THE BIOLOGICAL AND CHEMICAL SYNTHESIS OF POLYSACCHARIDES

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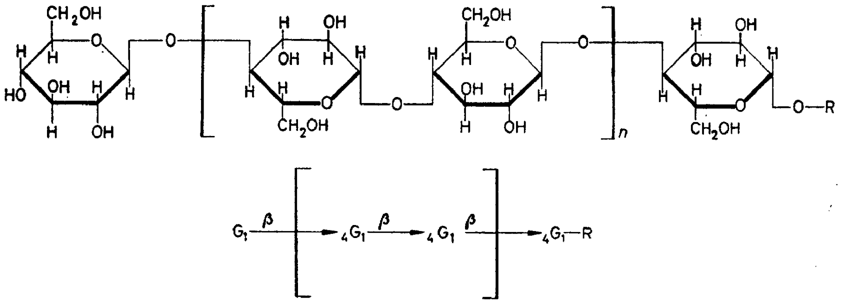
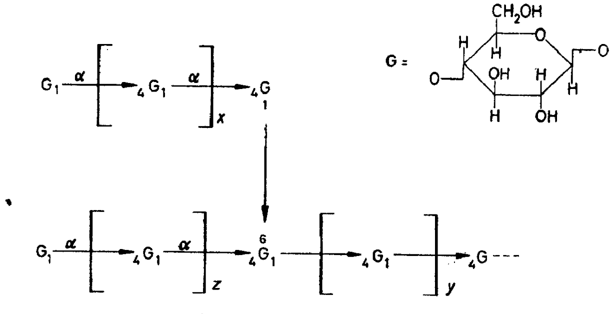
Polysaccharides are high molecular weight condensation polymers of monosaccharide residues which are glycosidically linked to form non-cyclic polymeric chains. With the synthesis of new types of carbohydrate polymers, it is likely that this definition will require modification. The degree of polymerization may vary from ~ 10 to above 10^4. On complete hydrolysis by acids or by enzymes, one or more monosaccharides may be liberated. Homopolysaccharides may be defined as containing essentially one sugar, whereas the complex heteropolysaccharides may contain up to six different component monosaccharides which may be neutral, acidic or basic in character[1].

Some carbohydrate polymers contain, in addition, non-carbohydrate material, such as phosphate, sulphate, acetate, pyruvate, malonate and



Cellulose (I)

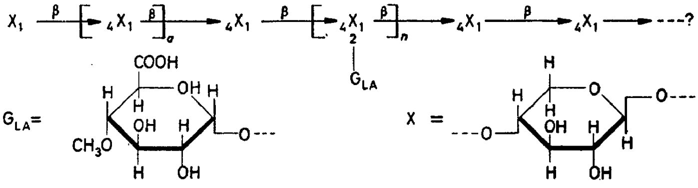


Amylopectin (II)

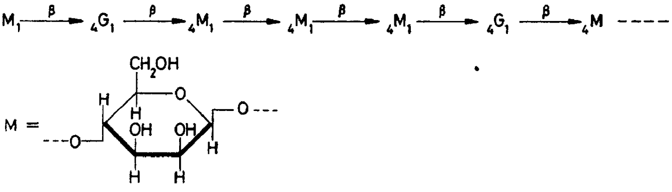
other esters[2]. Peptide chains may also be present as an additional complication[3]. The constituent monosaccharides may be arranged to give linear or branched chain polysaccharides which may also contain sugars in the pyranose and/or furanose forms.

Two common polymers of glucose which differ in having different types of linkage are cellulose (I) and amylopectin (II). Xylan (III) from land plants is a hetero-type polymer. It is of the branched-chain type and contains 1→4-β-linked D-xylopyranose residues and 4-*O*-methyl-D-glucuronic acid which is α-linked through C–2 of D-xylopyranose. L-Arabinofuranose residues are also encountered as part of some types of xylan molecule[4]. A glucomannan (IV), which is found in wood, contains 1→4-β-linked D-gluco- and D-mannopyranose residues which are joined to one another in a random fashion[5]. These polymers are not physically homogeneous, and are probably not chemically homogeneous, but consist of families of closely related polymers. The pneumococcal polysaccharide (Type VIII) (V) is a heteropolymer and is ordered; it consists of residues of α- and β-D-glucose, β-D-glucuronic acid and α-D-galactose all in the pyranose form.

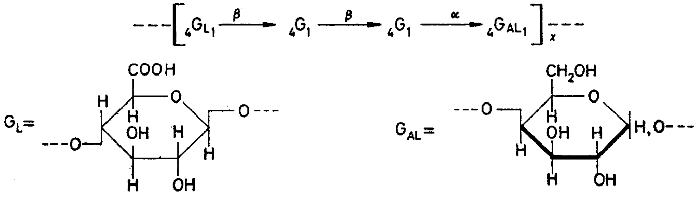
Recent work indicates that all the sugars found in polysaccharides probably arise initially from a D-fructose derivative which itself originates



Xylan (III)



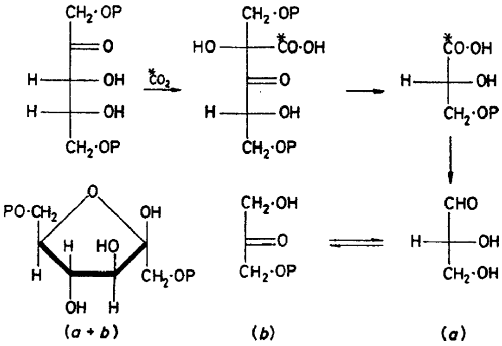
Glucomannan (IV)



Pneumococcus type (VIII)

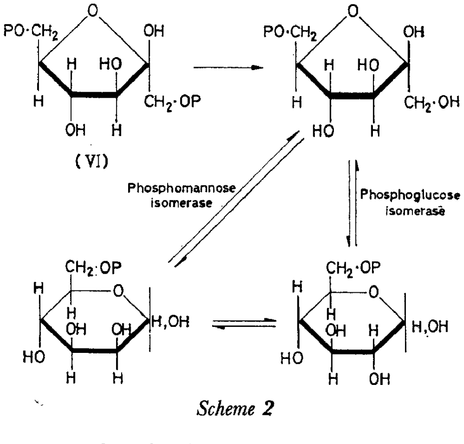
(V)

from carbon dioxide in the photosynthetic cycle[6]. This transformation is illustrated in simplified form in *Scheme 1.*



Scheme 1

The fructose 1,6-diphosphate (VI) derivative may be converted in plants and animals into D-glucose or into D-mannose phosphate derivatives by specific enzyme systems as illustrated in *Scheme 2*[7].

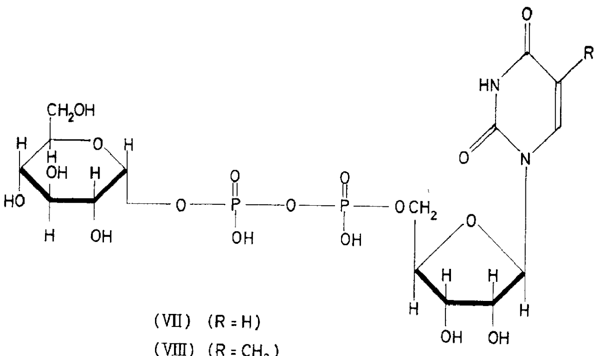


Scheme 2

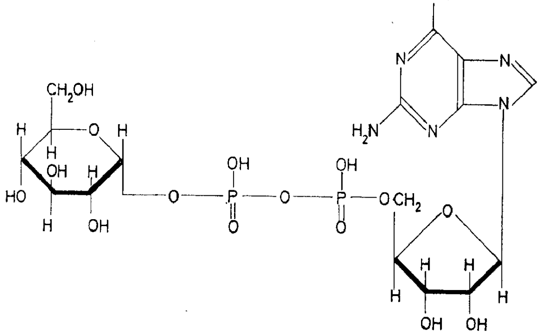
It is these precursors, after further reaction, which appear to be involved in the synthesis of some glucans, mannans, and possibly some glucomannans.

The work of Leloir[8], Hassid[9], their collaborators and others have indicated that uridine-diphosphate glucose (VII, R = H) is a key intermediate in the synthesis of sucrose, trehalose, uronic acids, pentoses and some polysaccharides. The major lines of polysaccharide synthesis appear to involve either a pyrimidine (*e.g.* VII and VIII) or a purine derivative (*e.g.* IX) *or* the transfer of sugars from one glycoside (relatively small) to another polymer molecule which is relatively large. These methods of polysaccharide formation are known as transglycosylation[10]. The exact mechanism of this transfer is not clear.

Polysaccharides are synthesized in plants and animals in huge quantities annually. Three well known polymers are cellulose, starch and glycogen.



(VII) (R = H)
(VIII) (R = CH_3)



(IX)

Cellulose is a structural polysaccharide, it is a linear polymer, and once formed it is metabolically inert. Starch, a food reserve, is usually a mixture of at least two types of polymers. One is linear, and named amylose; the other major component, amylopectin, is branched. Glycogen, the food reserve of animals, is also branched, and like amylose and amylopectin it is an α-glucosidically linked polymer.

Despite both the industrial and biological importance of cellulose its mode of formation is still obscure. Some bacterial organisms will synthesize cellulose from D-glucose-*Acetobacter xylinum* for example. It has been suggested that it may be possible in the future to make certain special types of cellulose this way. Colvin[11] has shown that aqueous ethanolic extracts of *Acetobacter xylinum* contain a compound which is rapidly converted into cellulose. In this conversion neither D-glucose-1-phosphate nor uridine-diphosphate glucose appear to be involved as an immediate precursor, however, either may form a fragment of a much larger molecule which is a precursor. Other workers[12] have shown that D-glucose labelled with ^{14}C may be incorporated directly into cellulose in the cotton plants. On the other hand, it has been shown that some of the D-glucose residues are broken down and re-synthesized from the fragments before the sugar is incorporated into the cellulose molecule[13].

Amylose was the first polymer to be synthesized *in vitro* by the use of enzymes. It was prepared by Hanes[14] from D-glucose-1-phosphate and the

Table 1. Nucleoside diphosphate sugar derivatives isolated from natural sources

Nucleoside	*Sugar*	*Configuration*
Uridine (*cf.* VII, R = H) Guanosine (*cf.* IX) Thymidine (*cf.* VII, R = CH_3)	Glucose	D
Uridine Guanosine	Galactose	D
Uridine Thymidine	Rhamnose	L
Uridine	Arabinose	L
	Xylose	D
	Glucuronic acid	D
	Galacturonic acid	D
	N-acetylglucosamine	D
	N-acetylgalactosamine	D
Guanosine	Galactose	L
	Mannose	D
	Fucose	L
	Fructose	D
	3,6-dideoxygalactose	L
	Glycero-D-mannoheptose	D
Cytidine	Ribitol	
	Sialic acid	D

enzyme, phosphorylase; a primer, which may be a tetrasaccharide or a larger molecule, is required for efficient polymer formation, This mechanism of synthesis is now believed by some to be a minor pathway, and it is thought that amylose synthesis proceeds *via* uridine diphosphate glucose which has been found in seeds of plants and in the starch granule. In this connection it may be mentioned that Stacey[15], some years ago, suggested that the traces of nitrogen found in some polysaccharides may be the vestiges of the enzymes responsible for their synthesis. The resulting linear amylose polymers may then be converted by another enzyme, the *Q*-enzyme[16], which causes group transfer, into the branched chain polymer, amylopectin.

Glycogen was, at first, thought to be synthesized from D-glucose-1-phosphate by phosphorylase, it is now known to be formed *via* uridine diphosphate glucose, D-glucose-6-phosphate being a catalyst in this reaction. This mode of synthesis is energetically favoured. The steps in these synthesis are outlined in *Scheme 3.*

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{D-Glucose-6P} + \text{ADP}$$

$$\text{D-Glucose-6P} \xrightarrow{\text{Phosphoglucomutase}} \text{D-Glucose-1P}$$

$$\text{UDP} + \text{ATP} \xrightarrow{\text{Nucleoside diphosphokinase}} \text{UTP} + \text{ADP}$$

$$\text{UTP} + \text{D-Glucose-1P} \longrightarrow \text{UDPG} + \underset{\text{(from UTP)}}{\text{PP}}$$

$$\text{UDPG} \xrightarrow{\text{UDPG-glycogen transferase}} \text{Glycogen} + \text{UDP}$$

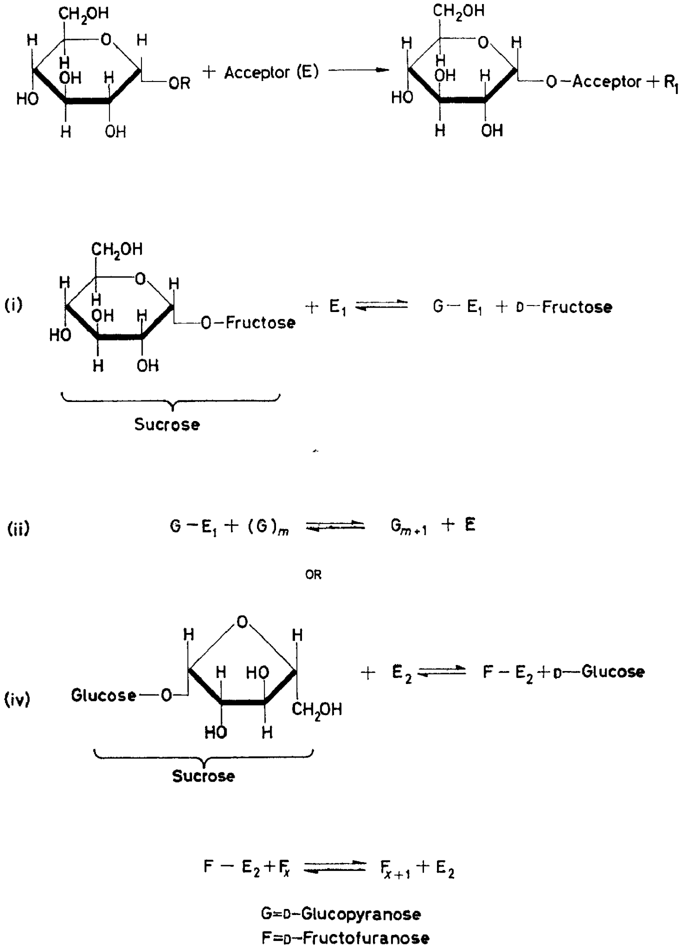
SUM

$$\text{D-Glucose} + 2\,\text{ATP} = \text{Glycogen} + 2\,\text{ADP} + \text{PP}$$
$$\Delta F^\circ = -11{,}700\ \text{cal}$$

Scheme 3

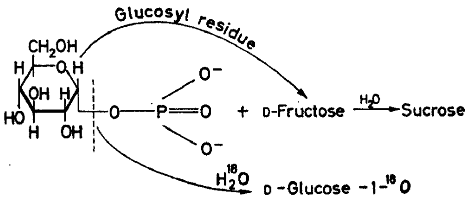
The enzyme, muscle phosphorylase, exists in two forms, *a* and *b*, which are interconvertible: it has been shown that both enzymes *a* and *b* are involved, together with uridine diphosphate glucose, in the transfer of sugar residues from uridine diphosphate glucose to glycogen[17]. Hassid[18] and his co-workers have shown that similar mechanisms probably hold in the synthesis of arabans, galactans, glucans and xylans. The nucleoside carbohydrate derivatives which have so far been isolated from natural products are listed in *Table 1.* In most of these experiments extracts from bean seedlings were used. It is very probable that the same or similar metabolic pathways hold in other plants and animals.

The general reaction by which monosaccharides are converted into oligo- or polysaccharides consists of a reaction in which a glycosyl derivative acts as a monosaccharide donor and donates a sugar molecule to an acceptor (the *trans*-glycosylation reaction), *Scheme 4.* For example, if the glycosyl residue is D-glucose and R is D-fructose (*i.e.*, sucrose) the resulting product may be dextran[19]. On the other hand, if the glycosyl residue is D-fructose and R is D-glucose then the polymer formed may be inulin (1→2 linkage) or



Scheme 4

levan (2→6 linkage)[20]. The inulin molecule is terminated by a sucrose residue indicating that sucrose was the original starter in the reaction. Another enzyme, amylosucrase, will convert the D-glucose moiety of sucrose into an amylose-type polymer. In all these reactions the energy associated with a pre-formed glycosidic linkage is used to form a new link by exchange of the originally substituted radical with a new one. Each step in polysaccharide synthesis does not necessarily involve the *direct* exchange of the glycosidic linkage in the product for that of the substrate, an enzyme–glycoside product probably intervenes.



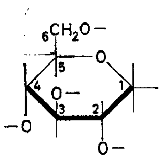
Scheme 5

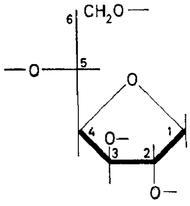
It has been shown by use of water labelled with isotopic oxygen that in the conversion of D-glucose-1-phosphate to sucrose it is the bond between carbon and oxygen, and not the one between oxygen and phosphorus that is split, *i.e.* the D-glucose is transferred as $C_6H_{11}O_5$ and not as $C_6H_{11}O_6$ (*Scheme 5*).

In some enzyme systems (*e.g.* invertase) it has been postulated that water may function as an acceptor and the enzymes may then act as hydrolases

Table 2. Reversion products of D-glucose

Disaccharide	*Position of substitution*	*Isolator*
Trehalose	$1 \longrightarrow 1$	Wolfrom
Isomaltose	$1 \xrightarrow{\alpha} 6$	Fischer (1890)
Gentiobiose	$1 \xrightarrow{\beta} 6$	Berlin (1926)
Maltose	$1 \xrightarrow{\alpha} 4$	Fetzer (1953)
Cellobiose	$1 \xrightarrow{\beta} 4$	Wolfrom (1954)
Nigerose	$1 \xrightarrow{\alpha} 3$	Pazur (1956)
Sophorose	$1 \xrightarrow{\beta} 2$	Wolfrom (1954)
5—*O*—β—D—Glucopyranosyl—D—glucose	$1 \xrightarrow{\alpha} 5$	Sowden (1956)

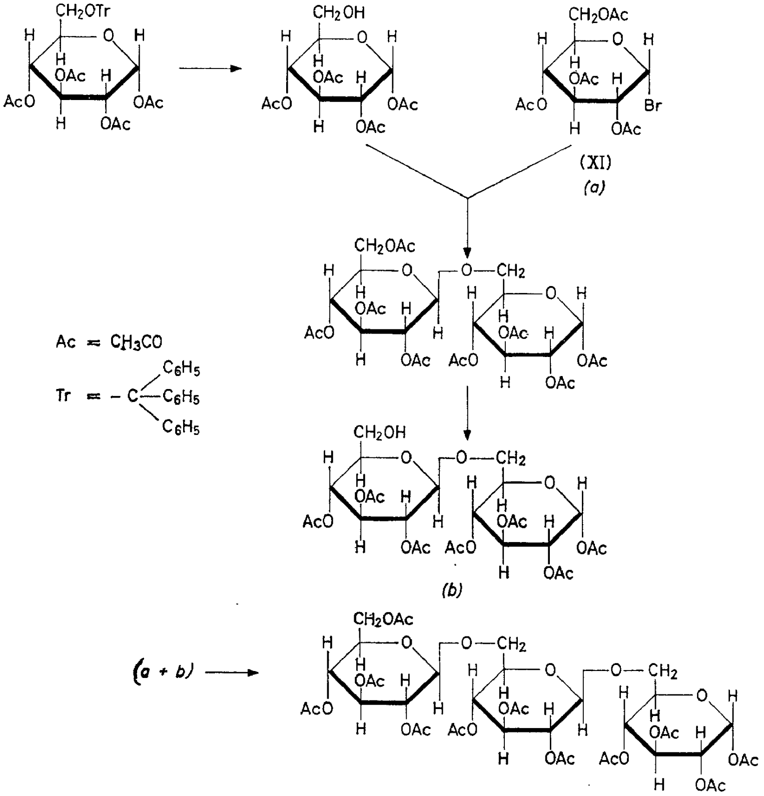




or as transferases, *e.g.*, carbohydrases behave in this fashion. Usually these enzymes are inefficient in their synthesis of polymers. The action of these enzymes resembles that of acids on sugars; like acids they can cause the formation of higher saccharides from lower saccharides under suitable conditions. Whelan has synthesized D-galactose containing oligosaccharides from D-galactose and galactosidase[21], and Peat[22] has converted D-glucose to oligosaccharides by use of almond *emulsin* β-glucosidase. This process is analogous to the synthesis of polysaccharides by purely chemical procedures. When a polysaccharide is heated with an acid in aqueous solution monosaccharides are produced; this is a reversible process, and, under certain conditions, reducing sugars are converted into polymers in the presence of acid.

When any aldohexose is heated in aqueous acidic solution disaccharides and oligosaccharides are produced in small quantity. For example when D-glucose was heated in acid solution the disaccharides shown in *Table 2* were isolated and identified[23].

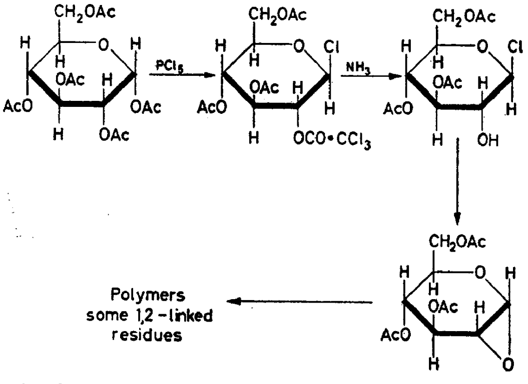
If a sugar solution is evaporated in the presence of traces of catalyst such as boric acid, thionyl chloride, hydrofluoric acid, phosphorous acid, hydrochloric or sulphuric acid, or if the reaction is carried out in a non-aqueous solvent such as dimethyl sulphoxide, relatively high molecular weight polymers may be



Scheme 6

produced[24]. Unfortunately these preparations are not easily reproducible. The products are mixtures and contain α- and β-glycosidically-linked residues which are joined through any of the hydroxyl groups. A linkage through the oxygen at C–5 with consequent formation of a furanosidic linkage has been found by Dutton[25] in a synthetic glucose polymer.

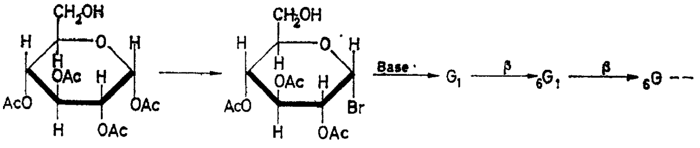
Several attempts have been made to produce polymers of known structures by purely chemical means, so far without significant success. For example, attempts have been made to build up a polymer in a stepwise manner, but the yields were low and a tetrasaccharide was the highest polymer so far produced. These condensations usually proceed through acetobromo-sugars (XI) and yields are seldom better than 33 per cent at each stage. Thus, after four steps, the over-all yield is less than 1 per cent. Zemplen[26] achieved the synthesis of oligosaccharides of the gentiobiose series as shown in *Scheme 6*.



(XII)

Scheme 7

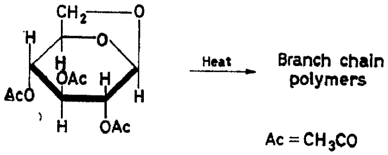
Whelan[27] has polymerized 3,4,6-tri-*O*-acetyl-1,2-anhydro-D-glucose (XII) (*Scheme 7*). He has also obtained a polymer of low molecular weight by shaking 2,3,4-tri-*O*-acetyl-α-D-glucosyl bromide (XIII) with silver oxide in an inert solvent (*Scheme 8*).



Ac = CH₃CO

(XIII)

Scheme 8



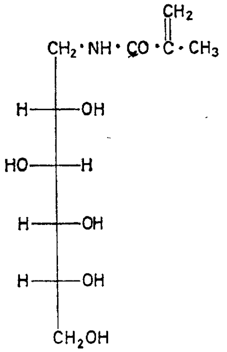
Ac = CH₃CO

(XIV)

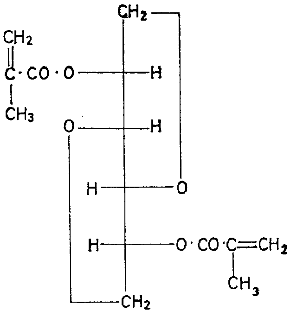
Scheme 9

Pringsheim[28], Schuerch[29], Wolfrom[30] and others have obtained polymers by the action of heat or acid catalysts on 1,6-anhydro-D-glucopyranose (XIV) (*Scheme 9*). The products are branched chain polymers of relatively high molecular weight which contain α- and β-glucosidic links. When starch is heated either alone or with acids it is converted into dextrins. The change in physical properties which results is due in part to the breaking of the glucosidic linkages and the subsequent formation of new ones.

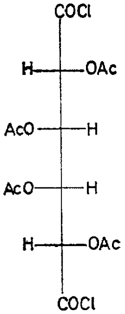
Synthetic carbohydrate polymers have also been made by polymerization of sugar derivatives such as 1-methylacrylamido-1-deoxy-D-glucitol (XV)[31], 2,5-dianhydro-D-mannitol dimethacrylate (XVI), the condensation of diamines with tetra-*O*-acetylgalactaroyl dichloride (XVII)[32], and the condensation of methylene glucitol derivatives (XVIII) with adipic acid.



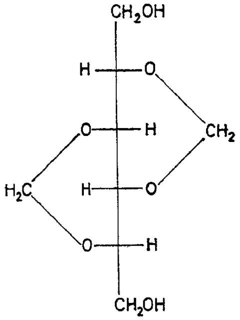
(XV)



(XVI)

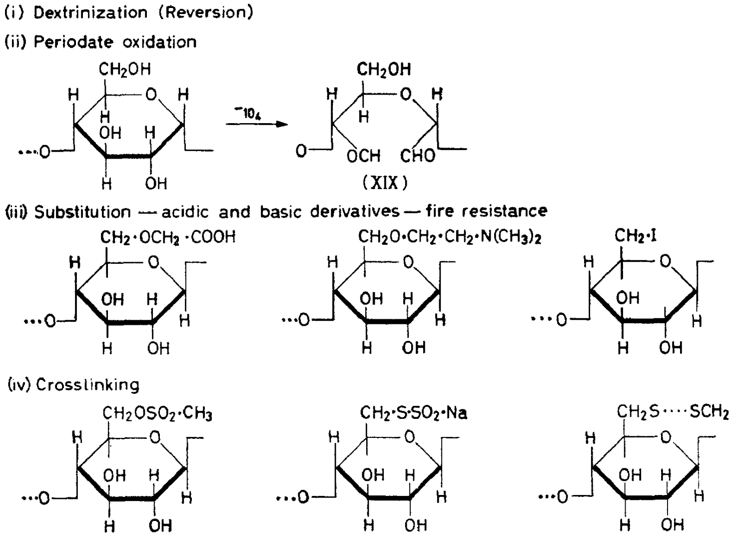


(XVII)

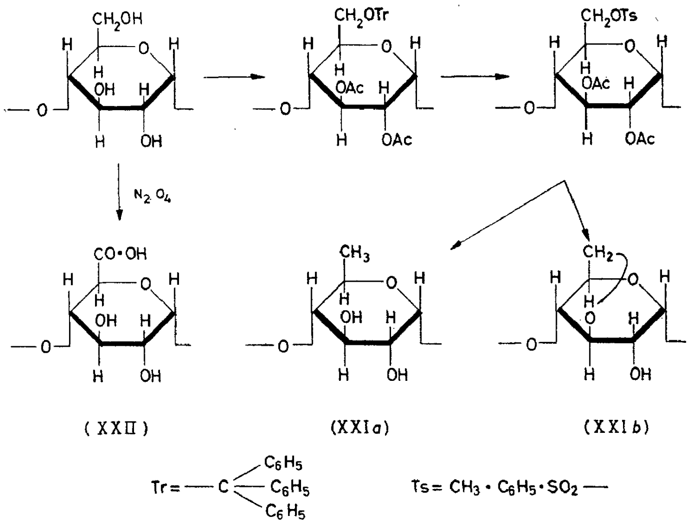


(XVIII)

The possibilities of forming new carbohydrate polymers by polymerization of unsaturated substituents are very great indeed. Many new types of polymers may be produced by modification of a preformed polysaccharide (*Schemes 10–12*). These are common industrial processes and have led to the commercial production of esters, ethers, dextrins, and oxidation products of polysaccharides. Perhaps the latest industrial derivative to be produced commercially is periodate oxidized starch (XIX). Smith[33] has shown that the action of methanolic hydrogen chloride on (XIX) converts it into a relatively alkali-stable polymer which is probably crosslinked.

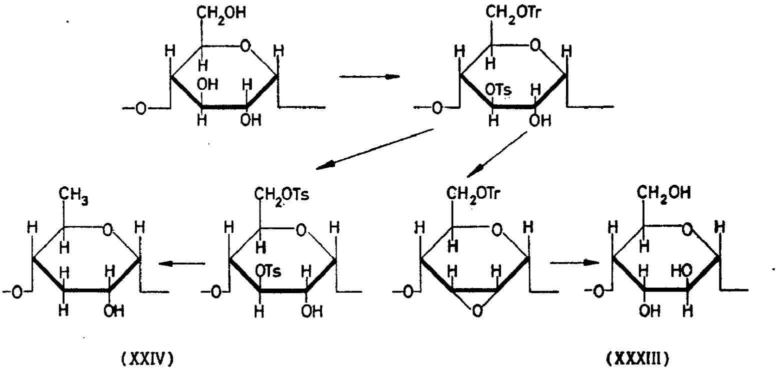


Scheme 10



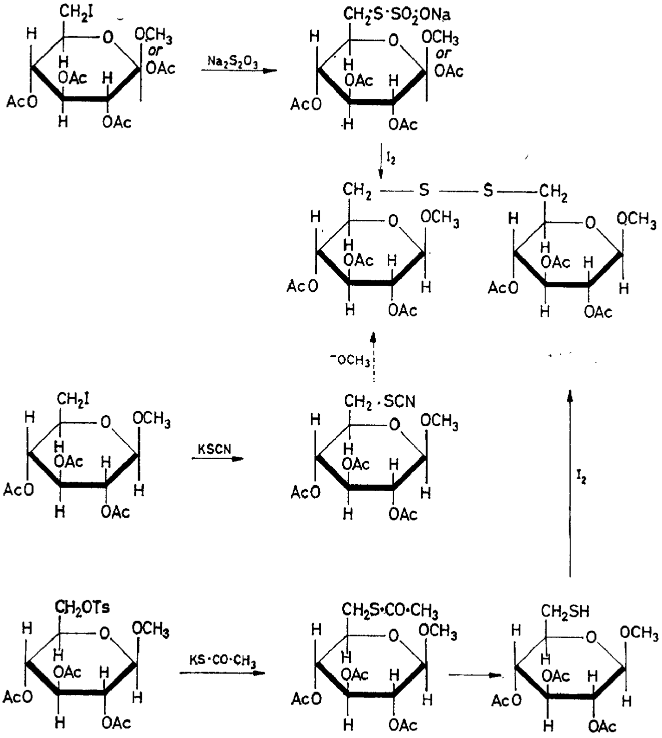
Scheme 11

Recently, modified polysaccharides have been prepared by converting primary alcohol groups into the methyl groups as in deoxy compound (XXI*a*), or by formation of cylic ethers (XXI*b*). These modifications cause the polymer to become acid-labile. If the primary alcohol group is oxidized to a carboxyl group (XXII) then a more acid-stable polymer results (*Scheme 11*).



$Ts = CH_3 \cdot C_6H_5 \cdot SO_2—$
$Tr = —C(C_6H_5)_3$

Scheme 12

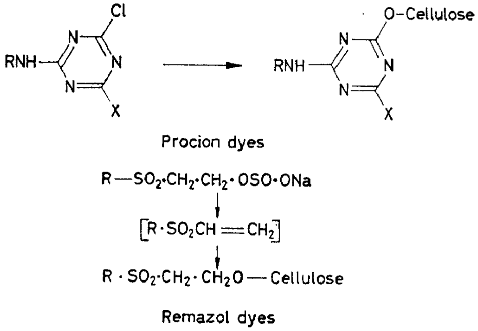


$Ts = CH_3 \cdot C_6H_5 \cdot SO_2—$ $Ac = CH_3CO$

Scheme 13

The configurations of the hydroxyl groups on C–2 and C–3 of amylose have been transposed by Whelan (as shown in *Scheme 12*) to yield an altrose derivative (XXIII) which has unusual properties: it is hydrolyzed with great difficulty, and then only by trifluoroacetic acid[34]. From the intermediate required to carry out this transformation a 3,6-di-deoxyhexosan, which is very acid labile, may be prepared (XXIV). This substance may have importance in immunology. Perlin showed that an initially water-soluble arabinoxylan became insoluble when the arabino-furanosidic residues were removed from the main chain by careful hydrolysis with acids. Barker and Stacey have demonstrated that a reverse reaction, the addition of D-xylose residues to a main chain of D-glucose residues, can be carried out enzymically. Cellulose has been crosslinked and converted into a derivative with crease and flame resistance[35]. Dicyanates, formaldehyde, and divinyl sulphone derivatives have been used for this purpose. Pacsu has put an —S—S—bridge into cellulose by the type of reactions shown in *Scheme 13* and has made fire resistant cellulose by exchange of primary hydroxyl group on C–6 for iodine (*Scheme 10*).

New dyestuffs for cellulose have been used, these include the vinyl sulphone type (Remazol dyes) and the Procion dyes[36] (*Scheme 14*).



Scheme 14

Thus we see that biologists and chemists have reached a stage when it may be possible to make tailor-made polysaccharides with desired properties in the not too distant future.

Summary

Polymeric pentosans and hexosans occur abundantly in nature and are of great biological and industrial importance. These materials are built up of monomeric sugar units which are united through hemiacetal-type linkages to form polymers. The structures of many of these substances have been investigated and, in some cases, the finer structural details are known with some precision. The biochemical mechanisms by which some of these materials are formed are fairly well understood in some cases—in others, nothing is known of the method by which they are produced.

Natural polymers may be converted into synthetic, unnatural polymers by suitable chemical or biochemical modification of their structure. Many attempts have been made to synthesize chemically, polymeric carbohydrates of known structure with but little success. However, the syntheses of randomly branched polysaccharide mixtures has been achieved.

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