents on carbon atoms β to each other.

All the monosaccharides discussed above are 'aldoses'—a term derived from the aldehydic character of their reducing groups on C_1. When the monosaccharide (e.g. D-fructose [14]) has a carbonyl group as its reducing or potentially reducing group, it is classified as a ketose. More detailed nomenclature of monosaccharide derivatives can be found in the 'Editorial Report on Nomenclature', 1952.



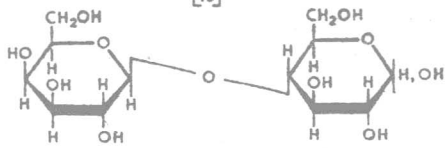
[14]

4. Oligosaccharides and polysaccharides

An oligosaccharide is a compound which, on complete acidic hydrolysis, gives monosaccharides only—generally from 2 to *c.* 20 per molecule. Where the numbers of monosaccharide units produced on hydrolysis are 2, 3, and 4, etc., per molecule they are termed di-, tri-, and tetra-saccharides, etc., respectively.

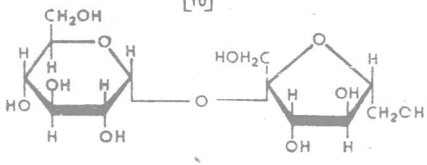
Disaccharides can be classified as reducing, e.g. lactose, maltose, cellobiose, etc., and non-reducing, e.g. sucrose and the trehaloses, etc. In the former the reducing group of only one of the monosaccharides is engaged in glycosidic linkage (α- or β-); in the latter the reducing group of both monosaccharide units are mutually engaged in glycosidic linkage ($\alpha\alpha$-, $\alpha\beta$-, or $\beta\beta$-). Besides the trivial names given to these disaccharides, usually before the structure was determined, they can be named from their component monosaccharides:

[15]



Lactose: 4-*O*-*β*-D-galactopyranosyl-D-glucopyranose

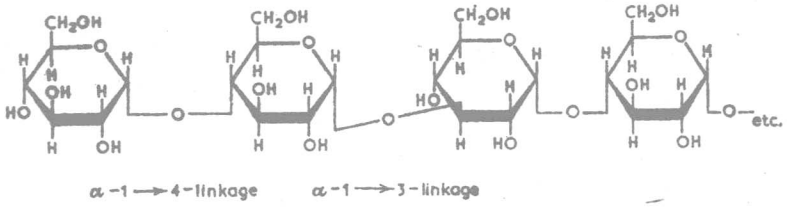
[16]



Sucrose: *β*-D-fructofuranosyl *α*-D-glucopyranoside or
α-D-glucopyranosyl *β*-D-fructofuranoside

Polysaccharides, of which the most common examples are starch and cellulose, and oligosaccharides are 'condensation' type polymers which can be regarded as being formed by elimination of water molecules between a series of monosaccharides; the 'glycosidic' —OH group on carbon atom no. 1 is invariably involved in this elimination when the monosaccharides are aldoses. The linkages between carbon atom 1 of one saccharide can engage any one of the carbon atoms 2, 3, 4, or 6 of the next hexopyranose unit. In this way many beautiful, complex patterns are built up with additional variation coming from the presence of *α*- or *β*-type linkages. Similar patterns are built up from ketoses, e.g. D-fructose where the attachment always involves carbon atom 2. From present knowledge of both the mechanism of enzymic synthesis of polysaccharides and of the structure of polysaccharides it appears that such patterns, although complex, are in a precise and ordered arrangement. A polysaccharide containing one of the simpler patterns is nigeran which is elaborated by certain species of *Aspergillus niger*. It has the structure given in [17]—having long chains of more than 300 D-glucose units alternately linked *α*-1:3 and *α*-1:4.

[17]

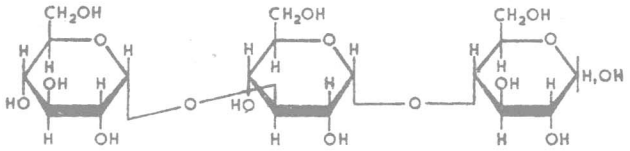


To illustrate the application of carbohydrate nomenclature to oligosaccharides, the polysaccharide depicted in [17] gives, on partial acid hydrolysis, the following oligosaccharides:

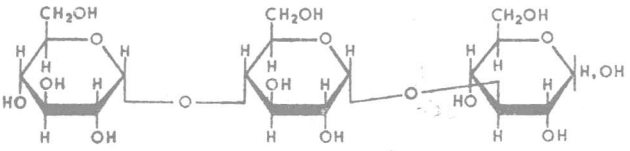
Disaccharides: maltose (4-*O*-α-D-glucopyranosyl-D-glucopyranose)
nigerose (3-*O*-α-D-glucopyranosyl-D-glucopyranose)

and the two trisaccharides shown in [18] and [19], T_1, *O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 4) D-glucose and T_2, *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose.

[18] T_1



[19] T_2



REFERENCES

ASPINALL and ZWEIFEL (1957) *J. Chem. Soc.*, p. 2271.
'Editorial Report on Nomenclature' (1952) *J. Chem. Soc.*, p. 5108.
WHIFFEN (1956) *Chem. and Ind.*, p. 964.

II

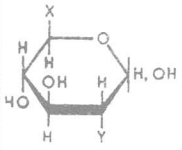
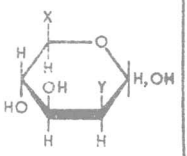
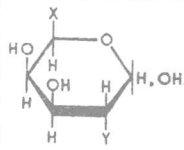
MONOSACCHARIDE COMPONENTS OF POLYSACCHARIDES

1. Occurrence

At least twenty monosaccharides have been recognized as constituents of bacterial polysaccharides. The structures of these monosaccharides (Table 2) reveal, however, that the majority of them can be grouped into three families of compounds having the glucose, mannose, and galactose configurations. It is quite remarkable that derivatives of only three of the eight possible types of hexoses should constitute, so far as is known, such a vast proportion of naturally occurring monosaccharide substances.

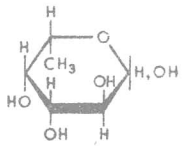
TABLE 2

Monosaccharide components of polysaccharides

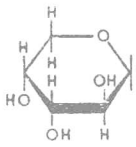
			
When			
X = —CH_2OH Y = —OH	D-glucose	D-mannose	D-galactose
X = —COOH Y = —OH	D-glucuronic acid	D-mannuronic acid	D-galacturonic acid
X = —H Y = —OH	D-xylose	D-lyxose (not found)	L-arabinose
X = —CH_2OH Y = —N(H)—C(=O)—CH_3	*N*-acetyl-D-glucosamine	*N*-acetyl-D-mannosamine (not found)	*N*-acetyl-D-galactosamine
X = —CH(OH)—CH_2OH Y = —OH	D-*glycero*- and L-*glycero*-D-*gluco*heptose (not yet found)	L-*glycero*-D-*manno*heptose, D-*glycero*-D-*manno*heptose	D-*glycero*-D-*galacto*heptose

In Table 2 the monosaccharides are written in the pyranose form and so far most aldoses appear to occur in polysaccharides in this way. Several, however, e.g. D-arabinose in *Mycobacterium tuberculosis* (Haworth, Kent, and Stacey, 1948), D-galactose in *Penicillium charlesii* G. Smith (Haworth, Raistrick, and Stacey, 1937), and L-fucose in *Aerobacter aerogenes* A3 (S1) (Aspinall, Jamieson, and Wilkinson, 1956), have also been found in the furanose form. D-Fructose has so far been found in the furanose form only in polysaccharides. D-Arabinose, L-fucose, and D-fructose written in the pyranose form (see below) bear a partial relationship to D-altrose (having the same configuration on three of the carbon atoms). Two main amino sugars (2-amino 2-deoxy sugars) occur in bacterial polysaccharides. The most common of these is D-glucosamine which is found particularly in the chitin of crustacea and the higher fungi.

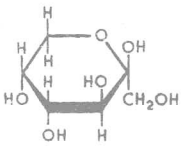
TABLE 2 (*cont.*)



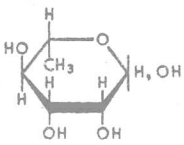
L-Fucose
(6-deoxy L-galactose)



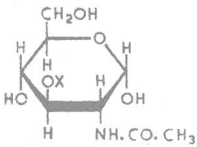
D-Arabinose



β-D-Fructose

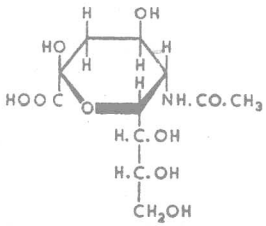


L-Rhamnose
(6-deoxy L-mannose)

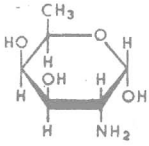


(X = CH(CH$_3$).COOH)

N-Acetyl muramic acid

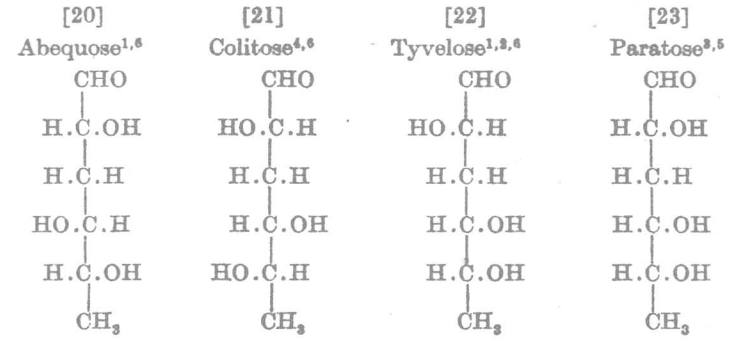


N-Acetyl neuraminic acid



D-Fucosamine
(6-deoxy-D-galactosamine)

The four 3:6-dideoxy hexoses illustrated below ([20]–[23]) have been found in specific polysaccharides extracted from various species of *Salmonella* (food poison bacteria).



[1] Westphal, Lüderitz, Fromme, and Joseph (1953). [2] Pon and Staub (1952). [3] Davies, Fromme, Lüderitz, Staub, and Westphal (1958). [4] Lüderitz, Staub, Stirm, and Westphal (1958). [5] Fouquey, Polonsky, Lederer, Westphal, and Lüderitz (1958*b*). [6] Fouquey, Lederer, Lüderitz, Polonsky, Staub, Stirm, Tinelli, and Westphal (1958*a*).

Although *N*-acetyl D-mannosamine has not been found as such in polysaccharides it occurs in the synthesis of *N*-acetyl neuraminic acid which is found in colominic acid, a polysaccharide produced by *Escherichia coli* K235 (Barry and Goebel, 1957). Comb and Roseman (1958*a*) have effected the synthesis of *N*-acetyl neuraminic acid by condensing together *N*-acetyl D-mannosamine and pyruvic acid in the presence of extracts of *Clostridium perfringens* (see [24]). (Plate 1.) The hexosamine moiety of *N*-acetyl neuraminic acid has now been firmly established as mannosamine rather than glucosamine (Kuhn and Brossmer, 1958).

2. Monosaccharides in antibiotics

Besides those listed above, the majority of which are elaborated by all living cells whether animal, plant, microbial, etc., a