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RECENT ADVANCES IN THE STRUCTURAL ANALYSIS OF POLYSACCHARIDES

By D. J. MANNERS, M.A., PH.D., F.R.I.C.

The past few years have seen a remarkable increase in our knowledge of the molecular structures of naturally occurring high polymers. This is especially true of the polysaccharides, and the purpose of this review is first, to describe some of the techniques and methods which have made this progress possible, and second, to illustrate (on pp. 15–35) their use, with an account of studies on certain glucose-containing polysaccharides.

The polysaccharides are high-molecular-weight polymers of anhydromonosaccharide units which are glycosidically linked to form non-cyclic polymeric chains. The degree of polymerization ($\overline{DP}$) may vary from *ca* 30 to $\sim 10^5$, and on complete hydrolysis one or more monosaccharides may be liberated. Homopolysaccharides may be defined as being composed of essentially one monosaccharide, whereas the more complex heteropolysaccharides may contain 2–6 different component sugars. In addition, small amounts of non-carbohydrate constituents may be present, *e.g.* ester groups derived from phosphate, sulphate, malonate or pyruvate. The constituent sugars may be arranged to give linear or branched structures (Fig. 1).

Progress in this field may be divided into a number of phases. The first, covering the nineteenth century, was concerned with the isolation, purification and composition of polysaccharides. Detailed chemical studies were limited, since the ring structure of the various monosaccharides had not been adequately characterized. The second phase (1900–1940) saw the establishment of the *basic* structures of the commonly occurring plant and animal polysaccharides by the classical method of methylation (p. 4) and by a limited number of physico-chemical methods (*e.g.* measurement of the viscosity and osmotic pressure of certain polysaccharide derivatives). The work of Sir Norman Haworth and his many collaborators, at Birmingham, was predominant during the latter part of this period. The third and fourth phases, which are developing concurrently, are concerned with the determination of the *fine* structure of polysaccharides by specialized techniques, and with the biological synthesis and degradation of these polymers.[1] The major part of this review will be devoted to certain aspects of these last two phases.

The first and often the most difficult stage in the structural examination of a polysaccharide is its isolation in an unmodified and undegraded state. The conventional methods of selective extraction with water, dilute salt, acid or alkali, followed by fractional precipitation with organic solvents, are adequate in only a few instances. Other methods involve the selective precipitation of certain types of polysaccharide as copper or

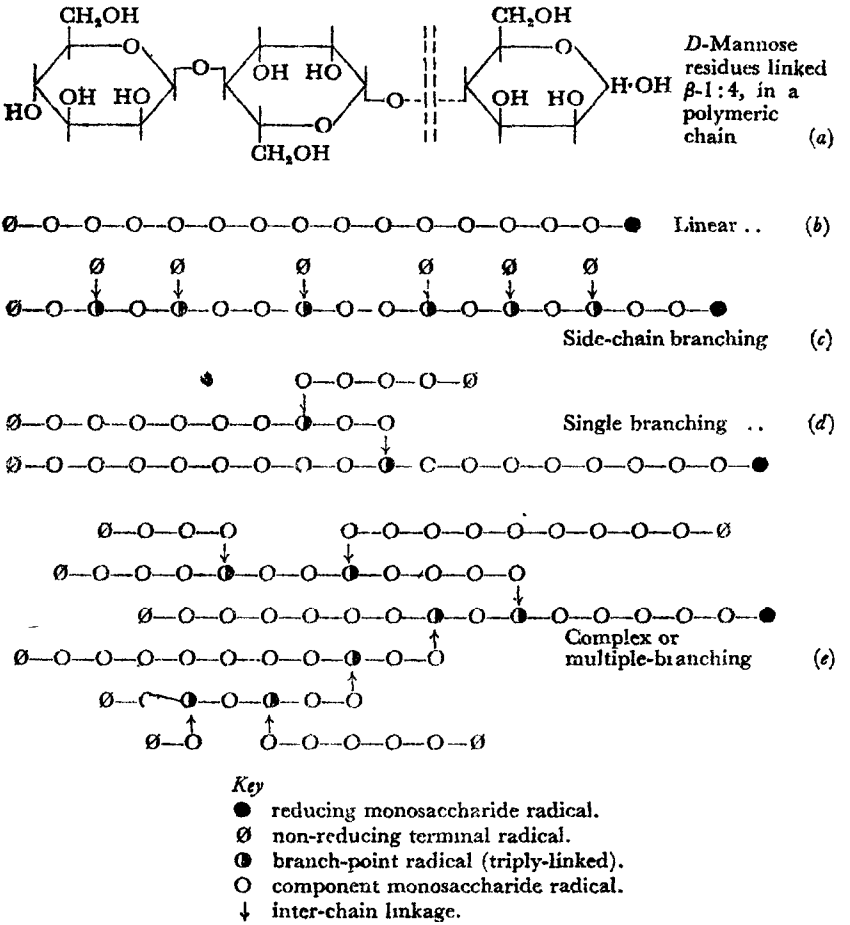


FIG 1. Types of polysaccharide structure

iodine complexes or by the use of Cetavlon or barium hydroxide. Ionophoretic methods are becoming increasingly important, and a small-scale preparative column has now been developed[2] from an earlier analytical method. However, most carbohydrate chemists would agree that the development of new methods for the isolation of polysaccharide material free from lignin and protein, and the separation of individual polysaccharides, merits a high priority, particularly in view of recent findings on the degradative action of dilute alkali and oxygen on many polysaccharides.

For the structural analysis of a purified polysaccharide, a number of different techniques are required. Firstly, the component sugars are identified by suitable examination of an acid hydrolysate. Secondly, application of the methylation procedure may provide, in many instances, information on the ring structure of the constituent sugars, the C atoms

involved in the repeating glycosidic linkage(s), and the proportion of non-reducing terminal groups. By analysis of a partial acid hydrolysate, the anomeric configuration of the repeating linkage(s), the nature of inter-chain linkages and, in some cases, the order of monosaccharide residues in the polymeric chains can be determined. Finally, estimates of the molecular size and shape may be obtained by certain physico-chemical methods.

Polysaccharides containing uronic acid residues or sulphate ester groups require special methods, since they tend to resist hydrolytic attack and methylation. These difficulties may be partly overcome by reduction of carboxylic acid groups to primary alcohol groups with lithium aluminium hydride and by desulphation (using methanolic hydrogen chloride) to yield modified polysaccharides composed of unsubstituted monosaccharide residues.

The outstanding advance in the field of structural polysaccharide chemistry has been the application of chromatographic methods. It is now possible to separate by column chromatography a mixture of five or six different monosaccharides or their methyl ethers in an acid hydrolysate of a polysaccharide or the corresponding methylated derivative. Further, the quantitative separation of milligram quantities of sugars can be effected by paper chromatography. In contrast, the earlier separations of monosaccharides, by selective precipitation of derivatives or of methylated sugars by fractional distillation, required gram quantities of material and were seldom quantitative. The application of chromatographic methods to carbohydrate chemistry has been described in detail by Hirst, Hough and Jones.[3]

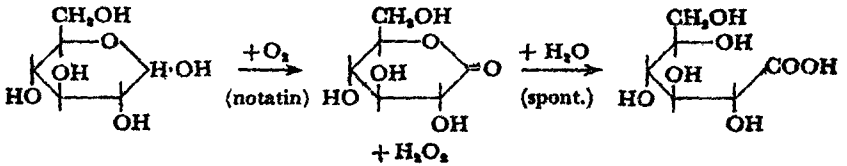
A second technique of increasing importance is zone electrophoresis (ionophoresis).[4] In many instances it provides a method for separating mono- and oligo-saccharides and their derivatives with similar chromatographic mobilities. By a combination of chromatographic and zone-electrophoretic methods, many of the more complex mixtures of sugars have been separated.

THE COMPOSITION OF POLYSACCHARIDES

In this analysis, the polysaccharide is hydrolysed with hot mineral acid under conditions whereby loss of monosaccharides by decomposition or by 'acid-reversion' is minimal. The conditions for hydrolysis depend upon the nature of the polysaccharide; for example, fructosans and glucosans require heating in 0·05N- and 1·5N-sulphuric acid for 10 and 90 min. respectively. The neutralized hydrolysate is examined qualitatively by paper chromatography, and the component sugars then separated quantitatively by chromatography on a column of cellulose, starch, charcoal–Celite or ion-exchange resin, and characterized by the measurement of physical constants and the preparation of crystalline derivatives.

Hydrolysates containing D-glucose and certain other monosaccharides may be analysed by enzymic methods. The oxidation of D-glucose to

D-gluconic acid is catalysed by notatin (glucose oxidase)[5] and the reaction may be followed by (*a*) manometric determination of the oxygen



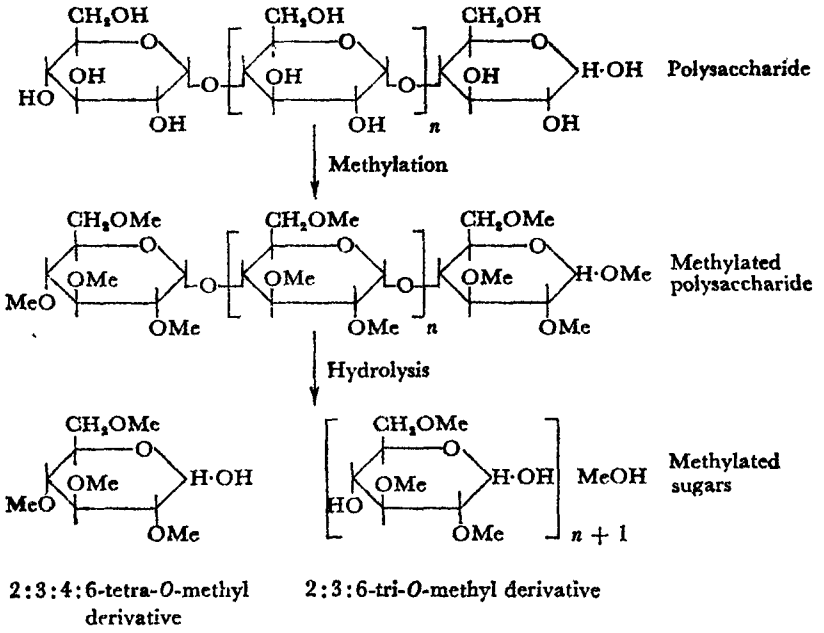
uptake,[5] (*b*) titration of the D-gluconic acid with alkali,[6] and (*c*) measurement of the change in reducing power.[7] Using method (*a*), the glucose content of hydrolysates of various fructosans has been determined,[8] and method (*c*) has been applied to *Brachychiton diversifolium* gum[9] and to protozoal polysaccharides.[7]

ANALYSIS BY METHYLATION

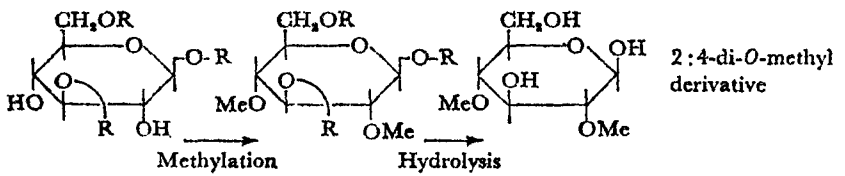
The methylation procedure, which was devised by Purdie and Irvine, and developed by Haworth and Hirst, is the most important analytical method in polysaccharide chemistry. Experimentally,[10] the method (Fig. 2) involves complete methylation of the polysaccharide (dimethyl sulphate and sodium hydroxide are normally used), hydrolysis with acid and quantitative separation and estimation of the resulting mixed methylated sugars. As mentioned previously, the application of chromatographic methods has greatly improved and facilitated the separation and has also markedly reduced the amount of methylated polysaccharide required for investigation.

As illustrated in Fig. 2 (*a*), the repeating linkage of the component sugars can be determined, the nature and proportion of non-reducing terminal groups examined (*i.e.* end-group assay), and, since each polysaccharide chain contains only one end-group, the average chain length ($\overline{CL}$) can then be measured. A comparison of this with the $\overline{DP}$ reveals the linear or branched nature of the polysaccharide. If the polysaccharide is branched, then the nature of the inter-chain linkages may be determined, as indicated in Fig. 2 (*b*).

The method has some limitations. The configuration of the glycosidic linkages, the order of monosaccharides in the polymeric chains of a heteropolysaccharide or the types of branching (side-chain, single or multiple) are not revealed. In practice, complete methylation of highly branched polysaccharides (*e.g.* glycogen, plant gums and mucilages) is difficult, and the hydrolysate may contain mono- or di-methyl sugars arising from undermethylation of non-terminal residues; a small amount (*ca* 2–3 per cent) of demethylation may also occur during the hydrolysis of the methylated polysaccharide. Both processes may therefore prevent the unambiguous identification of inter-chain linkages. Despite these limitations, methylation remains the fundamental method of structural analysis, and many examples of its application are given by E. L. Hirst in his recent reviews on the chemistry of fructosans,[11a] hemicelluloses,[11b] seaweed polysaccharides[11c] and plant gums.[11d]



(*a*) Methylation of a linear polysaccharide (repeating linkage 1:4-glucosidic)



(*b*) Methylation of a branched polysaccharide (R = chain of monosaccharide residues)

FIG. 2. Methylation Analysis

ANALYSIS BY PARTIAL ACID HYDROLYSIS

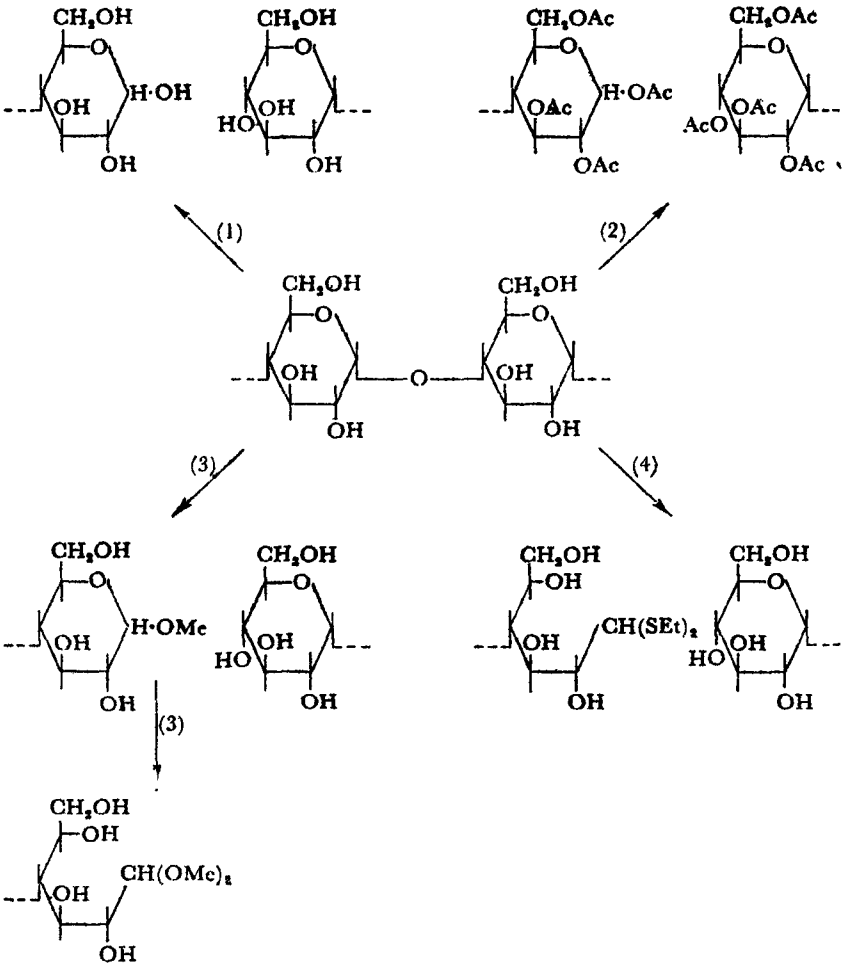
A methylation study of a polysaccharide may be supplemented by an examination of the products of partial acid hydrolysis, involving chromatographic separation and chemical identification of the mixed oligosaccharides. The success of this method, termed 'linkage analysis' by Peat and his co-workers[12] is largely dependent on the development of charcoal–Celite column chromatography[13,14,15] whereby most mixtures of mono- and oligo-saccharides can be readily separated. The resolving power of such columns may be improved by the presence of borate[16] or molybdate.[17]

An essentially linear homopolysaccharide yields, on linkage analysis, the constituent monosaccharide and a homologous series of oligosaccharides of increasing $\overline{DP}$, each containing the repeating linkage. Thus, amylose[14] gives glucose and maltosaccharides (α-1:4-linkage) and pustulan[18] (from the lichen *Umbilicaria pustulata*) yielded glucose and

gentiosaccharides (β-1 : 6-linkage). In the case of branched homopolysaccharides, the nature of the repeating and inter-chain linkages is revealed. In yeast glycogen these linkages are of the α-1 : 4- and α-1 : 6-glucosidic type, respectively, since glucose, maltose, isomaltose and panose were the products of partial hydrolysis.[18] However, the most useful application of the method is to heteropolysaccharides, since it provides unambiguous proof of the presence of two or more component sugars in the *same* polysaccharide. For example, gum ghatti[19] yields two aldobiouronic acids—*O*-β-D-glucuronosyl-(1 → 6)-D-galactose and *O*-β-D-glucuronosyl-(1 → 2)-D-mannose—whereas from the S. VIII pneumococcal polysaccharide, the aldotetraouronic acid *O*-β-D-glucuronosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 4)-D-galactopyranose has been isolated.[20]

Linkage analysis has several advantages. The configuration of the glycosidic linkages can be determined, and the presence of 'anomalous' linkages or minor components detected. The presence of both α- and β-glycosidic linkages in the S. VIII polysaccharide is of interest, whilst examination of partial hydrolysates of laminarin[21] provided the first evidence of the occurrence of mannitol and β-1 : 6-glucosidic linkages, in addition to β-1 : 3-linkages (see p. 17). The method may permit the order of glycosidic linkages to be determined. For example, methylation studies have shown that nigeran (an intracellular polysaccharide synthesized by *Aspergillus niger*) is linear and contains equal numbers of α-1 : 3- and α-1 : 4-glucosidic linkages. A partial hydrolysate contained maltose, nigerose (*O*-α-D-glucopyranosyl-(1 → 3)-D-glucopyranose) and the isomeric trisaccharides *O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucose and *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose. Maltotriose and 'nigerotriose' were absent. This indicates that the α-1 : 3- and α-1 : 4-linkages are arranged alternatively in the polysaccharide chain.[22]

Some modifications of the method of partial acid hydrolysis have been introduced (Fig. 3). Certain insoluble polysaccharides may be more conveniently degraded by means of a mixture of acetic anhydride, acetic acid and sulphuric acid (partial acetolysis). The free sugars are then obtained by deacetylation of the corresponding acetates. This procedure has provided evidence for the presence of a small number of α-1 : 4-linked D-mannose residues in ivory-nut mannan, in addition to β-1 : 4-linked sugars.[23] Mercaptolysis and methanolysis are alternative methods in which the polysaccharides are depolymerized by mixtures of hydrochloric acid and ethyl mercaptan or methyl alcohol. The resulting diethyl mercaptals or methyl glycosides (or dimethyl acetals) can be readily isolated and crystallized. These last two techniques are particularly useful in studies on the complex seaweed polysaccharides that contain labile anhydro-sugars. For example, mercaptolysis of a polysaccharide from *Chondrus crispus* yielded[24] the diethyl mercaptal of the rare sugar 3 : 6-anhydro-D-galactose (Fig. 4). Araki and his co-workers have examined the products of the mercaptolysis of agar in some detail[25]; these included the diethyl mercaptals of D-galactose, DL-galactose, 3 : 6-anhydro-L-galactose and a disaccharide—agarobiose. The constitution

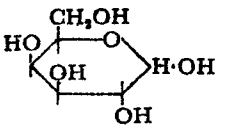


(1) Acidic or enzymic hydrolysis
(2) Acetolysis
(3) Methanolysis
(4) Mercaptolysis

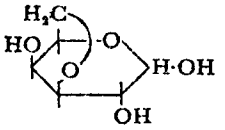
For convenience, the degradation of *glucosidic* linkages by methanolysis and mercaptolysis is shown; in practice, these procedures are normally applied to polysaccharides containing anhydro-sugars.

FIG. 3. Methods of degradation of glycosidic linkages

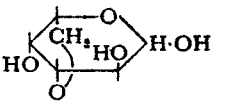
of the latter (*O*-β-D-galactosyl-(1 → 4)-3 : 6-anhydro-L-galactose) characterizes one of the repeating linkages in agar. Methanolysis studies have been equally important in this field. An interesting example is the isolation from agar of the dimethyl acetal of a disaccharide linked in ketal form to pyruvic acid.[26] This has been characterized as 4,6-*O*-1′-carboxyethylidene - β - D - galactopyranosyl - (1 → 4) - 3 : 6 - anhydro - L - galactose (Fig. 4).



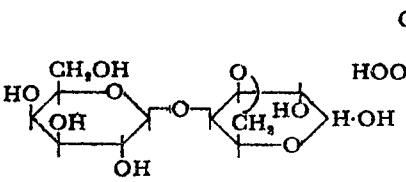
D-galactose



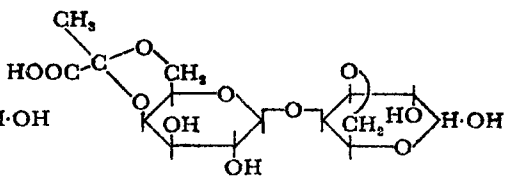
3:6-anhydro-D-galactose



3:6-anhydro-L-galactose



Agarobiose



Pyruvic acid derivative of Agarobiose

FIG. 4. Some components of seaweed polysaccharides. (In carbohydrate formulae >H·OH represents the equilibrium mixture of the α- and β-anomers.)

The method of linkage analysis does not provide quantitative information on the number of branch points (or end-groups), or on the proportion of various linkages. Further, the structural significance of the presence, in minute quantities, of certain oligosaccharides in partial hydrolysates (*e.g.* 0·001 per cent nigerose from glycogen[27]) is uncertain, owing to the possible 'acid-reversion' of monosaccharides[28] or acid-catalysed transglucosylation of oligosaccharides (see p. 34). Nevertheless, the combined use of methylation and partial hydrolysis enables the main structural features of a polysaccharide to be determined, provided that *gram quantities* of material are available.

ANALYSIS BY PERIODATE OXIDATION

The field of polysaccharide chemistry now includes materials from bacteria, protozoa, algae, lichens and fungi. Many of these are available only in very small quantities, and there has been a natural trend towards the development of semi-micro analytical methods. One such method, namely, periodate oxidation, has been widely used, and its application to the analysis of α- and β-glucosans by the writer and his collaborators forms part of this review.

A study of the periodate oxidation of a polysaccharide may, in many instances, provide quantitative information on the nature of the repeating and inter-chain linkages and enable the $\overline{CL}$ and, or, the $\overline{DP}$ to be determined. The reagent was introduced by Malaprade during studies on polyhydric alcohols and was first applied to carbohydrates in general by C. S. Hudson. During the past 13 years, the method[29] has been successfully applied to structural investigations of polysaccharides[30] by Hirst, Jones, Bell, Smith and many other workers.

In aqueous solution, periodate will oxidize compounds containing α-β diol- or α-β-γ triol-groups with the formation of aldehydes, iodate and, in certain cases, formic acid, formaldehyde and carbon dioxide (Fig. 5).

The arrangement of diol or triol (α-glycol) groups may be deduced from measurements of the reduction of periodate and the nature of the oxidation products. The required analyses are semi-micro in scale, and usually simple in operation, so that small quantities (10-1,000 mg) of polysaccharide can be readily examined. The experimental procedures are (*a*) reduction of periodate, by titration with thiosulphate or arsenite[29] or spectrophotometrically[31]; (*b*) production of formic acid, by titration with dilute alkali; (*c*) production of formaldehyde, gravimetrically as the dimedone derivative[29] or colorimetrically with chromotropic acid[32] or phenyl hydrazine-ferricyanide reagents (Schryver's method)[33]; (*d*) production of carbon dioxide, by use of a Warburg-type manometer.[34]

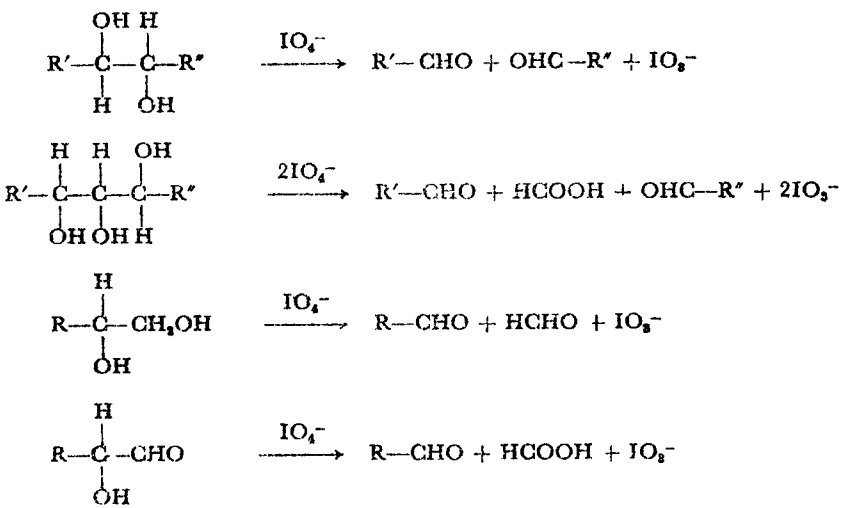


FIG. 5. Oxidation of Polyhydroxy Compounds by Metaperiodate

The rate and extent of periodate oxidation are controlled by the reaction conditions, and the equations in Fig. 5 represent ideal Malapradian-type oxidations. If the oxidation is carried out in presence of a large excess of oxidant, at an elevated temperature (>20°), in alkaline solution or in sunlight,[35] then 'over-oxidation' will occur, *i.e.* the oxidation proceeds beyond the simple Malapradian reaction with the reduction of further quantities of periodate, and the normal products of oxidation (formic acid, formaldehyde) will themselves be oxidized. A careful control of oxidation conditions is therefore essential.

The periodate oxidation of reducing groups in oligo- and polysaccharides may involve (*a*) normal Malapradian oxidation of diol or triol groups, with the production of a formyl ester, (*b*) slow hydrolysis of this ester followed by further oxidation, or (*c*) oxidation of activated H-atoms in malondialdehyde-type structures (Fig. 6). (The mechanism of the latter oxidation is uncertain: free iodine may appear, and iodate rather than periodate may be reduced.) Hough and Perry[36] have shown that all three reactions occur readily at pH 8·0, and that 1:3- or

1 : 4-linked aldohexose polymers are completely oxidized, by a step-wise process, with the production of 1 mol. formaldehyde per hexose residue. Oxidation under these conditions thus makes it possible to detect the presence of 1 : 6-linkages in a 1 : 3- or 1 : 4-linked polymer.

Recent studies on starches,[37] glycogens,[38] dextrans,[39] lichenin (p. 15) and laminarin (p. 17) provide examples of the above type of periodate oxidation analysis.

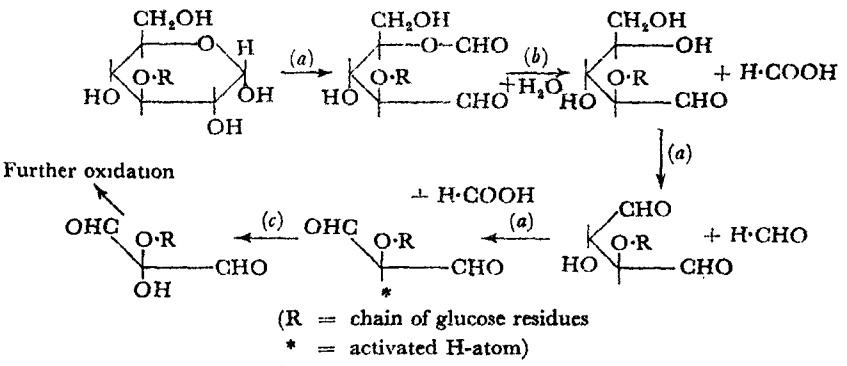


FIG. 6. The Periodate Oxidation of a Reducing Glucose Residue

Alternative periodate oxidation methods involve examination of the residual oxidized polysaccharide. Barry and his co-workers[40] have studied the interaction with carbonyl reagents. On warming in phenylhydrazine acetate, condensation takes place at residues containing dialdehyde groupings, the adjacent glycosidic linkage is ruptured and glyoxal bisphenylhydrazone is produced. With 1 : 3-linked glucosans (*e.g.* laminarin, yeast glucan), the reaction yields a polysaccharide from which the non-reducing terminal group has been removed. Repetition of the oxidation and phenylhydrazine acetate treatment results in the step-wise degradation of the polymeric chain. By contrast, a polysaccharide containing adjacent 1 : 3- and 1 : 4-linkages is fragmented, the former linked residue appearing as the monosaccharide osazone whilst the latter gives glyoxal bisphenylhydrazone. This Barry degradation has given new structural information on several polysaccharides, *e.g.* snail galactogen[41] and arabic acid.[42]

The Barry degradation method has been extended recently to polysaccharides containing either 1 : 5-linked pentofuranose or 1 : 6-linked hexopyranose residues.[43] In these, the glycosidic linkage from a non-oxidized residue to an adjacent oxidized pentose or hexose residue was found to be resistant to cleavage by phenylhydrazine. The products therefore included the osazones of di- or tri-saccharides rather than glyoxal bisphenylhydrazone and residual polysaccharide. The sequence of glycosidic linkages and the arrangement of inter-chain linkages in beet araban and yeast mannan have been examined by this method.

Alternatively,[44] isonicotinhydrazide or thiosemicarbazide can be condensed with the periodate-oxidized polysaccharide, and analysis (for S

and/or N) of the resulting polymer yields data on the proportion of α-glycol groups (*see* p. 15).

Acid hydrolysis of a periodate-oxidized polysaccharide is a useful procedure for the identification of monosaccharide residues devoid of α-glycol groups.[45] These are liberated as the free monosaccharide. However, the oxidized polysaccharides are readily *decomposed* by acid, and Smith and his co-workers[30] recommend reduction to the corresponding polyalcohol prior to acid hydrolysis, which is then quantitative. This method has been used to detect 1 : 3-linkages in starches and dextrans, and in plant gums and mucilages.

ANALYSIS BY ENZYMIC METHODS

The application of enzymic methods to the structural analysis of polysaccharides is a recent development in polysaccharide chemistry. Progress has been greatest in studies on starches and glycogens.[46] The hydrolysis of a polysaccharide by a purified and tested enzyme preparation has the advantage of specificity, and the degradative action may follow paths which cannot be traced out by present chemical methods. For example, α-glucosidases have no action on β-glucosans, whilst hydrolysis by β-amylase (β-amylolysis) is confined to the α-1 : 4-glucosidic linkages in the *exterior* chains of glycogen and amylopectin. In contrast, hydrolysis of glucosans by acid is an essentially random process.

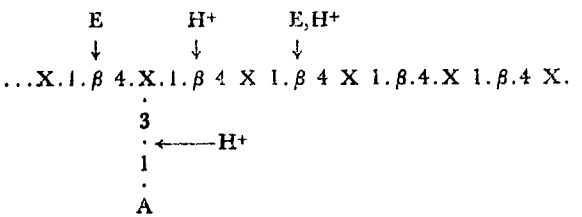
Enzymic analysis may provide information on (*a*) the anomeric configuration of the repeating glycosidic linkages, (*b*) the nature of interchain linkages and sequence of monosaccharide residues, (*c*) the location of branch points in a branched polysaccharide, and (*d*) qualitative and quantitative evidence of multiple branching. The latter two types of analysis are mainly confined to branched α-1 : 4-glucosans, and are considered in detail on pp. 26–34.

Examples of (*a*) and (*b*) may be cited from recent studies on hemicelluloses. Whistler and Smith[47] examined the degradation of guaran by an enzyme preparation from guar seeds. The products included the trisaccharide mannotriose (7·5 per cent) and the disaccharide *O*-α-D-galactopyranosyl-(1 → 6)-D-mannopyranose. These findings confirmed previous conclusions based on chemical methods that guaran is a linear β-1 : 4-mannan, to alternate residues of which are attached, at C_6, an α-galactopyranose residue. This polysaccharide structure is therefore of the type shown in Fig. 1 (*c*).

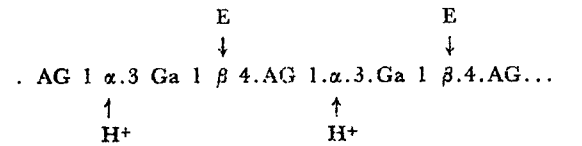
Proof that L-arabofuranose residues are true components of wheat-straw xylan was provided by Bishop and Whitaker[48] who isolated a trisaccharide containing two D-xylopyranose and one L-arabofuranose residues from the products of enzymic hydrolysis. Partial hydrolysis with acid was quite unsuitable in this case, since arabofuranosidic linkages are extremely acid-labile, especially when compared with xylopyranosidic linkages.

A further example of the specificity of enzymic hydrolysis was reported recently by Araki and Arai.[49] The main polysaccharide fraction of agar, named agarose, was treated with an agar-digesting bacterium. The

products included a disaccharide (neoagarobiose) and a tetrasaccharide (neoagarotetraose) which contain 3.6-anhydro-L-galactose residues α-linked to C_3 of D-galactose. This result supports the linear structure of agarose as an alternating polymer of 1·3-linked β-D-galactopyranose and 1:4-linked 3:6-anhydro-α-L-galactopyranose residues. In contrast to enzymic hydrolysis, acids selectively hydrolyse the α-glycosidic linkages, giving agarobiose as the main product (Fig. 7).



(*a*) Wheat straw xylan



(*b*) Agarose

Key

X = D-Xylopyranose residue
A = L-Arabofuranose residue
AG = 3:6-Anhydro-L-galactopyranose residue
Ga = D-galactopyranose residue
E = linkage hydrolysed by enzyme
H+ = linkage cleaved by acid

FIG. 7. The Partial Hydrolysis of Polysaccharides

In the field of fructosan chemistry, enzymic methods[50] have enabled the fructosan synthesized by *Acetobacter aceligenum* to be characterized as a levan, *i.e.* β-2:6-fructosan; a related polysaccharide from the fungus *Aspergillus sydowi* was shown to be of the inulin type, *i.e.* β-2:1-fructosan. The specific fructosanase preparations were culture filtrates from various micro-organisms grown on either inulin or the Acetobacter fructosan. The former had no action on *Bacillus subtilis* levan and the bacterial fructosan, whereas the latter did not hydrolyse inulin or the *Aspergillus* fructosan.

Other examples of partial enzymic depolymerization are the isolation of isomaltose and isomaltotriose from dextran–dextranase systems,[51] of di- and tri-galacturonic acid from the degradation of pectic acid,[52] and of xylose, xylobiose (*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose), rhodymenabiose (*O*-β-D-xylopyranosyl-(1 → 3)-D-xylopyranose) and higher xylosaccharides from the action of rumen bacteria on *Rhodymenia palmata* xylan.[53]

It is clear from this short survey, and the examples reported elsewhere,[1] that enzymic methods provide a valuable means for the controlled depolymerization of polysaccharides, with the proviso that experimental conditions do not allow the synthesis of oligosaccharides by transglycosylation reactions to occur. An illustration of this possible artefact is provided[54] by the synthesis of laminaribiose, gentiobiose and higher β-glucosaccharides during the action of a barley β-glucosidase preparation on cellobiose.

ANALYSIS BY PHYSICO-CHEMICAL METHODS

Marked improvements have been made recently in the experimental techniques for the measurement of the size and shape of high polymers. In the earlier studies, viscometry and osmometry were among the principal methods for the investigation of polysaccharides (usually as their acetylated or methylated derivatives).[55] These methods have been improved; new types of viscometer and osmometer have been designed,[56] and membranes with known permeability characteristics prepared,[57] thereby extending the range of molecular-weight determinations.

New techniques have been introduced into this field, a number of which can be applied to unsubstituted polysaccharides. This is an important advantage, since the possibility of inadvertent degradation of a polysaccharide during the preparation of the corresponding acetate or methylated derivative is considerable. The development of the ultracentrifuge by Svedberg and his collaborators[58] has permitted the sedimentation properties of polysaccharides to be determined. From these, estimates of the polydispersity can be obtained and, with a knowledge of the diffusion constant, the weight-average molecular weight and molecular-weight distribution calculated. The application of light-scattering[59] is particularly useful for the higher-molecular-weight polymers, in the range 10^5–10^7. The availability of commercial instruments, *e.g.* the electrically-driven ultracentrifuge, and several designs of light-scattering photometer, has greatly facilitated the application of these procedures, both of which are now in routine use for the determination of the size and shape of polysaccharides and of the effect on these properties of certain chemical reagents and enzymes.

The use of the ultracentrifuge and light-scattering photometer may be illustrated by recent studies on glycogen.[60] The sedimentation constants of 23 different samples were measured; the majority of these were polydisperse, and the main components had molecular weights in the range $3–9 \times 10^6$. Glycogen isolated by hot water extraction or by the classical Pflüger method (using 30 per cent potassium hydroxide solution) had similar molecular weights; however, hot dilute alkali rapidly degraded the polysaccharide. The polydisperse nature of many of the glycogens was confirmed by light-scattering which gave much higher molecular-weight values. In contrast, the results with samples showing no polydispersity were in good agreement with the sedimentation data (Table I). It was therefore suggested that unambiguous proof of polydispersity

could be obtained by a comparison of sedimentation and light-scattering measurements.

TABLE I

A COMPARISON OF MOLECULAR WEIGHT VALUES OF SOME GLYCOGENS

Sample	Sedimentation-diffusion		Light-scattering
	10^{-6}M	Other components	10^{-6}M
Ascaris lumbricoides	2·8	F	8 9
Brewer's yeast	3·7	—	4·4
Cat liver I	4·4	F,S	13·6
„ „ IV	4·9	F,S	13·4
„ „ VI	5·9	F,S	17·9
Commercial I	0·7	—	1·9
„ II	4·0	—	5·4
Rabbit liver II	5·5	—	7·8
Rabbit muscle	4·6	—	4·1

F = Fast component. S = Slow component.

The method of isothermal distillation[61] is proving to be particularly valuable in studies on polysaccharides in the molecular-weight range 3,000–20,000 which are not amenable to investigation by conventional osmometry or sedimentation methods. The numerous polysaccharides of the hemicellulose group fall into this category, and much information is now being collected.[62]

Although the measurement of the size and shape of polysaccharides has been adequately reviewed,[63] the use of other physico-chemical techniques for the determination of the type and anomeric configuration of the repeating glycosidic linkages has not often been described. These techniques include infra-red spectroscopy,[64] the comparison of the optical rotations of certain polysaccharides in aqueous and cuprammonium solutions,[65] and of the corresponding tricarbanilates in pyridine and morpholine,[66] and, for starch-type polysaccharides, the measurement of iodine binding-power by differential potentiometric titration.[67] In Table II are summarized some data for a number of glucosans. Thus, polysaccharides containing β-glucosidic linkages, *e.g.* bacterial cellulose and luteose, give an infra-red absorption band at 891 $\pm$ 7 cm^{-1} of moderate or strong intensity (designed type 2*b* by Barker and his co-workers[64]), do not show 'type 2*a*' absorption (at 844 $\pm$ 8 cm^{-1} as shown by polymers of α-D-glucose) and have only very weak 'type 3' absorption, at *ca* 770 cm^{-1}. The correlation between the nature of the glycosidic linkages and the infra-red absorption spectra provides a speedy micro-method for the *preliminary* examination of a polysaccharide, despite certain experimental difficulties, *e.g.* adequate drying of the material without structural modification. The complexing reaction between cuprammonium and carbohydrates results in a change of optical rotation, which is characteristic of the type of glycosidic linkage. Studies of this rotation change have provided the first evidence of a small proportion of 1 : 2-glucosidic linkages in certain dextrans.[68]

TABLE II

PROPERTIES OF SOME GLUCOSANS[64,65,66]

Polysaccharide	Main glucosidic linkage	Infra-red absorption peaks, frequencies (cm^{-1})			[α] 436 mμ of Polysaccharide		[α]D of Carbanilate	
		Type 1	Type 2a or 2b	Type 3	H_2O or NaOH	Cuprammonium	Pyridine	Morpholine
Crown-gall	β-1.2	919	888,880	—	−23	+960	+50	−11
Laminarin	β-1 3	917	890	—	−29	+34	−63	−63
Cellulose	β-1:4	914,933	894	(766)	−20	−1,200	−152	−85
Amylose	α-1.4	938	857,838	756	—	—	−83	−7
Amylopectin	α-1:4†	931	840	756	+375*	−715*	−62	−4
Glycogen	α-1:4‡	928	840	760	+366	−597	−32	+4
Dextran	α-1:6§	919	840	768	+297	−128	Insol.	+343

*Data for soluble starch.
† Contains 4% α-1:6-glucosidic linkages.
‡ Contains 8% α-1:6-glucosidic linkages.
§ May contain a small proportion of 1:3- and 1:4-glucosidic linkages.

The biological chemist may require a knowledge of the three-dimensional organization of the polysaccharides as laid down in plant-cell walls. This type of information has been obtained by use of the electron microscope and by X-ray analysis. Recent advances in this specialized field have been reviewed by Preston [69] and Northcote.[70]

FINE STRUCTURE OF GLUCOSE-CONTAINING POLYSACCHARIDES

The remainder of this review deals with certain aspects of the fine structure of the following glucose-containing polysaccharides: lichenin, laminarin, the starch components (amylose and amylopectin) and glycogen.

LICHENIN

Lichenin, a glucose-containing polysaccharide, occurs in various lichens, including Iceland moss (*Cetraria islandica*). Early methylation studies[71] indicated that the molecule was essentially linear and contained both β-1:3- and β-1:4-glucosidic linkages. These conclusions have been confirmed and extended by periodate oxidation.[72]

On oxidation with sodium metaperiodate, 0·73 mol. per anhydroglucose residue was reduced. Since 1:3-linked residues do not contain α-β diol groups, this suggests that *ca* 30 per cent of these residues are present. Treatment of periodate-oxidized lichenin with isonicotinhydrazide[44] gave an insoluble product containing 11·6 per cent N. Under these conditions, 1 mol of isonicotinhydrazide reacts with each oxidized glucose residue; this reaction, together with one possible structure for the product, is shown in Fig. 8. The observed N-content indicates the presence of *ca* 66 per cent diol groups, *i.e.* 1:4-linked glucose residues, and hence, *ca* 34 per cent of 1:3-linked units. The agreement between this figure and that from the reduction of periodate shows that the lichenin molecules contain *both* types of linkage, and are not a *mixture* of two distinct polysaccharides, one a β-1:3-glucosan, and the other, of the β-1:4-cellulose-type.[44] If lichenin was such a mixture, the