114. *Quantitative Aspects of the Acid Reversion of Glucose.*

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The products formed when glucose is heated in aqueous sulphuric acid, with or without pretreatment with formic acid, have been separated and estimated. The major products are 1 : 6-anhydro-β-D-gluco-pyranose and -furanose, while the remainder consists of the group of glucose disaccharides, in which isomaltose and gentiobiose predominate. The amounts of sugars so formed provide a guide to the quantities of reversion products likely to be encountered as artefacts in the examination of the structures of polyglucoses by the method of partial acidic hydrolysis.

WHEN exposed to acid sugars undergo inter- and intra-molecular elimination of water, between the hemiacetal and (mainly) the primary hydroxyl group. This acid-catalysed reversion of sugars, and of glucose in particular, has recently been studied, first with respect to the synthesis of large polymers with dextran-like properties,[1-3] and secondly as a factor in the linkage analysis [4] of polysaccharides by partial acidic hydrolysis. The present work is concerned with the second aspect.

Thompson, Wolfrom, and Quinn [5] found that when glucose was heated in 0·082N-hydrochloric acid at 97° for 10 hr. isomaltose and gentiobiose were formed in amounts which were approximately equal and in linear relation to the concentration of glucose over

[1] Pacsu and Mora, *J. Amer. Chem. Soc.*, 1950, **72**, 1045.
[2] Ricketts, *J.*, 1954, 4031.
[3] Kent, *Biochem. J.*, 1953, **55**, 361.
[4] Peat, Whelan, and Edwards, *J.*, 1955, 355.
[5] Thompson, Wolfrom, and Quinn, *J. Amer. Chem. Soc.*, 1953, **75**, 3003.

the range 0·4—10%. Previously, Wolfrom and his collaborators [6] had isolated isomaltose from an acid hydrolysate of waxy-maize starch and Thompson *et al.*[5] concluded that the isomaltose found in the polysaccharide hydrolysates could not be a reversion artefact since the yield was 200 times that obtained from the reversion of glucose under comparable conditions. Results reported by Bacon and Bacon [7] in respect of isomaltose formed from glucose and from glycogen under the same acidic conditions were similar except that the yield of isomaltose from glycogen was only about eight times that formed from glucose.

While isomaltose and gentiobiose are the major disaccharide products of reversion it is known that hydroxyl groups other than primary can condense with the reducing group. There are, in all, eleven disaccharides which could be formed from two glucopyranose units and Thompson, Anno, Wolfrom, and Inatome [8] isolated seven of these (as the crystalline octa-acetates) from a glucose–acid reaction mixture. These were isomaltose, gentiobiose, maltose, cellobiose, nigerose, sophorose, and ββ-trehalose. In addition, the product of an intramolecular elimination of water, namely, 1 : 6-anhydro-β-D-glucopyranose (lævoglucosan), was obtained.

Our interest in this problem arises from structural studies on glucans (cf. ref. 4) in which sulphuric acid or formic acid–sulphuric acid has been used to hydrolyse the polysaccharides. Accordingly, we have examined the formation of reversion products from glucose under conditions identical with those used to hydrolyse the polysaccharides. A scheme of fractionation, based on charcoal and paper chromatography and paper electrophoresis, has made possible the separation and estimation of the products, and has provided further information on the products of intramolecular elimination of water.

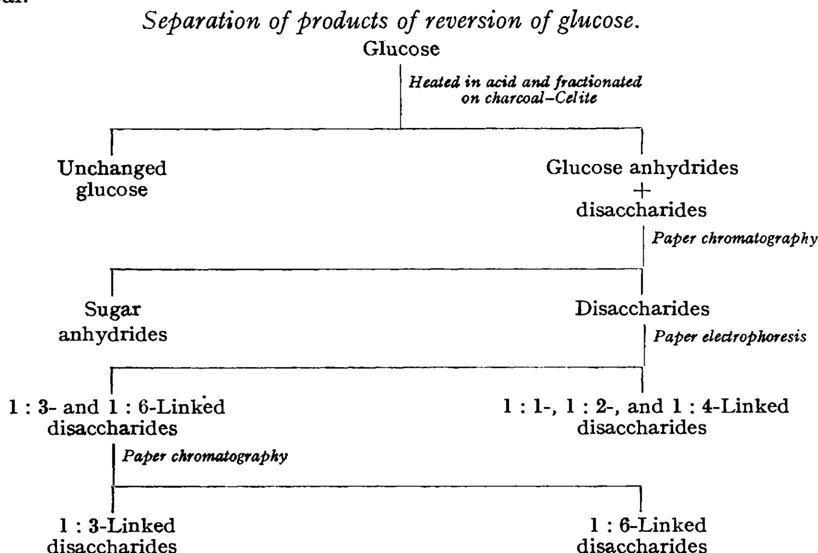
Conditions of Acid Reversion.—Two sets of conditions were chosen. The reaction studied in greater detail was the reversion occurring in 0·33N-aqueous sulphuric acid at 100°. These conditions have been used by us for the hydrolysis of amylose,[9] laminarin,[10] lichenin,[11] isolichenin,[12] dextran,[13] and yeast glycogen.[4] These polysaccharides are soluble in aqueous acid. Yeast glucan, however, is insoluble in this medium but can be dissolved and degraded in hot formic acid to water-soluble fragments, and the hydrolysis can then be continued in 0·33N-sulphuric acid.[14]

The fractionation scheme, which is summarised below, involved the following steps: (1) The neutralised solution of acid-treated glucose was passed through charcoal–Celite; irrigation with water then removed salts and unchanged glucose; thereafter the reversion products were displaced from the column with 25% aqueous ethanol. [In some experiments the column was eluted first with 7·5% ethanol, to displace glucose anhydrides and disaccharides and then with 15% ethanol to displace trisaccharides. The amounts of the latter formed under these conditions were, however, negligible.] (2) The reversion products were separated by thick-paper chromatography into sugar anhydrides and disaccharides. (3) The disaccharide mixture was separated into two fractions by electrophoresis on thick paper in borate buffer. It was known from earlier studies [15] that the fraction of lower M_G value would contain 1 : 1-, 1 : 2-, and 1 : 4-linked diglucoses, if these had been formed, whereas the faster-moving fraction would contain the 1 : 3- and 1 : 6-linked sugars. (4) The fraction of higher M_G value was further resolved by paper chromatography into two sub-fractions, the first of which would contain the 1 : 6-linked anomers, isomaltose and gentiobiose, and the second, the 1 : 3-linked, nigerose and laminaribiose. These anomers were not separated in the case of the sulphuric acid reversion but with the

[6] Wolfrom, Tyree, Galkowski, and O'Neill, *J. Amer. Chem. Soc.*, 1951, **73**, 4927.
[7] Bacon and Bacon, *Biochem. J.*, 1954, **58**, 396.
[8] Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309.
[9] Whelan, Bailey, and Roberts, *J.*, 1953, 1293.
[10] Peat, Whelan, and Lawley, *J.*, 1958, in the press.
[11] Peat, Whelan, and Roberts, *J.*, 1957, 3916.
[12] Peat, Whelan, and Morgan, to be published.
[13] Turvey and Whelan, *Biochem. J.*, 1957, **67**, 49.
[14] Peat, Whelan, and Edwards, to be published.
[15] Peat, Whelan, and Hinson, *Nature*, 1952, **170**, 1056; *Chem. and Ind.*, 1955, 385.

products formed by the combined action of formic acid and sulphuric acid the subfractionation on paper was carried further in that isomaltose was separated from gentiobiose on charcoal.

Separation of products of reversion of glucose.



Quantitative Estimation of the Products of Reversion.—(a) *Glucose anhydrides*. This product was first encountered, during trial experiments with formic acid–sulphuric acid, in the fraction (of negative optical rotation) eluted from the charcoal immediately before the disaccharide fraction. The material had an R_F value almost twice that of glucose and could not be located on paper by reducing-sugar sprays but was by silver nitrate–sodium hydroxide,[17] with which it reacted slowly. This is characteristic of a non-reducing sugar. The non-reducing character was confirmed by quantitative measurement with an alkaline copper reagent. The suggestion that this substance might be lævoglucosan was made by Professor E. J. Bourne. The specific optical rotation (−41·4°) was, however, appreciably less negative than that of 1 : 6-anhydro-β-D-glucopyranose (−66·3°) [18] and it seemed possible that some of the furanose form [19] (+42·5°) might be present. A ratio of pyranose : furanose of 77 : 23 would accommodate the observed $[\alpha]_D$ values. This surmise was confirmed when the substance was oxidised with periodate. (The pyranose form consumes 2 moles of periodate and releases 1 mole of formic acid; the furanose form is not oxidised.[19]) On the assumption that only lævogluco-pyranosan and -furanosan were present in this fraction the periodate consumption and formic acid liberation corresponded to pyranose : furanose ratios of 78 : 22 and 80 : 20, respectively, in good agreement with the calculation based on optical rotation. Accordingly, in the later quantitative studies of acid reversion, the weight yield of the anhydride fraction was estimated by acidic hydrolysis to glucose (measured by copper-reducing power) and its composition by measurement of $[\alpha]_D$ value. The results are shown in the Table.

(b) *Disaccharides*. The weights of sugars present in all fractions were estimated by acidic hydrolysis to glucose. In four cases it was possible to calculate the weights of individual sugars. As has been indicated, the 1 : 6- and 1 : 3-linked disaccharides were obtained in anomeric pairs, *viz.*, isomaltose + gentiobiose and nigerose + laminaribiose. The members of each pair have widely different $[\alpha]_D$ values and therefore the amounts of the individual sugars could be accurately assessed by comparing the $[\alpha]_D$ value of the mixture with those of the anomers. Incidentally, for preparative purposes, the disaccharide anomers are easily separable by charcoal–Celite fractionation; the α-linked form is eluted first.

The fraction containing the 1 : 1-, 1 : 2-, and 1 : 4-linked sugars was not analysed for

Products of reversion of glucose by acid (% basis).

	Glucose (1·0%) heated in 0·33N-sulphuric acid			Glucose heated in (i) formic acid and (ii) aqueous sulphuric acid
	2 hr.	5 hr.	10 hr.	
Glucose anhydrides *	0·375 (53 : 47)	0·430 (69 : 31)	0·510 (66 : 34)	1·35 (82 : 18)
Disaccharides:	0·164	0·234	0·307	1·37
Isomaltose	0·052	0·074	0·105	0·249 †
Gentiobiose	0·032	0·053	0·071	0·246 ‡
Nigerose	0·014	0·015	0·025	0·119
Laminaribiose	0·011	0·013	0·008	0·087
Maltose + cellobiose	0·021	0·040	0·054	0·185
Kojibiose + sophorose + trehaloses	0·034	0·039	0·044	0·487
Total	0·539	0·664	0·817	2·72

* Figures in parentheses are the ratios of pyranose : furanose forms.
† $[\alpha]_D$ +119°. ‡ $[\alpha]_D$ +12°.

the individual components, although suitable separative methods were available if larger quantities of material had been produced. Measurement of the copper-reducing power of the mixture gave, however, an estimate of 1 : 4-linked sugars, the amount of 1 : 2- and 1 : 1-linked sugars being obtained by difference. [Trehaloses (1 : 1-linked) have no reducing group, while the 1 : 2-linked sugars, kojibiose [16] and sophorose, although possessing a free reducing group, do not reduce Somogyi copper reagent.[16, 20]]

The Table shows that the relative amounts of reversion products obtained when glucose is heated (for two hours) with (*a*) aqueous sulphuric acid alone and (*b*) formic acid followed by aqueous sulphuric acid, are considerably different. Thus the percentage yield of reversion products with (*b*) is 5 times that obtained with (*a*). This may possibly be related to the concentration of the glucose. In the first case the concentration of glucose in 0·33N-sulphuric acid was 1·0%. In the second case, the initial concentration of the glucose in 90% formic acid was 6%; its final concentration, after the addition of the aqueous sulphuric acid, was, however, only 0·4%. A striking feature is the large proportion of anhydroglucose formed in each case. With aqueous sulphuric acid, it constitutes 70% of the total reversion products and with formic acid, 50%. This is in contrast to the observations of Thompson *et al.*[8] that lævoglucosan (only the pyranose form was detected) is a relatively minor product of the reversion which occurs in 0·082N-hydrochloric acid. The concentration of glucose used by these authors was, however, 30% and it is to be expected that the higher the concentration of the sugar, the greater will be the tendency for inter-, rather than intra-, molecular condensation to occur.

In the group of disaccharide reversion products, the 1 : 6-linked sugars, isomaltose and gentiobiose, preponderate, whichever method of acid treatment is used. It is perhaps noteworthy that the 1 : 1- and 1 : 2-linked disaccharides together constitute 6% of the total reversion products produced by aqueous sulphuric acid whereas in the formic acid treatment these disaccharides constitute 18%.

The validity of the method of linkage analysis of a polysaccharide by partial acid hydrolysis is dependent upon the demonstration that the oligosaccharides formed are not products of reversion synthesis and this demonstration is not complete when the control experiments are concerned, as here, with reversion synthesis from the monosaccharide. The disaccharide reversion products from glucose are formed by reaction between the reducing group of one glucose molecule with a hydroxyl group of a second and obviously the concentration of reducing groups will be much less during the partial hydrolysis of a polyglucose than when the equivalent of glucose is being treated under the same conditions.

[16] Barker, Bourne, Grant, and Stacey, *Nature*, 1956, **178**, 1221; Haq and Whelan, *ibid.*, 1222.
[17] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.
[18] Haq and Whelan, *J.*, 1956, 4543.
[19] Dimler, *Adv. Carbohydrate Chem.*, 1952, **7**, 37.
[20] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.

In consequence, the amounts of disaccharides formed from glucose will exceed those formed by reversion during acid hydrolysis of the polysaccharides.

There is, however, another factor which would work in the opposite direction and increase the reversion tendency with the polysaccharide as against the monosaccharide. When a glycosidic linkage is severed by acid the two saccharide fragments may combine with water or, alternatively, combine with each other or with other fragments. In the latter case, the energy associated with the original linkage could be available to accelerate the formation of reversion products. An analogy exists in the case of enzymic synthesis of glucosidic linkages. The " transfer " reactions catalysed by, for example, phosphorylase or D-enzyme,[21] wherein linkage-scission precedes linkage-synthesis, occur much more rapidly than does the dimerisation of glucose by the agency of a glucosidase.[15]

There is, therefore, some uncertainty as to whether control experiments with a monosaccharide substrate provide a reliable guide to the true extent of acid reversion during polysaccharide hydrolysis, and it is clear that a disaccharide found in the partial acid hydrolysate of a polysaccharide can be regarded as a structural unit of the latter only if isolated in a yield many times as great as that obtained in the monosaccharide control experiments.

In conclusion it is suggested that a better method of providing control data for reversion products occurring during linkage analysis would be to study the formation of such substances from the disaccharide containing the main polymeric linkage of the polysaccharide, rather than from the monosaccharide; *i.e.*, in an investigation of laminarin, laminaribiose should be treated under the same conditions. In any case, reversion synthesis must be kept at a minimum by maintaining, so far as is practicable, minimal temperature and concentrations of polysaccharide [5] and acid during hydrolysis.

Experimental

Evaporation was at 40° under diminished pressure, unless otherwise stated, and the pH was maintained at 5—6 by adjustment with sodium hydroxide or acetic acid. Optical rotation was measured in 4-dm. tubes.

Test for Purity of Glucose.—Anhydrous D-glucose (30 g.; B.D.H. " AnalaR " quality) was dissolved in water and adsorbed on charcoal–Celite which was eluted with water and then with 25% (v/v) ethanol as described below. The ethanolic eluate was evaporated to dryness and the residue dissolved in water (25 ml.). After treatment with Somogyi's deproteinising reagents [25] (total vol. 9 ml.), a 10 ml. portion of the solution was heated with 3N-sulphuric acid (10 ml.) at 100° for 3 hr. The reducing power (as glucose) was measured as described by Pirt and Whelan.[22] A second 10-ml. portion was treated in the same way but was not heated with the acid. When the reducing power of this solution was measured, 20 minutes' heating with copper reagent [20, 22] was necessary to allow full development of reducing power by disaccharides. The difference in reducing power between the hydrolysed and unhydrolysed solutions corresponded to the presence in the whole fraction of 36·8 mg. of disaccharide or 18·4 mg. of glucose anhydride (expressed in terms of glucose). That is, the " AnalaR " glucose was contaminated with 0·123% of disaccharide or 0·062% of glucose anhydride. Paper chromatography showed that the contaminants consisted largely of sugar anhydride with a little 1 : 6-linked disaccharide (isomaltose and/or gentiobiose). When assessing the amounts of reversion products (below) no correction was applied for the presence of these contaminants for the reason that they would be partly hydrolysed in the reversion reaction. Glucose disaccharides are about 25% hydrolysed during 2 hr. at 100° in 0·33N-sulphuric acid.

Treatment of Glucose with 0·33N-*Sulphuric Acid.*—A solution of chromatographically pure anhydrous D-glucose (30 g.) in 0·33N-sulphuric acid (3 l.) was heated in a boiling-water bath. Three experiments were performed, in which the heating times were 2, 5, and 10 hr., respectively. Each solution was then cooled quickly in running water, neutralised with 3N-sodium hydroxide, concentrated under reduced pressure to about 600 ml., and adsorbed on charcoal–Celite (100 × 4 cm.), prepared as described by Whelan *et al.*[9] Water (5 l.) was passed through

[21] Peat, Whelan, and Rees, *J.*, 1956, 44.
[22] Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224.

the column under pressure to remove salts and unchanged glucose. The reversion products were then eluted with 25% (v/v) aqueous ethanol (2·5 l.), and the solution evaporated to dryness. Inorganic matter and colloidal charcoal were removed by filtering a solution of the syrup in hot 80% (v/v) aqueous methanol through a " Sterimat " (GS grade).

Successive Treatment of Glucose with 90% *Formic Acid and* 0·33N-*Sulphuric Acid*.—" AnalaR " anhydrous D-glucose (21·3 g.) was heated, with mechanical stirring, in " AnalaR " 90% formic acid (342 ml.) at 98° for 25 min., more acid (114 ml.) was then added and heating continued for 15 min. The hot solution was then poured into hot 0·44N-sulphuric acid (4·56 l.) and heated for 1·5 hr. The cooled solution was neutralised with 6N-sodium hydroxide and concentrated until crystallisation began. The solution was then applied to charcoal–Celite (90 × 8·5 cm.) which was washed with water (13 l.) and then with 50% aqueous ethanol until all optically active material was desorbed. The ethanolic eluate was evaporated to dryness and freed from inorganic matter as described above.

Fractionation of the Reversion Products.—The weighed syrup was diluted with water to a concentration of about 160 mg./ml., and 80-mg. quantities (0·5 ml.) were applied to lines (46·5 cm. long) ruled parallel to the shorter sides of sheets of Whatman No. 3MM paper (18¼ × 22½ in.) which were irrigated in butan-1-ol–acetic acid–water (4 : 1 : 5, by vol.) until control strips showed a separation to have been achieved between lævoglucosan, the traces of glucose always present, and the reversion disaccharides. The papers were left to dry at room temperature for 24 hr. Thereafter guide strips (1·5 cm. wide) were cut from the sides and middle of the sheets and sprayed with silver nitrate–sodium hydroxide [17] to locate the three sugar zones. The disaccharide and lævoglucosan areas were then cut out from the unsprayed portions. Control experiments with a sample of 1 : 6-anhydro-β-D-glucofuranose, kindly supplied by Dr. R. J. Dimler, had shown the impossibility of locating this substance (which is not oxidised by periodate [19]) on a paper-chromatogram with spray reagents capable of locating non-reducing sugars. Since the anhydride fraction was likely to contain the pyranose as well as the furanose form, the whole of each chromatogram sheet from the bottom edge of the paper to the lower edge of the glucose zone was cut off; these portions of the papers were extracted together by being shaken with water (500 ml.) until the paper disintegrated, the mixture filtered, and the pulp twice more extracted with 250 ml. portions of water. The combined extracts were evaporated to dryness, the residue dissolved in hot 80% methanol, and the solution filtered and re-evaporated.

The sugar anhydride fraction was dissolved in water (50 ml.), and portions (3 ml.) removed for determination of sugar concentration after acidic hydrolysis to glucose.[22] The optical rotation of the anhydride fraction was measured and by using the $[\alpha]_D$ values of −66·3° [18] and +42·5° [19] quoted for 1 : 6-anhydro-β-D-glucose-pyranose and -furanose, respectively the proportion of the two forms present was calculated. The copper-reducing power of the fraction was also measured.[20] It was never more than 1·3% of that of glucose.

The disaccharide fraction, isolated in the same way, was dissolved in water (1 ml.), and applied to a line 46·5 cm. long, parallel to the shorter side of a sheet of Whatman No. 3MM paper (18¼ × 22½ in.) and about 7 in. from one end. The paper was supported horizontally in an apparatus [23] for ionophoresis in borate buffer, pH 8·7 (0·2M-boric acid + 0·05M-sodium borate; 2 : 3; v/v), the starting line being remote from the anode. A potential of 390 v was applied for 24 hr., after which the paper was dried at room temperature and guide strips removed for spraying with benzidine–trichloroacetic acid [24] (note that silver nitrate–sodium hydroxide does not react with sugars on alkaline borate-impregnated paper). Two zones were always detected, one of M_G 0·6—0·7 containing 1 : 6- and 1 : 3-linked sugars, the other of M_G 0·3 containing the remaining disaccharides. The unsprayed zones were cut out, with the inclusion of ample margins on either side of the sugar-areas. The sugars were then extracted with water (3 × 450 ml.), the paper pulp being filtered off between each extraction. To avoid alkaline decomposition, the pH was brought below 7, in the first extraction, by adding N-sulphuric acid. The salts were removed, and the sugars concentrated by passing the combined extracts through charcoal–Celite (20 × 2·5 cm.) which was washed with water until free from buffer and then eluted with 50% aqueous ethanol (200 ml.), this extract being finally evaporated to dryness.

[23] Latner, *Biochem. J.*, 1952, **51**, xii.
[24] Bacon and Edelman, *Biochem. J.*, 1951, **48**, 114.
[25] Somogyi, *J. Biol. Chem.*, 1945, **160**, 69.

The disaccharides of M_G 0·3 were dissolved in water (10 ml.) and treated with Somogyi's deproteinising reagents [25] (4 ml.) to remove substances eluted from charcoal–Celite which interfere with cuprimetric determinations,[9] and 10 ml. of the centrifuged solution were diluted to 25 ml. The carbohydrate content was determined by acid hydrolysis and the reducing power (as maltose) was measured with Somogyi's reagent.

The disaccharides having M_G 0·6—0·7 were freed from buffer salts as described above and refractionated, each on one sheet of Whatman No. 3MM paper in butan-1-ol–acetic acid–water, and the separated zones (1 : 6- and 1 : 3-linked sugars) located and extracted as before. The separated sugars were finally dissolved in 25 ml. of aqueous solution, their concentration measured by acid hydrolysis, and the optical rotation determined. On the assumption that these fractions were binary mixtures the amount of each component was calculated from the specific optical rotation, the following values of $[\alpha]_D$ for the pure substances being assumed: nigerose [26], +136°; laminaribiose [27], +18·6°; isomaltose [13], +122°; gentiobiose [15], +9·6°. A modification of the above method was used in the case of the disaccharides having M_G 0·6—0·7 obtained in the formic acid–aqueous sulphuric acid treatment of glucose. After adsorption of the dissacharide on charcoal–Celite and removal of buffer, the column (75 × 3 cm.) was subjected to gradient elution,[28] a reservoir of water being connected to the column and the level maintained in the reservoir by feeding in 15% ethanol through a constant-head device. Fractions (100 ml.) were collected under diminished pressure and their optical rotation measured. Positive-rotating material was detected in fractions 22—30 which were combined and evaporated. A further six fractions were collected and combined with the eluate obtained on subsequently washing the column with 20% ethanol. This second fraction was then separated by paper chromatography as described above for the disaccharides with M_G 0·6—0·7. In this way isomaltose (fractions 22—30) was separated from gentiobiose. The isomaltose fraction was treated with deproteinising reagents and its specific optical rotation calculated from the rotation of its solution and the sugar concentration as measured by acid hydrolysis (see Table). The same measurements were made on the gentiobiose and the mixed 1 : 3-disaccharide fractions, the solutions being first "deproteinised." This purification procedure was later found to be unnecessary for sugars eluted from paper and was reserved for products eluted from charcoal.

Preparation and Analysis of Sugar Anhydrides.—Glucose (30 g.) was heated at 100° in 90% formic acid (111 ml.) for 25 min., a further 37 ml. of acid were then added and the heating continued for 15 min. Sulphuric acid (0·44N; 1480 ml.) was added and heating at 100° was continued for 3 hr. The cooled solution was neutralised and the sugar anhydride fraction isolated by charcoal–Celite and paper chromatography as previously described. The product was taken up in water (100 ml.). The concentration of sugar anhydride was measured by acidic hydrolysis to glucose (2 hr. at 100°) and measurement of copper-reducing power by Pirt and Whelan's method.[22] Heating for a longer time (4 hr.) did not increase the reducing power. The yield of anhydride $\{[\alpha]_D -41\cdot4°$ (*c*, 0·18)$\}$ was 181 mg. and a portion equivalent to 3·62 mg. had no measurable copper-reducing power.[20] The volume of the remaining solution was reduced to one-third and the concentration redetermined (4·99 mg./ml.). A portion (5 ml.) was stored in the dark with 0·37M-sodium metaperiodate (10 ml.). The consumption of periodate was measured by adding a portion (1 ml.) to a solution of potassium iodide (0·5 g.) in 2N-sulphuric acid (5 ml.) and titrating the liberated iodine with 0·05N-sodium thiosulphate. Formic acid production was measured by mixing a 1 ml. portion with ethylene glycol (0·3 ml.) and after 10 min. adding potassium iodide (0·2 g.) and titrating with neutral 0·005N-thiosulphate. Measurements were made on the oxidation mixture and on a control containing water in place of sugar anhdride solution. The consumption of periodate was 1·56 mol. after 4 hr. and after 21·5 hr., and the amounts of formic acid liberated were 1·59 and 1·60 mol., respectively.

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[26] Barker, Bourne, and Stacey, *J.*, 1953, 3084.
[27] Bachli and Percival, *J.*, 1952, 1243.
[28] Alm, *Acta Chem. Scand.*, 1952, **6**, 1186.