Dihydroisomammein Dimethyl Ether (XII).—Methylation of dihydroisomammein (XI) with dimethyl sulfate and potassium carbonate in acetone solution was carried out as described above and after recrystallization from dilute isopropyl alcohol led to dihydroisomammein dimethyl ether (XII), m.p. 82–84°, depressed by 15° upon admixture with dihydromammein dimethyl ether (VII); $\lambda_{max}^{CS_2}$ 5.70, 5.82 and 6.17 μ, λ_{max}^{EtOH} 246 and 297 mμ (log ε 4.08, 4.12); λ_{min}^{EtOH} 238 and 269 mμ (log ε 4.06, 3.90).

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51; OCH_3, 15.43. Found: C, 71.59; H, 8.51; OCH_3, 15.50.

Ozonolysis of Mammein (I).—Ozone was passed at room temperature through a solution of 500 mg. of mammein in 10 cc. of glacial acetic acid until the exit gas showed the presence of ozone as indicated by moist starch iodide paper (30 min.). After stirring for one hour with 1 g. of ferrous sulfate in 15 cc. of water, the solution was steam distilled and the distillate (200 cc.) passed directly into a solution of 1.0 g. of 2,4-dinitrophenylhydrazine in 80 cc. of water and 20 cc. of concd. sulfuric acid. The resulting yellow 2,4-dinitrophenylhydrazone (163 mg., 51% yield) was purified by filtering in benzene solution through Merck acid-washed alumina and recrystallizing from hexane giving orange crystals, m.p. 125–126°, undepressed upon admixture with acetone 2,4-dinitrophenylhydrazone; the infrared spectra were also identical.

No evidence for the formation of other dinitrophenylhydrazones was obtained and under identical conditions a blank run did not furnish any detectable precipitate with the 2,4-dinitrophenylhydrazine reagent.

In a subsequent run with 927 mg. of mammein in 20 cc. of acetic acid[17] over a period of 78 minutes, the ozone consumption was measured quantitatively and found to equal 1.1 moles before ozone could be detected in the exit gases. The ozonide was decomposed by stirring for 1 hr. with 2.0 g. of ferrous sulfate in 20 cc. of water, but instead of steam distilling, 200 cc. of water was added and the product was extracted with ether. After washing with water and evaporating, there was obtained 868 mg. of the yellowish solid aldehyde XIV which was decolorized in ether solution with Norit. Chromatographic purification on Merck acid-washed alumina, elution with benzene–ether mixtures and recrystallization from aqueous methanol gave colorless crystals, m.p. 136–144°. The melting point was not improved on further recrystallization from ether–hexane or chloroform–hexane. The aldehyde gave a red-brown color with ferric chloride and in contrast to mammein showed a positive reaction with Tollens reagent; $\lambda_{max}^{CS_2}$ 3.0(w), 3.59(w) (not present in mammein), 5.70(w) and 5.82(m) μ; $\lambda_{max}^{CHCl_3}$ 3.01(w), 5.70(m), 5.80(s), 6.06(s) and 6.16(s) μ; λ_{max}^{EtOH} 293 mμ (log ε 4.30), λ_{min}^{EtOH} 249 mμ (log ε 3.50), $\lambda_{max}^{1\ N\ EtOH-KOH}$ 327 mμ (log ε 4.58) and shoulder at 380 mμ (log ε 4.29), $\lambda_{min}^{1\ N\ EtOH-KOH}$ 293 mμ (log ε 4.55).

(17) The aldehyde XIV could not be isolated when the ozonization was conducted in ethyl acetate solution at −70°.

Anal. Calcd. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40; O, 27.72; mol. wt., 346. Found: C, 65.54; H, 6.66; O, 27.36; mol. wt., 351 (electrometric titration in 66% DMF, initial *p*H 5.9, pK_a' 7.5).

A sample of mammein dimethyl ether (III) was ozonized in acetic acid solution as described above for mammein and yielded 35% of acetone 2,4-dinitrophenylhydrazone, uncontaminated by other dinitrophenylhydrazones. Treatment of dihydromammein (V) with ozone for 30 minutes showed that ozone could already be detected in the exit gases within 2–3 min. No steam-volatile products were formed and 45% of pure dihydromammein (V) could be recovered from the residue after ozonization and steam distillation.

Ozonization of Isomammein (VIII).—Since the ultraviolet spectral comparison (Figs. 1 and 2) between mammein (I) and isomammein (VIII) showed a marked change in the absorbing system, it was necessary to show by ozonization that the position of the reactive double bond was not altered in this transformation (I → VIII). Treatment of 498 mg. of isomammein (VIII) in 15 cc. of glacial acetic acid solution (yellow rather than colorless as with mammein) with ozone exactly as described above for mammein furnished 195 mg. (60%) of acetone 2,4-dinitrophenylhydrazone and 430 mg. of the crude aldehyde XV as a brownish solid. Decolorization with Norit in ether solution and recrystallization from ether–hexane gave transparent, pale yellow plates, m.p. 130–132°; $\lambda_{max}^{CS_2}$ 3.00(m), 3.60(w) (not present in isomammein), 5.70(m), 5.75(s), 5.81(s) μ; $\lambda_{max}^{CHCl_3}$ 3.02(m), 5.80(s), 6.15(s) μ; λ_{max}^{EtOH} 281, 332 and 369 mμ (log ε 4.45, 4.07, 4.20), and a shoulder at 388 to 398 (log ε 4.14); λ_{min}^{EtOH} 246 and 310 μ (log ε 3.81 and 4.01); $\lambda_{max}^{1\ N\ EtOH-KOH}$ 222.5, 305 and 408 mμ (log ε 4.56, 4.25, 4.24); $\lambda_{min}^{1\ N\ EtOH-KOH}$ 270, 340 and 371 mμ (log ε 4.12, 4.01, 4.11). While isomammein gives a gray-green color with ferric chloride and does not react with Tollens reagent, the aldehyde XV gave a green color and reduced Tollens reagent.

Anal. Calcd. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40; O, 27.72; mol. wt., 346.4. Found: C, 65.40; H, 6.37; O, 28.05; mol. wt., 364 (electrometric titration in 66% DMF, initial *p*H 5.7, pK_a' 7.6).

It was not possible to convert the aldehyde XIV, derived from mammein, by alkaline isomerization into the aldehyde XV, possibly due to other competing reactions since no crystalline product was isolated.

DETROIT, MICHIGAN
MAYAGUEZ, PUERTO RICO

[CONTRIBUTION FROM CORN PRODUCTS REFINING COMPANY'S MULTIPLE FELLOWSHIP, MELLON INSTITUTE, AND THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF PITTSBURGH]

Polymerization of α-D-Glucose in the Solid State, in the Presence of Metaboric Acid[1]

BY HARRY W. DURAND,[2] MALCOLM F. DULL AND R. STUART TIPSON

RECEIVED NOVEMBER 16, 1957

The polymerization of crystalline α-D-glucose by heat in the presence of powdered metaboric acid has been studied. Optimal conditions for conducting the reaction are given, and the properties and evidence of the structural nature of typical polymeric products are described. One such product has been examined chromatographically and indicated to be a poly-D-glucoside of branched structure built up of both 1 → 6- and 1 → 4-acetal linkages.

Introduction

In 1945, Leuck[3] disclosed the successful polymerization of α-D-glucose in essentially the solid state, by heating of an intimate mixture of the crystalline anhydrous sugar with powdered boric acid or an anhydride thereof, in such a way that practically no fusion occurs. To the naked eye, the original shape of the sugar crystals is largely maintained throughout the reaction, but X-ray diffraction patterns show that the products are actually amorphous.

Leuck considered typical products to be polymeric on the basis of their infusibility, their lowered solubility in aqueous organic solvents, and the

(1) Based on a thesis submitted by Harry W. Durand to the Graduate School of the University of Pittsburgh, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, February, 1956.

(2) Corn Products Refining Co., Argo, Ill.

(3) G. J. Leuck, U. S. Patent 2,375,564 (May 8, 1945); *C. A.*, **39**, 4508 (1945).

cryoscopic and viscometric behavior of their aqueous solutions. He also described the polymers as retaining practically none of the sweetness of the original sugar, as relatively non-reducing to Fehling or Benedict solution, and as substantially unfermentable by yeast. (Insusceptibility to salivary and blood amylases has, more recently, also been demonstrated.[4])

We have found that the polymers obtained under the mildest reaction conditions specified by Leuck (t = 135 to 140°) are completely soluble in water. However, a gradual increase in temperature during the process, to 180–200°, ultimately leads to products which are largely water-insoluble. Whether water-soluble or not, the polymers are readily hydrolyzed by dilute mineral acids to afford a degree of reducing power equivalent to that of the original α-D-glucose.

The process shows an advantage over procedures using strong mineral acids or acidic salts in that there is relatively little tendency for decomposition and color formation, and the products are readily isolated and freed from impurities. The boric acid can be removed almost completely from the products by extraction with cold methanol, suggesting that its function is primarily catalytic.

Although Leuck[3] assumed the products to be D-glucans, he gave no evidence regarding their structure. We have now repeated his work, have established useful procedures for preparing reproducible polymeric samples, and have more thoroughly investigated the properties of typical products and the structure of one of them.

Experimental

Reaction Conditions.—A preliminary study of the reaction indicated that smoothness of operation requires adherence to the following experimental conditions: (a) at least initially, the temperature should be held within the range of 135–140°; (b) metaboric acid is to be preferred as the additive over either boric acid or boron trioxide, and the minimum amount used should be about 5% of the weight of the sugar; (c) the ratio of the particle size of the D-glucose to that of the metaboric acid should be relatively large; (d) in order to minimize tendency toward fusion, the dry blend of anhydrous D-glucose and metaboric acid should be heated in a thin, fairly uniform layer on a shallow container. Use of agitation or vacuum is unnecessary, but it is important that the reaction mixture have a sufficient surface exposed to permit the rapid escape of evolved water; otherwise retained moisture may lower the melting point of the mixture and cause liquefaction.

Materials.—The α-D-glucose was from a commercial source (anhydrous dextrose, Corn Products Refining Co.) and was prepared for use by manual screening through standard 8-in. copper sieves to collect the portion passing through 50-mesh and retained by 60-mesh. This fraction was rescreened on a 70-mesh sieve to remove occluded fines. Repeated reductimetric analyses showed >99.0% of D-glucose; for $[\alpha]_D$ (in water) see Table I.

The metaboric acid was prepared from boric acid (Eimer and Amend U.S.P. powder) having the following analysis: non-distillable from methanol <0.10%; boric acid, based on alkali titration, ≧99.0%. The boric acid was heated for 12–24 hr. in an air-circulating oven at 110–125°. The product was ground and screened through a 200-mesh copper sieve. Repeated preparations were analyzed by titration with standard alkali and showed ≧98% metaboric acid.

The standard blend chosen for polymerization experiments comprised 100 parts of the sugar plus 5 parts of the metaboric acid by weight. This mixture was blended by agitation on a can roll for 6 hr.

(4) Private communication from the late Dr. Lawrence Greenman of Children's Hospital, Pittsburgh, Pa.

TABLE I

MOLECULAR WEIGHT OF POLYMERS IN RELATION TO REDUCING POWER

Polymer	$[\alpha]^{23}D$ (degrees)	Mol. wt. (number-average)[a]	D.P.[b]	Reducing sugar, % Calcd.[c]	Found
GP30	+57.5	920	5.7	17.5	3.6
GP28	+64	1050	6.5	15.4	1.5
GP33	+63	2765	17.1	5.8	1.3
α-D-Glucose	+99.5 → 53[d]	180[e]	..	..	99.0

[a] Determined by the cryoscopic method applied to aqueous solutions. [b] Degree of polymerization, calculated as the value x in a polymer of the assumed formula $(C_6H_{10}O_5)x$. [c] Calculated on the basis of an assumed simple linear formula of the D.P. given, containing one reducing end-group per molecule. [d] Signifies the mutarotation of the α-D-glucose from the value obtained at 5 minutes to that obtained 6 hr. after preparation of the solution; in the case of the polymers, no mutarotation was observed. [e] Formula weight of $C_6H_{12}O_6$.

Course of Reaction at 140°.—The heating chamber was a Cenco–DeKhotinsky cylindrical oven of gravity-convection type (Catalog No. 95050); according to the manufacturer, the maximum variation of temperature at different points within this oven is ±3° at a setting of 110°. The oven control was set at 140°. Replicate samples (10.000 g.) of the standard blend were weighed into Pyrex-glass Petri dishes (95 mm. diameter), spread fairly evenly to layers of about 0.5-cm. depth, and then placed within the oven on a level about 1 in. below the tip of the oven thermometer. Samples were removed periodically, weighed, and analyzed for reducing-sugar content, using Schoorl's iodide method. Figure 1 summarizes the data relating reducing power and loss of weight to the time of reaction. If the decrease of reducing power be accepted as an index of the course of polymerization, the results show that duplicability of the process can be obtained merely by operating the reaction for at least 12 hours.

Polymer Preparations.—The oven was of a mechanical convection type (model 1204, Electric Hotpack Co., Philadelphia, Pa.). The amount of standard blend treated was 500 g. per batch, weighed into a rectangular, Pyrex-glass baking dish (8 × 13 × 1.5 in.), and spread to a fairly uniform layer of about 0.5-cm. thickness. The oven was operated with a minimum of mechanical convection (to avoid surface fusion and caramelization), and an auxiliary thermometer was mounted inside the oven chamber with its mercury tip within 0.5 in. of the surface of the blend.

Polymerization runs were conducted in duplicate under (I) mild and (II) relatively rigorous conditions, in accordance with the following heating cycles: for batch I, 16 hr. at 140 ± 0.5°; and for batch II, 16 hr. at 140 ± 0.5°, followed by gradual increase of temperature over an 8-hr. period to 150°, and a final 28 hr. at 150 ± 1.0°. The products were analyzed.

Batch no.	Yield, % (based on standard blend)	Reducing-sugar content, % (based on D-glucose)	HBO_2 content, %
I	89.4, 89.8	6.0, 6.3	3.8, 4.0
II	88.3, 88.5	1.52, 1.59	3.6, 3.7

The separate products of each batch were combined and exhaustively extracted with anhydrous methanol at room temperature. The methanol-insoluble residues were suction-filtered, air-dried at room temperature, and finally vacuum-dried at 105° to constant weight. The products ("glucose polymers," GP) were analyzed.

Product	Yield, % (based on crude polymer)	Reducing-sugar content, %	HBO_2 content, %
GP30[a]	86.0	3.6	0.06
GP28[b]	95.5	1.50	0.15

[a] From batch I. [b] From batch II.

In addition to the above preparations, the following coarse fractionation was carried out: Polymer GP28 (200 g.) was dissolved in 200 ml. of distilled water. Anhydrous methanol (400 ml.) was added in 100-ml. portions with agitation, the mixture was stirred for another 2 hr., and

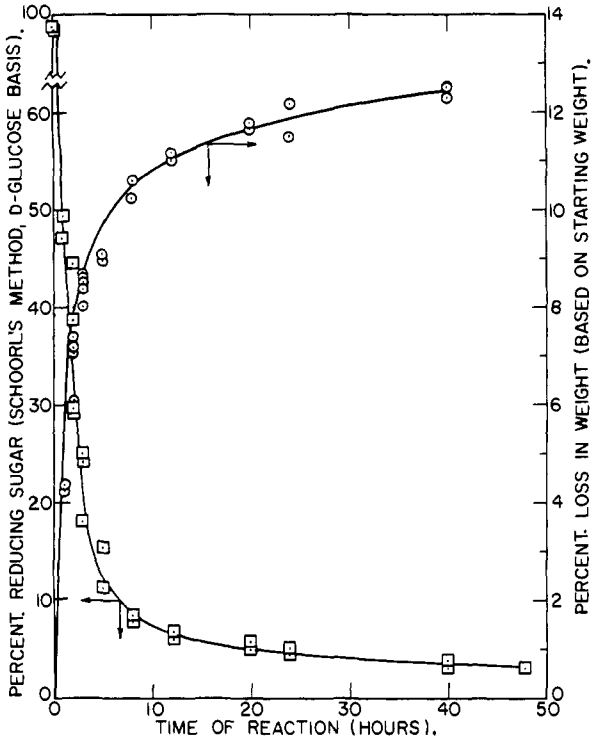


Fig. 1.—Effect of heating anhydrous α-D-glucose containing 5% of metaboric acid at 140°: reaction time *vs.*: ⊡, % reducing-sugar content, and ⊙, % loss in weight.

was then allowed to stand 24 hr. at room temperature; during this time, a clean separation into two liquid layers occurred. The top layer was removed by pipet decantation. To the remaining (heavier) phase was added 100 ml. of distilled water to restore a single solution, followed by another 200 ml. of anhydrous methanol. After being stirred for 3 hr., the mixture was allowed to stand for 48 hr., and again the top layer was decanted. The sirupy residue was dried under vacuum, initially at 50° and finally at 105°, to constant weight. A yield of 106 g. of product (GP33) was obtained. *Anal.* 1.3% reducing sugar; 0.09% metaboric acid.

On paper-chromatographic examination, the above three polymeric products gave no evidence for the presence of unreacted D-glucose.

Polymer Properties.[5]—**The X-ray diffraction powder pattern** of polymer GP30 was compared with that of the α-D-glucose used as the starting material and indicated complete loss of crystallinity as a result of the polymerization. On the other hand, microscopic comparison of the particulate state of the unrefined polymer used to make product GP30 with that of the crystalline α-D-glucose showed that, although some rounding of edges occurred as well as a slight increase in particle size, the general shape of the original sugar crystals was retained over the course of the polymerization.

The molecular weights of polymers GP30, GP28 and GP33 were determined cryoscopically, with water as the solvent (Table I). Only the "rough" fractional product GP33 showed a relationship between molecular weight and reducing-sugar content approaching that required by the assumption that the polymerization reaction simply involves acetal-bond formation.

The limitations of the cryoscopic procedure prevent any precise statement as to the molecular size of the polymers examined.

The apparent lack of mobility shown by polymer GP30 in the paper chromatograms described later indicates[6] that, despite its low cryoscopic molecular-weight value of 920, the product is almost entirely composed of polymeric species containing more than nine anhydro-D-glucose residues and having molecular weights greater than 1500. It would therefore appear that the polymeric products of this investigation are rather highly heterogeneous in molecular size: the cryoscopic values are probably much lower than would be obtained by a "weight-average" method.

Specific rotation ($[\alpha]_D$) values for polymers GP30, GP28 and GP33, as well as for the α-D-glucose source material (Table I), were determined at 23°, using water as the solvent and a concentration in each case of 1.00 g. per 100 ml. of solution; the length of the polarimeter tube was 2 dm. The $[\alpha]_D$ values for all three polymers were in about the same range, regardless of molecular weight, and were much less dextrorotatory than polymers obtained in previous investigations[7] concerned largely with self-condensation of D-glucose in the presence of hydrochloric acid.

It has been pointed out[8] that high dextrorotatory power in a sugar or polysaccharide is evidence that the structure is based on α-D-linkages, whereas a high degree of levorotation signifies the presence of β-D-linkages. Therefore, the polymers of this investigation may differ from those of the cited prior cases in having a much lower ratio of α-:β-linkages. There is also the possibility that the presence of strongly levorotatory terminal groups, resembling β-D-glucosan, for example, could act to reduce the dextrorotatory power of the final polymer.

As would be expected, the **viscosities** (Fig. 2) of aqueous solutions of the three polymeric products increased in the order of their increasing molecular weight. The determinations were made at 25°, using a Fenske-modified Ostwald viscometer.

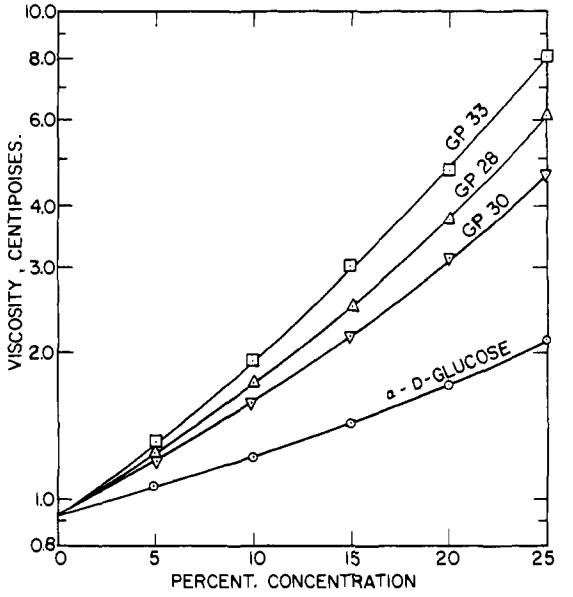


Fig. 2.—Viscosity of metaboric acid-catalyzed α-D-glucose polymers in aqueous solution at 25°.

Infrared absorption spectra were recorded for polymers GP30 and GP33, as well as for α-D-glucose, β-D-glucose and corn starch, by means of a Baird infrared spectrophotometer, using a sodium chloride prism and the Nujol mull technique. The polymers both gave ill-defined absorption throughout the critical frequency range[9] of 730-960 cm.$^{-1}$, whereas the

(5) Acknowledgment is given to the Departments of Research in Physical Chemistry and in Chemical Physics, Mellon Institute, for the X-ray diffraction powder photographs, the cryoscopic molecular weights and the infrared absorption spectral data referred to in this paper.

(6) Private communication from Research Dept., Corn Products Refining Co., Argo, Ill.

(7) P. A. Levene and A. Ulpts, *J. Biol. Chem.*, **64**, 475 (1925); E. Pacsu and P. T. Mora, THIS JOURNAL, **72**, 1045 (1950); A. Thompson, K. Anno, M. L. Wolfrom and M. Inatome, *ibid.*, **76**, 1309 (1954); P. W. Kent, *Biochem. J.*, **55**, 361 (1953); C. R. Ricketts, *J. Chem. Soc.*, 4031 (1954).

(8) W. N. Haworth, "The Constitution of Sugars," Edward Arnold & Co., London, 1929, p. 85.

(9) S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *J. Chem. Soc.*, 171 (1954).

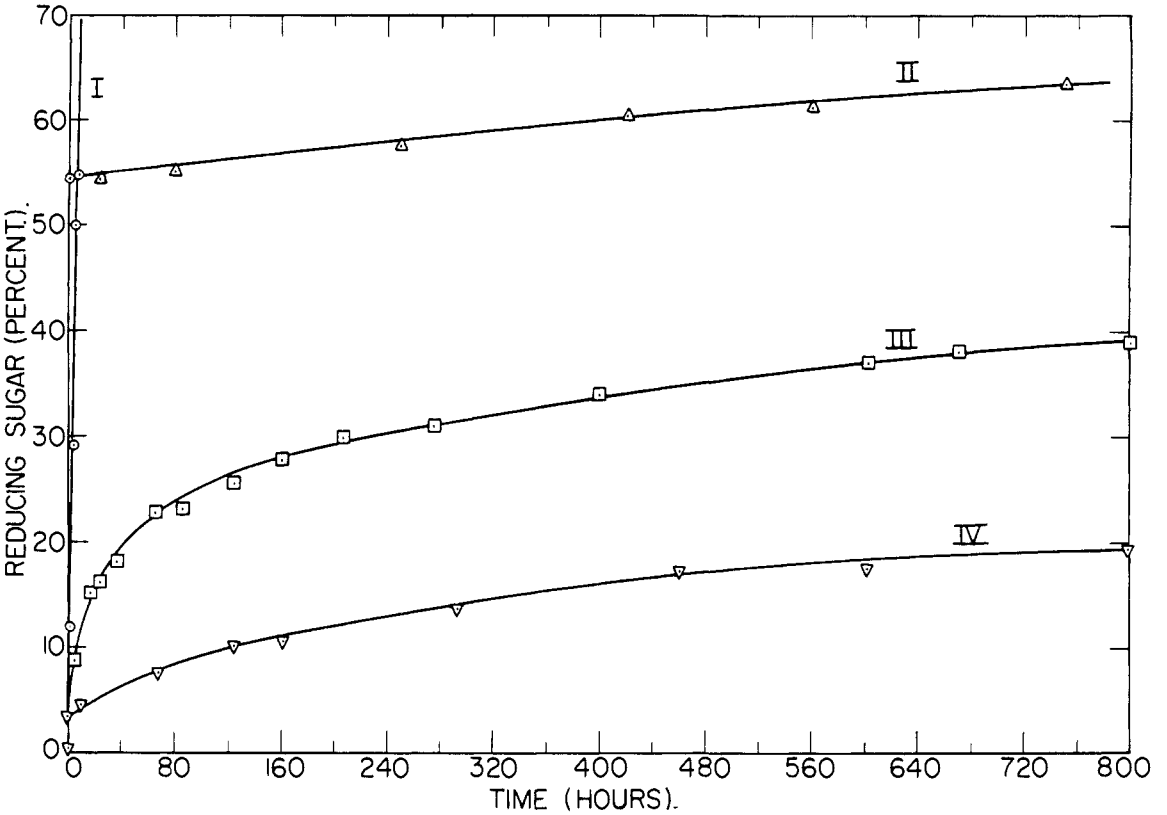


Fig. 3.—Rates of increase of reducing-sugar content at 25° for 1.00% solutions of: (I) sucrose in 0.100 *N* HCl; (II) maltose in 1.000 *N* HCl; (III) polymer GP30 in 1.000 *N* HCl; and (IV) polymer GP30 in 0.100 *N* HCl.

other products gave reasonably well-defined bands as follows: α-D-glucose, 3 bands: 912, 837 and 755 cm.$^{-1}$; β-D-glucose, 1 band: 898 cm.$^{-1}$; corn starch, 3 bands: 920, 855 and 764 cm.$^{-1}$. The results indicate that the polymers obtained in this investigation are too complex and too heterogeneous to permit use of infrared spectral data for their structural assignment.

The hydroxyl content of polymer GP30 was determined indirectly by acetylation at elevated temperatures using two different methods,[10] followed by hydrolysis of the resulting acetates and titration of the acetic acid liberated.[11] (Attempts to acetylate the polymer with acetic anhydride in cold pyridine failed due to lack of solubility of the polymer in pyridine, even at room temperature.) The acetylations were carried out using hot acetic anhydride with (1) anhydrous sodium acetate and (2) pyridine. In method 1, 20 g. of GP30 yielded 28 g. of acetylated product (GP30-Ac1), $[\alpha]^{25}D$ +66.5° (*c* 1.0, in chloroform); in method 2, 10 g. of GP30 yielded 16.5 g. of acetylated product (GP30-Ac2), $[\alpha]^{25}D$ +65° (*c* 1.0, in chloroform). The acetyl contents of the products also were determined: Standardized sulfuric acid (100 ml. of 0.252 *N*) was pipetted into a 250-ml., round-bottomed flask containing 0.500 g. of the polymer acetate, and the flask was fitted with a water-cooled condenser. The mixture was heated at reflux temperature for 5 hr. After cooling the mixture, 25 ml. of *N* sodium hydroxide was pipetted in. The resulting partially neutralized solution was then titrated with 0.1003 *N* potassium hydroxide. The results of duplicate analyses are summarized in Table II, and show that the two different acetylation procedures gave products having practically identical acetyl contents. The values obtained correspond closely to the theoretical amounts contained in a completely acetylated D-glucan containing three hydroxyl groups per unit residue; the number of hydroxyl groups actually found was 2.9.

(10) F. J. Bates and associates, "Polarimetry, Saccharimetry and the Sugars," Circular C440, U. S. Government Printing Office, Washington, D. C., 1942, pp. 486, 488.

(11) *Ibid.*, p. 495, method 2.

For the determination of the **rates of hydrolysis** (Fig. 3) solutions of polymer GP30 were prepared at 1.00% concentration in aqueous hydrochloric acid (1.000 and 0.100 *N*) and subjected to constant agitation at 25°. Aliquot portions of the solutions were withdrawn periodically,

TABLE II

ACETYL CONTENTS OF POLYMER (GP30) ACETATES

Acetate sample	0.1003 *N* KOH, ml.	Acetyl content Meq.	G.	%	% of theoretical[a]
Blank	3.3	...	....	..	..
GP30-Ac1- (0.500 g.)	50.0, 50.0	5.015	0.2156	43.1	96.2
GP30-Ac2- (0.500 g.)	50.2, 50.2	5.035	0.2165	43.3	96.6

[a] Based on the D-glucan formula $(C_6H_{10}O_5)x$ (theoretical acetyl content 44.8%).

neutralized by addition of aqueous sodium hydroxide of appropriate normality and analyzed for reducing-sugar content by Schoorl's iodide method (D-glucose basis). For the sake of comparison, similar experiments were carried out using maltose and sucrose. Data on the behavior of maltose in 0.100 *N* acid and of sucrose in 1.000 *N* acid are not included; in the former case, practically no increase of reducing-sugar content was observed under the experimental conditions used, whereas with sucrose, hydrolysis proceeded extremely rapidly: to 87% of completion in 2 hr. and to 97% in 5 hr. of reaction. Although the results with the polymer are only indirectly an index of rate of hydrolysis or of stability under acid hydrolytic conditions, it is certainly obvious that the polymer is much more stable than sucrose and, except during the early stages of reaction, behaves more like maltose. On this basis,[12] it may be concluded that the predominant unit structure of the polymer is

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

of D-glucopyranosidic rather than D-glucofuranosidic nature.

The rapid increase in reducing-sugar content occurring during the early stages of hydrolysis of GP30 with 1.000 *N* mineral acid is of interest. The effect is not accounted for as being one of concentration, nor is it likely that the later leveling off is due to reversion, since D-glucose was found to undergo no polymerization under similar conditions. Absence of the effect in the more dilute (0.100 *N*) acid precludes an explanation based on an assumption of the presence of a small percentage of D-glucofuranose residues. The possibility of the existence of D-glucosan terminal groups already has been considered. If such groupings were present, it seems reasonable to suppose that their dissociation might proceed more rapidly than intermolecular cleavage under acid hydrolytic conditions. Thus, the simultaneous occurrence of the two reactions could account for the effect noted.

Paper Chromatographic Studies of Polymer GP30. (A) Apparatus and Materials.—The procedure was a qualitative descending technique using the Fisher Scientific Co. strip-paper chromatographic apparatus. The paper was Whatman No. 1 in the form of stripping 1.5 in. wide, cut to sufficient length to allow 50 cm. of effective development. As many as six strips could be accommodated by the apparatus in a single experiment. The developments were conducted at room temperature over periods of 16 to 30 hr.

For the examination of the **partial acid hydrolyzates of GP30**, the developing solvent was the miscible system: 1-butanol–1-propanol–water, in the volume ratio of 2:5:3 (solvent BPW)[6]; the spray reagent consisted of a 5:5:1 mixture, by volume, of a 4% solution of aniline in ethyl alcohol, a 4% solution of diphenylamine in ethyl alcohol, and 85% phosphoric acid, respectively[13]; the reference sugars used were α-D-glucose, sucrose, maltose, cellobiose, gentiobiose and isomaltose. (Authentic samples of the last three were generously supplied by Dr. Roy L. Whistler of Purdue University.)

For the examination of the **acid hydrolyzate of the methylated GP30**, the solvent used for development was the partially miscible system 1-butanol–ethanol–water, in the volume ratio of 4:1:5, respectively (solvent BEW)[14]; the spray reagent was aniline hydrogen phthalate (prepared by adding 930 mg. of aniline and 1.6 g. of phthalic acid to 100 ml. of water-saturated 1-butanol)[15]; in addition to α-D-glucose, D-xylose, L-rhamnose and D-ribose, various *O*-methyl-D-glucoses were used for reference. (Authentic samples of these were generously supplied by Dr. J. K. N. Jones of Queen's University, Dr. Fred Smith of the University of Minnesota, and Dr. Alva Thompson of The Ohio State University.)

(B) Acid Hydrolysis of Polymer GP30. (1) **At 25°.**—A 16-hr. development with solvent BPW was carried out on strips spotted with the following solutions of GP30 at 1% concentrations: (a) in water, (b) in 0.100 *N* hydrochloric acid after 400 hr. (reducing-sugar content 16%, Fig. 3), (c) in 1.000 *N* hydrochloric acid after 600 hr. (reducing-sugar content 37%, Fig. 3). The results showed: (a) no movement, indicating that the unhydrolyzed polymer consists almost entirely of species built up of more than nine anhydro-D-glucose residues each[6]; (b) slight smearing but no resolution, indicating that very little breakdown to simple sugars had occurred, despite the fairly high reducing-sugar content that was attained (Fig. 3), a result consistent with the hypothesis that glucosan termination is involved in the polymer structure; and (c) heavy smearing, with resolution of one spot (R_f 0.33–0.35) identified as D-glucose. Resolution in (b) was not improved by increasing the time of development to 28 hr.

(2) **At 100°.**—Initially, a 1% solution of GP30 in 0.100 *N* hydrochloric acid was held at 100°, and spot samples were taken at 10-minute intervals. Chromatographic examination revealed no distinguishable entity except D-glucose, and this was seen only after a hydrolysis time of 40–60 minutes. (A reducing-sugar analysis on the 60-minute hydrolyzate showed 96.0–98.5% conversion to D-glucose.) However, when the concentrations of both the acid and the polymer were increased, an intermediate disaccharide of about the same R_f value as that of maltose was detected. That the intermediate was not a product of reversion was evidenced by the fact that it was detectable only during the first 40 minutes of hydrolysis.

The following experiment was therefore carried out: Polymer GP30 (2.00 g.) was dissolved in 50 ml. of 1.000 *N* hydrochloric acid and heated as rapidly as possible to boiling (2 minutes was required to reach reflux temperature). Samples (5 ml.) of the boiling solution were withdrawn by pipet at 0, 2, 4.5 and 8.0 minutes after the start of refluxing. Each sample was immediately neutralized by the addition of excess silver carbonate, cooled and filtered. Small amounts of Celite were added to the filtrates, excess Ag^+ was precipitated by hydrogen sulfide and the mixtures were centrifuged. The decanted liquors were aerated to remove excess hydrogen sulfide, and the samples were chromatographed along with control strips spotted with the various sugars used for reference. The results (Table III) strongly indicated the unknown disaccharide intermediate to be either isomaltose or gentiobiose, or both. In view of the fact that D-glucose was the only product detected on long hydrolysis under these conditions, it may be concluded that the polymer is largely of 1 → 6-polyglucosidic structure.

TABLE III

PAPER-CHROMATOGRAPHIC RATE DATA[a] FOR POLYMER GP30 ACID HYDROLYZATES[b] AND FOR VARIOUS CONTROL SUGARS

Strip	R_f Of D-glucose	R_f Of non-D-glucose	R_G[c] Of non-D-glucose
Hydrolyzate (0 min.)	0.33	0.18 (very faint)	0.55
Hydrolyzate (2 min.)	.34	.19	.56
Hydrolyzate (4.5 min.)	.35	.19	.54
Hydrolyzate (8 min.)	.34	.19	.56
Maltose, D-glucose	.35–0.37	.23–0.24	.65–0.66
Cellobiose, D-glucose	.34– .37	.22– .24	.63– .64
Gentiobiose, D-glucose	.37– .38	.20– .22	.55– .57
Isomaltose, D-glucose	.35– .37	.20– .21	.56– .58
Sucrose, D-glucose	.36– .37	.29– .30	.80– .83

[a] Using Whatman No. 1 paper strips with 1-butanol–1-propanol–H_2O (2:5:3, by volume) as solvent at 25°. [b] After various periods of time in *N* HCl at 100°. [c] Rate with reference to D-glucose.

(C) Methylation of Polymer GP30.—Polymer GP30 (50 g.) was acetylated, using the hot acetic anhydride–sodium acetate procedure, and the product was purified by treatment with activated carbon in 95% ethyl alcohol, reprecipitated, and dried to constant weight at 80°. The yield of acetylated polymer (GP81) was 61 g., $[\alpha]^{25}D$ +66.5° (*c* 1.0, in chloroform); 42.6% acetyl content, equivalent to 2.8 acetyl groups per anhydro-D-glucose residue.

The acetate GP81 (25 g.) was dissolved in 50 ml. of acetone containing 10 ml. of water[16] and subjected to methylation by a modified Haworth procedure.[17] The yield of product (GP83) was 16 g., $[\alpha]^{25}D$ +64.5° (*c* 1.0, in chloroform). (The methoxyl content was not determined at this stage, but the product was assumed to be suitable for subsequent exhaustive methylation, because of its complete solubility in methyl iodide.)

The Haworth-methylated product GP83 (10 g.) was now subjected to six successive methylations using Purdie reagents.[18] The yield of final product (GP94) was 11 g., $[\alpha]^{30}D$ +62.5° (*c* 1.0, in chloroform). *Anal.* Calcd. for $C_6H_7O_2(OCH_3)_3$: C, 52.9; H, 7.8; OCH_3, 45.6. Found: C, 52.3; H, 7.8; OCH_3, 44.2. The degree of methylation achieved was therefore about 97% of the theoretical (the product isolated after the first Purdie methylation gave a value of about 70%), corresponding to about 2.9 methoxyl groups per anhydro-D-glucose residue.

(13) J. L. Buchan and R. I. Savage, *Analyst*, **77**, 404 (1952).

(14) E. L. Hirst and J. K. N. Jones, *Disc. Faraday Soc.*, **7**, 271 (1949).

(15) S. M. Partridge, *Nature*, **164**, 443 (1949).

(16) W. N. Haworth, E. L. Hirst and H. A. Thomas, *J. Chem. Soc.*, 821 (1931).

(17) W. N. Haworth, *ibid.*, **107**, 8 (1915); R. S. Tipson and P. A. Levene, *J. Biol. Chem.*, **129**, 578 (1939).

(18) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1026 (1903); H. Hibbert, R. S. Tipson and F. Brauns, *Can. J. Research*, **4**, 221 (1931).

(D) Chromatography of the Methylated Polymer.[19]—The methylated polymer GP94 (0.25 g.) was placed in a small Carius tube, together with 2.5 ml. of a 4% soln. of hydrogen chloride in anhydrous methanol. The tube was sealed, and then heated for 16 hr. at 100°. After cooling, the seal was broken, the solution was transferred to a test-tube, and 5 ml. of *N* hydrochloric acid was added. The mixture was heated under reflux at 100° for 3 hr., cooled, and neutralized by addition of 2 g. of silver carbonate. The mixture was filtered through Celite-coated paper on a Hirsch funnel, and the residue was washed with a little distilled water. The filtrate plus washings was heated to boiling with 1 g. of Amberlite resin IR-120 (to remove excess Ag^+) and again filtered. The filtrate was evaporated *in vacuo* at 70° to constant weight. The residue (0.245 g.) was taken up in 2.5 ml. of distilled water, and the solution was used for spotting of paper strips. The strips were developed, using the solvent BEW, over 24–28 hr., sprayed with aniline hydrogen phthalate indicator, and heated for 10 min. at 100–110° to bring out the positions of the resolved products.

Seven spots of varying intensity were thereby obtained, but all were sufficiently sharply defined to allow differentiation; R_f values for the resolved spots were determined and compared with those obtained for various control sugars (Table IV). Spot 7 was then easily identified as being occasioned by the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose; therefore R_{tg} values were also determined with reference to the position of spot 7, and similarly compared with R_{tg} values for the control sugars, also listed in Table IV.

TABLE IV

CHROMATOGRAPHIC RATE VALUES[a] FOR VARIOUS SUGARS AND *O*-METHYL-D-GLUCOSES

Sugar	Present results R_f	Present results R_{tg}[b]	Previously reported R_{tg}[b]
D-Glucose	0.20	0.24	0.09
D-Xylose	.28	.34	.15
D-Ribose	.33	.40	.21
2-*O*-Methyl-D-glucose	.33	.40	.22
3-*O*-Methyl-D-glucose	.35	.42	.26
6-*O*-Methyl-D-glucose	Unavailable	..	.27
L-Rhamnose	0.41	.50	.30
2,6-Di-*O*-methyl-D-glucose	.49	.59	.51
3,6-Di-*O*-methyl-D-glucose	.50	.61	Not given
4,6-Di-*O*-methyl-D-glucose	.50	.61	0.46
3,4-Di-*O*-methyl-D-glucose	.52	.63	.52
2,3-Di-*O*-methyl-D-glucose	.54	.66	.57
2,4,6-Tri-*O*-methyl-D-glucose	Unavailable	..	.76
2,3,6-Tri-*O*-methyl-D-glucose	0.69	.84	.83
2,3,4-Tri-*O*-methyl-D-glucose[c]	.72	.88	.85
2,3,4,6-Tetra-*O*-methyl-D-glucose	.82	1.00	1.00

[a] Using Whatman No. 1 strips and 1-butanol–ethanol–H_2O (4:1:5, by volume) at 25°. [b] Rate with reference to 2,3,4,6-tetra-*O*-methyl-D-glucose. [c] Regenerated from the aniline derivative by the method of G. W. Huffman and F. Smith, THIS JOURNAL, **77**, 3141 (1955).

This procedure enabled tentative identification of the sugars in the hydrolyzed methylated polymer, in the order of increasing R_f and R_{tg}:

	Sugar found	Chromatographic intensity
(1)	α-D-Glucose	Very weak
(2)	2-*O*-Methyl-D-glucose	Strong
(3)	2,6-Di-*O*-methyl-D-glucose	Weak
(4)	2,3-Di-*O*-methyl-D-glucose	Strong
(5)	2,3,6-Tri-*O*-methyl-D-glucose	Strong
(6)	2,3,4-Tri-*O*-methyl-D-glucose	Very strong
(7)	2,3,4,6-Tetra-*O*-methyl-D-glucose	Very strong

(19) E. L. Hirst, J. Hough and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).

This conclusion subsequently was strengthened by the results of simultaneous development of three strips spotted by the hydrolyzate of the methylated polymer and of three strips spotted by a control aqueous solution containing each of the above methyl-D-glucoses at equal concentrations (1%): the six strips appeared to be substantially identical, insofar as coincidence of spot positions was concerned.

These data permit the following inferences to be drawn as to the structure of the unmethylated polymer (GP30): (a) The strength (intensity) of the spot attributed to the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose indicates a rather high proportion of non-reducing end-groups in the original polymer. Therefore, the polymer cannot be of a cyclic type,[20] but must be of a highly branched structure. (b) The high intensity of the spot attributed to the presence of 2,3,4-tri-*O*-methyl-D-glucose shows that the polymer structure contains a large proportion of 1 → 6-acetal linkages, a result in agreement with the findings obtained on examination of partial hydrolyzates of the unmethylated polymer. (c) The fact that the spot attributed to the presence of 2,3,6-tri-*O*-methyl-D-glucose is of rather high intensity indicates that the 1 → 4-acetal linkage is also an important structural entity. (d) The presence of 2,3-di-*O*-methyl-D-glucose then is explained easily as originating in the D-glucose units engaged in the branch points of the polymer (linked by 1 → 6 and 1 → 4 bonds). This compound also could have been formed from a reducing end-group linked from C4 to an adjacent anhydro-D-glucose unit and having its reducing group involved in a 1 → 6-D-glucosan linkage. (e) The presence of 2,6-di-*O*-methyl-D-glucose is not readily explained; it may possibly be an artifact, a degradation product of 2,3,6-tri-*O*-methyl-D-glucose,[21] formed during the hydrolysis. The same could apply in regard to the presence of 2-*O*-methyl-D-glucose and the evident trace of D-glucose. In support of this hypothesis, the presence of free D-glucose was *not* detected when the time of hydrolysis was shortened, from 16 to 4 hours.

Summary.—The results of this investigation permit these conclusions as to the structure of the polymeric products synthesized by heat treatment of a dry blend of anhydrous α-D-glucose with metaboric acid: (1) the metaboric acid is not inherently a part of the polymeric structure (from the fact that it can be almost completely removed by methanol extraction); (2) the polymers are non-crystalline (from X-ray photographic evidence); (3) the polymers are heterogeneous, both as to size (from cryoscopic molecular-weight data pertaining to two unfractionated products and to a single "rough" fraction) and as to bond structure (from infrared absorption data and from paper-chromatographic results); (4) the polymers are polyglucosides, being simply built up of anhydro-D-glucose units connected by acetal bonds (from data on hydroxyl content and from the fact that only D-glucose was detected chromatographically on complete acid hydrolysis); (5) the ring structure of the anhydro-D-glucose units of the polymers is predominantly pyranose (from the relatively slow, over-all rate of acid hydrolysis at 25°; (6) the reducing end of the polymers may be largely involved in D-glucosan formation (from the fact that a single fractionation of one of the polymers resulted in a product of over twice the molecular weight of the starting material but with no appreciable change in reducing-sugar content, from the low values of specific rotation obtained, and from the non-uniformity of rate curves relating to acid hydrolysis at 25°); (7) the polymers are not cyclic but are highly branched and contain 1 → 6- and 1 → 4-bonds as the predominant modes of acetal linkage (from paper-chromatographic data).

(20) K. Freudenberg and M. Meyer-Delius, *Ber.*, **71**, 1596 (1938).

(21) S. Peat and J. Whetstone, *J. Chem. Soc.*, 276 (1940).

No information was obtained either as to the anomeric disposition of the acetal bonds (except for the polarimetric evidence indicating the presence of a fairly high ratio of α- to β-linkages) or as to the proportions of branch points and non-reducing end-groups. Consequently, it is not yet possible to set forth a structural formula of any fundamental significance, particularly in view of the apparent heterogeneity of bond structure evidenced by the experimental data.

Acknowledgment.—We are indebted to the Corn Products Refining Co. for permitting this investigation and for the generous contribution of necessary materials and facilities.

ARGO, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

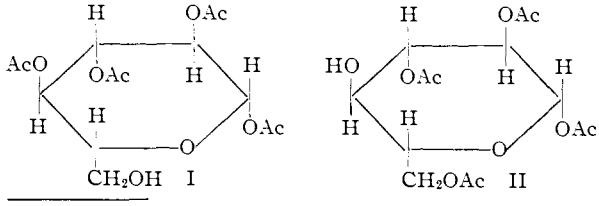
On the Structure of 1,2,3,6-Tetra-*O*-acetyl-β-D-glucose

BY WILLIAM A. BONNER

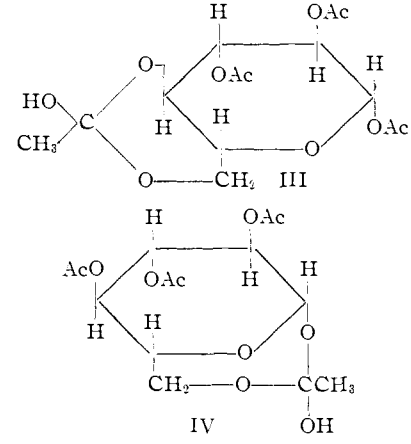
RECEIVED FEBRUARY 21, 1958

The action of very dilute alkali on 1,2,3,4-tetra-*O*-acetyl-β-D-glucose (I) leads to the formation of an isomeric tetra-*O*-acetyl-β-D-glucopyranose whose chemical behavior has been interpreted in terms of both the structures 1,2,3,6-tetra-*O*-acetyl-β-D-glucose (II) and 2,3,4-tri-*O*-acetyl-β-D-glucose 1,6-(orthoacetate) (IV). A distinction between these possibilities, favoring structure II, can be made on the basis of labeled-acetyl exchange experiments on the penta-*O*-acetyl-β-D-glucopyranose obtainable from II, and on the basis of infrared carbonyl absorption intensities. Both I and II were acetylated at −5° with radioactive acetic anhydride, affording essentially optically pure samples of monoacetyl labeled penta-*O*-acetyl-β-D-glucopyranose. When these pentaacetates were placed in a 1:1 mixture of acetic anhydride and acetic acid, containing sulfuric acid catalyst, they underwent anomerization but *failed* to exchange labeled acetyl, indicating the absence of labeled acetyl at the anomeric center in each case. The infrared carbonyl absorption intensities of both I and II established the presence of four normal acetyl groups in each molecule. These data are in accord with II and eliminate IV as the structure of the alkali engendered isomerization product of I.

In 1926, Helferich and Klein first observed[1] polarimetrically the sensitivity of 1,2,3,4-tetra-*O*-acetyl-β-D-glucose (I) to traces of alkali, and a new isomeric tetra-*O*-acetyl-β-D-glucopyranose was isolable from such an alkali-catalyzed isomerization. The latter substance has more recently been obtained both by Lewis-acid catalyzed isomerizations[2] of I, as well as by hydrolysis, followed by partial acetylation, of 1,2,3-tri-*O*-acetyl-4,6-benzylidene-β-D-glucose.[3] The isomerized acetate, although then similar in melting point to the known 2,3,4,6-tetra-*O*-acetyl-β-D-glucose, showed a mixed melting point depression with the latter,[1,6] and therefore did not have its free hydroxyl group at C1. Also, the isomerized tetraacetate could be tosylated to give a product which, after cautious saponification, yielded a mono-*O*-*p*-toluenesulfonate different from the known 3-*O*-*p*-toluenesulfonyl-D-glucose of Freudenberg and Ivers,[4] an observation which was interpreted as eliminating the possibility of a free hydroxyl at C3 in the isomerized acetate. Of the remaining structural possibilities, *i.e.*, free hydroxyl at C2 or C4, Helferich and Klein suggested the C4 alternative II as the most likely structure, although the rearranged acetate underwent some reaction with phenylhydrazine.[1] Both I and II readily yielded penta-*O*-acetyl-β-D-glucopyranose on acetylation with acetic anhydride and pyridine, thus establishing their ring size and anomeric similarity. On methylation of II with methyl iodide and silver oxide the known methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside resulted in low yield,[1] a reaction clearly involving additional acetyl migration.



On the basis of the observation that both I and II as well as 2,3,4,6-tetra-*O*-acetyl-β-D-glucose all gave methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside on methylation, and of the assumption that the proposed ortho-acid intermediate[5] III involved in a C6–C4 acetyl migration was unlikely because of the *trans* disposition of the groups at C4 and C5, Haworth, Hirst and Teece later cast doubt[6] on the correctness of Helferich's formulation II for the rearranged tetraacetate. Their methylation data, as well as the properties of other known tetra-*O*-acetyl-D-glucoses led them to suggest the stable



(1) B. Helferich and W. Klein, *Ann.*, **450**, 219 (1926); **455**, 173 (1927).

(2) H. Bredereck and G. Höschele, *Ber.*, **86**, 1286 (1953).

(3) A. L. Raymond, *J. Biol. Chem.*, **113**, 375 (1936).

(4) K. Freudenberg and O. Ivers, *Ber.*, **55**, 937 (1922).

(5) E. Fischer, *ibid.*, **53**, 1624 (1920).

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