Preparation and Structure of Synthetic Hexose Polymers.

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A polyglucose can be prepared by deliquescence of glucose crystals over concentrated hydrochloric acid (Ricketts, *J.*, 1954, 4031) or more conveniently by the action of dry gaseous hydrogen chloride on glucose monohydrate. The infrared absorption spectrum resembles that of unbranched dextran and indicates the presence of α-1 : 6-linkages. This was confirmed by paper chromatography and ionophoresis in borate buffer of polyglucose hydrolysates and by the isolation of *iso*maltose as its crystalline octa-acetate. Ionophoresis applied to the polymerisation mixture showed the presence of maltose as well as *iso*maltose, so that α-1 : 4-linkages probably occur in the polyglucose. Polymers have been prepared from other sugars. The polymer from maltose closely resembles polyglucose, suggesting that an equilibrium of all available glucosyl groups contributes to the polymerisation and that appreciable amounts of intact disaccharide are not incorporated into the polymer.

THE action of hydrochloric acid on glucose yields a mixture containing disaccharides, higher saccharides, lævoglucosan, and various decomposition products. Choosing conditions giving the maximum yield of disaccharides, so as to reveal the relative proportions of each type of linkage, Thompson, Anno, Wolfrom, and Inatome (*J. Amer. Chem. Soc.*, 1954, **76**, 1309) found *iso*maltose and gentiobiose to be the major products, thereby confirming the work of earlier investigators. Cellobiose and maltose were formed in moderate amounts, so was lævoglucosan. Small amounts of sophorose and trehalose were found. Neither laminaribiose nor nigerose was detected. Thus it appears that the principal types of linkage to be expected in polymers formed from glucose under the catalytic influence of hydrochloric acid are the 1 : 6- and 1 : 4-linkages in α- and β-forms. The possibility that other linkages and anhydro-rings occur in polyglucose cannot be discounted.

The kinetics of the reaction of glucose in hydrochloric acid solution have been investigated several times but in connection with the experiments described in this paper Moelwyn-Hughes's observation (*Trans. Faraday Soc.*, 1928, **24**, 321) that the rate of reaction increases with glucose concentration is significant. Experimental evidence indicated that the reaction was of the pseudo-unimolecular type.

Myrbäck, Hammarstrand, and Gelinder (*Arkiv Kemi*, 1950, **1**, 235) isolated tetrasaccharides containing 1 : 6- and other types of linkage from a solution of glucose in hydrochloric acid. Pacsu and Mora (*J. Amer. Chem. Soc.*, 1950, **72**, 1045) obtained saccharides consisting of about 40 glucose units by evaporation of a solution of glucose in hydrochloric acid to dryness. During the latter stages of this evaporation the molecules may be pictured as being brought closer together in the presence of acid, bonds being formed between them. An alternative means of achieving the conditions of polymerisation was reported in a preliminary communication (Ricketts, *J.*, 1954, 4031). Crystalline anhydrous glucose was allowed to deliquesce in an atmosphere containing water vapour and hydrochloric acid. Under these conditions the molecules, which are as close together as they can be in the crystal, move further apart and pass through the optimum distance for bond formation while under the catalytic influence of acid. A saccharide containing about 20 glucose units and having predominantly 1 : 6-linkages was obtained in 17% yield. The investigation has now been extended to other sugars and a more satisfactory method of carrying out the reaction has been found.

Crystalline glucose monohydrate was found to absorb dry gaseous hydrogen chloride rapidly. By starting with glucose monohydrate the initial water content was defined. The reaction was conveniently carried out in a rotary mixer. Dry hydrogen chloride was passed into about 100 g. of glucose monohydrate, the crystalline powder forming a plastic mass in about 30 minutes. Polymerisation then continued without further addition of hydrogen chloride. Preliminary experiments showed that the maximum degree of

polymerisation was reached in between 1 and 5 days and the latter time was adopted for preparative purposes. The total yield of soluble non-dialysable material was about 45%. The Table shows the properties of two typical polyglucose preparations, P 2 and P 3.

*Properties of hexose polymers.**

	Yield				
	g.	%	$[\alpha]_D^{20}$	$[\eta]$	Reducing value
Polyglucose (P 2)	26·6	32·5	+111° (+52·7°)	0·035	18·4
Polyglucose (P 3)	24·6	30·0	+107 (+52·7)	0·032	16·9
Polyglucose (P)	—	—	+125 (+52·7)	0·033	19·7
Polygalactose	2·8	6·3	+168 (+80·2)	0·032	24·9
Polymaltose	16·0	36·1	+133 (+137)	0·036	20·1
Polylactose	9·9	21·1	+140 (+55·4)	0·034	29·7
Polyglucose-galactose	7·3	21·7	+139	0·036	38·2

* Mutarotation equilibrium values for the original sugar are shown in parentheses. Yields are corrected for moisture content as determined by heating at 100°/0·1 mm. to const. wt. The reciprocal of the reducing value is the reducing power relative to glucose (Shaffer and Hartman, *J. Biol. Chem.*, 1921, **45**, 377).

The products from five such polymerisations were combined and fractionally precipitated from ethanol–water to eliminate short-chain saccharides. The polyglucose (P) so obtained had a somewhat higher optical rotation than the other samples, see Table. The infrared spectrum of polyglucose (P) showed absorption of type 2*a* at 834 cm.$^{-1}$ (Barker, Bourne, Stacey, Whiffen, *J.*, 1954, 171) and no absorption of type 2*b* (890 $\pm$ 5 cm.$^{-1}$), from which it was concluded that the links between glucose units were of the α-form. The absorptions of type 1 at 914 cm.$^{-1}$ and type 3 at 760 cm.$^{-1}$ showed that the links involved positions (1) and (6) of the pyranose ring. No 1 : 3-links were detected, there being no absorption maximum at 793 $\pm$ 3 cm.$^{-1}$; also no absorption maxima at *ca.* 800 cm.$^{-1}$ associated with furanose rings or 3 : 6-anhydroglucopyranose derivatives were detected. The spectrum was very similar to that of a dextran containing no 1 : 3-linkages.

Polyglucose (P) yielded on oxidation with sodium metaperiodate, 0·72 mole of formic acid per anhydroglucose unit. The interpretation of this result is difficult since the reducing end-group yields several mols. of formic acid, necessitating a large correction for a polymer with relatively short chain-length. The yield of formic acid is less than the theoretical, one mol. for a chain with 1 : 6-links only, by 0·28 mol. Therefore it seems reasonable to assume that at least 28% of the glucose residues are involved in linkages other than the 1 : 6-link. Such linkages may occur at a branching point, in the main chain, or in an anhydro-ring.

Partial hydrolysis of polyglucose in acid, followed by paper chromatography of the de-salted hydrolysate, showed spots at R_G values corresponding to glucose, *iso*maltose, *iso*maltotriose, and *iso*maltotetraose. Dextran hydrolysates, prepared in the same way, were used as controls on the chromatogram. A control experiment showed that under the conditions of hydrolysis only a trace of disaccharide and no higher saccharides were formed from glucose. Some of the higher saccharides from dextran were clearly defined on the chromatograms but the corresponding higher saccharides from polyglucose could not be resolved in repeated attempts. Measurements of electrical conductivity showed that the solutions had been adequately de-salted. This lack of chromatographic resolution may be interpreted as an indication that in the higher saccharides there is an increasing complexity due to the permutation of several types of linkage. Such complexity is necessarily absent from the lower saccharides in a polyglucose hydrolysate and does not arise to the same extent in natural polysaccharides on account of the repeating patterns formed by enzymes.

To obtain supporting evidence the hydrolysis was repeated on a larger scale and the products were separated on a charcoal column (Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 607). Glucose, characterised as the β-penta-acetate, and *iso*maltose, characterised as the crystalline octa-acetate, were isolated. A third fraction was obtained which on paper chromatography showed spots corresponding to *iso*maltotriose and *iso*maltotetraose. Hence blocks of four consecutive 1 : 6-linked glucose units occur in polyglucose.

Information about other types of linkage likely to be present in polyglucose was obtained by subjecting the products of incomplete polymerisation to separation on a charcoal column. On elution with 8% v/v ethanol and freeze-drying a white solid was obtained. Paper chromatography showed a spot with an R_F value identical with that of glucose and a second spot with an R_F value close to that of *iso*maltose. Electrophoresis of the same material in borate buffer showed four spots with M_G values corresponding to glucose, gentiobiose, *iso*maltose, and maltose. Thus, by examination of the products of incomplete polymerisation, the presence of linkages so sensitive to acid hydrolysis that they escaped detection in hydrolysates was revealed. For instance, maltose is hydrolysed four times as rapidly as *iso*maltose (Wolfrom, Lassettre, and O'Neil, *J. Amer. Chem. Soc.*, 1951, **73**, 595).

No polymeric material could be obtained from xylose, fructose, or sucrose on account of the rapid decomposition of pentoses and ketoses under strongly acidic conditions.

Extension of the investigation to disaccharides presents interesting possibilities since the action of hydrochloric acid could lead to incorporation of the intact disaccharide into the polymer or to hydrolysis of the disaccharide to an equilibrium mixture in which all available glycosyl groups participate.

The polymer from maltose was compared with polyglucose. The optical rotation and chain length of polymaltose were slightly greater than the corresponding values for polyglucose as shown in the Table. Paper chromatography of polymaltose hydrolysates showed spots with R_F values corresponding to *iso*maltose and *iso*maltotriose. Oxidation with sodium metaperiodate yielded 0·71 mole of formic acid per anhydroglucose unit, very close to the yield obtained from polyglucose under the same conditions. A lower value would be expected if polymaltose contained more 1 : 4-linkages than polyglucose. The infrared spectrum of polymaltose showed absorption of type 2*a* at 837 cm.$^{-1}$ associated with α-glucopyranose links. Absorption of type 1 at 918 cm.$^{-1}$ showed that long sequences of α-1 : 4-links did not occur. The type 1 absorption together with the type 3 absorption at 765 cm.$^{-1}$ provided a strong indication that the principal link was α-1 : 6 in type. Again there was no absorption maximum at 793 $\pm$ 3 cm.$^{-1}$ corresponding with 1 : 3-links. Thus the spectrum of polymaltose was similar to, but not quite identical with, that of polyglucose.

Polymers were readily obtained from galactose, from lactose, and from a mixture of galatose with glucose, as shown in the Table. Their structures are being examined.

We are greatly indebted to Dr. M. Heidelberger who tested the serological reactivity of some of these polysaccharides (cf. Heidelberger and Aisenberg, *Proc. Nat. Acad. Sci. U.S.A.*, 1953, **39**, 453; Heidelberger, Aisenberg, and Hassid, *J. exp. Med.*, 1954, **99**, 343). Polymaltose reacted more heavily with pneumococcus Type XII antiserum than polyglucose but both left some antibody reactive with glycogen. There were no precipitates with the Type II antisera used. Polygalactose showed a small but definite precipitate in Type IV antiserum only after long standing and centrifugation; possibly the quantity of polygalactose used was too large in this experiment.

Experimental

Paper Chromatography.—All chromatograms were run on Whatman No. 1 or No. 54 paper in butanol–pyridine–water (6 : 4 : 3 by vol.) (Myrbäck and Willstaedt, *Arkiv Kemi*, 1953, **6**, 417) and sprayed with aniline hydrogen phthalate (Partridge, *Nature*, 1949, **164**, 443).

Polyglucose.—Finely ground glucose monohydrate (9·14% of H_2O) (100 g.) was placed in the rotary mixer into which a delivery tube passed through a glass–glass socket lubricated with Silicone grease. Dry hydrogen chloride was passed through while the mixer rotated for 30 min. The gas was rapidly absorbed and the powder became sticky, adhered to form pellets, and finally became a plastic mass. Rotation was then stopped and the apparatus closed for 5 days. The viscous mass was then dissolved in ice-cold N-sodium hydroxide solution, neutralised, dialysed, and decolorised with charcoal. On evaporation under reduced pressure to 92 ml. a small quantity of insoluble material separated and was rejected. Addition of ethanol (92 ml.) precipitated a syrup which was hardened in ethanol, washed with ether, and dried *in vacuo* over phosphoric oxide, yielding polyglucose (P 2) (see Table). The supernatant solution yielded on evaporation more polyglucose (12·0 g.) consisting of short-chain material, bringing the total yield of soluble non-dialysable polymer to 45%.

Polyglucose (P 3) (see Table) was prepared in the same way, the total yield of polymer being 46%.

Polyglucose (P) (see Table) was obtained by combining the products of five similar preparations. By careful fractional precipitation it was obtained free from saccharides mobile on a paper chromatogram.

Polygalactose.—Galactose (0·75% of H_2O) (50 g.) in a thin layer covering a dish 16 cm. in diameter was placed in a desiccator containing concentrated hydrochloric acid. The crystals slowly deliquesced to a pale brown syrup. After 7 days at room temperature the syrup was worked up as above, yielding a brown polygalactose (10·18 g.). Evaporation of the supernatant solution yielded a further 3·6 g., bringing the total yield to 31%.

The first fraction (10·18 g.) was reprecipitated from ethanol–water, yielding a fraction (5·1 g.) containing all the brown colour and a further fraction (2·8 g., dry basis) (see Table) free from coloured impurity.

Polymaltose.—Maltose monohydrate (6·21% of H_2O) (50 g.) was treated as described for galactose and the polymer was isolated in the same way, yielding polymaltose (16·0 g., dry basis) (see Table), and from the supernatant solution a further 3·0 g. (total yield, *ca.* 47%).

Polylactose.—Lactose (0·29% of H_2O) (50 g.) yielded, under the conditions described for galactose, polylactose (9·9 g., dry basis) free from coloured impurity (see Table). A further 1·6 g. from supernatant solution brought the total yield of polymer to *ca.* 27%.

Polymer from Glucose–Galactose Mixture.—Finely ground galactose (20 g.) was moistened with water (2 ml.) and after storage in a closed container for 20 hr. to attain, as far as possible, a uniform distribution of moisture, the mixture was ground with glucose monohydrate (20 g.). The mixture was placed in the rotary mixer, and dry hydrogen chloride was passed through it for 30 min. The product was isolated as described for polyglucose. At 50% v/v ethanol a fraction (7·3 g., dry basis) was precipitated. From the supernatant solution a further 4·2 g. was isolated (total yield of non-dialysable polymer, *ca.* 36%).

Polymerisation of Glucose for 19 Hours and Examination of Oligosaccharides.—Glucose monohydrate (100 g.) was exposed to anhydrous hydrogen chloride as described above. After 19 hr. the mixture was dissolved in excess of cold N-sodium hydroxide, and the solution neutralised, decolorised with charcoal, and concentrated to 37 ml. After removal of the insoluble matter (0·424 g.), polyglucose was precipitated as a syrup by the addition of ethanol (74 ml.). The syrup was ground in alcohol, washed twice with alcohol and twice with ether, and dried *in vacuo* (yield 24·51 g.; $[\alpha]_D$ +88°; reducing value 5·4).

Polyglucose (5 g.), dissolved in water (10 ml.), was passed down a column, 33 cm. long and 2 cm. in diameter, of a 1 : 1 mixture of washed B.D.H. activated charcoal and Hyflo Supercel (Whistler and Durso, *loc. cit.*). The column was washed with water and then aqueous ethanol (8%), and eluted fractions were collected and their optical rotations measured.

From the eluate a freeze-dried white powder (0·267 g.) was obtained which on paper chromatography gave a spot with an R_F value identical with that of glucose (0·43) and a second spot with an R_F value of 0·28 close to that of *iso*maltose and different from that of the laminaribiose in a laminarin hydrolysate. Electrophoresis of the same material in 0·2M-sodium borate (Foster, *loc. cit.*) showed four spots with M_G values 1·03, 0·75, 0·70, and 0·32, identical with those from a mixture of glucose, gentiobiose, *iso*maltose, and maltose.

Partial Hydrolysis of Polyglucose (P) and Chromatography.—A solution of polyglucose (0·6 g.) in water (6 ml.) was centrifuged to remove the small amount of insoluble matter. The solution (1 ml.), mixed with 2N-sulphuric acid (1 ml.), was heated in a sealed ampoule at 100° for 62 min. The cooled hydrolysate was neutralised with excess of barium carbonate, and the solid filtered off and washed. The combined washings and filtrate were concentrated to 1 ml.; the electrical conductivity was $1·05 \times 10^{-3}$ μmhos. The hydrolysate showed on paper chromatography at 35° for 18 hr. spots with R_G 1·00, 0·57, and 0·26, and the controls (dextran hydrolysate) spots with R_G values 1·00, 0·57, 0·30, and 0·15.

Effect of Conditions of Hydrolysis on Glucose.—Glucose (0·1 g.), dissolved in 2 ml. of N-sulphuric acid, was heated in a sealed ampoule at 100° for 75 min. The solution was de-salted with barium carbonate and concentrated to 0·5 ml. Paper chromatography showed only a very faint trace of disaccharide and no higher saccharides. Polyglucose hydrolysate chromatographed on the same paper showed a readily detectable quantity of disaccharide as well as of higher saccharides.

Partial Hydrolysis of Polyglucose and Separation of Oligosaccharides.—Polyglucose (P) (10·00 g.) was fractionated further by reprecipitation from its aqueous solution (30 ml.) with absolute alcohol (43·5 ml.). The precipitated syrup was poured into excess of alcohol (500 ml.),

and the white solid washed with ether and dried *in vacuo* (P_2O_5) (yield 6·38 g.; $[\alpha]_D$ +115·5°; reducing value 34·8). No saccharides mobile on a paper chromatogram could be detected.

A solution of this purified polyglucose (5·81 g.) in water (58 ml.) was heated with stirring on a boiling-water bath for 30 min. Sulphuric acid (58 ml.; 2N) was added dropwise during 5 min. The mixture was heated for a further 65 min. and then cooled rapidly. $[\alpha]_D$ was then +69·4° (*c*, 5). The hydrolysate was immediately passed down a column, 33 cm. long and 4·5 cm. in diameter, of a 1 : 1 mixture of B.D.H. activated charcoal and Hyflo Supercel. After adsorption of the hydrolysate the column was washed with water followed by water–ethanol mixtures of increasing ethanol content. After most of the sulphuric acid had been washed off the column (before the saccharides), excess of barium carbonate was added to the eluate fractions [which were slightly acid (pH 4—5·5)]. Measurement of optical rotation of the eluate showed three main peaks, and the corresponding fractions were combined, filtered, and distilled to dryness under reduced pressure. The residues were extracted successively with hot methanol and hot water (products I and II), or hot ethanol and hot water (product III). The alcoholic and the aqueous extracts were combined and from the extract of product I a white solid crystallised (810 mg.). Products II (388 mg.) and III (898 mg.) were isolated as freeze-dried powders. Product I when chromatographed on paper gave a single spot corresponding to glucose and was further characterised by conversion into β-glucose penta-acetate, m. p. and mixed m. p. 130—131°. Product II was shown by paper chromatography and borate electrophoresis (Foster, *J.*, 1953, 982) to be *iso*maltose. A portion (0·151 g.) was heated with fused anhydrous sodium acetate (0·800 g.) and acetic anhydride (1·50 ml.) at 100—110° for 1 hr. (cf. Barker, Bourne, Bruce, Neely, and Stacey, *J.*, 1954, 2395) to give a product which on repeated recrystallisation from absolute ethanol gave a crystalline acetate, m. p. 147·6—148·0° (*iso*maltose octa-acetate prepared from *iso*maltose obtained from a dextran hydrolysate had m. p. 146·2—147°). Product III when subjected to paper chromatography and to ionophoresis in borate buffer gave two spots corresponding to the *iso*maltotriose and *iso*maltotetraose in a dextran hydrolysate.

Partial Hydrolysis of Polymaltose.—Polymaltose (0·10 g.) was heated in N-sulphuric acid (2 ml.) at 100° for 75 min. in a sealed ampoule. The cooled hydrolysate was neutralised with barium carbonate, filtered, and concentrated to 0·5 ml. A spot of this solution was chromatographed on Whatman No. 54 paper and compared with dextran hydrolysate, polyglucose hydrolysate, and maltose on the same paper. Polymaltose hydrolysate showed spots with R_G values 1·00, 0·54, 0·27, and 0·16; polyglucose hydrolysate 1·00, 0·55, 0·26, and 0·15; and dextran hydrolysate 1·00, 0·52, 0·25, and 0·14; maltose showed R_G 0·73.

Periodate Oxidation of Polyglucose and Polymaltose.—Polyglucose (0·0995 g.) which had been freeze-dried and dried to const. wt. *in vacuo* (P_2O_5 at 100°) was oxidised in 100 ml. of 0·0355M-sodium metaperiodate. The formic acid produced (Jeanes and Wilham, *J. Amer. Chem. Soc.*, 1950, **72**, 2655) and the sodium metaperiodate consumed (Fleury and Large, *J. Pharm. Chim.*, 1933, **17**, 107, 196) were determined.

Polymaltose (0·0660 g.) was freeze dried and dried to const. wt. in the same way.

Polyglucose: The number of mols. of formic acid produced per anhydroglucose unit was at: 22·7 hr. 0·65; 46·0 hr. 0·71; 71·5 hr. 0·73. And the mols. of sodium metaperiodate consumed were at: 22·8 hr. 1·63; 46·2 hr. 1·59; 71·0 hr. 1·62.

Polymaltose: Corresponding figures were: for formic acid at: 22·4 hr. 0·63; 45·8 hr. 0·70; 71·3 hr. 0·72 mol.; for sodium metaperiodate consumed at: 22·5 hr. 1·57; 45·9 hr. 1·55; 70·8 hr. 1·57 mols.

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