group the absorption with the C–H deformations in Table IV (as D).

The IV, A, bands can be assigned to C–H absorption characteristic of an isolated ring hydrogen grouping. The normal range is 900–860 cm.$^{-1}$,[22] In the 2-nitro and 2,4-dinitro series the band is shifted slightly above 900 cm.$^{-1}$ by the nitro substituent. The grouping of 2 adjacent ring hydrogens, which is given the range 860–800 cm.$^{-1}$, occurs in the estrone series and in the 4-nitroestrones. The generally strong band (IV, B) observed in that range can thus also be assigned to C–H deformation. (The nitro group in 4-nitroestrone, not being coplanar with the ring, does not shift the frequency appreciably.) In the spectra of these compounds in KBr, there is an additional characteristic band (IV, C) near 790 cm.$^{-1}$.

The 2-nitroestrones are distinguished from the 2,4-dinitroestrones in having strong absorption at 660 cm.$^{-1}$ (IV, E).

It is interesting to note, finally, that the C–H absorptions, like the C=C stretching bands, are markedly weakened in the acetates.

Acknowledgment.—Grateful appreciation is expressed to R. Hart for his assistance with the preparation of this paper.

CHICAGO 37, ILL.

(22) Bellamy, ref. 9, pp. 67–69.

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Synthetic Polysaccharides. I. Polycondensation of Glucose[1]

BY PETER T. MORA AND JOHN W. WOOD

RECEIVED SEPTEMBER 20, 1957

The polycondensation of α-D-glucose to high molecular weight polymers was studied in the temperature range of 140–170° under vacuum and in the presence of 0.164% phosphorous acid. Under the carefully controlled conditions used, no decomposition was observed and glucose was the only product recovered upon subsequent acid hydrolysis. A melt polymerization with infrared heat gave some insoluble gel indicating condensation at other than the glucosidic hydroxyl, but a melt polymerization plasticized with tetramethylene sulfone, and a process where the solidified polymer was powderized and heated in a second stage gave only water-soluble polyglucoses. The mechanism apparently follows that of the $A{-}R{-}B_{f-1}$ polycondensation in which the glucosidic hydroxyl (A) can react only with one of the non-glucosidic hydroxyls (B). The influence of temperature and method of polymerization on residual reducing power (unreacted A end group, indicating 5,000–30,000 number average molecular weight), on intrinsic viscosity (0.02–0.07), optical rotation (+58° to +80°), precipitation behavior by alcohol and percentage of non-dialyzable polymer (70–80%) was studied.

The hydrolysis of polysaccharides is a reversible process and there have been many reports on low molecular weight reversion products, mainly disaccharides, formed in aqueous acid solution.[2] It was also reported that low molecular weight "polyglucosans" can be formed when glucose is dissolved in water-free acids.[3] In a preliminary note Pacsu and Mora have shown that polycondensation of glucose and other simple sugars to high molecular weight polysaccharides can be achieved using an acid catalyst, and applying high vacuum to eliminate water produced during condensation.[4] Subsequently there were reports from other investigators on similar results.[5–8] The scope of the general method for polycondensation of carbohydrates and the early preferred methods for the polymerization of glucose appeared in the patent literature,[9] but there is no detailed description of methods of preparation of polyglucoses with high conversion and without decomposition. Only the macromolecular investigations were published on the solution behavior of high molecular weight branched polyglucoses.[10]

The present studies were undertaken to find new methods of producing polyglucose in optimum yield, without decomposition and free of insoluble gel, and also to study the influence of temperature on the polycondensation and on the resulting polymer. A high degree of polymerization of glucose will be favored by (1) an acid catalyst at a concentration which does not cause side reactions (*i.e.*, decomposition) at the reaction temperature, (2) high temperature, (3) effective elimination of the water produced during the condensation, (4) high concentration of glucose, (5) sufficient mobility of the monomer and of the low molecular weight polymers, (6) absence of oxygen and other conditions which may cause degradation, and finally (7) uniform reaction conditions throughout the reaction medium. This paper describes in detail polymerization experiments in which these conditions were fulfilled. It is necessary to adhere scrupulously to the given set of conditions, since relatively minor deviations can cause great differences in the product and may result in excessive decomposition.

We used three methods designed to achieve both good control of reaction variables and uniform reaction conditions: (1) a melt polymerization where infrared heat was applied; (2) a melt polymerization plasticized with an inert solvent (tetramethylene sulfone); and (3) a two-stage process in which

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(1) Presented before the Polymer Division of the American Chemical Society at the 131st National Meeting in Miami, Fla., April 5, 1957.

(2) See, for example, C. N. Tarton, A. Bebbington, S. Dixon and E. Pacsu, THIS JOURNAL, **77**, 2565 (1955); A. Thompson, K. Anno, M. L. Wolfrom and M. Inatome, *ibid.*, **76**, 1309 (1954); K. Myrbäck, M. Hammerstand and H. Gelinder, *Arkiv. Kemi*, **1**, 235 (1949).

(3) *Cf.* B. Helferich and S. Böttger, *Ann.*, **476**, 150 (1929); H. M. Schlubach and E. Luhrs, *ibid.*, **547**, 73 (1941).

(4) E. Pacsu and P. T. Mora, THIS JOURNAL, **72**, 1045 (1950).

(5) P. W. Kent, *Biochem. J.*, **55**, 361 (1953).

(6) C. R. Ricketts, *J. Chem. Soc.*, 4031 (1954).

(7) C. R. Ricketts and C. E. Rowe, *ibid.*, 3809 (1955).

(8) C. T. Bishop, *Can. J. Chem.*, **34**, 1255 (1956).

(9) P. T. Mora and E. Pacsu, U. S. Patent 2,719,179 (1955); E. Pacsu and P. T. Mora, Canadian Patent, 530,079 (1956).

(10) P. T. Mora, *J. Polymer Sci.*, **23**, 345 (1957).

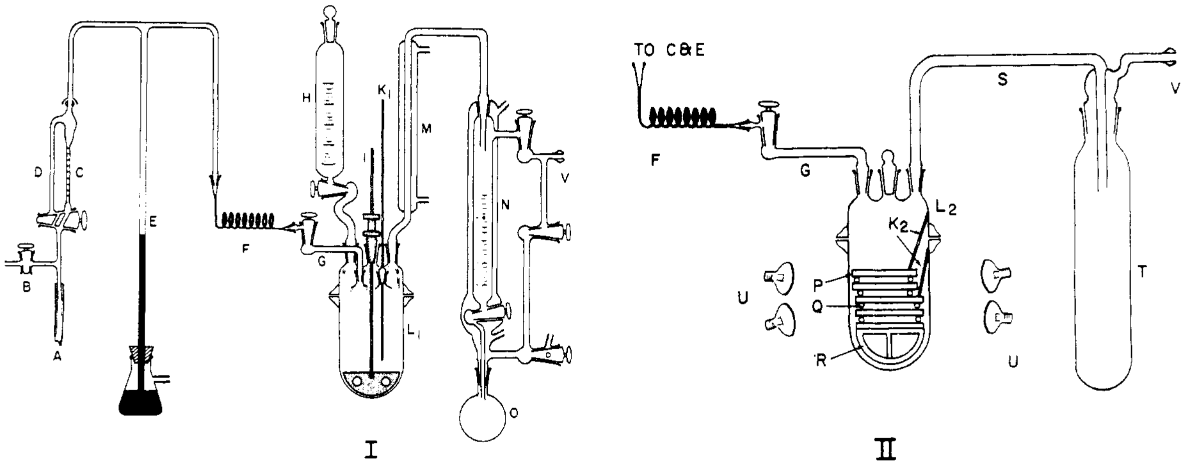

Fig. 1.—I, apparatus for "solution melt" type and "two-stage" polymerization of glucose; II, apparatus for "infrared" polymerization: A, to tank nitrogen; B, pressure release stopcock; C, flowmeter (Fischer and Porter No. 08F-1/16-08-4) with 3-way stopcock and spherical joint; D, flowmeter by-pass; E, mercury manometer 0–1 atm.; F, flexible helical connection of copper tubing with two Ŧ 14/35 outer brass joints; G, adapter tube with vacuum stopcock and a Ŧ 24/40 and a 14/35 inner joint; H, addition funnel, 200-ml. cap., with vacuum stopcock (4 mm.) and Ŧ 24/40 inner joint; I, stainless steel stirrer and shaft, and stainless steel vacuum stuffing box with Ŧ 24/40 inner joint (Scientific Glass App. Co. No. J-2164); K$_1$, thermometer with Ŧ 10/30 joint; K$_2$, Anschütz thermometers; L$_1$, Pyrex resin flask (1- to 2-l. sizes used) with a cover having four Ŧ 24/40 and one Ŧ 10/30 outer joints; L$_2$, resin flask (2 l.) with cover having three Ŧ 24/40 necks; M, reflux condenser (inner tube 22 mm. o.d. × 40 cm.) and adapter with Ŧ 24/40 joints; N, water-cooled vacuum receiver (grad., 250 ml.) with two Ŧ 24/40 joints, and one spherical 28/12 inner joint, with four vacuum stopcocks (6 mm.); O, receiving flask; P, Petri dishes (9 cm.); Q and R, glass rod supports; S, adapter (22 mm. o.d. tubing) to trap, with a Ŧ 24/40, a Ŧ 45/50 and a spherical 28/12 inner type joint; T, Dry Ice–methyl Cellosolve cold trap (7.5 cm. o.d. × 40 cm.) with Ŧ 45/50, outer joint; U, infrared therapeutic lamps, 250 watts size; V, to K of vacuum manifold, described in ref. 11.

the solidified low molecular weight polymer from the first stage was cooled, powdered and reheated in a second stage. All experiments were carried out under vacuum and with phosphorous acid as a catalyst.

Experimental and Results

Preparation of Glucose–Catalyst Mixture.—It is necessary to distribute the acid catalyst uniformly, employing minimal amounts of water. A mixture of 11.024 g. of phosphorous acid (Purified, Fisher, 33.737 weight % H$_3$PO$_3$, determined by titration) and 10 ml. of distilled water was added dropwise into 2,267.7 g. of α-D-glucose (anhydrous, reagent, Merck), while mixing the glucose in a porcelain mortar. The flask containing the acid was washed with an additional 10 ml. of water which was added in a similar manner to the glucose. The resulting mixture which had a final H$_3$PO$_3$ concentration of 0.164 weight % was then milled in a porcelain ball mill for 17 hours. Portions of the fine powder were used in all the experiments, except in the case of a control experiment without catalyst, in which the phosphorous acid was replaced by an equivalent amount of water.

Vacuum System.—It is essential to eliminate oxygen as completely as possible before heating. Also, it is necessary to maintain a high vacuum during the polycondensation to accomplish rapid removal of the water.

The reactions were carried out in large resin flasks connected by means of wide bore adapters to a vacuum manifold similar to that described by Calvin, *et al.*[11] Two traps employing Dry Ice and methyl Cellosolve refrigerant were used in tandem between the reaction flask and the manifold. A McLeod vacuum gauge and a thermocouple type vacuum gauge (used mainly as a rapid leak detector) were placed in the vacuum system. The entire system was evacuated by means of a mechanical fore pump and a vapor diffusion pump.

In order to assure complete removal of oxygen, a nitrogen flushing system was constructed. It incorporated a manometer, a flow meter with by-pass, suitable vacuum stopcocks and a cylinder of water-pumped nitrogen gas. The system was designed in a manner which made it possible to alternately evacuate to 0.1 mm. with a fore pump and flush with nitrogen close to atmospheric pressure. This procedure was routinely carried out five times before each polymerization experiment. The interchangeable use of vacuum and of the flushing system with the three different polymerization vessels required suitably installed standard taper or semi-ball joints. All the joints and stopcocks were good quality high vacuum type and before use they were "lapped in" with No. 600 grade of Carborundum powder, then thoroughly cleaned, and finally lubricated with heavy Silicone high vacuum grease. Figure 1 illustrates the nitrogen flushing system and the different set-ups used in the polymerization experiments.

Polymerization Methods. I. "Infrared" Method.—The heat necessary to melt and polymerize the glucose mixture was obtained from infrared lamps. A resin flask with a three-neck cover was connected to the vacuum manifold and to the nitrogen flushing system. Five Petri dishes each containing 20 g. of the powdered glucose–catalyst mixture were placed in the flask and were spaced at equal distance from each other by means of glass supports. Two small glass thermometers were placed in the flask, so that their mercury bulbs were covered by the glucose–catalyst mixture. After the aforementioned flushing operation the flask was evacuated.

Experiment 1, 140°.—Heat was supplied by placing four pairs of therapeutic infrared lamps at a distance of 7 in. from the sides of the flask, and with each pair at right angles to the axis of the flask and to the adjacent pairs. The whole set-up was covered with a sphere formed from crinkled aluminum foil which acted as a reflector. Voltage was applied simultaneously to each lamp from variable transformers (115 v., 7.5 amp.) and it was increased gradually to produce the temperature reported in Table I. The time necessary to attain this temperature is also given. A setting of 47 v. was reached in 6.5 hr. and maintained for the rest of the experiment.

The powdered glucose–catalyst mixture started to melt at 121° and the whole mass melted at 130°. Violent bubbling of the melt indicated the evolution of the water of condensation. The bubbling gradually subsided in about 3 hr., the viscosity of the melt increased, and after

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[11] M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and F. P. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 141 (Fig. 55).

TABLE I

POLYMERIZATION OF GLUCOSE

Expt.	Glucose[a] used, g.	Temperature, °C.		Time, hr.	Vacuum,[b] $\times 10^{-4}$ mm.	Yield of crude polymer, g.	Description of crude polymer
		Bath	Flask				
			Infrared method				
1	100.0		R.t.[c]–121[d]	0.5	170	86.9	Light amber, frothy solid
			121–142	23.7			
			Av. 140				
2	100.0		R.t.–199[d]	1.0	170	86.0	Light to dark amber, frothy solid
			119–148	3.3			
			148–156	17.3			
			Av. 150				
			Solvent melt method				
3	100.0	R.t.–139		0.8	650–2000[e]		Light amber brittle resin
		139–148		2.3			
		148–158		21.0			
		Av. 155					
4	100.0	R.t.–170		0.5	1600–3200[e]		Light to dark amber, brittle resin
		170–178		21.5			
		Av. 175					
			Two-stage powder method: stage I				
5	400.0	77[h]–161		0.5	50	348.3	Amber, "honey-combed" solid
		161–173		1.3			
		137–145		25.8			
		Av. 140					
			Two-stage powder method: stage II				
6	168.0[f]	69[h]–120	R.t.–160	0.4	50	162.9	Light tan, free flowing powder
		120–150	60–113	1.4			
		148–157	113–125	22.0			
		Av. 150					
7	168.0[f]	150[h]–170	R.t.–92	0.4	0.05–0.10	161.8	Medium tan, free flowing powder
		170–174	92–124	0.7			
		168–176	124–138	21.5			
		Av. 170					
			Two-stage powder method: stage I				
8	400.0	102[h]–145		0.2	0.01–0.05	356.0	Light to medium amber, "honeycombed" solid
		138–144		20.0			
		Av. 140					
			Two-stage powder method: stage II				
9	346.0[f]	81[h]–160	R.t.–96	1.0	0.01–0.10	338.5	Tan, free flowing powder
		160–163	96–140	2.3			
		159–163	140–145	20.9			
		Av. 160					
			Control, without catalyst: stage I				
10	400.0[g]	95[h]–136		0.5	0.5–1.0	363.1	Dark brown, low melting "caramel"
		136–144		1.0			
		144–153[d]		26.5			
		Av. 150					

[a] Contains 0.164% phosphorous acid. [b] Based on McLeod gauge readings in all experiments except 3 and 4 where a thermocouple type gauge was used. [c] Room temperature. [d] Mixture begins to melt. [e] Pressure maintained within these limits by "bleeding" in nitrogen *via* a flowmeter. [f] Used powdered crude polymer from preceeding stage I. [g] Water, 5.3 ml., added. [h] Bath preheated to these temperatures.

about 5 hr. the mixture had solidified to a light amber, frothy mass which remained unaltered to the end of the polymerization, a period of 23.7 hr. During most of this period the temperature was 140 ± 2°. Finally the flask was cooled to room temperature under vacuum. The polymer was a brittle, frothy, light amber glass, which was easily powdered in a mortar.

Experiment 2, 150°.—Conditions were similar to experiment 1, except that two additional infrared lamps were added, one focused directly over the cover of the flask and the other upward from the bottom. All the lamps were gradually increased to 50 v. in 2 hr. to produce an average temperature of 150°. The changes during the polymerization and the appearance of the polymer were similar to experiment 1, except that the product was of a darker amber shade. Also, there were localized brown portions, where the heat was obviously more concentrated. In spite of the careful attempt to distribute the heat uniformly by the symmetrical focusing of the bulbs and the use of the aluminum reflector, homogeneous temperatures could not be maintained by this method.

II. "Solution Melt" Method.—In this method the glucose catalyst mixture was plasticized by tetramethylene sulfone.[12] This chemical is a fairly good solvent of glucose

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[12] Supplied generously by the Shell Development Co., Emeryville, Calif.

and of the low molecular weight polyglucoses. It does not react with glucose or with the polymers, and it is sufficiently stable and not too volatile at the temperatures, pressures and acid concentrations employed. In addition, it aided in the removal of water from the reaction by forming an azeotropic mixture which was distilled off and collected in a trap.

The reaction was carried out in a 1-l. resin flask with a cover having four ₮ 24/40 and one 10/30 outer joint necks. The flask was fitted through a stuffing box vacuum seal with a stainless steel stirring assembly. Sufficient power to stir the viscous polymerization mixture and to overcome the friction developed in the stuffing box was obtained by using a small reduction gear motor. To reflux the tetramethylene sulfone and to distil the azeotrope, the flask was provided with a wide bore (inside diam. 18 mm.) vertical water jacketed condenser joining a downward bent adapter which led to the first trap of the vacuum manifold. Since tetramethylene sulfone crystallizes at 27° the temperature of the circulating water in the condenser jacket was adjusted to 28–30°. The tetramethylene sulfone which was lost through distillation was replaced from a supply contained in a dropping funnel. The fourth outlet of the flask was connected to the nitrogen flushing system.

Experiment 3, 155°.—The glucose–catalyst mixture, 100 g., was covered with 150 ml. of melted tetramethylene sulfone and the flask was flushed and evacuated. A thermostatically controlled oil-bath (Dow-Corning Silicone oil no. 550) was used to supply the necessary heating. As the bath temperature was increased and the tetramethylene sulfone began to reflux, nitrogen was let into the system through the flow meter by manipulation of a suitably grooved vacuum stopcock, to establish a pressure which at the desired temperature of 155° allowed only slow distillation of the azeotropic mixture. As the polymerization proceeded (*ca.* 2–3 hr.) the less soluble polymer began to come out of solution as a very light amber colored soft gel, which became progressively more viscous until, finally, stirring was prevented. A total of 268 ml. of tetramethylene sulfone was added in small portions to replace the loss by distillation. The reaction conditions are reported in Table I. At the end of the reaction the mixture was allowed to cool under vacuum to 30° and the solvent was decanted from the brittle hard resin-like polymer.

Experiment 4, 175°.—Conditions were similar to experiment 3, except that 300 ml. of tetramethylene sulfone was used initially, and an additional 150 ml. added during the experiment; temperature was maintained at 175°.

III. "Two-stage" Method.—The reaction vessel set-up was similar to that of the solution melt method, except that a flask of 2-l. capacity was used which was not provided with addition funnel, reflux condenser or thermometer (a thermometer was used in the second stage). The flask was connected to the first trap of the vacuum manifold system by means of a short wide tube (18 mm. diam.).

Stage I, Experiments 5 and 8, 140°.—In stage I of this procedure 400 g. of glucose–catalyst mixture was introduced into the resin flask and the system was flushed. The mixture was heated under vacuum with stirring but without the bleeding in of nitrogen. The mixture melted and bubbled as in the "infrared" runs. The reaction mixture remained in this melted stage usually for several hours (1.5–3) depending on the rate of the heating, after which it began to froth and solidify into a hard porous mass which was impossible to stir. Heating was continued, however, for additional periods for a total time of 20–28 hr. (see Table I). The reaction mixtures were cooled to room temperature while under vacuum.

Stage II, Experiments 6 (150°), 9 (160°) and 7 (170°).—The polymer from stage I was finely powdered in a mortar. Various allotments of this intermediary polymer were placed back in the same reaction vessel as used in stage I. In the second stage the polymer did not change its powdery consistency and stirring was used throughout the whole period. The conditions of the different experiments are summarized in Table I.

Control Experiment Without Catalyst, Stage I, 150°, Experiment 10.—An attempt was made to polymerize glucose under conditions parallel to those described under stage I, expts. 5 and 8, except that the phosphorous acid was replaced by an equivalent weight of water. After the usual nitrogen flushings and evacuation, the temperature was raised to 140° and maintained there for 1 hr. Melting did not occur until the temperature was raised to 150°. The molten glucose darkened rapidly and soon became a black sirup, which bubbled violently and remained in liquid form until the end of the heating period (26.5 hr.). After cooling under vacuum the product was a low melting black and hygroscopic mass.

Properties of Unfractionated Polyglucoses. "Turbidity Point," Alcohol Precipitation.—In all experiments the catalyst was neutralized by making a 2% solution of the polymer in 0.1 *N* sodium bicarbonate. To this solution sodium chloride was added in 0.5% (w./v.) concentration. Sodium chloride was found to speed up sedimentation of the fine emulsion of sirupy precipitate which formed when ethanol was added to the solution during the later fractionation. The aqueous solutions of experiments 1, 3–6 and 8–10 were clear, amber colored, while 2 and 7 had suspended gel. The gel was collected by centrifugation, and dried to constant weight in vacuum at 100°.

Aliquots of the clear supernatants were stirred at 26.3°, and ethyl alcohol was added from a buret to produce the first slight permanent turbidity when viewed in strong light. The volume percentage alcohol necessary to produce this turbidity is called "turbidity point."

Other aliquots of aqueous supernatants were agitated at 40°, and varying amounts of ethyl alcohol were added in a thin stream. The slightly turbid solutions were allowed to cool in a water-bath to room temperature overnight and then kept at 15° for three days. During this period a sirupy sediment settled. The supernatants were decanted and the sediments were dissolved in water and freeze dried to a voluminous white powder. Figures 2 and 3 report the yields plotted against alcohol concentration (v.).

To separate the polymers of experiments 3 and 4 from the occluded tetramethylene sulfone and to prepare them in a form suitable for weighing the following precipitation method was used: A 2% solution of polyglucose was prepared (based on theoretical yield) in 0.1 *N* sodium bicarbonate, and 0.5% sodium chloride was added. This solution was poured in a thin stream into well stirred excess ethyl alcohol (cooled in an ice-bath), ten times the volume of the aqueous solution. A fine white powder precipitated. This was collected by centrifuging or by filtration. The powder was dissolved and a second similar precipitation was carried out. Finally, the second precipitate was taken up in water, freeze-dried and dried to constant weight (24 hr.) at 60° in vacuum. Similar precipitation procedures can be carried out on any of the other polymers, and the recovery is about 95%. Only those impurities are eliminated which are soluble in 90% alcohol, such as the phosphorous acid and the very low molecular weight polymers. The products of Experiments 1, 2 and 5–9 were used in their original powdered form.

Dialysis.—The white powder was dissolved in water to give a 1% solution. This solution was dialyzed in Visking Cellophane tubing against running distilled water. After a 24-hr. dialysis, the solution inside the bag was freeze-dried and dried as above. The percentage non-dialyzable material is reported in Table II.

For the following measurements samples were prepared by freeze-drying portions and drying at 60° for 24 hr. under vacuum to constant weight.

Number Average Molecular Weight ($\bar{M}_n$) by Determination of Reducing End Group.—A method based on the reduction of alkaline copper citrate reagent as modified by Isbell[13] was employed. The only changes in the procedure were that the thiosulfate was 0.02 *N* and a 10-min. boiling time and 1-min. cooling in ice–water-bath was used. For polyglucoses below $\bar{M}_n$ of 10,000, 0.5-g. samples were used; the polyglucoses above $\bar{M}_n$ of 10,000 required 1-g. samples for accurate measurement. $\bar{M}_n$ values were computed on the basis of standardization with gentiobiose, assuming that equimolar quantities of polyglucose and gentiobiose have approximately the same reducing power.

Intrinsic Viscosity.—Viscosities were measured in modified Ubbelohde viscometers at 26.5°. Kinetic energy corrections were not applied. Solutions were filtered through sintered glass filters with fine or medium porosity.

Optical Rotation.—Samples were dissolved in 1 *N* HCl and the rotation was measured within 15 min. There is no detectable change in optical rotation at room temperature

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[13] H. S. Isbell, W. W. Pigman and H. L. Frush, *J. Research Natl. Bur. Standards*, **24**, 241 (1940).

TABLE II

PROPERTIES OF UNFRACTIONATED POLYGLUCOSES

Method of polymn. (expt. no. in Table I)		Average temp., °C.	"Turbidity point"	% pptd. by 70% (vol.) EtOH	% non-dialyzable	$\overline{M}_n$[a]	$[\eta]$, dl./g.	$[\alpha]^{25}D$ in 1 N HCl
Infrared	(1)	140	54.5	47.5	74.1	7,300	0.04	+65.0°
Infrared	(2)	150	46.4[c]	39.0	82.4	15,150	.06	68.2
Solution melt	(3)	155	59.2	24.2	81.6	10,850	.05	67.0
Solution melt	(4)	175	55.1	32.9	82.3	28,800	.07	80.5
Two-stage, I	(5)	140[b]	56.4	..	71.9	5,250	.05	60.5
Two-stage, I	(8)	140	58.2	..	69.1	3,250	.04	58.0
Two-stage, II	(6)	150	53.3	45.6	75.8	8,100	.05	62.7
Two-stage, II	(9)	160	51.4	..	79.1	11,800	.06	68.0
Two-stage, II	(7)	170	49.2[d]	50.2	77.8	13,200	.06	63.9
Control (no acid)	(10)	150	93.0	..	14.8	<400	..	41.8

[a] Assuming one reducing end group per molecule. [b] Overheated to 170° for 1.3 hr. [c] Contains 43% insoluble gel which was centrifuged off. [d] 3.4% gel.

for the first hour in 1 N HCl, and the value is only very slightly higher than in water. Hydrochloric acid was used merely as a matter of convenience, since some of the solutions were subsequently hydrolyzed in this solvent as reported below.

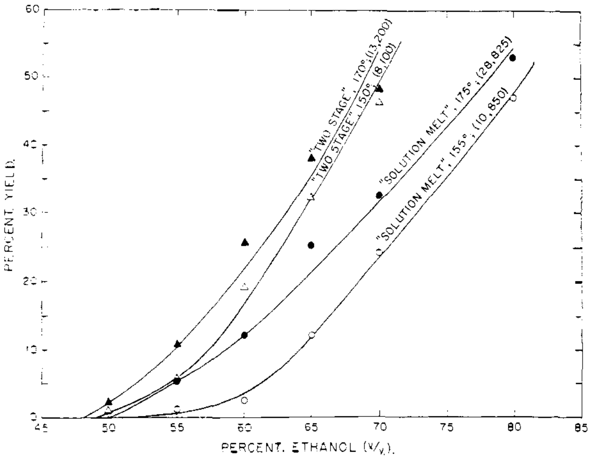

Fig. 2.—Cumulative yields of polyglucoses precipitated by alcohol. Numbers in parentheses indicate $\overline{M}_n$ of starting product, determined by reducing end-group method.

Hydrolysis, Recovery of Glucose.—Glucose and the polymers produced in the highest temperature experiments by each method (expts. 2, 4 and 7) were heated in 1 N HCl solution in a boiling water-bath for 3 hr. Aliquots taken at different intervals were neutralized and titrated by the modified Isbell method for determination of glucose equivalent. After 90 min. hydrolysis of the polyglucoses, 96–100% reducing power of equivalent glucose was recovered.[14] The glucose was identified as glucosazone.

Paper Chromatography Investigation for Decomposition Products.—Use of a developing solvent mixture consisting of butyl alcohol–ethyl alcohol–water (4:1:5) and of aniline hydrogen phthalate as a spray showed the presence of a large amount of glucose (R_f 0.17–0.19) in the hydrolysate and a small amount of (1–1.5%) dimer (R_f 0.06–0.07) as reversion product. There was no difference between the chromatograms of hydrolyzed polyglucoses and of glucose treated by acid under similar conditions. Only glucose and dimer were detected and in the few cases where traces of other reducing compounds appeared to show up, the concentration of these was below 0.5%; and in no case was there more unidentified reducing component in the polyglucose hydrolysates than in the parallel experiment with glucose. Similar results were obtained with silver nitrate spray on hydrolysates neutralized by passing through Amberlite IR-45 ion exchange resin.

Unneutralized hydrolysates were developed by ethyl acetate–acetic acid–water (3:1:1) and sprayed with water-saturated phenol freshly mixed with 0.05 N sodium hydroxide, to detect levulinic acid.[15] Comparison with authentic levulinic acid showed the formation of this compound to be about 0.25% from the glucose treated with acid, and only 0.12% from the polymers hydrolyzed under the same conditions. Similar results were obtained by a brom cresol green indicator spray.

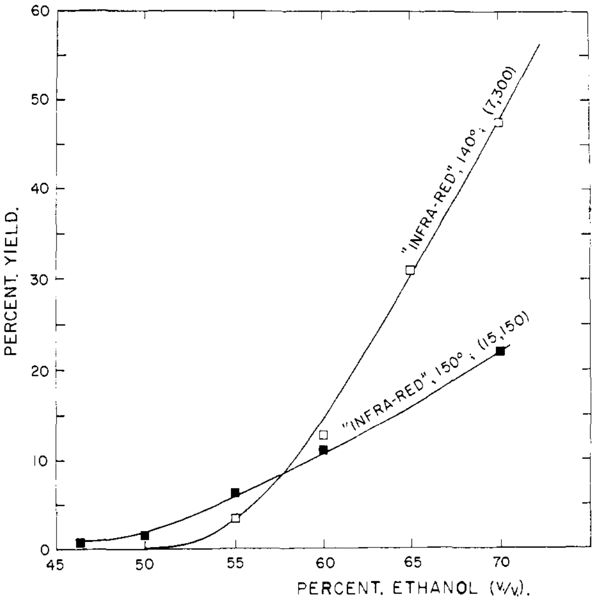

Fig. 3.—Cumulative yields of polyglucoses precipitated by alcohol, $\overline{M}_n$ in parentheses.

In testing the hydrolysates in different concentration using either phloroglucinol[16] spray or aniline hydrogen phthalate[17] spray, 5-hydroxymethylfurfural (compared with authentic sample) was detected in quantities below 0.25%, in both acid-treated glucose and polyglucoses.

Using sodium metaperiodate–nitroprusside spray, sensitive for glycols and deoxy sugars,[18] no positive indication of these products can be obtained.

The acid-catalyzed polycondensation products of glucose, without hydrolysis, gave no detectable moving reducing component over 0.5% by any of the above techniques, indicating that no unpolymerized monomer remained in substantial quantity.

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[14] On earlier samples only 92% was recovered by a ferricyanide method developed for the determination of polyglucose in blood and urine: D. D. Van Slyke and F. M. Sinex, *Proc. Soc. Exptl. Biol. Med.*, **79**, 163 (1952). Apparently, our method of drying a fine freeze-dried powder leads to more nearly complete dehydration.

[15] J. E. Loffler and E. H. Reichl, *Mikrochem. Acta*, 79 (1953).

[16] R. H. Horrocks and G. B. Manning, *Lancet*, **256**, 1042 (1949).

[17] S. M. Partridge, *Nature*, **164**, 443 (1949).

[18] J. T. Edwards and D. M. Waldron, *J. Chem. Soc.*, 3631 (1952).

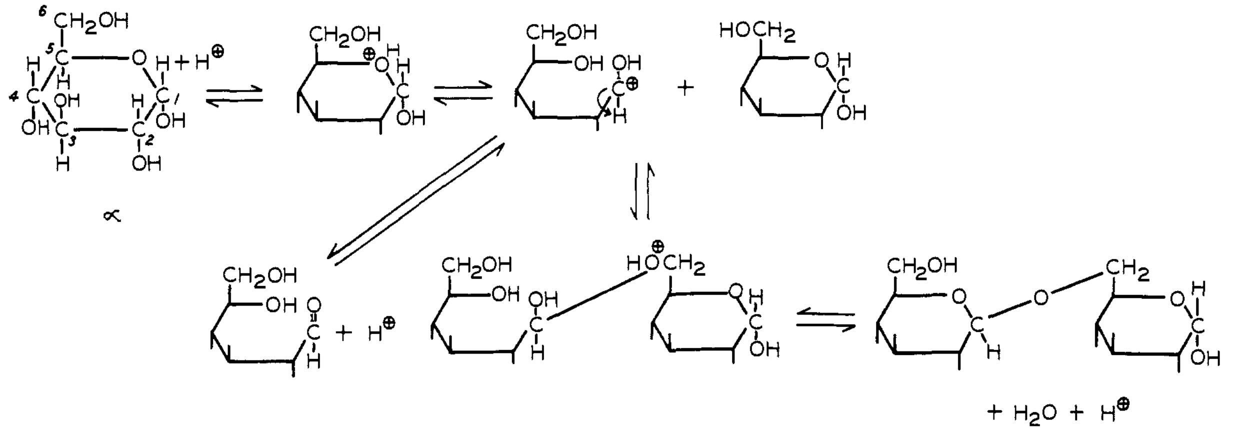

Fig. 4.—Mutarotation, hydrolysis and reversion of glucose.

The undialyzable portions of the 170° "two-stage" product (experiment 7) and of the control run without acid catalyst (experiment 10) were freeze-dried and dried to constant weight at 60° in vacuum for 16 hr. They were hydrolyzed in 2% solution in 1 *N* HCl for 3 hr. in boiling water-bath. The hydrolysates were passed through Amberlite IR 45 ion exchange resin and freeze-dried. Glycols or deoxysugars were not detectable by the above reported spraying reagents, and the amount of 5-hydroxymethylfurfural was about 0.25–0.5% in the product of expt. 10 and 0.25% in the product of experiment 7. Dimers were present in 1–1.5% in both cases; the concentration of levulinic acid was in the 1% range, but considerably higher in the unneutralized residue of expt. 10.

In the unhydrolyzed end product of polymerization expt. 10 the amount of chromatographically separated glucose was 15%.

Discussion

The ability of glucose readily to undergo acid and thermal degradation has to be taken into account when devising conditions for efficient polymerization. The presence of acid catalyst is necessary as shown in the control experiment where polymerization was attempted without phosphorous acid (expt. 10): there was considerable decomposition and only a low degree of polymerization.

For the purpose of discussion of polymerization mechanism we may choose a well known interpretation of mutarotation, hydrolysis and reversion and apply it to the polycondensation of α-D-glucose. This is illustrated schematically in Fig. 4, showing the role of the hydrogen ion and water in these processes. It is generally accepted that the rate-determining step in mutarotation is the formation of the open chain aldehyde intermediate, which is present only in very small equilibrium quantity (0.2 mole %),[19] and which rapidly recyclizes to the much more stable α- or β-form. However, the carbonium ion at the first carbon might react with any of the hydroxyls of another glucose molecule such as the primary (6th) hydroxyl as illustrated in the right of Fig. 4. The chance that this will occur is small when the molecules are far from each other, as in a dilute solution, but will increase with increased concentration of the monomer. Continuation of the downward reaction in Fig. 4 can be facilitated according to the law of mass action by the elimination of water, leading to polycondensation, the reverse process of hydrolysis.

It was shown that the mutarotation of glucose proceeds by a concerted displacement mechanism,[20,21] and that both an acid and a base must be present in order for the reaction to proceed.[22,23] Phosphorous acid has a low dissociation constant for the third hydrogen (4.8×10^{-13}) and the twice ionized acid can be considered as a relatively more nucleophilic reagent than the undissociated acid, which is the electrophilic reagent. Then the phosphorous acid may have a dual role during polycondensation at elevated temperatures in proton transfer by a concerted mechanism. The concentration of phosphorous acid used (0.164%) was found empirically to cause the least decomposition and the highest conversion to polymer in the temperature range investigated just above the melting point of glucose. Of course, any other Lewis acid can catalyze the polymerization,[9] but phosphorous acid is the most convenient one, probably because of its postulated dual role as well as its relative non-volatility and non-oxidative property at higher temperatures. It is interesting to note that glucose is most stable at about *p*H 3 in aqueous solution at elevated temperatures[24] which is the *p*H of the polymerization mixture when dissolved in water. The phosphorous acid concentration and the temperature range employed for the polymerization are sufficient to activate condensation while side reactions such as levulinic acid or 5-hydroxymethylfurfural production do not occur, as indicated by the hydrolysis and chromatography experiments.

Careful balancing of the reaction parameters is necessary when new reaction conditions are developed for the polymerization of a sugar. Obviously, higher acid concentrations will require lower temperatures and shorter heating time and, conversely, higher temperatures or longer heating time is needed with lower acid concentration. Relatively long heating time is required to bring the polymerization to a high degree of conversion since the mobility of the monomers, low molecular weight polymers and the evolving water is rather low. In general, the higher the vacuum the more rapidly and completely the water is eliminated, the more

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(19) S. M. Cantor and Q. P. Peniston, THIS JOURNAL, **62**, 2113 (1940).

(20) C. G. Swain, *ibid.*, **72**, 4578 (1950).

(21) C. G. Swain and J. F. Brown, *ibid.*, **74**, 2534 (1952).

(22) T. M. Lowry and G. F. Smith, *J. Chem. Soc.*, 2539 (1927).

(23) T. M. Lowry and I. J. Falknev, *ibid.*, 127, 2883 (1925).

(24) W. Kroner and H. Kothe, *Ind. Eng. Chem.*, **31**, 248 (1939).

effective the polymerization is, and the less the oxidative decomposition that occurs. Decomposition can be detected by the beginning of yellowing or darkening of the melt; the temperature should then be lowered and the vacuum increased.

If the reaction conditions are sufficient to cause condensation only through the most reactive hydroxyl (the so called aldehydic or glucosidic hydroxyl) at the first carbon producing glucosidic linkage, then the monomer has a functionality of the type A–R–B_{f-1}. Here A (glucosidic hydroxyl) may condense with B (non-glucosidic hydroxyls), but reactions between B functional groups are forbidden. The mechanism of such a three-dimensional polyfunctional condensation was discussed by Flory.[25,26] Making the usual assumptions, reactivities of groups are not changed by the reaction of one or more of the other functional groups in the same molecule (the principle of equal reactivity of functional groups at different polymer size), and assuming that ring formation is negligible, then the formation of insoluble gel is impossible since the branching probability may approach but never reach the critical value for incipient formation of infinite structures.[25] Erlander and French[27] developed similar statistical treatment[28] when the B groups are not equally reactive,[29] which is the actual situation with glucose.[30] If A–A reactions are also excluded, each n-meric species will contain $n(f-1)+1$ unreacted B groups and only one unreacted A. The latter is the basis of the number average molecular weight determination by reducing end group. Actually, there is no energetic reason to advance an argument for the absence of the A–A (1-1′-glucosidic or trehalose-like) linkages.[31] However, agreement was shown previously between number average molecular weights determined by osmotic pressure and by reducing end-group method.[10] A probable explanation is that the A–A linkages which are formed during condensation are products of an equilibrium similar to the transglucosidation during hydrolysis, and after they are formed they might break again. Meanwhile, however, on the two parts of the polymer which they connect, the number of available free B groups increases with growth in molecular size, and the probability that an A–A link be formed again through the available two single A groups will be lower. This might be the cause of the statistically insignificant concentration of A–A linkage in polymers above a given molecular weight. A–A links can reopen nearly as frequently as A–B links, since the activation energy for the hydrolysis of an A–A dimer (trehalose, which consists of two 1-1′-linked α-D-glucoses) is 40.180 cal./g. mole, only slightly higher than the activation energy range for the hydrolysis of the A–B links in dimers (30,000–38,600 cal./g. mole).[32]

The absence of gel in experiments 1, 3–6, 8, 9 is proof that no B–B reaction occurs, and that the linkages are A–B type. Insoluble products in experiments 2 and 7 can be due to B–B linkages or to other side reactions. In experiment 2, where a large amount of gel was produced, decomposition was obvious by darkening of portions of the product where the infrared heat was apparently more concentrated in spite of the careful symmetrical focusing of the infrared bulbs. In our experience, the "infrared" method is the least practical and controllable of the three methods compared. The formation of the small amount of gel in experiment 7 (170° second-stage run) shows that this temperature is probably the borderline where B–B linkage or other side reactions may start.

The figures reported in Table II show increase of number average molecular weight with increase in temperature. However, it should be kept in mind that the figures indicate only fewer free reducing end groups and can be used as true indication of molecular weight only if each molecule has a single reducing end group.[33]

The critical temperature at which dextran fractions of known molecular weight and branching become turbid in water–methanol mixture (40.3% by volume) was used in studying the intrinsic viscosity and molecular weight relationship.[34] Ethyl alcohol was used to fractionate dextrans by molecular weight.[35] We used the amount of ethyl alcohol sufficient to produce initial turbidity as an empirical value to compare the presence of high molecular weight products in the molecular weight distribution of the different polymers. Higher temperature experiments required less alcohol, indicating the presence of higher molecular weight products. The yield of precipitated polymers with 70% (v.) ethanol gave an approximate indication of the relative amount of medium molecular weight products. Higher temperature runs gave more precipitate, except in the case of the "infrared" 150° run (expt. 2), where the amount of glucose which formed the gel reduced the yield below that of the 140° run (expt. 1). This is the reason also for the cross-over of the yields of these two runs (Fig. 3). The low yield of the "solution melt" products (expts. 3 and 4) and the less steep precipitation curves (Fig. 2) is due either to differences in the reaction mechanism or to different solubility caused by difference in configuration (branching). The first might be the consequence of the decreased chance of condensation between monomeric units and more frequent re-enclosure into the glucopyranose ring of the intermediary open chain form before condensation can take place. Ideally, no diluent should be present; the melt, however, solidifies rapidly due to the high melting point of the intermediary polymers, and plasticized melt polymerization method was

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[25] P. J. Flory, THIS JOURNAL, **74**, 2718 (1952).

[26] P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 347–398, Chapter IX.

[27] S. Erlander and D. French, *J. Polymer Sci.*, **20**, 7 (1956).

[28] For general probability theory of the condensation of A–R–B_2 units see E. S. Allen, *ibid.*, **21**, 349 (1956).

[29] For review of relative reactivities of hydroxyl groups of carbohydrates see J. M. Sugihara, *Adv. Carbohydrate Chem.*, **8**, 1 (1953).

[30] H. Frahm, *Ann.*, **555**, 187 (1944).

[31] *Cf.* discussion by A. Katchalsky and P. T. Mora in reference 10.

[32] E. A. Moelwyn-Hughes, *Trans. Faraday Soc.*, **25**, 503 (1929).

[33] For further data and discussion on fractions see Part II of this series, P. T. Mora, J. W. Wood, P. Maury and B. G. Young, THIS JOURNAL, **80**, 693 (1958).

[34] M. Wales, P. A. Marshall and S. G. Weissberg, *J. Polymer Sci.*, **10**, 229 (1953).

[35] F. R. Senti, N. N. Hellman, N. H. Ludwig, G. E. Babcock, R. Tobin, C. A. Glass and B. L. Lamberts, *ibid.*, **17**, 527 (1955).

developed to maintain higher mobility of the monomers and of the n-mers and to assure more uniform temperature throughout. Differences in branching in the fractions of the melt polymerization products will be discussed later.[33]

Intrinsic viscosities of the higher temperature polymers were generally higher. Both intrinsic viscosity and its increase by molecular weight are small in polyglucose, due to the high degree of branching.[10]

Between 70 and 82% of the polymer did not dialyze through cellophane, but if polymerization was attempted without acid catalyst there was only 14.8% non-dialyzable product and 15% of the glucose remained unpolymerized. Furthermore, there was sign of extensive decomposition. The polyglucoses prepared in the presence of phosphorous acid contained more higher molecular weight products when polymerized at higher temperatures. The large percentage of non-dialyzable product in all the polyglucose preparations and the low figures for the number average molecular weights indicate broad molecular weight distribution. The presence of a relatively small amount, but very low molecular weight product will lead to a low molecular weight figure by number average, but the major amount of the polymer is much above this molecular weight. A considerable amount of polyglucose can be present in the vicinity of 100,000 molecular weight, as was indicated by other macromolecular data.[10]

It is expected that the amount of α- and β-glucosidic linkages built into the polymer will correspond to the equilibrium concentration of the glucose isomers under the applied conditions of polymerization (temperature, acid concentration, etc.). The high positive optical rotation and the decrease down to the value for glucose during hydrolysis were used to imply higher concentration of α-linkages,[6] but we do not have sufficient information to state that the β-linkage is absent. The presence of absorption of radiation at 834 cm.$^{-1}$ and the absence of absorption at 890 ± 5 cm.$^{-1}$ were taken as proof that α-linkages are present, and β-linkages are absent.[7] The infrared spectra of different polyglucose preparations are nearly flat in the region 700–950 cm.$^{-1}$ where the absorption band characterizing polysaccharide linkages occur.[36] In our opinion, the very slight indentations on the curves are insufficient to allow any quantitative deduction. In a random polycondensation such as this, the presence of many linkages is expected, and their effect may cancel each other in this region. Higher temperature runs generally gave polymers with higher optical rotation, with the exception of the 160° and 170° "solution melt" products.

It is expected also that the amount of the linkages through the different B groups will correspond to the relative reactivities of the different non-glucosidic hydroxyls under the polymerization conditions but that their distribution will be random, and that branching may occur up to the functionality of glucose when all of the B groups react on the same monomeric unit. In 40% aqueous hydrogen chloride at 20° the primary (6th) hydroxyl is three times as reactive in condensation as the 2-, 3- and 4-hydroxyls,[30] and it may be expected that this linkage (1–6′) will be the most frequent, at least in lower temperature polymerization experiments.[10] At higher temperature, when the activation energy to form other A–B linkages (1–2′, 1–3′ and 1–4′) will be reached more frequently, there will be a smaller proportion of 1–6′-linkage and greater probability for simultaneous reaction at the other B groups; consequently a greater degree of branching can be expected. Periodate oxidation figures to show differences in branching and other pertinent data on fractions will be given in the second part of this series.[33]

The method of polycondensation is general for aldoses, except that the optimum reaction conditions will be different, taking into account the differences in reactivities and in sensitivity to acid destruction of the different sugars. Several other aldoses have been polymerized to high molecular weight polysaccharides. The conditions for these polymerizations will be presented in due course.

Acknowledgment.—The authors thank B. G. Young, P. Maury and M. M. Trail for experimental assistance. P. T. M. wishes to express his gratitude to Professor Eugene Pacsu of Princeton University for his invaluable help and suggestions, and also to acknowledge the collaboration of several colleagues during 1950–1954 at the Experimental Station, E. I. du Pont de Nemours and Co. Inc., Wilmington, Del. The early research on polyglucose was done at Princeton University in 1948–1950, while this author served as Post Doctoral Fellow in the Chemistry Department.

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