Chemical Synthesis of Hexose Polymers

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It has long been recognized that, under suitable conditions, disaccharides may be formed chemically from monosaccharides. Fischer (1890, 1898) reported that heating of glucose with concentrated hydrochloric acid resulted in the formation of a mixture of substances including disaccharides. This process, termed 'reversion', appeared to proceed under conditions favouring the reversal of the acid hydrolysis of polysaccharides. Drew & Haworth (1927) obtained a polymer of ten units by dehydration of 2:3:4-trimethyl arabinolactone, whilst more recently Deriaz (1949) and Overend, Shafizadeh & Stacey (1951) found that distillation of methyl furanoside of 2-deoxyribose and 2-deoxygalactose furnished polymeric substances.

Reversion products, known to be formed during the acidic treatment of carbohydrates, e.g. starch, may bear little structural similarity to the parent substance, and the identification of the linkage in a disaccharide obtained by chemical hydrolysis does not indicate, without further evidence, the occurrence of that linkage in the original polysaccharide.

The formation of reversion products can be represented diagrammatically as follows:

Reaction I represents the common hydrolytic cleavage of polyglycosides, favoured by low concentrations of carbohydrate, low concentration of acid and low temperatures. Sadovyl (1939) showed that the rate of hydrolysis was proportional to the concentration of acid present and the evaluation of the rate constants agreed well with experimental findings.

The condensation reaction II A, was studied by Moelwyn-Hughes (1929) using glucose and normal hydrochloric acid between 60 and 70°. In all cases an equilibrium between monosaccharide and condensation products was attained, the composition of which appears to be dependent on the initial concentration of glucose. Similar investigations were carried out by Seaman (1944), and it was pointed out by Silin & Sapegina (1939) that the elimination of water in reaction II A was an important step,

permitting the application of a modified bimolecular equation to the system. The monosaccharides could be recovered by dilute acidic hydrolysis of the 'reversion products' (reaction II B). Under more drastic conditions irreversible intramolecular breakdown of the monosaccharide occurs (reaction III).

The researches of Berlin (1926) and Georg (1930) on reversion products of glucose indicate the presence of 1:6-linked disaccharides, particularly isomaltose (glucosido- α -1:6-glucose), whilst the occurrence of other disaccharides is discussed by Pringsheim (1924, 1926), Hurd & Cantor (1938) and others. The presence of non-reducing disaccharides and trisaccharides was shown by Hurd & Edwards (1949), and an extensive investigation of the condensation of partly substituted glucoses was made by Frahm (1941, 1944). It was found that whereas 1:2, 1:3 and 1:4 glucosidic linkages were formed at about the same rate, 1:6 linkages were formed with markedly greater readiness. By the action of 40% hydrochloric acid on glucose, Myrbäck, Hammarstrand & Gelinder (1949) obtained a mixture of tetrasaccharides in which 1:6, 1:2 and/or 1:3 linkages were found.

Reversion products of a high degree of polymerization were described by Fredenhagen & Cadenbach (1933) who showed that cellulose reacted readily with liquid hydrogen fluoride in anhydrous conditions, forming glucosyl fluoride. In the presence of traces of water or removal of the hydrogen fluoride by evaporation, a fluorine-free polysaccharide material was obtained different from the initial cellulose. This new polymeric material termed 'cellan' (Helferich & Böttgers, 1929; Helferich, Stärker & Peters, 1930; Helferich & Peters, 1932) had weak reducing properties, was soluble in water and was strongly dextrorotatory. It was claimed that cellan was formed also by the action of hydrogen fluoride on glucose (Fredenhagen & Helferich, 1929). More recently Pacsu & Mora (1950) reported the formation of polymers of high molecular weight, non-dialysable in cellophan membranes by concentration of glucose with hydrochloric acid.

We have now synthesized polymers from a number of hexoses and mixtures of hexoses in order to study their physical and biochemical properties, in view of the interesting possibilities which the materials present.

EXPERIMENTAL

Conditions of polymerization. The monosaccharide was mixed with SOCl_2 by being ground in a mortar and the reactants, packed in a glass vessel, were heated at various temperatures in a thermostatically controlled oven ($\pm 2^\circ$). Whilst still hot the resulting pale-yellow liquid was dried in a vacuum and the crude product was obtained as a brittle sponge-like mass, which was water-soluble.

Reducing values (R.V.). The reducing values were determined by the method of Somogyi (1952) and expressed as the ratio of the weight of polymer to the weight of anhydrous glucose of the same reducing power.

Optical rotations. Measurements were made on a Hilger Standard microptic polarimeter in sodium light, readings being accurate to $\pm 0.01^\circ$.

Chromatography. Examinations were carried out on Whatman no. 1 paper using butanol:ethanol:water (4:1:5, v/v) as a descending eluting agent as described by Partridge (1946). The behaviour of components is described in terms of R_f values (Hough, Jones & Wadman, 1949).

Fractionation by ethanolic precipitation. The material was dissolved in 50 ml. of water and ethanol was added dropwise, with stirring, to the solution at room temperature. The precipitate was removed centrifugally (3000 rev./min.) and was dried over absolute ethanol and ether. The addition of ethanol to the supernatant was resumed until a second precipitate was observed. This, and subsequent fractions obtained in the same way, was separated and dried.

Preparation of non-dialysable glucose polymers. (a) 18 g. glucose monohydrate were ground with 3 ml. of SOCl_2 , heated at 105° for 45 min. After drying, the fraction precipitated by 3 vol. of ethanol was dried and heated at 50° for 1 hr. with 2 ml. HCl. The syrup so formed was dissolved in 100 ml. of water and dialysed in cellophan against distilled water for 36 hr. and again precipitated by 3 vol. of ethanol, yielding 2.1 g. of product (R.V., 52.8, $[\alpha]_D^{21} + 100^\circ$ in water, c, 0.6).

(b) 3 g. of glucose polymers (R.V. 10) were mixed with 0.5 ml. SOCl_2 and heated at 105° for 45 min. The product dried *in vacuo* to a pale-yellow dry friable foam which, dissolved in 13 ml. of water, was dialysed in cellophan for 48 hr. and isolated by freeze drying. Yield, 0.4 g. (R.V. 33.2, $[\alpha]_D^{21} + 100^\circ$ in water, c, 0.6.) Material (designated polyglucose A) prepared in this manner was used for further examination.

Hydrolysis with dilute mineral acid. 80 mg. of polyglucose A were heated at 100° with 10 ml. of 0.01 N-HCl. No change in reducing power or in optical rotation was observed after 3 hr. nor could monosaccharides be found by chromatographic analysis. The concentration of the acid was increased to 1 N and the rotation changed $[\alpha]_D^{19} + 101^\circ \rightarrow +72^\circ$ (constant value) in 90 min.

Paper chromatography of the hydrolysate revealed a single reducing substance with properties corresponding to glucose, run simultaneously. Glucose was identified in the hydrolysate by the formation of glucosazone.

Sedimentation measurements. These were made over a range of concentrations using polyglucose A, in 0.1 M-NaCl, and the results are reported separately by Baldwin (1953).

Diffusion coefficient. This was determined by Mr J. R. Hall with polyglucose A using the Gouy diffusometer as described by Ogston (1949).

Viscosity. This was determined at $25^\circ (\pm 0.1^\circ)$ in an Ostwald viscosimeter using a range of concentration of the fractions.

Action of enzymes. (a) *Amylase.* Freshly collected saliva was diluted with five volumes of 0.85% saline and centrifuged for 30 min. at 2000 rev./min. Samples of polyglucose A were incubated at 37° with 1 ml. of the enzyme preparation and the amount of reducing sugar generated was determined at intervals using the Somogyi method (1952). Control digests in which the polyglucose was substituted by corresponding amounts of whole potato starch were carried out simultaneously. (b) *Invertase.* Fresh baker's yeast (5 g.) was autolysed in the presence of toluene (10 ml.) for 8 days. The resulting mixture was centrifuged and the aqueous layer containing invertase was dialysed in cellophan and centrifuged before use. (c) *Potato phosphorylase.* Fresh potato juice at pH 6 was centrifuged for 20 min. at 0° before use.

RESULTS

In order to arrive at suitable conditions for the polymerization of hexoses, three series of experiments were performed.

(a) Glucose monohydrate was heated with varying amounts of SOCl_2 at 105° for 30 min. The results are shown in Table 1.

Table 1. *Action of thionyl chloride on D-glucose monohydrate (18 g.)*

(105° for 30 min.)			
Preparation	Vol. SOCl_2 (ml.)	Yield (g.)	Reducing value
1	1.0	10.2	4
2	2.0	10.7	4.3
3	3.0	10.5	5.9
4	4.0	10.2	5.0
5	4.6	9.8	6.7
6	5.0	10.2	6.0

(b) Glucose monohydrate (18 g.) was heated at 105° with SOCl_2 (3 ml.) for varying times. The resulting fractions (Table 2) were dissolved severally in 25 ml. of water and the material, precipitated by the addition of 75 ml. of ethanol, was collected and dried.

Table 2. *Polymerization of glucose at varying times*
(105°, 18 g. glucose, 3 ml. SOCl_2 .)

Preparation	Time (min.)	Yield of material precipitated with 3 vol. ethanol (g.)	Reducing value
7	30	13.7	10.8
8	45	12	9.9
9	60	11	11.8
10	90	10.7	7.8

(c) The effect of temperature on the polymerization was investigated, each experiment being carried out on 18 g. glucose monohydrate mixed with 3 ml. of SOCl_2 (Table 3).

Table 3. *Polymerization of glucose at various temperatures*(18 g. glucose, 3 ml. SOCl_2)

Preparation	Temp. (°)	Yield of material precipitated with 3 vol. ethanol (g.)	Reducing value
11	37	7.5	2.1
12	60	12.7	2.36
13	100	11	4.82
14	105	12	6.78
15	130	9.1	7.1
16	158	4.8	8.2

Paper-chromatographic examination of the crude polymeric material (preparation 3, Table 1) showed the presence of unchanged glucose and some disaccharide-like products, though the main bulk of material was immobile.

Fractionation by ethanolic precipitation showed that polymers constituting about 30 % of the whole, with a mean chain length of ten units, were precipitated by 3 vol. of ethanol (Fig. 1a), and that products of higher degrees of polymerization (D.P.) could be separated from this fraction by precipitation by graded amounts of ethanol (Fig. 1b). A considerable increase in the degree of polymerization was found and products which did not dialyse through cellophan resulted, when small-chain polymers (preparation 3, Table 1), fractionated with 3 vol. of ethanol and dried, were retreated with thionyl chloride under the same conditions as the initial polymerization. Yields of the dialysed product (polyglucose A) varied between 7 and 20 % of the small-chain polymers used. The product was a pale-yellow amorphous solid with a D.P. of 33.2. In an examination of the physical properties of polyglucose A, the diffusion constant (D_w^{20}) was found to be 12.6×10^{-7} and the sedimentation constant (S_w^{20}), 0.80×10^{-13} . Assuming $\bar{V} = 0.62$ these values

combine to give a value of 4100 ($\pm 5\%$) for the molecular weight. This value is in keeping with the

Fig. 1. (a) Fractionation of crude polysaccharide (18 g.). (b) Fractionation of polysaccharide (18 g.) with *n.v.* 10. Hatched portions refer to reducing values.

magnitude of the intrinsic viscosity (0.018) of the material. The polymer was chlorine- and sulphur-free and when it was hydrolysed by 1N-HCl, only glucose could be identified (chromatographically,

Table 4. *Properties of synthetic hexose polymers*

Preparation	Weight of sugars (g.)	Vol. SOCl_2 (ml.)	Temp. (°)	Time (min.)	Fractionations (vol. ethanol)	Yield (g.)	Reducing value	$[\alpha]_D$
17	Galactose, 9 g. (+1 ml. water)	1.5	105	30	2	4.6	9.3	+70
					3	1.6	6.2	+93.5
18	D-Glucose monohydrate, 3 g., L-rhamnose, 3 g.	1.0	105	30	7	0.3	10.5	+171
19	Lactose, 9 g. (+0.5 ml. water)	1.5	105	30	2	5.1	12.1	+86
					3	0.7	7.9	+72
20	D-Mannose, 3 g. (+0.1 ml. water)	0.5	105	60	2	1.0	12.4	+64
					3	0.3	10.7	+60
21	D-Glucose monohydrate, 9 g.	3.0	105	30	2	10.3	10.8	+76
	D-Galactose, 9 g.				3	1.8	—	+107
22	D-Glucose monohydrate, 1.0 g. D-Glucurone, 0.5	0.2	105	30	7	0.16	4.5	+79

as the osazone and as the benziminazole derivative). The polymer was not degraded by salivary amylase under conditions which hydrolysed a similar amount of starch in 5 min. The enzyme was not inactivated by the polyglucose. Similarly, yeast invertase and potato phosphorylase were without detectable action on polyglucose A.

By the same process used for the formation of polyglucose A, a number of simple and mixed hexose polymers were synthesized and characterized (Table 4).

DISCUSSION

The results indicate that the polymerization is facilitated by the presence of small amounts of water, and retarded by traces of alcohols or glycosides. When anhydrous hexoses were used, there was no evidence of polymerization to any marked extent. At temperatures below 100°, the rate of polymerization was slow and most effective results were obtained at the higher temperatures. Above 150° decomposition of the sugar occurred readily. The higher degrees of polymerization resulted from a re-treatment of small-chain polymers. Attempts to keep the sugar in contact with the catalyst for longer periods by means of hydrocarbon solvents (toluene, xylene) gave results little different from the repolymerization experiments.

Ethanol-water fractionation proved useful in separating the more highly polymerized material (D.P. 10 and greater) from unchanged glucose, disaccharides and other products. The degrees of polymerization have been calculated by measurement of residual reducing power in isolated fractions, in terms of glucose, and this value represents the D.P. only if the molecule has a single reducing end. These values have been taken as an index of the extent of polymerization. In the ultracentrifuge a broad peak was observed and certain consistency of properties was observed in the fractionation procedure, but no claim is made that the polyglucose A is a single polymeric species. Myrbäck *et al.* (1949) observed that a synthetic tetrasac-

charide preparation was a mixture of at least three substances.

The identification of glucose as the sole product of acidic hydrolysis substantiates the view that in the polymer this sugar is glycosidically linked and that internal elimination of the elements of water forming anhydro-sugar units (Newth, Overend & Wiggins, 1947; Peat, 1946) does not occur. The stability of the polymer towards dilute acids is more characteristic of pyranoside than furanoside or acyclic acetal linkages, and the change in optical rotation ($[\alpha]_D^{20} + 101^\circ \rightarrow +72^\circ$) during hydrolysis suggests the occurrence of some α -linkages. The polymer was remarkably stable towards enzymic hydrolysis by salivary amylase, invertase and potato phosphorylase. The enzymes were not inhibited by the polymeric material.

Insufficient evidence is available to shed much light on the mechanism of polymerization but the dehydrating conditions required suggest that the concentration of water plays a significant part. The acidic catalyst may lead to the formation of glycosyl complex as in the case described by Pacsu & Hillier (1949). The number of simple and mixed polymers synthesized from hexoses other than glucose indicates that the reaction is of a general nature.

SUMMARY

1. Conditions of temperature, time and concentrations are described under which glucose, in the presence of thionyl chloride, forms polymeric products.

2. Non-dialysable glucose polymeric material (molecular weight, 4100) was found to have D_w^{20} 12.6×10^{-7} , S_w^{20} 0.80×10^{-13} , and $[\eta]$ 0.018.

3. The polymer ($[\alpha]_D^{21} + 100^\circ$ in water) was hydrolysed completely by 1N-hydrochloric acid to glucose, but was not degraded by salivary amylase, invertase or potato phosphorylase.

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The Component Acids of Milk Fats of *Balaenoptera physalus* (Finner Whale)

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Although the component acids of the milk fats of many land animals, especially ruminants, are characterized by the presence of acids of lower molecular weight than those present in their respective depot fats, the component acids of milk fats of marine mammalia so far examined are qualitatively and to a greater or lesser extent quantitatively similar to those of their blubber fats. Thus Schmidt-Nielsen & Frog (1933) were unable to identify any acids of lower molecular weight than lauric in the component acids of milk fat of finner whale (*Balaenoptera physalus*) and showed that the proportions of the chief groups of acids (saturated and unsaturated together) were: C₁₄, 5.5; C₁₆, 22; C₁₈, 32; C₂₀₋₂₂, 39. These results, taken in conjunction with the iodine value of the oil (137.8) and the presence of palmitoleic, oleic, gadoleic, and 'clupanodonic' acids, were considered to indicate the close similarity between the milk and blubber fats. Similar figures reported by Klem (1935) for the milk fat of the blue whale (*Balaenoptera musculus*) are in agreement with this view. The values obtained by Klem are nevertheless somewhat speculative, since he reported the loss of a fraction comprising about 24 % of the mixed acids, and was obliged to make tentative assumptions as to the composition of this fraction. In spite of this indeterminacy the results obtained by Klem are in good agreement with those obtained in the present work. Meara (1952), however, showed that although the component acids of seal milk fat were broadly similar to those of seal blubber fat there were certain significant differences notably in the palmitic acid contents.

The present investigation, which was made possible by Dr J. C. D. White, who kindly placed four specimens of finner-whale milk at our disposal, was carried out to determine how far the broad

similarity between the milk and blubber fats holds, and to determine whether any significant differences similar to those which obtain in the case of seal milk and blubber fatty acids also occur in whale milk and blubber fats respectively.

METHODS AND RESULTS

The following data supplied by Dr White indicate the origin of the milk from which the fats were extracted. For each finner whale the length, and the time after death at which the sample was taken, are given. A: 72 ft. (23.5 m.), 7 hr. 45 min.; B: 68 ft. (22.1 m.), 6 hr.; C: 69 ft. (22.3 m.), 16 hr. (sample obtained from nipple and probably purest sample); D: 73 ft. (23.7 m.), 19 hr. (whale appeared to be drying off; a little blood present from external source).

The samples were viscous, resembling condensed milk in consistency, pale pink in colour, and appeared to be in good condition, with the exception of specimen D, which had a highly rancid fishy odour and was contaminated with blood and aqueous medium. Each sample was extracted in the manner described by Meara (1952) for seal milk fat, the extraction data together with the chemical characteristics of the extract being given in Table 1.

The component acids of each milk fat were determined as reported by Meara (1952). Unsaponifiable matter was removed from the soaps after saponification of the fat. Steam distillation of the regenerated fatty acids, followed by titration of the steam distillate, indicated the absence of the lower molecular weight steam-volatile fatty acids. The recovered acids were resolved by low-temperature crystallization into groups which consisted of mainly saturated, mainly monoethenoid, and mainly polyethenoid acids, respectively, each group being methylated (Bjarnason & Meara, 1944) and fractionally distilled. From the analytical data obtained the composition of each ester fraction was computed (Hilditch, 1947, pp. 498-510); thence the composition of each group of acids, and finally the composition of the whole fat was deduced.

The final results of these analyses, together with data on related fats, are given in Table 2.