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ADVANCES IN THE SYNTHESIS OF POLYSACCHARIDES

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I. INTRODUCTION

The synthesis of high-molecular-weight polysaccharides from various monoses or low-molecular-weight polyoses is one of the most complex problems of modern carbohydrate chemistry and also one which holds out most promise. The considerable interest in this problem is due to the following reasons.

1. The efforts to synthesise polysaccharides analogous in structure and properties to the more important natural polysaccharides, in particular cellulose and amylose, in which the glucose units are united by α- or β-(1 → 4)glycosidic linkages. Successful syntheses of this kind will help elucidate many problems associated with the structure and properties of these most important natural high-molecular-weight compounds and their derivatives.
2. The possible synthesis of new synthetic materials with valuable properties, which could be used for various purposes, particularly in medicine and biology. By changing the molecular weight, chemical structure (for instance the type of linkage between the elementary units of the macromolecule) and especially the branching of synthetic polysaccharides the properties of this new type of synthetic polymers can be varied within wide limits and new fields for their application found.
3. Simulation of many complex biological systems, particularly with a view to studying complex biological and biochemical processes and the physicochemical and biological properties of mucopolysaccharides (heparin, chondroitin, etc.).

Although work on the synthesis of polysaccharides (the so-called "reversion") started as early as the seventies of the last century, the most important results in this field were obtained in the last few years.

By using the basic methods and principles of modern synthetic polymer chemistry, particularly polycondensation and conversion of cyclic compounds into linear polymers, it was possible to obtain polysaccharides with molecular weights from several thousand to one million. Application of modern physicochemical and physical methods for the study of high-molecular-weight compounds has led to great advances in the field of these typical polyelectrolytes and their derivatives.

The problem of the preparation of synthetic analogues of cellulose and amylose, which have a linear, stereoregular structure, and whose macromolecules contain but one type of glycosidic linkage [α- or β-(1 → 4)], has so far remained unsolved. Obviously the synthesis cannot be effected by using monomeric monoses, containing 4–5 reactive functional groups, as starting material in the homopolycondensation. Other methods, starting from bifunctional monomers, must be used.

Systematic studies of this kind are only just starting, in the USSR as well as abroad. We think, nevertheless, that very interesting results have already been obtained in this new and complex branch of the chemistry of synthetic polymers. We have therefore undertaken to review briefly the current state of this problem.

II. METHODS FOR THE PREPARATION OF SYNTHETIC POLYSACCHARIDES

We already pointed out that two methods are available at present for the synthesis of polysaccharides, *i.e.* polycondensation and conversion of cyclic compounds into linear polymers.

A. SYNTHESIS OF POLYSACCHARIDES BY POLYCONDENSATION

Of all the various modifications of polycondensation, only homopolycondensation can be used in the synthesis of polysaccharides, since every monose required in this reaction contains two types of functional groups: hydroxyl groups and the aldehyde group in the form of a hemiacetal.

Interaction between these functional groups results in the glycosidic linkages between the units of the polymer macromolecule. Depending on the position of the hydroxyl group in the monose molecule, various types of glycosidic linkages may be formed (1 → 1, 1 → 2, 1 → 3, 1 → 4, 1 → 6).

The polycondensation of monoses can be effected not only in anhydrous media (as in the case with other polymers), with continuous elimination of water formed in the reaction, but also in aqueous solutions in the presence of acid catalysts, as for instance in the hydrolysis of crude polysaccharides. In contrast to homopolycondensation proceeding without a catalyst, *e.g.* of polyamides, where the only factor which controls the reaction rate and the molecular weight of the product is the temperature, the synthesis of polysaccharides is always carried out in the presence of acid catalysts.

1. Polycondensation in Solution

(a) The polycondensation of monoses in aqueous solutions in the presence of acid catalysts has been known for a long time. In the seventies of the last century it was observed that glucose can undergo polycondensation to form high-molecular-weight polysaccharides. In 1872 Musculus[1] described the dextrin-like products formed by the action of concentrated mineral acids on glucose. Other research workers reported similar results[2-5]. Tollens (ref.6, p.10) showed somewhat later that polycondensation (reversion) occurred in 20% aqueous glucose solutions in the presence of hydrochloric acid. The work of Wohl[7] and Ost (ref.6, p.11) belongs to the same period. In their study of the products of polycondensation obtained by the action of concentrated hydrochloric acid upon 25% aqueous glucose solution, the authors isolated a product, $[\alpha] = +122^\circ$, with a reducing power 7–9 times lower than that of glucose, which they named "glucosin". On hydrolysis with diluted acid this product was converted quantitatively into glucose, but it resisted enzymic hydrolysis.

Interesting investigations of the structure of the products of glucose polycondensation in the presence of hydrochloric acid were carried out by Fischer[8] during this period. Willstätter and Zeichmeister[9] studied the reversion of glucose in hydrochloric acid solutions by measuring the change in the specific rotation. Silin and Sapegina[10] studied the polycondensation of monoses in the presence of dilute acids. All this work was of great practical importance for the development of methods of saccharification in the industry of starch and molasses. Smirnov[11] recently carried out investigations along the same lines. Sharkov and Liprandi (ref.6, p.12) studied the effect of 80% sulphuric acid on glucose by measuring the change in the reducing power of glucose with time. Sharkov and Sartania

(ref. 6, pp. 13–15) later investigated the effect of 80% sulphuric acid on glucose, galactose, mannose, fructose, and xylose. The polycondensation products had a reducing power 6–7 times smaller than the initial monoses. They showed that the higher the concentration of the monose in solution, the faster and the more efficient its polycondensation. In aqueous alcoholic solution the degree of polycondensation is considerably higher than in aqueous solution. Sharkov and Smirnova[12], who studied the polycondensation of glucose in the presence of concentrated sulphuric acid, arrived at a similar conclusion.

Odintsov and Preobrazhenskii[13,14] also studied the polycondensation of glucose in concentrated sulphuric acid and on the whole confirmed the results of Sharkov and his collaborators; they established in addition that the products of polycondensation are more stable to high temperatures in the presence of concentrated sulphuric acid than glucose itself. The average degree of polymerisation of the products of glucose polycondensation in sulphuric acid, according to end group analysis, was 4–5.

The kinetics of glucose polycondensation in the presence of hydrogen chloride of varying concentration have been studied in great detail by Fram[15,16]. He showed the reversible character of this reaction, made an attempt to determine the molecular size distribution of the reaction products, and studied the structure of the resulting polysaccharides. He carried out the polycondensation of methyl-substituted glucoses with a view to determining the reactivity of various hydroxyl groups in the glucose molecule. The methylated reaction products were fractionated by high-vacuum distillation. The primary alcoholic group at carbon atom six in glucose was established to be the one mainly engaged in polycondensation.

Pogosov and Rogovin[17,18] studied the polycondensation of glucose in hydrogen fluoride solutions. At hydrogen fluoride concentrations lower than 75% and at room temperature this process proceeds very slowly, the average degree of polymerisation of the resulting sugars, determined from the iodine number, being lower than 2. Polycondensation proceeds more readily at hydrogen fluoride concentrations 80% and higher. The polysaccharide obtained from these solutions had the average degree of polymerisation 10–12 (from end group analysis).

Ricketts[19] recently studied the polycondensation of glucose in concentrated hydrochloric acid containing a small amount of concentrated sulphuric acid. The reaction mixture was left at room temperature for seven days. After purification and dialysis the syrup-like product had an average degree of polymerisation of 20 and $[\alpha] = +106°$. On complete hydrolysis it yielded only glucose; isomaltose was isolated chromatographically after partial hydrolysis. Periodate oxidation established the presence of 70% of α-(1 → 6) glycosidic bond. Hurd and Cantor[20] and Fetzer[21] studied the products of polycondensation of glucose in the presence of aqueous acid solutions. By methylation and subsequent molecular distillation of methylglucose, Montgomery and others[22,23] isolated 40% of monoses, 28% of bioses and 4% of trioses. From the mixture of bioses Miller[24] isolated after acetylation gentiobiose acetate. In addition to gentiobiose he found a disaccharide of uncertain structure which did not ferment with yeast. Coleman later showed[25] this disaccharide to have the structure like gentiobiose but with an α-glycosidic linkage (isomaltose).

Moelwyn-Hughes[26] calculated the activation energy for the formation of the glycosidic bond to be 33 500 cal. Since this value is considerably higher than the energy of the cleavage of the glycosidic bond (28 000 cal), the rate of polycondensation increases faster with increasing temperature than the rate of hydrolysis.

The polycondensation of a number of carbohydrates in aqueous solutions in the presence of acid catalysts has been studied recently. The resulting products were analysed chromatographically. Bishop[27] studied the polycondensation of xylose in hydrochloric acid solution. The degree of polymerisation of the resulting xylose polymer was 10. Ball and Jones[28], who also studied the polycondensation of xylose, obtained the same results. Hough and Pridham[29] studied the polycondensation of arabinose.

Jones and Nicholson[30] investigated the composition of products formed by the polycondensation of L-arabinose and D-mannose in 6 *N* HCl during 96–168 h.

Separation of the reaction products in a cellulose chromatographic column, followed by the paper chromatography of separate fractions, showed them to be predominantly disaccharides with different types of glycosidic linkage between the monoses.

Taufel *et al.*[31,32] studied the polycondensation of D-glucose, D-xylose, D-galactose, D-arabinose, lactose, maltose, and cellobiose in acetic acid solution in the presence of small amounts of hydrochloric acid. By using paper chromatography to separate the polycondensation products, oligosaccharides were detected in addition to bioses, but their structure was not studied in detail.

Peat *et al.*[33] used paper chromatography to study the composition of the products of polycondensation of glucose formed in the presence of 0.33 *N* sulphuric acid and in 90% formic acid. Disaccharides of various structures predominated among the reaction products, which also contained laevoglucosan, formed by intramolecular elimination of water from glucose. Micheel and Groses[34] studied the polycondensation of glucose in dimethylsulphoxide solution in the presence of hydrogen chloride at room temperature. The products detected by paper chromatography were gentiobiose, maltose, isomaltose, cellobiose, other oligosaccharides, and polysaccharides. The polysaccharides were insoluble in methanol. The structure of the oligo- and poly-saccharides was not investigated.

Using absorption chromatography on charcoal-zeolite columns, Thompson and his collaborators[35] succeeded in isolating from the products of the polycondensation of glucose in 0.082 *N* hydrochloric acid the following bioses: isomaltose, gentiobiose, maltose, cellobiose, nigerose, sofrose, β-trehalose, and anhydromonose (laevoglucosan).

The analytical data on the quantitative composition of the biose fraction of the glucose polycondensation products show[33,35] that disaccharides formed by the condensation of the primary hydroxyl group at carbon atom 6 with the aldehyde group of another molecule of monose predominate in the mixture. This can be explained by the higher reactivity of primary hydroxyl groups in acid media, particularly in the formation of a glycosidic linkage[36].

It is clear from the foregoing that polycondensation in aqueous solutions does not yield products of very great molecular weight. The average degree of polymerisation of the polycondensation products did not exceed 15–20. Such results are fully understandable since the polycondensation is reversible and in aqueous solution the equilibrium is shifted towards the hydrolysis of the high-molecular-weight synthetic polysaccharides.

(b) The polycondensation of glucose in the presence of anhydrous acid catalysts with a view to shifting the reaction equilibrium towards the formation of high-molecular-weight polysaccharides was effected by a number of research workers. Schlubach and Lüers[37] were the first to show liquid hydrogen chloride to be, at room temperature, an active catalyst in the polycondensation of sugars. Under such conditions glucose yields polyglucosans, which on hydrolysis can be converted again to glucose; thus only glycosidic bonds exist between the units of the synthetic polysaccharide. Fructose gave the so-called polyfructosans[38] whose structure has not been studied in detail. Helferich and coworkers[39-41] studied the action of anhydrous hydrogen fluoride on glucose and trimethylglucose, but did not succeed in identifying the polycondensation products. In their study of the effect of anhydrous hydrogen fluoride on cellulose Freudenhagen and Cadenbach[42] obtained a polysaccharide formed by the polycondensation of the hydrolysis products (the so-called cellan). Cellan weakly reduced Fehling solution and was not hydrolysed by enzymes. The use of methylation to determine the structure of the polycondensation products was unsuccessful. Sharkov and his collaborators (ref. 6, p. 20) obtained similar results in their study of the action of hydrogen fluoride on cellulose.

By fractional precipitation of the products of glucose polycondensation in the presence of hydrogen fluoride, Rogovin and Posogov[18,36] recently obtained a series of fractions of different molecular weight and specific rotation. They studied the structure of the biose fraction and showed the bioses to contain primarily the α-(1 → 6) glycosidic linkages.

In all cases the average degree of polymerisation did not exceed 25, although products of higher molecular weight might have been expected in polycondensations carried out in the presence of anhydrous acid catalysts. It is clear, however, that high-molecular-weight products cannot be obtained without a continuous removal of water from the reaction system.

(c) New methods for the synthesis of polysaccharides by polycondensation have recently been suggested. Several amongst them, for instance the solid-phase reaction, have acquired great significance for the chemistry of synthetic polymers in general.

Polycondensation at elevated temperatures with continuous removal of water under high vacuum was the first method used for the preparation of synthetic polysaccharides of molecular weight 50 000 to a million. It should be noted that the polysaccharides obtained under these conditions have a higher molecular weight than other types of synthetic polymers obtained by the same method (*e.g.* polyamides, polyesters, etc.).

These new methods of synthesis may be divided into a number of groups, which are considered below.

2. High-Vacuum Polycondensation of Glucose

The preparation of high-molecular-weight branched polyoses, with a degree of polymerisation from 1 to 10, by high-vacuum polycondensation of various sugars was first described in the patent by Mora and Pacsu[43]. They showed that heating sugars individually or in mixtures, in an inert solvent or without solvent, in the presence of small amounts of an acid catalyst (*e.g.* 0.01–5 wt.% H_3PO_4 with respect to the glucose) between -80° and +110°, at pressures from 10^{-5} to 100 mm Hg (reaction times from 2 min to 72 h), yields vitreous polymers not containing any decomposition products. These polymeric carbohydrates had a molecular weight from 2500 to a million. Various polymer fractions were separated by dialysis or by fractional precipitation. These polymers were suggested for use as blood plasma substitutes instead of dextrans, which are synthesised enzymically.

In a series of communications Mora and his collaborators[44-46] described in detail the experimental technique and the composition of the resulting synthetic polysaccharides. They concluded[44] that to obtain polysaccharides of a high degree of polymerisation (> 300), the homopolycondensation should be effected under the following conditions: (*a*) catalyst concentrations at which there can be no decomposition of polysaccharide in a side-reaction; (*b*) uniform heating of the reaction mixture to fairly high temperatures; (*c*) high concentration of the monomer in the reaction mixture; and (*d*) the absence of oxygen or other reagents which could cause the decomposition of the resulting polymer.

The high-vacuum polycondensation, giving rise to products of high-molecular-weight and of varying degree of branching, has been carried out in three ways: (a) by the polycondensation of molten glucose heated by an infra-red lamp in the presence of an acid catalyst; (b) by the polycondensation of molten glucose as under (a) but in the presence of tetramethylsulphone which, being a plasticiser for glucose, lowers its melting point; and (c) by a two-stage polycondensation. In the last case the solid polymer formed in the first stage of the process is cooled, ground up, and heated again as under (b). In all three modifications phosphoric acid (0.168 wt.% with respect to the glucose) was used as a catalyst. To obtain a uniform reaction, small amounts of acid had to be distributed uniformly within the mass of monosaccharide by prolonged agitation of glucose with the acid in a ball mill.

The number-average molecular weights of certain polysaccharide fractions obtained under the above conditions are collected in Table 1.

TABLE 1. Number-average molecular weights and specific rotations of certain synthetic polysaccharide fractions obtained according to the Mora method[44].

Method of polycondensation	Temp., °C	Number-average mol. wt. (determined by the end-group method)	$[\alpha]_D^{25}$ in 1 *N* HCl, degrees
Modification (a)	140	15 000	71.1
	140	16 800	73.3
	150	22 750	79.0
	150	21 250	82.0
Modification (b)	155	16 200	71.2
	155	8 250	69.7
	175	32 800	85.2
	175	20 000	82.8
Modification (c)	150	17 500	63.6
	150	15 100	65.7
	170	20 000	67.8
	170	16 200	68.6

The branched polysaccharides, isolated by precipitation with methanol, were white and readily soluble in water. To separate the low-molecular-weight fractions, the polysaccharides were dialysed. They were also subjected to hydrolysis in 1 *N* HCl in order to determine the yield of glucose (96–100% of theoretical). Glucose was identified as the ozazone. The hydrolysis products were analysed chromatographically in order to determine the extent of partial decomposition and the formation of side-products during the polycondensation of glucose under the above conditions. Only traces of laevulinic acid[47,48] and hydroxymethylfurfural were detected on the chromatograms. The reaction mechanism proposed for the polycondensation of glucose is illustrated in Fig. 1, [44] which shows the role of hydrogen ions and water in mutarotation, hydrolysis, and polycondensation. Mutarotation is accompanied by the formation of a free aldehyde group, which at equilibrium is present to the extent of about 0.2 mole %.[49] The open-chain form rapidly undergoes ring-closure to give the stable α- or β-forms (only the α-form is shown in the first part of the scheme). It is clear that the carbonium ion at carbon atom 1 could react with any hydroxyl group in another molecule of glucose, for instance with the primary hydroxyl group at carbon atom 6 (see Fig. 1). The probability of such an interaction is very low in dilute solutions, but the process can be accelerated by the continuous removal of water from the system. It was shown earlier that the mutarotation proceeds according to a rearrangement mechanism[50,51], which applies in the presence of both acids and bases[52,53]. Phosphoric acid has a relatively low dissociation constant (4.8×10^{-3}) and the dissociated acid is more nucleophilic. It is very probable therefore that phosphoric acid may play a double role in the reaction and serve at high temperatures as an ion carrier. The concentration of phosphoric acid used in the synthesis (0.164%) was chosen empirically. Other acids could obviously also catalyse this process[43], but phosphoric acid has a number of advantages, being non-volatile and stable to oxidation at high temperatures. Glucose is most resistant to decomposition at pH 3. It is clear from Fig. 1 that the formation of the glycosidic bond proceeds according to the A-R-B_{f-1} scheme[54] (where A = glucosidic hydroxyl; B = non-glucosidic hydroxyl).

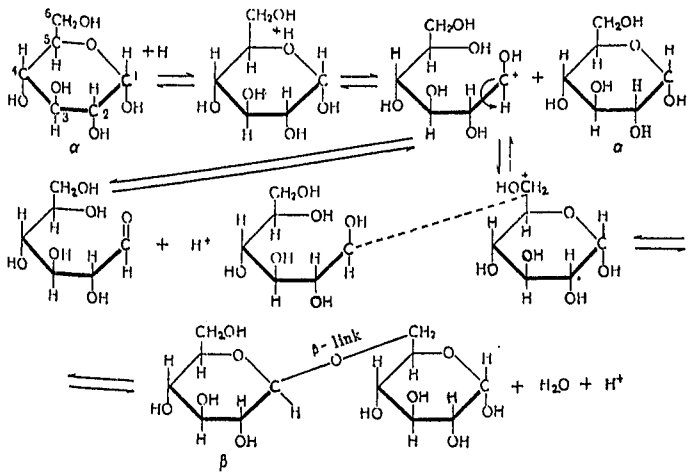


Fig. 1. Mutarotation, hydrolysis, and condensation (reversion) of glucose.

The formation of the 1 → 1 bond is energetically unfavourable, as already pointed out by Moelwyn-Hughes[26], and the bond is in fact not found in the reaction products.

Although it could be expected that the amount of α- and β-linkages in the synthetic polysaccharide should correspond to the equilibrium concentrations of glucose isomers (in accordance with Fig. 1), the high specific rotation of the preparations indicated the predominance of α-linkages between the units of the polysaccharide macromolecule.

Mora[45] suggested a structure for the polymer (see Fig. 2), based on his results of the periodate oxidation of the synthetic polysaccharides.

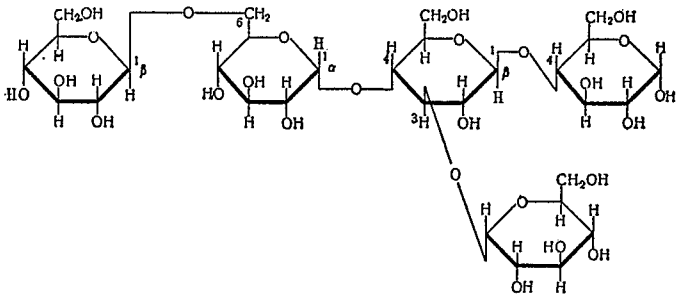


Fig. 2. The possible structure of the synthetic polysaccharide.

3. Solid-Phase Polycondensation of Glucose

In 1945 Lenk[55] described a method for the polycondensation of glucose in the solid phase, by heating it with boric acid or its anhydride. The product kept its crystalline structure throughout the reaction and weakly reduced Fehling solution. Lenk's data were recently confirmed by a number of workers[56] who used metaboric acid (HBO_2) as a catalyst. The polycondensation was carried out at 135°–140°. The resulting polyglucosans were completely soluble in water. The amount of catalyst used was 5 wt.% with respect to the glucose. To obtain polysaccharides, both the acid and monose had to be dry. The highest degree of polymerisation obtained under such conditions was 17, and the specific rotation was +64°. It was established by infra-red spectroscopy that the resulting polysaccharides contained both α- and β-linkages. The presence of gentiobiose and isomaltose was detected chromatographically in the products of partial hydrolysis. Methylation followed by hydrolysis gave a number of methyl-substituted glucoses, which were found by paper chromatography to contain a large proportion of 2,3,4-trimethylglucose. This is an indication of the predominance of the 1 → 6 linkage in the polymer.

Qualitative paper chromatography also detected considerable amounts of 2,3,6-trimethylglucose, *i.e.* 1 → 4 linkages are also present in these polysaccharides. The conclusions drawn from this work can be briefly summarised as follows.

The polysaccharides obtained by solid-phase polycondensation in the presence of metaboric acid do not contain chemically bound acid. Since hydrolysis yielded only glucose, the polysaccharide units are bound solely by glucosidic bonds. The polymer is considerably branched, and mainly contains 1 → 6 and 1 → 4 linkages between the units of the macromolecule. It contains both α- and β--glucosidic bonds, without either of them predominating.

4. Polycondensation in the Presence of Dehydrating Agents

A number of investigations in recent years were devoted to polycondensation of glucose in the presence of thionyl chloride. London and his collaborators[57,58] subjected glucose to polycondensation at 110° for one hour in the presence of thionyl chloride. Thionyl chloride served to bind the water formed in the reaction, generating hydrogen chloride, itself a catalyst in glucose polycondensation.

This method for the removal of water appeared, however, to be less effective than the continuous removal of water in a high vacuum. This is revealed by the fact that the average degree of polymerisation of the products did not exceed 20. Periodate oxidation showed that 58% of all the glycosidic bonds were of the 1 → 6 type. From infra--red absorption spectra, it followed that the units were mainly linked by α-(1 → 6) glucosidic bonds, less than 5% being of the α-(1 → 3) type. This polysaccharide (of relatively small molecular weight) has been used as an inhibitor of blood coagulation.

Kent[59] carried out the polycondensation of glucose at 100° – 150° in the presence of thionyl chloride containing traces of water or alcohol. The mean degree of polymerisation of the resulting polysaccharides was 25. The polymer is completely hydrolysed by 1 *N* HCl, yielding glucose.

Ricketts and Rowe[60] carried out the polycondensation of glucose, galactose, maltose, lactose, and a glucose–galactose mixture, by passing dry hydrogen chloride as a water-binding agent through powdered carbohydrates, and stirring. The resulting paste was left for five days. After neutralisation and dialysis, the degree of polymerisation was found to be 20. After partial hydrolysis by dilute acid, the polysaccharide was chromatographed on paper. The α-(1 → 6) bonds were found to predominate.

5. Polycondensation of Glucose in the Presence of Polymeric Cation-Exchangers

It was shown recently that cation-exchangers can be used for the hydrolysis of water-soluble polysaccharides[61,62]. Such investigations are very promising from the theoretical as well as the practical standpoint. Hydrolysis of polysaccharides in the presence of a polyelectrolyte, which is insoluble in the reaction mixture and can easily be removed from the reactions products, may become one of the most efficient industrial methods for the hydrolysis of water--soluble polysaccharides, *e.g.* starch. The amount of catalyst is practically unchanged in this process. Although the cation-exchangers catalyse the cleavage of the glycosidic bond, under a different set of conditions they may serve as catalysts for the formation of that bond. The synthesis of polysaccharides from monosaccharides in the presence of cation-exchangers was first carried out by the Hungarian scientists Zemplen and Kisfaludy[63]. If 280 g of glucose, 160 ml of water, and 20 g of polymeric benzenesulphonic acid resin are heated to 70° for 72 h, a polycondensation equilibrium is established. The authors separated gentiobiose from the complex mixture of reaction products.

Colla and Lee[64] used a series of polymeric cation--exchangers for the synthesis of polysaccharides from monoses, *e.g.* the following: Zeokarb-215, Zeokarb-225, IR-100(H), and IR-125(H).

The equivalent amounts of glucose and a cation--exchanger were mixed and heated to 100° for 15 min. In the solution of the resulting polysaccharides isomaltose was identified by paper chromatography. The yield of polysaccharides increased on increasing the reaction time. The composition of the reaction products has not been studied in detail.

Recently, Japanese workers[65] isolated a biose from the products of polycondensation of glucose in the presence of a cation-exchange resin.

B. SYNTHESIS OF POLYSACCHARIDES BY THE CONVERSION OF RINGS INTO LINEAR POLYMERS

In addition to the polycondensation of glucose in a high vacuum, another method has been developed for obtaining polysaccharides, namely the conversion of rings into linear polymers. By this method polymers are obtained from three-membered rings (ethylene oxide, ethyleneimine, epoxy compounds, etc.), as well as seven-membered rings, *e.g.* caprolactam. The principles of the conversion of cyclic compounds into linear polymers were systematically studied in the polymerisation of caprolactam and other heterocyclic compounds by Knunyants, Rogovin, Strepikheev *et al.*[66-68]. The same method was used for the synthesis of polysaccharides from anhydromonoses, *e.g.* laevoglucosan.

By this method branched polymers can be obtained owing to the presence in the monose molecule of several free hydroxyl groups. So far, only one type of strained-ring monose, laevoglucosan, has been employed in this reaction, but there is no reason why a series of different monoses, whose polymerisation would result in new types of synthetic polysaccharides, should not be used in a systematic study.

Pictet and his collaborators showed 40 years ago[69-79] that in a high vacuum, and in the presence of catalysts, anhydrosugars are capable of polymerisation. For instance, on heating laevoglucosan (1,6-anhydro-β-D-glucopyranose) in the presence of platinum black, dimers, octamers, and other polysaccharides are formed. It has been shown[74] that a small fraction of the reaction products does not pass through a semipermeable membrane and must consequently have a rather high molecular weight. Somewhat later the Pictet method was used for the synthesis of oligosaccharides from laevoglucosan[80,81]. At that time modern physicochemical methods for studying polymers were not available, and the high-molecular-weight fractions were therefore not investigated.

Carvalho *et al.*[82] recently made an attempt to polymerise laevoglucosan using procedures developed by Pictet[69-79] and other workers[80,81]. They carried out the polymerisation at 100° – 130°, and used 0.001 to 0.05 mole of catalyst per mole of monomer. The catalysts employed were HCOOH, CH_3COOH, HCl, oxalic acid, H_3PO_4, $ClCH_2COOH$, and $ZnCl_2$. Some experiments were carried out in the

presence of solvents (dimethyl sulphoxide or tetramethyl sulphone). At 120°, and in the presence of monochloroacetic acid, an amber-coloured polymer, readily soluble in water, was obtained. In spite of its relatively high molecular weight (38 000–90 000) the preparation had low viscosity, which was an indication of the spherical form of the macromolecule, *i.e.* of extensive branching. Investigation of the structure of this product by periodate oxidation gave various positions for the glycosidic bonds, 1 → 6 and 1 → 4 predominating. The specific rotation was +90°, which is evidence for the prevalence of α-glycosidic linkages. Carvalho *et al.*[82] suggested that the polymerisation of laevoglucosan proceeded *via* the formation of an intermediate anhydro-compound of the α- or β-(1 → 2)-anhydroglucopyranose type. This is consistent with the data obtained in other investigations on the conversion of cyclic compounds into polymers[83,84]. The mechanism of the polymerisation and the structure of the resulting synthetic polysaccharides suggested by Carvalho are given in Fig.3.

According to the scheme in Fig.3, on ring opening, reaction takes place between the resulting free bond and one of the three hydroxylic groups of another monomer molecule. As a result, as already shown by Pictet[70] in the hydrolysis of synthetic polysaccharides, only glycosidic bonds are formed between the polysaccharide units.

Wolfrom *et al.*[85] described a method for the thermal polymerisation of laevoglucosan in the absence of catalysts.

They showed earlier[86] that heat treatment converts the α-(1 → 4) glycosidic bond in starch into α- and β-D-(1 → 6) glycosidic linkages, a few β-D-(1 → 2) linkages being formed simultaneously. Considerable amounts of 1,6-anhydro-β-D-glucopyranose (laevoglucosan) were formed under such conditions. Since this process takes place almost instantaneously at 200° in the absence of water or acid, clearly neither hydrolysis nor polycondensation occur in this case. The polymer obtained in the thermal polymerisation of laevoglucosan was, after partial hydrolysis, subjected to chromatographic analysis. The acetates of the following sugars were isolated: gentiobiose, isomaltose, maltose, cellobiose, and laevoglucosan. It was thus established that the polysaccharides contained the following linkages: α- and β-(1 → 6); α- and β-(1 → 4); and β-(1 → → 2). A possible reaction scheme of the polymerisation of pyrodextrins is given in Fig.4.[85]

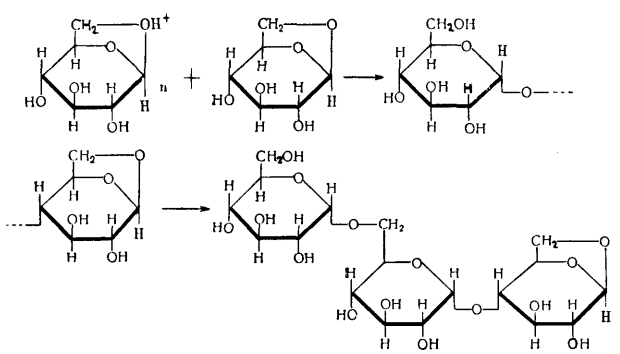


Fig. 3. Polymerisation of laevoglucosan in the presence of acid catalysts.

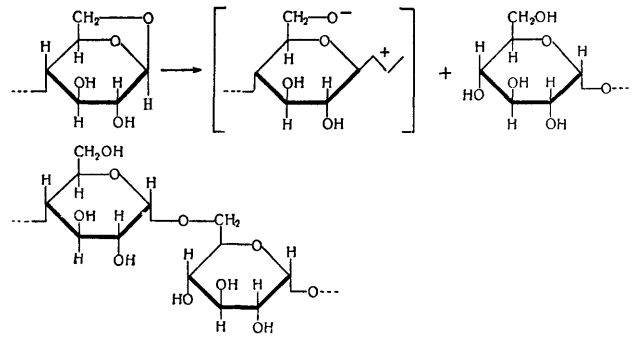


Fig. 4. A reaction scheme for the thermal polymerisation of laevoglucosan.

Korshak[172] recently studied the polymerisation of laevoglucosan and its ethers in dioxane solution in the presence of a series of catalysts. The most effective catalysts were ferric chloride, aluminium chloride, benzenesulphonic acid and the boron fluoride-ether compound. The work included a detailed investigation of the physicochemical properties of polymers and of the mechanism of polymerisation in the presence of the boron fluoride-ether compound.

To sum up, no method for the polycondensation of monoses or the polymerisation of anhydromonoses has as yet become available which would yield polysaccharides with a regular linear structure similar to that of cellulose, amylose, and other natural high-molecular-weight polyoses. By the methods available at present polysaccharides of high molecular weight, but also extensively branched are obtained. The formation of branched polysaccharides is fully understandable since the starting monoses contain 4–5 reactive functional groups. That is why new methods of synthesis should be developed for the preparation of linear polyoses containing only one type of linkage, *e.g.* 1 → 4, or monoses containing only two reactive functional groups should be used.

The highly branched polysaccharides contain exclusively glycosidic linkages between the units, which is confirmed by the composition of hydrolysis products.

Chromatographic and optical analyses showed that α-(1 → 6) glycosidic bonds predominate in synthetic polysaccharides. This corroborates the observation made previously[87] that in acid media the primary hydroxyl groups are more reactive than the secondary, and that in alkaline media the contrary is true. This is the reason for the prevalence of 1 → 6 linkages (isomaltose or gentiobiose type) in the polycondensation products.

Konkin[88] and others[18,89] showed that the 1 → 6 linkage is more resistant to the action of hydrolysing agents than the 1 → 4 linkage. The hydrolysis rate of disaccharides containing 1 → 6 linkages is almost half that of maltose. These data also show that the 1 → 6 linkage is more readily formed in polycondensation in acid media, and that is considerably more difficult to cleave than other types of glycosidic linkage.

Irrespective of their molecular weight, synthetic branched polysaccharides are readily soluble in water†. Synthetic polyoses are atactic polymers, in contrast to natural polysaccharides which have an isotactic or syndiotactic structure. It should be noted, however, that many of the preparations obtained have not been investigated by modern chemical and physicochemical methods.

III. NEW METHODS FOR THE INVESTIGATION OF SYNTHETIC POLYSACCHARIDES

Thanks to the application of new methods of study, the composition and structure of several synthetic polysaccharides have recently been determined. Although isomaltose was discovered by Fischer at the beginning of the twentieth century, the structure of this disaccharide has till recently been the subject of a good deal of controversy [91]. Only by selective partition in adsorption chromatographic columns or by the application of paper chromatography was it possible to show that the product which Fischer considered as a biose of strictly determined structure, and which he named isomaltose, was not a homogeneous product, but consisted of stereoisomeric (1 → 6)-bioses. Isomaltose was isolated by partition chromatography and its chemical structure was established [35,92].

For almost 50 years mainly the classical methods have been used for the demonstration of the structure of carbohydrates [92-94] (ref. 67, pp. 512–520).

Although these methods retain their significance today, and have scored considerable achievements in this complex field of organic chemistry, they have often been unable to provide the necessary data on the structure of various bioses and especially the high-molecular-weight polysaccharides.

Many new physicochemical and physical methods have recently come to be widely used in polymer chemistry for the investigation of the structure of various polysaccharides, particularly the synthetic ones. Below we discuss the results obtained by some of these methods in determining the chemical and physical heterogeneity of polysaccharides, their molecular form, molecular weight, and the type of linkage between the units.

(a) Determination of the chemical heterogeneity of bioses and higher polyoses. Separation by chromatography has at present acquired great significance. The chromatographic separation of various isomeric bioses formed in the polycondensation of glucose has lately been elaborated and used by a number of workers [29,95]. Especially good separation has been achieved in columns packed with activated charcoal or zeolite [96,98].

In certain cases solutions of polysaccharide acetates, rather than those of the polysaccharides themselves, were used, since the former are more readily separated. Oligosaccharides of high molecular weight were not simply and successfully separated in columns until 1957–1959, when cellulose preparations were used as sorbents [99,100].

Paper chromatography is the simplest method for determining the chemical heterogeneity of low-molecular-weight polysaccharides. Many workers have lately used exclusively this method for the analysis and identification of complex product mixtures obtained in the polycondensation of monoses [30-34,56,101]. A wide variety of solvents and developers was used. Irrespective of the composition of the reagents used, fairly accurate and reliable results were obtained. Good results were also obtained in the application of this method for the separation of the products of the partial hydrolysis of synthetic polysaccharides [60].

(b) Determination of the molecular size distribution in synthetic polysaccharides. The usual fractionation methods are employed in the majority of cases for the determination of the molecular size distribution in these polymers: (1) fractional precipitation by the addition of methanol or ethanol to aqueous solutions of synthetic polysaccharides (Mora *et al.* used this method with success [44,45,102]); (2) fractional dissolution (a sample of the preparation is treated with aqueous ethanol or methanol of varying concentration [103]); (3) dialysis for the separation of low-molecular-weight fractions.

Very few data are available so far on the solubility of various mono- and poly-saccharides in organic solvents and their mixtures. One paper [104] contains data on the good solubility of carbohydrates in dimethyl sulphoxide and tetramethyl sulphone. Without suitable solvents the synthetic polysaccharide preparations cannot be isolated nor purified for subsequent investigations. The separation of catalysts from the resulting synthetic polysaccharides presents special difficulties. The methods used by various workers were relatively complex and involved considerable losses of the reaction products. One of the authors of this review [105] recently worked out a simple method for the separation of mono- and poly-saccharides of various degrees of polymerisation from inorganic impurities by extraction with anhydrous pyridine.

(c) Determination of the form of the macromolecule. The products obtained in the polycondensation of monoses are highly branched and the determination of the form of the macromolecule is of considerable importance both from the viewpoint of the physical chemistry of these polymers, as well as biological simulation, since the form of the macromolecule has a considerable effect on the biological properties of polymers.

To determine the form of the macromolecules of his synthetic polysaccharides, Mora [106] used the usual methods of polymer chemistry for the determination of the degree of branching: (a) comparison of number-average molecular weights (determined by the osmotic and end-group methods) with weight-average molecular weights (determined by viscosimetric and light-dispersion methods); (b) oxidation of polymer preparations by periodic acid [107,108].

(d) Spectroscopic methods for the determination of types of linkage in synthetic polysaccharides. The type of linkage between the units in polyoses (α or β), and the presence of pyran or furan rings, as well as of free hydroxyl groups in them, have recently been determined simply and fairly accurately by physical methods [27,57,109].

It was established [57] that the characteristic absorption band of the α-glycosidic linkage is 844 ± 8 cm^{-1}, and of the β-glycosidic band 891 ± 7 cm^{-1}. These values have been checked on various synthetic glucosans and on natural polysaccharides.

† Using the same methods Mora *et al.*, [90] recently obtained a polymer of 2-deoxyglucose, which is sparingly soluble in water and does not hydrolyse. It has not been studied in detail.

In his study of the infra-red absorption spectra of dry synthetic polysaccharide films, Durand[56] established that the polymers obtained by solid-phase polycondensation of glucose in the presence of metaboric acid contain, alongside other types of glycosidic linkage, a considerable number of the α-(1 → 6)-glycosidic bond, with its characteristic absorption maximum at 760–960 cm^{-1}. Finan and Reed[110] recently described the use of the mass-spectroscopic method for the determination of α- and β-glycosidic bonds in various sugars (maltose, cellobiose, laminaribiose, etc.). It was shown that the energy of dissociation of methyl-β-D-glucopyranoside is 0.6 eV higher than the energy of dissociation of methyl-α-D-glucopyranoside.

Electrophoresis in the presence of borate buffers[33,111] gave good results in the separation of various isomeric bioses and polyoses.

Enzymic analysis can also be used for the determination of α- and β-glycosidic links between polysaccharide units, as well as the number of terminal aldehyde groups[112-114].

The most successful of all the chemical methods recently used is periodate oxidation combined with mild hydrogenation. Individual stable polysaccharide fragments have been obtained by this method. By further study of these fragments, the structure of the polyose could be determined. All these questions have recently been discussed in reviews[112,113].

IV. PRINCIPAL USES OF SYNTHETIC POLYSACCHARIDES

After the discovery of the most important biological polysaccharides such as heparin, chondroitin, etc., and the investigations of the role they play in living organisms, attempts were made to prepare their synthetic analogues and substitutes[116]. As a result of the systematic investigations of synthetic polysaccharides and especially their derivatives containing a sulphate or carboxyl group, a number of preparations were obtained which could find practical applications in the following fields: (1) as blood plasma substitutes; (2) as substitutes for heparin and chondroitin in biological processes (heparin can inhibit the coagulation of blood by forming an inactive complex with thrombokinase); (3) as inhibitors of a series of enzymic processes; (4) as selective adsorbents for the separation of proteins and other compounds; (5) as water-soluble polyelectrolytes.

The first polysaccharide preparations used for biological purposes were derived from cellulose. In 1936 Chargaff *et al.*[115] and later Karrer[116] prepared cellulose sulphates which had heparin-like properties (against thrombosis).

In 1943 Kenyon *et al.*[117] showed that the products of cellulose oxidation containing a carboxyl group in position 6 had styptic properties. A detailed investigation has been published recently on the preparation of monocarboxycellulose and its application in medicine[118]. The same preparations were also used as blood plasma substitutes and as carriers of various physiologically active preparations[119].

Since cellulose sulphates were found to be toxic, the soluble cellulose ethers, *e.g.* monoglycolic ether and others[120,121] have been tested. The sulphates of glycogen and starch[122,123], dextran[124], polyvinyl alcohol[125], xylan[126], and chitin[116] have similar properties.

The majority of these substances are toxic owing to their precipitating effect on fibrinogen. That is why, in addition to preparations obtained by enzymic processes (dextrans), attempts were made to synthesise the water-soluble polysaccharides, and obtain from them blood coagulation inhibitors and other biologically important compounds. London *et al.*[57] prepared a sulphate derived from synthetic polyglucosans with γ = 176. This preparation had the heparin-like property of acting as blood coagulation inhibitor.

Similar products were obtained by Kent and others[59,60]. Their preparations had small molecular weights (degree of polymerisation 20–30), and could only be used as blood anticoagulants. They were not suitable as plasma substitutes, where polymers with molecular weights of 70 000–100 000 are required[127]. In contrast to these preparations, the polysaccharides obtained by Mora *et al.*[45] had high molecular weights and a varying degree of branching. By their fractionation a series of preparations of varying molecular weight and degree of branching was obtained.

Since many biological preparations have a constant mean molecular weight (*e.g.* enzymes), the molecular weight of the synthetic polysaccharide used and the configuration of its macromolecule should obviously have a considerable effect on its biological properties. Mora[45] showed that polyglucosans whose molecular weight is less than 75 000 are inactive biologically and can only be used as blood anticoagulants.

In one of their communications, Mora and his collaborators[128] described synthetic polysaccharide preparations obtained by high-vacuum polycondensation, which had different molecular weight and degrees of branching and in which hydroxyl hydrogen was substituted by sulphuric acid residues to different extents. The preparation of dextran sulphates had been described earlier[124,129-132]. The preparations were obtained in the form of sodium salts and were converted to acids on ion-exchange resins, *e.g.* Amberlite IR-120(H). Preliminary investigations showed that they had the same anticoagulant action as heparin[128], under the same conditions[133,134].

On interaction with macroanions proteins form insoluble complexes[135-137]. The insoluble complexes between proteins and polysaccharide sulphates, which are macroanions, were used for the fractionation of the former[137]. Macroanions also react with enzymes and inhibit various biochemical processes[137].

Mora[138] explains this inhibiting effect by the formation of inactive complexes of the protein with polyelectrolyte, a process which seems to be similar to the precipitation of antigens by antibodies. By titrating a synthetic polysaccharide, (mol.wt. 18 300) containing three free sulphate groups in a single unit, with a protein containing a fixed amount of basic groups (*e.g.* polymixin B), it was established that the two reacted in a stoichiometric ratio.

Adrenocorticotropic hormone in aqueous solution at pH 3.4 was not precipitated on addition of sulphuric acid solution, but on titration with a solution of polysaccharide sulphate the precipitate of the hormone-polysaccharide complex appeared at pH 1. Precipitates are also formed in reactions of synthetic polysaccharide sulphates with protamine, lysozyme, ribonuclease, and neomycin B[138].

Carboxy-derivatives of synthetic polysaccharides were tested as blood anticoagulants[139]. They were prepared by oxidation of synthetic polysaccharide with sodium periodate, followed by chlorite oxidation of the resulting dialde-

hyde to dicarboxy-derivatives[140,141]. By changing the reaction conditions, preparations with varying amounts of carboxyl groups (from 3 to 28%) could be obtained. The preparations had weak anion-exchanging properties. The advantage of these preparations over polysaccharide sulphates is their lower toxicity. Their molecular weight is practically the same as that of the starting polysaccharides. The carboxy-derivatives of polysaccharides were also used as inhibitors of various enzymes, such as lysozyme, ribonuclease, and other polyampholytes.

Competitive inhibition of enzymes by derivatives of synthetic polysaccharides containing acid groups. The nature of the interaction between macromolecules carrying opposite charges, which alters the solubility of the initial polymers, is of great importance in certain enzymic reactions. This is the main basis of the inhibition of enzymes by polyelectrolytes[137], demonstrated clearly on lysozyme[142], ribonuclease[143-147], hyaluronidase[148-151], etc.[152-158]. Since polyelectrolytes react with proteins to form insoluble complexes, this method is widely used for the fractionation of proteins in serum[135,159-161]. Polyelectrolytes are often polyampholytes, and the pH of the medium is very important.

In recent years systematic investigations have been undertaken on the interaction of various synthetic polyelectrolytes with serum albumin[135,159-161], γ-globulin[160], β-lipoprotein[136,162], fibrinogen[163], and other proteins and nucleic acids[164-169].

This work established the importance of the influence of the chemical composition and properties of polyelectrolytes on the inhibition of enzymes *in vivo* and *in vitro*[137]. Synthetic polysaccharides of varying degree of polymerisation and branching obtained by varying the reaction conditions, were used in model studies of the competitive inhibition of enzymic processes. Mora *et al.*[170] made a study of the reaction of synthetic polysaccharide sulphates of varying molecular weight and extent of branching with lysozyme, ribonuclease, protamine, pancreatic peptide, polymixin B, neomycin, and cytochrome *c* from the albumin serum of man. The competitive inhibition of hyaluronidase and ribonuclease by these polysaccharide derivatives has also been studied. It was established that synthetic polysaccharide sulphates are stronger inhibitors than those used earlier[142], such as glutamyl peptide and DNA (deoxyribonucleic acid). Data on the inhibiting action of polyglucosan preparations on lysozyme activity are given in Table 2.

TABLE 2.* Inhibition of the activity of 60 g of lysozyme by synthetic polysaccharide derivatives[170].

Degree of substitution y	Polyglucosan sulphate ($PGSO_3^{2-}$)								Carboxy-derivs. of polyglucosan ($PGCOO^-$)	
	B		a		b		C		F	G
	300		270		120		60		amount of preparation, μg	% inhibition
	amount of preparation, μg	% inhibition	amount of preparation, μg	% inhibition	amount of preparation, μg	% inhibition	amount of preparation, μg	% inhibition		
	10	100	10	100	10	100	20	100	50	100
	5	80	5	98	5	68	10	91	25	70
	2.5	66	2.5	65			5	41		

* Preparations B, a, b, C, F, and G differ in molecular weight and extent of branching. For particulars about these preparations see refs. 45 and 128.

It is clear from Table 2 that with increased branching (*e.g.* preparation a) the inhibiting effect of these preparations increases. With a higher extent of branching preparations of lower molecular weight can be used to obtain complete inhibition. The inhibiting activity of several highly branched preparations is four times higher than that of heparin. Similar results were obtained in investigations of the effect of branching of synthetic polyoses on the inhibition of ribonuclease activity[171].

The inhibiting effect of synthetic polysaccharide sulphates, being a result of the interaction of acid groups in the polysaccharide with basic groups in the protein, can be removed by substituting the protein in the protein-polysaccharide sulphate complex with another, more basic protein. For instance, if the ribonuclease-polyglucose sulphate is acted upon by lysozyme, the inhibiting effect of the sulphate is counteracted owing to its interaction with lysozyme which is a stronger base. Free ribonuclease is thereby made available again, with unaltered enzymic activity.

Carboxy-derivatives of polysaccharides showed under similar conditions a lower inhibiting activity owing to their low degree of dissociation.

In vivo carboxy-derivatives of synthetic polysaccharide preparations cause detoxification of basic macromolecules, *e.g.* polymixin B, which is more effective the higher the extent of branching and the higher the content of carboxyl groups. The activity of adrenocorticotropic hormone increases in the presence of carboxyl-containing polyglucosans[140].

The studies made so far on the synthesis of polysaccharides and their biological uses have not exhausted the numberless possibilities for the preparation of synthetic polysaecharides nor the fields of their application. Systematic studies in this interesting and complex field of chemistry and biochemistry are still in the process of development, and will undoubtedly lead to further remarkable practical and theoretical achievements.

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THE CAUSES OF THE STABILITY OF EMULSIONS

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CONTENTS

I. Stability of emulsions stabilised by soaps
II. Stability of inverse emulsions
III. Stabilisation of emulsions by substances other than soaps

A great number of investigations has been devoted to the causes of the stability of emulsions to aggregation, nevertheless, at the present time, there is no single opinion even on the causes of the stabilility of the classical emulsions stabilised by ordinary soaps. An attempt is made in