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The Occurrence of Isomaltose among the Products of heating Glucose in dilute Mineral Acid

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Since the identification by Emil Fischer (1890, 1895) of the disaccharide 'isomaltose' among the products of action of cold concentrated hydrochloric acid on glucose there have been numerous reports of such condensations in the presence of mineral acids, both under conditions likely to produce dehydration (cf. Levene & Upts, 1925; Pacsu & Mora, 1950; Kent, 1953), and also in aqueous solution (cf. Brown & Millar, 1899; Harrison, 1914; Berlin, 1926; Georg & Pictet, 1926; Moelwyn-Hughes, 1928). The characterization of the products was, however, hindered by the lack of suitable methods for their separation.

Later authors (Frahm, 1941; McDonald, 1950; Pirt & Whelan, 1951) have measured changes in reducing power and optical rotation in solutions of glucose and mineral acids under various conditions, but only Frahm considered condensation as a contributory cause. Täufel & Reiss (1951) used paper chromatography to estimate the changes in glucose content of a 10% solution in 3N hydrochloric acid heated at various temperatures; they found a 17% loss of glucose after 3 hr. at 100° and also noted that material with smaller R_F values appeared.

Whitby (1952), using glucose oxidase, found a loss of 4% of glucose from a 2·5% solution heated for 2·5 hr. at 100° in N hydrochloric acid.

This paper describes qualitative and quantitative observations made on the products of heating glucose in N aqueous acid (sometimes called 'reversion products') by the use of paper chromatography. The isolation of one major component of this mixture by charcoal–Celite chromatography is described and evidence is presented that this component is isomaltose (6-*O*-α-D-glucopyranosyl-D-glucose).

The conception that the branched structures of glycogen and amylopectin arise through the presence of α-1:6-linkages has depended mainly upon the isolation of isomaltose derivatives from enzymic (Montgomery, Weakley & Hilbert, 1949) and acidic (Wolfrom, Lassettre & O'Neill, 1951; Wolfrom, Tyree, Galkowski & O'Neill, 1951; Thompson & Wolfrom, 1951) hydrolysates of the two polysaccharides. The discovery that mould-enzyme preparations can form the isomaltose type of linkage from maltose (Pan, Andreasen & Kolachov, 1950) has cast doubts on the reliability of evidence from enzymic digests, and it has been suggested that the isomaltose found in acid hydrolysates might have been formed by reversion from free glucose. Myrbäck (1941) produced evidence to show that condensation products were absent from his starch hydrolysates but the methods available to him would seem to have been inadequate to settle

this point since it is now clear that under his conditions some condensation must have taken place.

While the present work was in progress Wolfrom and his collaborators published the results of several years' investigation of this problem (cf. Wolfrom, Georges & Miller, 1949): after acetylating the products of heating glucose in dilute acid solution they have isolated equal quantities of the octaacetates of isomaltose and gentiobiose (Thompson, Wolfrom & Quinn, 1953) and also smaller amounts of the octaacetates of other disaccharides (Thompson, Anno, Wolfrom & Inatome, 1954). The quantities of isomaltose octaacetate isolated in these experiments were much smaller than those obtained from the acetylated hydrolysis products of glycogen (Wolfrom, Lassettre & O'Neill, 1951) and amylopectin (Wolfrom, Tyree, Galkowski & O'Neill, 1951). In earlier experiments they had detected no isomaltose by their method in comparable hydrolysates of amylose.

It appeared of interest to apply the quantitative techniques developed here to a more accurate assessment of isomaltose in glycogen hydrolysates. The yields of isomaltose from two glycogen hydrolysates were found to be considerably greater than the condensation products from glucose or maltose under the same conditions of acid treatment, confirming Wolfrom's conclusion that the isomaltose found in acid hydrolysates originates in the glycogen molecule and not by condensation.

METHODS

Reducing substances (RS). These were estimated colorimetrically using a copper method (Nelson, 1944; Somogyi, 1945). Extracts from paper give a low blank value with this method. The blank areas cut from a sheet carrying fifteen spots of solution for analysis have a total reducing power of less than 0·1 mg. RS. Glucose, recrystallized from ethanol, was made up as a standard solution in saturated benzoic acid. All reducing substances were compared with dilutions of this solution and are expressed as the weight of glucose having the same reducing power.

Paper-partition chromatography. Chromatograms were prepared by descending development in butanol–acetic acid–water solvent using the apparatus and methods employed by Bacon & Edelman (1951). Spraying reagents used were the benzidine–trichloroacetic acid spray described by those authors, the phloroglucinol spray for ketoses (Horrocks & Manning, 1949) and the alkaline silver nitrate spray of Trevelyan, Procter & Harrison (1950), which reacts slowly with trehalose. Solutions for quantitative analysis were applied to paper sheets with an Agla micrometer syringe (Burroughs Wellcome and Co., London) in three groups of five 5 μl. spots applied serially with single guide spots between the groups. A further series of spots could be applied when the first were dry.

To avoid any possibility of hydrolysis of the paper all solutions used in quantitative work were neutralized before application (see Results section). Consequently the sheets carrying these solutions contained relatively large amounts of salts, and appeared to hold unusually large quantities of acetic acid when dry. The acid nature of the extracts made from these papers interfered with the use of the Nelson copper reagent. This difficulty was avoided by drying the sheets below 80° and hanging them in the open laboratory for 1–2 days before cutting them up for extraction.

Charcoal–Celite chromatography. Either 'Activated Charcoal' (British Drug Houses Ltd., London, N. 1) or Active Carbon no. 130 (Sutcliffe–Speakman Ltd., Leigh, Lancs) mixed with an equal weight of Celite no. 535 (Johns-Manville Co. Ltd., London, S.W. 1) was packed wet in glass tubes. The columns were eluted either with certain concentrations of ethanol (Whistler & Durso, 1950) or, where specified, by a gradient elution technique using aqueous ethanol (Bacon & Bell, 1953).

Polarimetry. The optical rotations of solutions were read in 2 dm. tubes using a sodium lamp as light source.

Total hydrolyses. These were carried out in 0·5 N-H_2SO_4 in sealed ampoules for 18 hr. at 100° alongside a control glucose solution of similar concentration.

MATERIALS

Glucose. Apart from the preparation of standard solutions (see Methods) A.R. grade glucose (British Drug Houses Ltd., London, N. 1) was used without further purification.

Isomaltose. Isomaltose was prepared from the products of partial acid hydrolysis of bacterial dextran (cf. Wolfrom *et al.* 1949). 'Dextran Transfusion Solution' (200 ml. of 6%, w/v) in physiological saline (Benger Laboratories Ltd., Holmes Chapel, Cheshire) was heated for 5 hr. at 100° in 0·09 N-H_2SO_4. The mixture was neutralized and the residual polysaccharide precipitated with 3 vol. of ethanol. The filtrate was then concentrated under reduced pressure and put on a charcoal–Celite column (32 × 2·7 cm.) made from 80 g. of charcoal–Celite mixture. Glucose was eluted with water and then isomaltose with 2% (v/v) ethanol. From this eluate the disaccharide was obtained as a dry syrup containing traces of a second disaccharide. The octaacetate prepared from this syrup had m.p. 144° and m.p. 144–145° when mixed with an authentic specimen (cf. Wolfrom *et al.* 1949).

Gentiobiose. (6-*O*-β-D-*glucopyranosyl*-D-*glucose*). For comparison of R_F values on paper chromatograms a solution containing mainly gentiobiose was prepared by prolonged incubation of emulsin with 60% (w/v) glucose solution (Peat, Whelan & Hinson, 1952). A charcoal–Celite column was used to separate the disaccharide mixture from protein and glucose.

Maltose. 'Biochemical reagent' (Thomas Kerfoot and Co. Ltd., Vale of Bardsley, Lancs).

Cellobiose. (L. Light and Co. Ltd., Colnbrook, Bucks).

RESULTS

The action of aqueous acids on glucose

Qualitative observations. After 18% (w/v) glucose in N-H_2SO_4 had been heated for 3 hr. at 100°, 5 μl. run on a paper chromatogram and tested with benzidine–trichloroacetic acid spray showed a distinct spot (R_F 0·07) in the disaccharide region. This solution was freed from glucose on a column of charcoal–Celite mixture (see below) and the remaining

material was concentrated to one-fifth of the original volume. When 5 μl. of this solution was chromatographed (Fig. 1) other spots became visible (*C*, *D* and *E*) and if development was continued for 10 days the main spot separated into two components *A* and *B* (Fig. 2). Spots *A* and *B* had very similar R_F values, which corresponded to those of gentiobiose and isomaltose respectively. Spot *C* had R_F value similar to maltose; spot *D* had R_F value greater than maltose and gave a distinctly more yellow colour with the benzidine–trichloroacetic acid spray than did the other spots. Between these spots and the starting line was another series of spots (Fig. 1, *E*). A similar chromatogram sprayed with the alkaline silver nitrate reagent showed a sixth spot close to *C*, indicating that at least one trehalose was present. No spots reacted with the ketose spray.

Quantitative experiments. To follow the progress of condensation, samples of 18% (w/v) glucose solution in N-H_2SO_4 were removed from the boiling-water bath at intervals, the optical rotation measured and, after neutralization, a known volume applied to large sheets of paper. The papers were developed for 1–2 days in butanol–acetic acid–water to separate the condensation products from glucose; an area between the glucose spot and the base line, indicated by guide strips, was eluted and RS determined in this eluate. An extract from similar areas of a chromatogram of a sample taken at zero time was used as a blank. Fig. 3 shows the increase in optical rotation and in the RS of the total condensation products formed under these conditions.

In earlier experiments (Fig. 3, Expt. 1) samples were heated in separate volumetric flasks, each of which was cooled, neutralized and made to volume before measuring the optical rotation. Both NaOH and hot $Ba(OH)_2$ were tried as neutralizing agents but it proved difficult, particularly with $Ba(OH)_2$, to avoid occasional traces of ketoses arising by epimerization of aldoses and thus lowering the optical rotation. Hence in later experiments (Fig. 3, Expt. 2) the glucose solution was heated in bulk under a reflux condenser and samples withdrawn, cooled and examined for optical rotation before a known volume of the sample was neutralized for

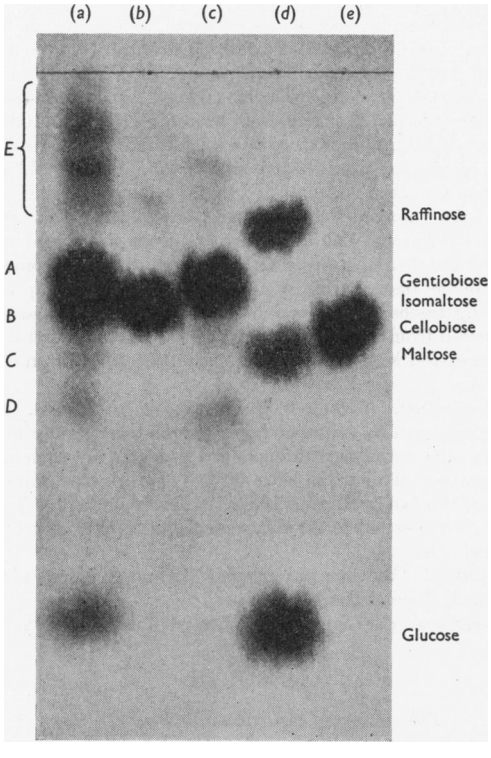

Fig. 1. Comparison of the acid-condensation products of glucose with various known sugars. (*a*) Condensation products (stage I) containing a little glucose, (*b*) isomaltose, (*c*) crude gentiobiose preparation, (*d*) mixture of glucose, maltose, and raffinose, (*e*) cellobiose. Chromatogram run descending for 6 days in butanol–acetic acid–water; sprayed with benzidine–trichloroacetic acid.

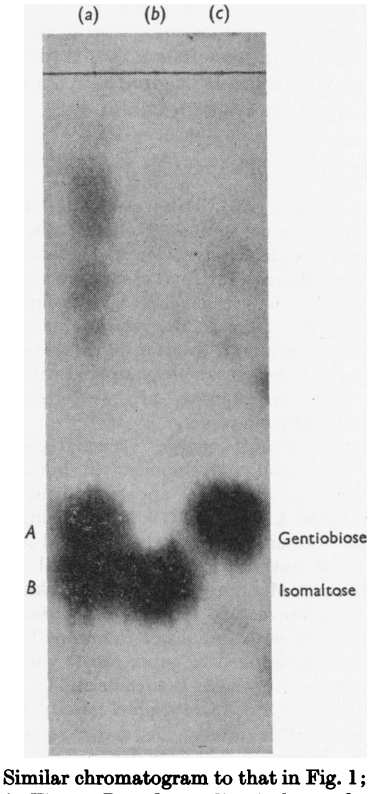

Fig. 2. Similar chromatogram to that in Fig. 1; (*a*), (*b*) and (*c*) as in Fig. 1. Run descending in butanol–acetic acid–water for 10 days; sprayed with benzidine–trichloroacetic acid.

chromatography. In these experiments NaOH was used for neutralization. The resulting salt travelled on the paper among the condensation products and suppressed the colour development with benzidine–trichloroacetic acid spray, but in practice reference spots were still visible when sprayed and the eluted salt did not interfere with RS estimation.

After 3 hr. at 100° the mixture of oligosaccharides present contained 0·75 g. RS/100 ml. Assuming that the mixture had 0·41 × reducing power of glucose (see below) this would correspond to the condensation of 1·83 g./100 ml. (10·2 %) of the original glucose, and to the loss of 1·08 g. RS/100 ml. The increase in optical rotation during this period amounted to 3·5 % of the initial value (cf. McDonald, 1950: 3·4 %), and closely followed the increase in oligosaccharide. Continued heating produced little further change; the darkening of the solution, which is not sufficient to prevent polarimetric observations in a 2 dm. tube at 3 hr., continued, and the appearance on chromatograms of a small amount of a spot with high R_F value and reacting in the cold with the benzidine–trichloroacetic acid spray (? a furfural derivative) suggested that some degradation of glucose took place.

With the same concentration of glucose and the same normality of acid, hydrochloric acid was at least as effective as sulphuric in oligosaccharide formation as judged from visual examination of chromatograms. Pirt & Whelan (1951) observed greater losses of glucose with HCl than with H_2SO_4. Oxalic acid was slightly less effective, while acetic acid produced a barely detectable effect. As reported by other authors, with lower concentrations of glucose or acid the proportion of glucose condensed was less (see Table 2). However, when 2 % (w/v) glucose heated in N-H_2SO_4 was freed from glucose and concentrated to 0·01 of the original volume, the same mixture of disaccharides was visible on a chromatogram.

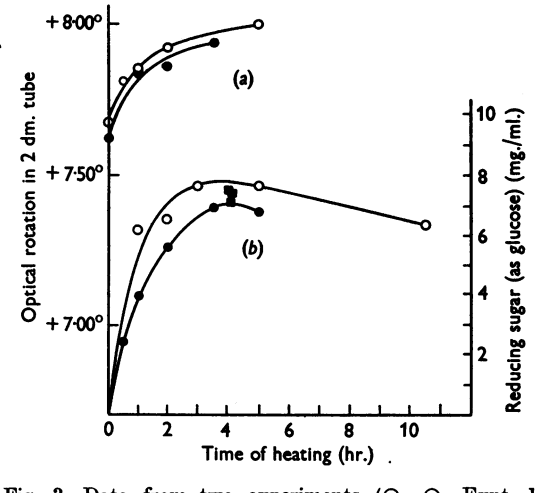

Fig. 3. Data from two experiments (○—○, Expt. 1; ●—●, Expt. 2) in which an 18% (w/v) solution of glucose was heated at 100° in N-H_2SO_4, showing (*a*) the increase in optical rotation of the whole solution and (*b*) the formation of oligosaccharides, separated from glucose by paper chromatography, and measured as RS. ■, Additional experiments under the same conditions.

Chromatography on mixtures of charcoal–Celite

Separation from glucose. Larger volumes of an 18 % (w/v) solution of glucose in N-H_2SO_4 were heated for 3 hr. at 100°, neutralized with NaOH, concentrated to a thin syrup, and placed on charcoal–Celite columns. If the columns were overloaded with respect to oligosaccharide some disaccharide was eluted by the water. A load of 6 g. original glucose, i.e. containing about 0·6 g. oligosaccharide, to an 80 g. column proved to be satisfactory. The glucose and salt were then eluted with water. When the effluent was free from benzidine-reacting material, water was replaced by 25 % (v/v) ethanol which eluted the remaining carbohydrates. Such eluates from several columns were combined and concentrated (stage I). Small amounts of glucose still present were removed by treatment on smaller columns (stage II). In this way 4 g. glucose yielded 0·43 g. mixed oligosaccharide as dry syrup, which on complete hydrolysis contained 0·38 g. glucose (corrected for reversion); this corresponded to 9·4 % of the original glucose. This mixture had $[\alpha]_D^{20} + 84°$ in water, assuming it to be all disaccharide. The ratio of RS before to that measured after hydrolysis was 0·41.

Separation of a single component. The mixed condensation products (stage I) were put on a charcoal–Celite column and washed with water to remove traces of glucose. Aqueous ethanol (1·5 %, v/v) was then passed through the column; even at this low ethanol concentration one component of the mixed oligosaccharides was readily eluted. This appeared chromatographically to be spot *B*. When the column was further washed with 25 % (v/v) ethanol and the eluate concentrated and chromatographed, spot *A* was the main component seen, accompanied by spots *C*, *D* and *E*; *B* was absent. When the gradient elution technique was used, spot *B* was the first component to emerge after glucose. Some preparations of *B* by both techniques have been contaminated with a second benzidine-reacting substance, giving the same characteristic pinkish colour as spot *B* with this spray but having R_F value similar to a trisaccharide. This second substance was not always seen and was no longer seen after the fractions had been passed through a fresh column. The silver nitrate spray showed that all preparations of spot *B* also contained some non-reducing material, probably a trehalose.

Table 1. *Properties of disaccharide fractions obtained from bacterial dextran and acid-condensation products*

	Disaccharide from bacterial dextran		Disaccharide from reversion products		
	Sample 1	Sample 2	Sample 1	Sample 2	Recorded values
Dry weight (mg.)	373	280	233	357	—
RS before hydrolysis (mg.) (*a*)	191	138	96	152	—
RS after hydrolysis (mg.) (*b*)	366	266	210	342	—
Reducing power (*a*/*b*)*	0·52	0·52	0·46	0·45	—
$[\alpha]_D^{20}$ in water, calc. from (*b*) (°)	+115	+113	+115	+114	+103·2 (Wolfrom *et al.* 1949) +120 (Montgomery *et al.* 1949) +122·9 (Barker & Carrington, 1953) +122·0 (Jeanes, Wilham, Jones, Tsuchiya & Rist, 1953)
M.p. of octaacetate (°)	144	—	146–147	—	143–4 (Wolfrom *et al.* 1949) 147†
Mixed m.p. with authentic sample from Dr M. L. Wolfrom (°)	144–145	—	147	—	—
M.p. of phenyl osazone (°)	160–161	—	144–150	—	140–153 (Fischer, 1890) 158 (Fischer, 1895)

* The correction required for loss of RS by reversion during hydrolysis was 5·5%; within the experimental error this balances the theoretical increase in weight consequent upon complete hydrolysis.

† Melting point measured on a sample provided by Dr Wolfrom.

By gradient elution from charcoal–Celite, 16·0 g. glucose yielded as dry syrup 0·78 g. of spot *B* and 0·88 g. of the other oligosaccharides.

Identification of isomaltose. The material which was eluted with 1·5% (v/v) ethanol and appeared on paper as spot *B* was compared in various respects with authentic isomaltose obtained from bacterial dextran. Some of these properties are summarized in Table 1. In addition it should be noted that both substances were eluted very readily from charcoal by 1·5% (v/v) ethanol.

The syrups were acetylated by being heated with fused sodium acetate and acetic anhydride, and the products crystallized from ethanol after seeding with a crystal of β-isomaltose ocataacetate kindly given to us by Dr M. L. Wolfrom of the Ohio State University.

The osazones were prepared by heating the materials with phenylhydrazine hydrochloride and sodium acetate; the crystals were washed well with water and ether and recrystallized twice from water. Crystals from the two sources appeared identical and exhibited the characteristic properties described by Fischer (1890, 1895), namely, high solubility in warm water and in ethanol, and the formation on drying of a brittle dark-brown caked mass which after pulverization formed a stable yellow powder of different crystalline appearance. Fischer (1895) pointed out that exhaustive purification was needed to get a sharp melting point at 158°.

The two disaccharides had the same R_F value in butanol–acetic acid–water, phenol–water, butanol–pyridine–water (Jeanes, Wise & Dimler, 1951), ethyl acetate–pyridine–water (Jermyn & Isherwood, 1949), and also with the benzylamine technique of Bayly & Bourne (1953).

Hydrolysis products of glycogen

Attempts to hydrolyse glycogen with H_2SO_4 under the conditions described by Wolfrom (Wolfrom, Lassettre & O'Neill, 1951) failed to give a sufficient degree of hydrolysis. Finally the hydrolyses were carried out as a 2% (w/v) solution in 0·056N-HCl heated under reflux at 98–100°. Under these conditions a sample of glycogen prepared from *Mytilus edulis* (by extraction with boiling water, deproteinization with picric acid, and repeated ethanol precipitation) was 42% hydrolysed (as measured by RS) after heating for 8 hr. and a sample of glycogen prepared from rabbit liver (by extraction with hot aqueous KOH followed by repeated precipitations by ethanol and acetic acid) was 83% hydrolysed in 24 hr.

After cooling and neutralization each hydrolysate was concentrated under reduced pressure and separated from most of the glucose in the same way as the acid-condensation products. The ethanol eluate was again concentrated (after adjusting pH to 6·0) and placed on a further charcoal–Celite column (80 g.) which was then eluted by the gradient elution technique using a very low gradient (15 or 20% (w/v) ethanol run into 1 l. water). The fractions were tested by paper chromatography and all those containing isomaltose were bulked, pH adjusted to 6·0, and taken to dryness under reduced pressure. Chromatography of concentrated solutions showed that the isomaltose so obtained from a partial hydrolysate of *Mytilus* glycogen contained a little material reacting as ketose. The isomaltose fraction from the more fully hydrolysed liver glycogen gave no reaction with ketose spray but with the silver nitrate spray was shown to contain a trace of non-reducing material, probably a trehalose.

Table 2. *Results of chromatographic separations of control and experimental mixtures of sugars*

The yields of combined sugars are expressed in terms of the glucose liberated on total hydrolysis, an appropriate correction being made for reversion. The recovery, where this is calculable, is given in parentheses.

Material subjected to chromatography	Material isolated: Glucose (g.)	Isomaltose (mg.)	Maltose (mg.)	Total oligosaccharide (mg.)
(1) 4·00 g. glucose + reversion products equivalent to 40 mg. glucose	3·90 (98%)	—	—	38 (95%)
(2) 2·00 g. glucose + 0·50 g. maltose monohydrate* + isomaltose equivalent to 119 mg. glucose	—	101 (86%)†	386†	—
(3) 25% (v/v) ethanol eluate (no sugars added)	—	—	—	Nil
(4) 4·00 g. glucose (untreated)	3·92 (98%)	—	—	5
(5) (*a*) 4·00 g. glucose heated in 200 ml. 0·056 N-HCl for 8 hr.	—	—	—	29
(*b*) as 5 (*a*)	—	—	—	29
(*c*) 4·00 g. *Mytilus* glucogen treated as 5 (*a*)	—	44†	436†	—
(6) (*a*) 4·00 g. rabbit liver glycogen heated in 200 ml. 0·056 N-HCl for 24 hr.	—	164†	—	—
(*b*) 4·20 g. maltose monohydrate* treated as 6 (*a*)	—	—	—	101
(*c*) 4·00 g. glucose treated as 6 (*a*)	—	—	—	47

* Commercial sample containing trisaccharide.
† After separation from glucose the total oligosaccharide mixture was fractionated by gradient elution.

Control solutions of glucose and commercial maltose were treated similarly with acid and a mixed oligosaccharide fraction obtained from each in the same way. The amount of carbohydrate material so obtained was however insufficient to fractionate further by gradient elution and instead the mixture was analysed in each case and carbohydrate content after total hydrolysis calculated on the assumption that the mixture was all disaccharide. The data so obtained, together with some recovery experiments in which known amounts of materials were separated on similar columns, are given in Table 2.

DISCUSSION

That glucose could be condensed with aqueous mineral acid has long been known, if not widely recognized. It is evident from our observations and those of Thompson *et al.* (1953, 1954) that with relatively dilute mineral acid isomaltose and gentiobiose are the main products, although a number of other disaccharides also arise. Chromatographic evidence and the fact that the reducing power is more than doubled by complete hydrolysis show that after treatment with concentrations of acid no greater than N some larger molecules are formed.

Quantitative studies indicate that of the free glucose disappearing from such solutions considerably more is incorporated into oligosaccharide than is destroyed. In an experiment described above 4 g. glucose gave rise to oligosaccharides containing 0·38 g. glucose (9·4% original glucose) with $[\alpha]_D$ approx. +84°. This would lead to an increase in the optical rotation of the whole solution of about 5%, whereas the observed rise was 3·5%, so that 1·5% of the original glucose may have been destroyed, compared with 9·4% condensed. Pirt & Whelan (1951), and other authors, have measured loss in reducing power when glucose is heated with dilute acid under various conditions. Their data give useful 'correction factors' but since most of the oligosaccharides produced are also reducing substances these factors are not a measure of the loss of glucose as such.

We were led to the present investigation by the observation that when 3% (w/v) maltose was hydrolysed at 100° with N-H_2SO_4 disaccharide material persisted even after 5 hr. Closer inspection showed this to be spot *A*, *B* (Figs. 1 and 2) and not maltose. Despite many references in the literature it still does not seem to be generally recognized that unless very dilute carbohydrate solutions and acids are used in total hydrolyses of carbohydrate material a correction for 'reversion' is necessary and control glucose solutions should always be used. Changes in strength and nature of acid used and in concentration of carbohydrate will make considerable differences to the amount of 'reversion' taking place in a given period of time.

A notable feature of the condensation process is the production of isomaltose. After heating 16 g. glucose for 3 hr. in N-H_2SO_4 it was possible to isolate 0·78 g. of a syrup consisting chiefly of this

disaccharide. The yield of total oligosaccharide in this experiment was 1·66 g. Even if an allowance of 10% is made for contaminating substances in the isomaltose fraction the figures show that nearly half the condensation product is isomaltose. Inspection of chromatograms suggests that the amount of gentiobiose formed is of a comparable magnitude, and this is confirmed by the isolation by Thompson *et al.* (1953) of equal amounts of the two octaacetates. The greater part of the condensation products evidently consists of these two disaccharides. Thus, as Peat *et al.* (1952) found in the case of glucose condensation catalysed by emulsin, the 1:6-link predominates, but while the enzyme specifically catalyses the formation of β-linkages, acid condensation produces both.

The recovery experiments (Table 2) demonstrate that the chromatographic methods used here do not permit the measurement of the whole of the isomaltose in an oligosaccharide mixture. However, they are probably better in this respect than the acetylation technique used by Wolfrom and his collaborators. Our figures for the yield of isomaltose on hydrolysis of glycogen are somewhat higher than those given by Wolfrom, Lassettre & O'Neill (1951). If one assumes that isomaltose amounts to about half the total oligosaccharide produced from glucose by condensation under the conditions used for glycogen hydrolysis, as in the more drastic conditions mentioned earlier, the yields recorded in our experiments are appreciably greater than those found by Thompson *et al.* (1953).

Thus our experiments bring the yields of isomaltose from glycogen closer to those produced by acid condensation under comparable conditions, but nevertheless the discrepancy between them is still so great that it must be concluded that the isomaltose structure is an integral part of the glycogen molecule.

SUMMARY

1. When glucose is heated in solution with N-H_2SO_4 the products include a complicated mixture of oligosaccharides ('reversion products') in which disaccharides predominate.

2. One component of this mixture is isomaltose.

3. The production of isomaltose and of the total condensation products has been examined quantitatively.

4. The degree of condensation depends upon the nature and concentration of the acid used, on the concentration of glucose and on the duration of heating.

5. The amounts of isomaltose appearing during hydrolysis of glycogen have been compared with those arising by condensation. The considerable excess produced from glycogen, over that expected from condensation, is taken to indicate that the isomaltose structure is an integral part of the glycogen molecule.

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