

POLYSACCHARIDE CHEMISTRY

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Foreword

In these pages are compiled the major results outlining the present status of our knowledge regarding the structure of natural high polymers of the carbohydrate type. These substances are elaborated in bewildering variety by plants and animals to serve as energy sources and as structural units. Great industries founded upon their utilization constitute a major portion of the civilization of our times. The starches and celluloses represent plant crops of high value produced in abundance, while in lesser abundance are found the microbial polysaccharides high in significance for the study of the problems of immunity. The glycogens of muscle and liver, in dynamic equilibrium with the whole complicated system of carbohydrate utilization, provide a center of interest for the physiologist and pathologist. Too often, the technologists in these various fields have little or no knowledge of their interrelations. Thus it is good to have these divergent results collected within the compass of one book.

The carbohydrate high polymers are entities whose behavior has been well characterized by modern physical methods. Thus no other known substance has been so extensively investigated as cellulose. Physical instrumentation is essential here for present and future progress. Constructed as they are in much simpler detail than are their sister compounds, the proteins, our present state of knowledge of carbohydrate polymers is far in advance of that of the proteins. Yet it is a perplexing fact that the carbohydrases are proteins, and thus the very nature of the interaction of proteins and carbohydrates poses a major problem in the elucidation of enzymatic action, upon which rests the entire structure of biology. We know protein enzymes that build up polysaccharides, and we know proteins that tear them down; their crystallization and characterization is progressing steadily. It is to be hoped that our knowledge of what they are may result eventually in a better definition of how they act.

The structural organic chemist is concerned with the nature and mode of linkage of those monosaccharide units from which the carbohydrate high polymers are constructed. Much progress has been made recently in the development of new methods suitable for attacking such problems. It is to be realized that the methylation techniques, so useful in a linear polymer, fall far short of their objectives when applied to the non-linear or branched polymers. We can now look forward to the rapid attainment of many new structures in this area.

The present summarizing compendium is thus recommended to the attention of the many scientists of divergent training concerned with the variegated aspects of carbohydrate high polymer science. Future advances in this field will be rapid, and we may confidently predict a series of future editions affording an even more definitive delineation of these significant natural entities.

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Preface

In recent years, knowledge of that broad but distinctive group of substances classified as the carbohydrates has been vastly augmented. Within the carbohydrate group and comprising a major part there occurs an array of macromolecules known as polysaccharides. Some of these are of great biological importance, and some have become the basis of large industries. In the early stages of characterization and the early practical application of polysaccharides, it was to be expected that some investigators became so concerned with the particular polymer under investigation that they excluded from their interest developments pertaining to other polysaccharides. Fortunately this phase has passed, and among investigators there has grown an awareness that expedited advance in one polysaccharide line can persist only if workers are cognizant of the known behavior and characteristics of all other polysaccharides.

This monograph is written with the sincere hope that into one unified compilation can be brought the entire field of polysaccharide chemistry, thus presenting for the first time an easily accessible comparison of the available knowledge of all known polysaccharides. Consequently much hitherto unassembled material is brought together, and each polysaccharide is dealt with in a uniform and critical manner. Although the monograph is primarily designed for the general use of research workers and graduate students, an attempt has been made to present the material in such straightforward style that it can be used to advantage by readers who are not engaged in research and who do not have an extensive acquaintance with polysaccharide chemistry. Consequently the first two chapters deal in general with polysaccharides, their occurrence, classification, and methods of handling and characterization. Standard carbohydrate nomenclature has been followed in naming the simple sugars and their derivatives. Since classification and nomenclature committees have not dealt specifically with the polysaccharide field, these matters have been handled in an arbitrary but hoped-for logical fashion. The authors would like to see uniform nomenclature brought into the polysaccharide field and have asked numerous carbohydrate chemists for their opinions regarding the possibility of change in the termination of polysaccharide names ending in "-in" to a uniform "-an" ending. The opinion of both American and European chemists was divided on this question of change. Hence the nomenclature change was made only for certain polysaccharides as indicated in the first chapter.

A complete literature survey was made for all polysaccharides, and it is hoped that all important papers have been cited except for such voluminous fields as those of cellulose, starches, and pectic substances which are too vast to be covered completely in a book of this type. Even in the case of

the other polysaccharides, the enormous number of pertinent research articles creates an acute problem to reviewers attempting a complete but yet discriminating and critical examination of both old and new literature. Extensive bibliographical footnotes have been included not only to document the statements but to provide the reader with starting points for more detailed reading of the scientific literature. Proper citation of old work is often very difficult, particularly when reviews are not available or when the early significance has been lost. All references cited were read in the original except for the articles cited through abstracting journals. The literature covered comprises the period to early 1952, but some still later work is included.

Despite serious efforts to eliminate errors, some may be found. It will be appreciated if such instances are brought to the attention of the authors.

Professor M. L. Wolfrom read the entire typewritten copy and gave many valuable critical comments. His comments and those of other persons mentioned added much to the improvement of the manuscript, but the authors alone take full responsibility for the statements made herein and no blame should befall the cooperating individuals. Excellent advice and constructive comments were obtained from Professor W. W. Pigman who read Chapters I and II, Drs. W. D. Maclay and H. S. Owen who read the chapter on pectic substances, Dr. W. W. Moyer who read the cellulose chapter, and Dr. S. Roseman who read the group of chapters of mucopolysaccharides. To Dr. F. W. Quackenbush we give sincere thanks for helpful comments and encouragement.

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CHAPTER I

OCCURRENCE, NOMENCLATURE AND CLASSIFICATION OF THE POLYSACCHARIDES

Polysaccharides are components of almost all living organisms. They are most abundant in the higher orders of land plants and in seaweeds where they constitute approximately three-quarters of the dry weight. They are abundant in fungi and in the exoskeletons of insects and crustaceans. They are important constituents of the capsules of microorganisms and of the slimes which are produced by numerous organisms. They are significant components of cartilage, of animal joint fluids, of fluid cancers, of skin and of mucosa. They are found in multitudinous places, usually in large amounts but often in small quantities. Polysaccharides fill diverse roles in the physiology of plants, animals, and microorganisms. Foremost, they serve as structural material in plants and in most organisms as fuel reserves which maintain uniformity to the flames of metabolism. They serve structurally in the exoskeletons of insects and crustaceans and protectively in the armor or capsular material of certain bacteria. As surface material they partially protect tissues from desiccation and as gums they are exuded from plants to seal and protect wounds. As thickeners they serve in physical or in mechanical roles in animals and as specific substances they are of importance in blood group specificity and in other immunological reactions. Still unfathomed are the roles of many polysaccharides.

In abundance, cellulose far transcends all other polysaccharides; in weight it alone may equal the combined weights of all other carbohydrates. Perhaps next in abundance is xylan and one or more of the yet unnamed and uncharacterized polysaccharides which make up the enigmatic hemicellulose group of polymers. In somewhat lesser amount than xylan occurs the seaweed polysaccharide, agar. Chitin of insect and crustacean shells and the amylose and amylopectin of starches are in great abundance. Pectic substances are plentiful and widespread though they are often in dilute amounts in plant tissue.

Polysaccharides containing only one kind of polymerized sugar unit (homoglycans) are more abundant than polysaccharides which contain two or more kinds of sugar units (heteroglycans) although the latter are more numerous. D-Glucose polymers, or glucans, are the most abundant since cellulose, laminaran, amylose, and amylopectin are members of this class.

D-Xylose constitutes xylan, whereas D-galactose makes up the galactans of pectin and certain seaweed polymers. Chitin is composed of 2-amino-2-deoxy-D-glucose (D-glucosamine) units. In polysaccharides containing aminosugars the amino group is usually monoacetylated. Of the approximately 130 known polysaccharides, about one-third to one-half contain uronic acid sugar units. Some polysaccharides are composed only of uronic acid units, as for example, alginic acid of seaweeds and the galacturonan of the pectic group of substances. Only two polysaccharides are known to contain methyl ether groups. All four of the methylated but otherwise unmodified sugar units encountered so far in nature are of the 3-methyl type.¹ Only one of these, 3-methyl-D-galactose, is found as a component of a polysaccharide, slippery elm mucilage. In the case of uronic acids only one natural methylated derivative has been detected; that is the 4-methyl-D-glucuronic acid unit of mesquite gum.

Structural polysaccharides, of which cellulose is the prime example, are almost always linear molecules, while polysaccharides which serve primarily as reserve food are commonly branched or, in the case of starch, a mixture of linear and branched polysaccharides with the branched type predominating (except in very rare instances). In general, branched polysaccharides are easily soluble in water and have immense thickening powers. Linear molecules, on the other hand, are excellent structural material because they pack closely and form many intermolecular secondary valence attachments which make the structure strong, rigid, and insoluble or at least difficultly soluble.

Polysaccharides are usually not formed in isolated pure condition but are deposited with other materials and may be inlaid and overlaid with other polysaccharides, proteins, fats, and many other biological substances. While cotton fiber is almost wholly cellulose, there are present pectin, protein, fat and traces of other material. Some plant exudates, or gums, and slimes of bacteria are nearly pure carbohydrate as is starch when it occurs in certain seeds and tubers. Polysaccharides produced and left in non-depot tissues are, as a rule, in complex admixture with many other compounds. During deposition of cell walls, for example, many substances are laid down simultaneously. As the structure develops, small holes and cracks are formed which may be later filled with new deposits of biochemical substances. Thus, a plant tissue section may be viewed as a complex matrix of giant polysaccharide molecules sometimes paralleling each other, sometimes weaving and entangling with other polysaccharide molecules and the whole fabric cemented over and through with lipids, proteins, lignins, salts, coloring matter and a host of other substances. Some polysaccharide molecules are so closely aligned over all or part of their lengths that they form numerous secondary valence links with each other or with

¹ See E. L. Hirst, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 323 (1951).

neighboring foreign molecules. Because of these forces or because some of the molecules are overlaid with different molecules and are mechanically trapped, they cannot be easily separated or purified from molecules of different types. In these instances the research worker trying to effect the separation often erroneously comes to the conclusion that the polysaccharides are chemically combined with each other or with difficultly removable non-carbohydrate material. Thus at one time or another, postulations have been made for the combination of polysaccharides with fatty acids, lignins, proteins, tannins, and many other substances. This problem of whether compound formation occurs between polysaccharides and other substances is one of the great unanswered questions of biochemistry. In many instances it is likely that primary valence linkages do exist between concomitantly occurring polysaccharides or a polysaccharide and another associated substance. It would seem probable that at least a certain number of such linkages would develop either during deposition of the material or later during modifying processes. It would also seem probable that uronic acid units could in some instances esterify with alcohol groups on neighboring polysaccharide chains. The aldehyde group at one end of a polysaccharide could combine glycosidically or as a full acetal with alcohol groups of juxtaposed chains. In any case it is very difficult to establish these points of attachment if they exist. Data either for or against such combinations should be examined critically.

When plants die they are attacked by a multitude of microorganisms. The attack may be preferentially on certain components. Thus, wood is sometimes decayed by initial predominance of carbohydrate-destroying organisms (brown rots) that leave the lignin while at other times there may develop an initial predominance of lignin-destroying organisms (white rots) that leave the cellulose. In no case is a component left completely intact. When a plant falls to the ground, its destruction is usually general and rapid, particularly if the plant becomes mixed with the soil. Even under such conditions of generalized destruction, certain chemical linkages and groups appear to be more resistant than others and accumulate in the soil. Uronic acids may be somewhat resistant, perhaps being protected in part by the resistance to hydrolysis of the glycuronosidic linkage, as is observed particularly in aldobiouronic acids.² Yet it must be kept in mind that although uronic acids may constitute³ 10–23% of the total carbon present in soils, the greater portion of the uronic acid is of microbial

¹ A disaccharide consisting of a uronic acid residue in glycosidic union with an un-oxidized sugar unit.

² A. G. Norman and W. V. Bartholomew, *Soil Sci.*, **56**, 143 (1943); E. C. Shorey and J. B. Martin, *J. Am. Chem. Soc.*, **52**, 4907 (1930); S. A. Waksman and H. W. Reuszer, *Soil Sci.*, **33**, 135 (1932); W. H. Fuller, *Soil Sci.*, **64**, 183 (1947); W. G. C. Forsyth, *Chemistry & Industry*, 515 (1948); *Biochem. J.*, **46**, 141 (1950); For a discussion of soil polysaccharides see W. G. C. Forsyth and D. M. Webley, *J. Gen. Microbiol.*, **3**, 395 (1949).

origin. Organic matter of the soil is largely waxes, nitrogenous compounds, lignin, and polysaccharides. Top soil may contain 5–20% hemicelluloses, 0–5% cellulose, and 5–15% uronic acid material all in various stages of degradation. Uronic acid content of the soil increases with depth, quickly reaches a maximum and then decreases at lower levels as the total organic matter decreases.

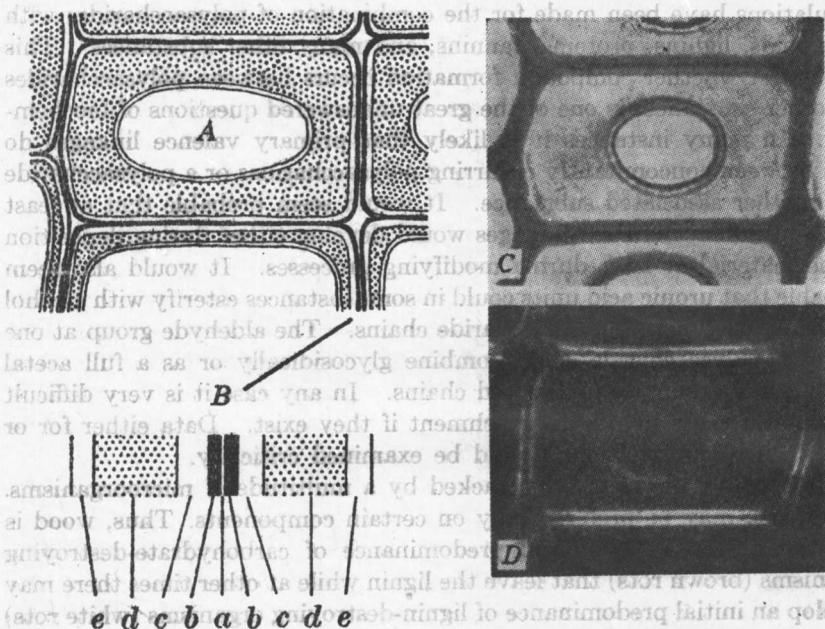


Fig 1. A. Transverse section through a plant fiber and parts of seven others. B. Section of adjacent walls more highly magnified; a. middle lamella; b. primary wall; c. outer layer of secondary wall; d. central layer of secondary wall; e. inner layer of secondary wall. C. Actual photomicrograph of transverse section of pine latewood ($\times 670$). D. Same as C but photographed with polarized light (after Bailey).⁴

1. Occurrence

A. Location of polysaccharides in a typical higher plant (*Spermatophyta*)

The majority of plant polysaccharides are components of the cell wall. It is the presence of a prominent wall which distinguishes the cellular structure from that in animals where the limits of the protoplast are less readily discernible. The cell wall is viewed as a secretion of the protoplast. The walls of the cells in a typical plant tissue consist of three morphologically distinct layers, namely the intercellular cement or middle lamella, the primary and the secondary walls as shown diagrammatically in Fig. 1.

⁴ I. W. Bailey, *Ind. Eng. Chem.*, **30**, 40 (1938).

In the dividing cell, polysaccharides are deposited in the initial membrane recognized as the cell plate. Possibly the first polysaccharide formed in predominance is pectin or calcium pectate though xylan and other hemicelluloses very likely are present with perhaps a submicroscopic frame of cellulose. During the early stages, the cell plate must also contain protoplasmic material and perhaps other non-carbohydrate substances. In any event, this primitive expanding plate reaching toward the walls of the mother cell is the middle lamella which, when cell division is complete, will serve, in part, as a binder or isotropic intercellular matrix which assists in holding together the two daughter cells. In the mature tissue this tenuous intercellular layer will contain a large amount of lignin, approximately 71% in the case of microdissected middle lamella of Douglas fir.⁵ There will also be present about 14% pentosan, probably xylan, uronic acid-containing material and a small amount of pectin.

The origin of the lignin is not known. The most plausible hypothesis is that in the aging tissue lignin is built up directly from simple sugars such as sucrose.⁶ Its postulated formation from pectin or other polysaccharides has never been experimentally verified. Lignin functions as an intercellular cement but its presence in high concentrations may cause the plant tissue to become unduly brittle. Thus corn stalks of high lignin content are found to break (lodge) more readily than varieties with lower lignin content. Since small amounts of lignin are found in quite young tissue, it is not solely a product of aged tissue.

The membrane formed on the surface of the cell plate is the initial stage of the primary wall. As cell enlargement continues this membrane thickens very slightly but remains plastic and adaptable to the changing volume of the cell. The primary wall is anisotropic to polarized light, a characteristic largely due to the presence of many fine cellulose threads in mesh-like but coherent arrangement and intricately woven and mixed with pectic substances and hemicelluloses as well as non-carbohydrate material. The primary wall can undergo changes in thickness and usually it is of uneven thickness. It contains many pits and holes and through it run protoplasmic threads (plasmodesmata). With attainment of final size and form in the cell, the primary wall may further thicken with the development of a laminated structure. In most seeds and many other organs the wall may attain considerable thickness and may contain a high ratio of hemicelluloses. Other polysaccharides such as mannans or galactans may be present in some plants.

Fibrous and other highly differentiated cells, after their mature size and shape are fully attained, undergo an irreversible loss of their poten-

⁵ A. J. Bailey, *Ind. Eng. Chem., Anal. Ed.*, **8**, 52 (1936); **8**, 389 (1936).

⁶ M. Phillips and M. J. Goss, *J. Agr. Research*, **61**, 301 (1935); M. Phillips, M. J. Goss, B. L. Davis, and H. Stevens, *J. Agr. Research*, **59**, 319 (1939).