384. α-1,4-*Glucosans. Part XIX.*[1] *The Action of Acid on Maltose and Starch-type Polysaccharides*

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When dilute solutions (0·4—1·0%) of maltose are heated with mineral acid, significant quantities of isomaltose and nigerose are produced; the yields are greater than that due to acid-reversion from D-glucose. The isolation of minute quantities of nigerose from partial acid hydrolysates of starch-type polysaccharides cannot, in the absence of other supporting evidence, be regarded as proof of the presence of α-1,3-glucosidic linkages.

Although it is now generally accepted that amylopectin is composed of chains of α-1,4-linked glucose residues interlinked by 4—5% of α-1,6-glucosidic interchain linkages,[2] the presence of a small proportion of α-1,3-glucosidic linkages has also been suggested. This was originally based on the isolation of *ca.* 0·13% of nigerose from a partial acid hydrolysate of waxy maize starch, under conditions in which its formation by "reversion" from glucose was negligible.[3] Partial acid hydrolysates of beef liver glycogen [4] and the algal glucan Floridean starch [5] also contain small quantities of nigerose (Table). However, nigerose has been prepared by the action of acid on a mixture of maltose and glucose,[6] whilst commercial starch dextrins are prepared by roasting starches in the presence of acidic reagents, which causes fragmentation and the formation of 1,3-glucosidic linkages by transglucosidation.[7] It therefore seemed possible that despite the difference in these

[1] Part XVIII, D. J. Manners and G. A. Mercer, *J.*, 1963, 4317.
[2] For a review, see W. J. Whelan, in "Encyclopedia of Plant Physiology," Springer, Berlin, 1958, vol. VI, p. 154.
[3] M. L. Wolfrom and A. Thompson, *J. Amer. Chem. Soc.*, 1956, **78**, 4116.
[4] M. L. Wolfrom and A. Thompson, *J. Amer. Chem. Soc.*, 1957, **79**, 4212.
[5] S. Peat, J. R. Turvey, and J. M. Evans, *J.*, 1959, 3223.
[6] J. H. Pazur and T. Budovich, *J. Amer. Chem. Soc.*, 1956, **78**, 1885.
[7] J. D. Geerdes, B. A. Lewis, and F. Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 4209; G. M. Christensen and F. Smith, *ibid.*, p. 4492.

experimental conditions, and those in the Table, limited acid-catalysed transglucosidation of maltose could account for the observed minute yields of nigerose. We now report evidence for the correctness of this view, a preliminary account of which has been published.[8]

The presence of nigerose in partial-acid hydrolysates of starch-type polysaccharides

Wt. of polysaccharide (g.)	Conditions of hydrolysis	Yield of nigerose (mg.)	Yield of nigerose (%)	Ref.
Waxy maize starch, 130	0·4% in 0·1N-HCl at 100° for 8 hr.	170 *	0·13	3
Beef liver glycogen, 92	0·4% in 0·1N-HCl at 100° for 5·8 hr.	1 *	0·001	4
Floridean starch, 12·5	1% in 0·33N-H_2SO_4 at 100° for 130 min.	35	0·28	5

* Isolated as twice this weight of octa-acetate.

Since a reference specimen of nigerose was required, and the fact that the sample prepared by Pazur and Budovich [6] differed markedly in specific rotation (+87°) from other literature values (+135 to +137°),[9] we have repeated the preparation of nigerose by the action of acid on maltose and glucose. A mixture of glucose (12 g.) and maltose (24 g.) was heated at 100° in 0·1N-sulphuric acid (90 ml.) for 5 hr., cooled, neutralised, and the product fractionated by charcoal–Celite column chromatography. The products were glucose (26·1 g.), isomaltose (0·19 g.), maltose (3·15 g.), and a mixture of nigerose and other sugars (1·98 g.). Part of this mixture was fractionated on a second column, as the borate complexes,[10] to give glucose, α,α-trehalose, kojibiose, maltose, and nigerose. The yield of the last, which had $[\alpha]_D$ +134°, in agreement with other literature values, corresponded to the presence of 0·388 g. in the original mixture, an amount similar to that (0·4 g.) recorded by Pazur and Budovich.[6]

The action of hot acid on a dilute solution of maltose was then examined, and in view of our previous interest in Floridean starch,[11] the conditions of Peat and his co-workers,[5] with a final concentration of 1% carbohydrate, were used. Paper chromatography showed the presence of five sugars with R_G 1·0, 0·70, 0·55, 0·37, and 0·28, corresponding to glucose, maltose, isomaltose, panose, and an oligosaccharide. The maltose spot was elongated and showed the probable presence of an additional sugar of slightly higher R_G. The mixture of sugars was neutralised, concentrated, and fractionated on a charcoal–Celite column. The products were glucose (8·48 g.), and mixtures of glucose and 1,6-anhydroglucose (0·05 g.), of disaccharides (2·50 g.), and of oligosaccharides (0·23 g.). One portion of the disaccharide mixture was chromatographed, as borate complexes, on charcoal. This gave traces of glucose and panose, isomaltose (65 mg.), and a mixture of nigerose and maltose which on preparative paper chromatography gave 12·5 mg. of a reducing sugar with the R_G of nigerose, the same electrophoretic mobility as laminaribiose, and whose acetate had m. p. 147° undepressed on admixture with β-nigerose octa-*O*-acetate kindly supply by Dr. J. R. Turvey. A second portion of the mixed disaccharides (0·65 g.) was fractionated by preparative paper chromatography, to give a further 9·7 mg. of nigerose, $[\alpha]_D$ +142° (in water). A third portion of the disaccharide mixture (0·56 g.) was analysed by quantitative paper chromatography, the various disaccharides being eluted with water, and estimated using a phenol–sulphuric acid reagent.[12] The apparent composition of the original disaccharide mixture was: maltose, 2344 ± 12 mg.; isomaltose, 72 ± 6 mg.; nigerose, 87 ± 6 mg. It is clear that substantial losses of nigerose had occurred during the various attempts to obtain a chromatographically pure specimen.

The oligosaccharides had the R_G values of maltotriose, panose, and isomaltotriose (0·44, 0·32, and 0·21, respectively). The last has been isolated from partial acid hydrolysates of glycogen, and assumed to be structurally significant.[4] This would mean

[8] D. J. Manners and G. A. Mercer, *Biochem. J.*, 1963, **89**, 34P.
[9] S. Haq and W. J. Whelan, *J.*, 1958, 1342.
[10] S. A. Barker, E. J. Bourne, and O. Theander, *J.*, 1955, 4276.
[11] I. D. Fleming, E. L. Hirst, and D. J. Manners, *J.*, 1956, 2831.
[12] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Analyt. Chem.*, 1956, **28**, 350.

that, in parts of the molecule, at least two branch points were linked together. This is not very likely in terms of the mode of action of branching enzyme,[13] and it is possible that this trisaccharide is also formed by transglucosidation, either from maltose (as above), or from isomaltose.

The fact that approximately similar yields of nigerose and isomaltose are formed from maltose was confirmed in small-scale experiments in which maltose (0·50 g.) was heated in 1% solution with dilute sulphuric acid, and the products analysed by quantitative paper chromatography. In one experiment, the relative yields of maltose, isomaltose, and nigerose were 392, 23, and 26 μg., respectively.

The effect of hot dilute hydrochloric acid on 0·4% solutions of maltose (cf. ref. 3) was also studied. In all experiments, paper chromatographic evidence for the formation of both isomaltose and nigerose was obtained; the yields were similar to that formed from 1% maltose by hot dilute sulphuric acid. During an 8 hr. heating period, isomaltose could be detected after only 1 hr.

We conclude that significant amounts of nigerose are formed by the action of hot dilute mineral acid on dilute (0·4—1·0%) solutions of maltose. Since these conditions are similar to those used for the linkage analysis of amylopectin-type polysaccharides (Table), the presence of nigerose cannot be regarded as uniquivocal evidence for the presence of α-1,3-glucosidic linkages. Other evidence for the presence of these linkages in amylopectin [14] was based on periodate oxidation studies, but was recently [1] shown to be inconclusive. Moreover, the fact that intestinal extracts hydrolyse nigerose does not, despite a suggestion to the contrary,[3] offer further support for the presence of 1,3-linkages in starch.

In the case of Floridean starch, the evidence for α-1,3-linkages is based on (a) the presence in partial acid hydrolysates of nigerose, and also of trisaccharides containing α-1,3-linkages, (b) the release of nigerose following enzymic degradation,[15] a process which does not, in this instance, involve transglucosidation reactions. The latter observation thus provides stronger evidence for the presence of α-1,3-linkages, and will be considered in detail in a later Communication.

Our conclusions differ from those of Wolfrom and his co-workers,[16] who have also considered the possibility that the nigerose was an artifact formed by the action of acid on maltose and glucose. A mixture of these sugars (114 and 30 g., respectively) was heated in 37 l. of 0·08N-hydrochloric acid for 10 hr. After separation of glucose by column chromatography, paper chromatography of the oligosaccharide fraction showed the presence of maltose, with smaller amounts of glucose, 1,6-anhydroglucose, " a significant amount of (1→6)-linked disaccharide, and a trace of nigerose." However, on acetylation of this mixed fraction, nigerose octa-acetate could not be isolated, and it was therefore concluded that the previous isolation of this derivative (*e.g.*, 2 mg. from 92 g. of glycogen [4]) was structurally significant. It must be noted that these workers have disregarded their own paper chromatographic evidence; moreover, neither the acetylation of sugars nor the chromatographic separation of sugar acetates is a quantitative procedure.

The absence of β-linked disaccharides in our experiments proves that isomaltose and nigerose are formed not by acid-reversion from glucose (which gives various sugars including gentiobiose and cellobiose [17]) but by a relatively rapid acid-catalysed transglucosidation reaction. This occurs with retention of configuration, and is not confined to maltose. For example, when methyl α-D-glucoside is heated with dilute acid, isomaltose is formed (cf. ref. 18), whilst, with methyl β-D-glucoside, gentiobiose is the product. When

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[14] J. K. Hamilton and F. Smith, *J. Amer. Chem. Soc.*, 1956, **78**, 5907, 5910.
[15] S. Peat, J. R. Turvey, and J. M. Evans, *J.*, 1959, 3228.
[16] M. L. Wolfrom, A. Thompson, and R. H. Moore, *Cereal Chem.*, 1963. **40**, 182.
[17] (*a*) A. Thompson, K. Anno, M. L. Wolfrom, and M. Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309; (*b*) S. Peat, W. J. Whelan, T. E. Edwards, and O. Owen, *J.*, 1958, 586.
[18] W. G. Overend, C. W. Kees, and J. S. Sequeira, *J.*, 1962, 3429.

isomaltose in 0·4% solution was heated with acid, maltose and a higher oligosaccharide could be detected.

Additional evidence for the formation of oligosaccharides from maltose was obtained by hydrolysing amylopectin in the presence of [^{14}C]glucose, and comparing the radioautogram with that of (a) maltose and (b) glucose, each of which was heated in dilute acid solution with [^{14}C]glucose. The formation of radioactive oligosaccharides was much greater from amylopectin and maltose than from glucose. This confirms the view [17b] that, in the linkage analysis of polysaccharides, the action of acid on the constituent disaccharide(s) rather than the component monosaccharide(s) should be used for control purposes.

EXPERIMENTAL

Methods.—(a) *Paper chromatography*. Ascending and descending chromatograms were developed using *A*, ethyl acetate–pyridine–water (10 : 4 : 3, v/v); *B*, butan-1-ol–pyridine–water (6 : 4 : 3, v/v); *C*, butan-1-ol–ethanol–water (40 : 11 : 19, v/v); *D*, butan-1-ol–acetic acid–methanol–water (517 : 100 : 219 : 166 v/v) [19] as solvents, together with aniline phthalate, and silver nitrate spray reagents.

(b) *Electrophoresis*. Separation of oligosaccharides on Whatman No. 1 paper was effected using an apparatus similar to that of Foster,[20] at 750 v and 10 mA. Borate [20] or germanate [21] buffers (pH 10·7) were used.

(c) *Column chromatography*. Charcoal–Celite columns were prepared from Ultrasorb 120—240 charcoal [22] and Celite 545, and oligosaccharides eluted with increasing concentrations of aqueous ethanol. In some experiments, borate complexes of oligosaccharides were eluted [10]; column fractions were then acidified to pH 3 and extracted with isopentyl alcohol for 24 hr. to remove borate ions.

(d) *Estimation of reducing sugars*. Glucose and disaccharides were determined by the method of Park and Johnson,[23] or by the phenol–sulphuric acid reagent of Dubois and his co-workers.[12] For disaccharides, a calibration curve against maltose was used.

Materials.—Glucose and maltose were commercial samples which had been purified by three recrystallisations. The glucose was chromatographically pure. The maltose sample contained a trace of maltotriose (identified by α-amylolysis to maltose and glucose), but no other disaccharides. Maltose was chromatographed on Whatman 3MM paper, and the leading and trailing edges of the band of sugar separated and rechromatographed in solvent *C* for 7 days. On spraying with both aniline oxalate and silver nitrate reagents, only maltose was present. For the experiments involving quantitative paper chromatography, maltose which had been freed from maltotriose by charcoal–Celite chromatography was used.

Authentic samples of isomaltose, isomaltotriose, and panose were available from previous studies.[24]

Action of Acid on a Mixture of Maltose and Glucose.—A mixture of glucose (12 g.) and maltose (24 g.) was dissolved in 0·1N-sulphuric acid (90 ml.) and heated at 100° for 5 hr. The solution was cooled, neutralised (barium carbonate), centrifuged, and the precipitate washed three times with water. The supernatant solution and washings were combined and concentrated; paper chromatography showed the presence of at least five sugars. The concentrate was applied to a charcoal–Celite column (100 × 6 cm.) which was eluted with water (5 l.) and then 10% ethanol (25 l.). The aqueous eluate did not contain carbohydrate; the ethanol eluate was collected in 500 ml. fractions, each of which was concentrated to *ca.* 1 ml. before chromatography in solvent *B* (two ascents on Whatman 3MM paper).

Fraction O (column fractions 11—19). This material crystallised, yield 26·1 g. The sugar had $[\alpha]_D$ +52° in water, the R_G value of glucose in solvents *A* and *B*, and the derived acetate had m. p. 130—131°. D-Glucose had $[\alpha]_D$ +52·6°, and the penta-acetate had m. p. 131°.

Fraction A (column fractions 20—24). Chromatography in solvent *B* showed the presence of one major and two trace components, with R_G values of isomaltose, glucose, and maltose,

[19] H. C. Silberman, *J. Org. Chem.*, 1961, **26**, 1967.
[20] A. B. Foster, *J.*, 1953, 982.
[21] B. Lindberg and B. Swan, *Acta Chem. Scand.*, 1960, **14**, 1043.
[22] R. C. Hughes and W. J. Whelan, *Chem. and Ind.*, 1958, 884.
[23] J. T. Park and M. J. Johnson, *J. Biol. Chem.*, 1949, **181**, 149.
[24] W. A. M. Duncan and D. J. Manners, *Biochem. J.*, 1958, **69**, 343.

respectively. The isomaltose was purified by preparative paper chromatography (yield 195·2 mg.). It had $[\alpha]_D$ +122·8° in water, and the derived acetate had m. p. 142° (cf. authentic values of $[\alpha]_D$ +122° and m. p. 143°, respectively).

Fraction B (column fractions 25—32). This material crystallised (yield 3·15 g.) but contained a mixture of glucose and maltose. A portion was fractionated by preparative paper chromatography to give glucose (34 mg.) and maltose (303 mg.) which had $[\alpha]_D$ +132·9° and gave an acetate with m. p. 158° (cf. authentic values of $[\alpha]_D$ +136° and m. p. 158—159°). Fraction B thus contained 315 mg. of glucose and 2·835 g. of maltose.

Fraction C (column fractions 33—50). Concentration to dryness gave 1·98 g. of material which, by chromatography in solvent *B*, contained three sugars with R_G values similar to those reported by Pazur and Budovich[6] for nigerose, maltose, and isomaltose. Chromatography with solvents *A* and *B*, using both descending and multiple-ascent techniques, failed to give an adequate resolution for quantitative separation.

A portion of fraction C (170 mg.) was applied to a charcoal–Celite column (60 × 5 cm.) previously washed with water and then with borate buffer pH 8·7,[10] and the column eluted with borate buffer (6 l.) and an ethanol gradient of 0—20%. Fractions (50 ml.) were collected, and their carbohydrate content estimated on a 1-ml. aliquot using the phenol–sulphuric acid reagent.[12] Various fractions were combined, concentrated, freed from borate, evaporated to dryness, and redissolved in known volumes of water for measurement of optical rotation and disaccharide content.

Fraction C/A (column fractions 8—23) (yield 1·2 mg.). The sugar had the R_G value of glucose, and $[\alpha]_D$ +50° in water.

Fraction C/B (column fractions 24—27) did not contain carbohydrate.

Fraction C/C (column fractions 28—35) (yield 5·4 mg.) had $[\alpha]_D$ +186° in water and did not react with Fehling's solution, aniline oxalate, 3,5-dinitrosalicylic acid, or triphenyltetrazolium chloride.[25] This suggests a 1,1-linked disaccharide; α,α-trehalose has $[\alpha]_D$ +178°.

Fraction C/D (column fractions 36—43) (yield 9·4 mg.) had $[\alpha]_D$ +136° in water, did not react with Fehling's solution, aniline oxalate, and triphenyltetrazolium chloride, but reduced 3,5-dinitrosalicylic acid. These reactions are characteristic of 1,2-linked disaccharides; kojibiose has $[\alpha]_D$ +133 to +140°.[26]

Fraction C/E (column fractions 44—59) (yield 33·3 mg.) had $[\alpha]_D$ +134° in water. The R_G and M_G value in both borate and germanate buffer were the same as that of nigerose isolated from a partial acid hydrolysate of isolichenin.[27] The derived β-acetate (prepared by the sodium acetate–acetic anhydride method) had m. p. 148° (lit.,[9] $[\alpha]_D$ +134 to +139°, m. p. 147—153°).

Fraction C/F (column fractions 60—90) (yield 97·5 mg.) had $[\alpha]_D$ +135° in water, and had the R_G and M_G values of maltose. The derived β-acetate had m. p. 159° (lit., $[\alpha]_D$ +136°, m. p. 159°).

From the above results, the composition of fraction C was calculated to be: glucose, 14 mg.; α,α-trehalose, 63 mg.; kojibiose, 110 mg.; nigerose, 388 mg.; maltose 1136 mg.

The ratio of isomaltose : maltose : nigerose in the reaction products was measured in a separate experiment in which glucose (2 g.) and maltose (4 g.) were heated in 0·1N-sulphuric acid (15 ml.). After neutralisation, the combined solutions were diluted to 50 ml. with distilled water, and aliquots (10 μl.) chromatographed on Whatman No. 1 paper using solvent *C* (which was not known to us during the previous experiment) for 8 days. Appropriate areas of the chromatograms were eluted, and the disaccharide content measured by Park and Johnson's method.[23] The relative yields were: isomaltose, 14 μg; maltose, 129 μg; nigerose 13 μg; the last is equivalent to the formation of 390 mg. nigerose in the large scale experiment.

Action of Acid on Maltose.—(a) 1% *Maltose and sulphuric acid.* Maltose (12·5 g.) was dissolved in 0·33N-sulphuric acid (1250 ml.), preheated to 100°, and heated for 125 min. at 100°. The cooled solution was neutralised (barium carbonate), centrifuged, and the precipitate washings and supernatant solution were combined. Paper chromatography (solvent *B*, two ascents) showed the presence of five sugars with the R_G values of glucose, maltose, isomaltose,

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[26] S. A. Barker, E. J. Bourne, P. M. Grant, and M. Stacey, *Nature*, 1956, **178**, 1221; S. Haq and W. J. Whelan, *ibid.*, p. 1222.

[27] N. B. Chanda, E. L. Hirst, and D. J. Manners, *J.*, 1957, 1951; S. Peat, W. J. Whelan, J. R. Turvey, and K. Morgan, *J.*, 1961, 623.

panose, and an oligosaccharide. The maltose spot was elongated, suggesting the probable presence of an additional sugar of slightly higher R_G value. The solution was concentrated to 50 ml. and applied to a charcoal–Celite column (45 × 7 cm.) which was eluted with 12 l. of aqueous ethanol (1—20%, as a linear gradient). Fractions (50 ml.) were collected, the carbohydrate content determined on 1-ml. aliquots, and after concentration, appropriate fractions combined after paper-chromatographic analysis (solvent *B*, two ascents).

Fraction	Column fraction	Wt. (g.)	Probable identity
A	4—12	0·001	—
B	13—69	8·390	Glucose
C	70—82	0·085	Glucose
D	83—103	0·048	Glucose, 1,6-anhydroglucose
E	104—163	2·454	Maltose, isomaltose, nigerose *
F	164—173	0·049	Maltose, isomaltose, nigerose, panose *
G	174—193	0·113	Maltose, maltotriose, panose *
H	194—230	0·117	Maltose, maltotriose, isomaltotriose *

* Traces of glucose were also present.

Fraction B had $[\alpha]_D$ +53° in water, and the R_G value of glucose in solvents *A* and *C*, and the acetate had m. p. 128° (cf. $[\alpha]_D$ +52·6° and m. p. 131° for glucose).

Fraction C also had the R_G value of glucose.

Fraction D was a mixture of glucose and 1,6-anhydroglucose. The relative yields of these sugars, by quantitative paper chromatography, were 18·7 and 26·2 mg., respectively.

Fractions E and F were combined, and one half was applied to a charcoal–Celite column (30 × 5 cm.) previously washed with borate buffer, and eluted with 10 l. of buffer with a linear gradient of 0—20% of ethanol. Fractions (*ca.* 40 ml.) were collected, analysed, and combined as appropriate, as follows.

Fraction EF/A (column fractions 15—22) was non-carbohydrate.

Fraction EF/B (column fractions 36—46) was a trace of glucose.

Fraction EF/C (column fractions 47—65) (yield 64·7 mg.) had the R_G value of isomaltose in solvents *A* and *C*, and $[\alpha]_D$ +117° in water (cf. +122° for isomaltose).

Fraction EF/D (column fractions 66—220) was a mixture of maltose and nigerose (chromatography in solvents *A* and *C*). These chromatograms were slightly streaked, and attempts to remove inorganic ions by treatment with ethanol caused the loss of some disaccharides. The mixture was fractionated by preparative paper chromatography (Whatman 3MM paper, solvent *C*, 4 days), to give 12·5 mg. of a disaccharide which had the R_G value of nigerose, an M_G value similar to that of laminaribiose, and formed a crystalline acetate with m. p. 147° undepressed on admixture with authentic nigerose β-octa-*O*-acetate.

Fraction EF/E (column fractions 221—269) contained a trace of panose (paper chromatography).

A second portion of fraction EF (650 mg.) was fractionated by preparative paper chromatography to give 9·7 mg. of a disaccharide with the R_G and M_G values of nigerose, and $[\alpha]_D$ +142° in water (lit., +134 to +139°). From a third portion of fraction EF (150 mg.), maltose was isolated by preparative paper chromatography, and identified by R_G and M_G values, and $[\alpha]_D$ +140° (lit., +136°).

Fractions G and H were combined, and examined by paper chromatography in solvent *B*. Three sugars with the R_G values of maltotriose, panose, and isomaltotriose were present.

(b) *Ratio of disaccharides formed from maltose.* The remainder of fraction EF (560 mg.) was dissolved in water (25 ml.), and 5 μl. portions chromatographed on sheets using solvent *C* for 9 days. Appropriate areas of the sheets were eluted and analysed by the phenol–sulphuric acid method. The calculated yields in EF were: isomaltose, 72 ± 6 mg.; maltose, 2344 ± 12 mg.; and nigerose, 87 ± 6 mg.

The similarity in the yields of isomaltose and nigerose was confirmed in a second experiment in which maltose (0·50 g.) was heated in 0·33N-sulphuric acid (50 ml.) for 130 min., cooled, neutralised (barium carbonate), and the solution and washings were adjusted to 10 ml. Portions were chromatographed as above for 7 days, and appropriate areas estimated by Park and Johnson's method.[23] The relative yields were: isomaltose, 23 μg.; maltose, 392 μg.; nigerose, 26 μg.

(c) 0·4% *Maltose and hydrochloric acid.* Maltose (500 mg.) was heated in 0·4% solution in 0·1N-hydrochloric acid for 8 hr., cooled, and neutralised by passage through a column of Duolite A-4 resin.[3] The solution was adjusted to 50 ml., and portions (0·2 ml.) chromatographed against authentic nigerose using solvent *D* for 4 days. Nigerose was detected, and amounted to *ca.* 2% of the total carbohydrate. Nigerose was also formed in similar experiments in which (a) the hydrochloric acid was neutralised with silver carbonate, and (b) 1% maltose was heated in 0·33N-sulphuric acid.

Considerable difficulty arose in these and the previous experiments in effecting complete separation of maltose and nigerose. In some experiments the following bands were eluted: isomaltose, maltose plus nigerose, and glucose; the mixed fraction was then rechromatographed in solvent *D* for 4 days. Even this technique did not always give chromatographically pure nigerose; hence, the quoted figures for paper-chromatographic analyses are not considered to be strictly quantitative.

When 0·4% solutions of maltose were heated in 0·1N-hydrochloric acid, isomaltose was not present after 30 min. but could readily be detected after 1 hr.

Action of Acid on other Glucosides.—(*a*) Isomaltose (70 mg.) was heated in 0·4% solution in 0·1N-hydrochloric acid for 4 hr., cooled, neutralised (Duolite A-4), concentrated, and chromatographed. In addition to glucose and isomaltose, small quantities of sugars with R_G values of a di- and tri-saccharide were present. The latter were eluated from a sheet of paper, and tentatively identified as maltose and isomaltotriose, by borate electrophoresis and R_G value, respectively.

(*b*) Methyl α-D-glucoside (500 mg.) was heated in 2N-hydrochloric acid (25 ml.) at 70° for 2 hr., cooled, neutralised (De-Acidite FF resin column), and concentrated to *ca.* 3 ml. Part of the solution was chromatographed on Whatman 3MM paper, and guide strips showed the presence of isomaltose or gentiobiose. This sugar was eluted (yield 4 mg.); it had the same M_G value as isomaltose, and was not hydrolysed by an almond β-glucosidase preparation. When methyl β-D-glucoside was heated similarly, the product was hydrolysed by the enzyme preparation (whose specificity had been tested against known substrates), and is therefore identified as gentiobiose.

Acid-transglucosidation Experiments using [^{14}C]*Glucose.*—Generally labelled [^{14}C]glucose (0·1 mc, 0·37 mg. in 0·5 ml. of water, supplied by the Radiochemical Centre, Amersham) was used. Solutions were chromatographed on Whatman No. 1 paper, air-dried, and placed on sheets of Ilford Fast Industrial-G *X*-ray film, which were developed after a suitable period. Exposure for up to 8 days failed to reveal any radioactive oligosaccharides in the sample of glucose.

Maltose (10 mg.), [^{14}C]glucose solution (0·1 ml.), in 0·33N-sulphuric acid (0·9 ml.) were heated for 130 min., cooled, neutralised (barium carbonate), and the solution and washings were concentrated to 0·1 ml. Radioautography showed the presence of radioactive sugars with the R_G values of 1,6-anhydroglucose, isomaltose, and an elongated maltose spot. The intensities of the radioactive disaccharides were much greater than that of a similar solution prepared from 10 mg. of glucose. When potato amylopectin (9 mg.) was hydrolysed under these conditions, radioactive disaccharides with the R_G values of maltose, isomaltose, and nigerose were formed in amounts similar to that from maltose. (In all the radioautograms, 5 μl. portions of solutions were used.)

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