1.38 g. (0.060 mole) of sodium and 50 cc. of absolute alcohol were added 8.38 g. (0.060 mole) of glycine ester hydrochloride and then 7.89 g. (0.030 mole) of 5-β-bromoethyl-5-ethylbarbituric acid. The mixture was allowed to stand in an ice–salt-bath 12 hours and then to come to room temperature overnight. Concentration of the filtrate followed by recrystallization of the product from alcohol gave 1.8 g. of the product XVI, m.p. 163–165°.

Anal. Calcd. for $C_{20}H_{29}O_8N_5$: mol. wt., 467; N, 14.98. Found: mol. wt. (Rast), 453; N, 14.87.

The anilide XVII was prepared by heating the above ester with freshly distilled aniline, m.p. 197–199°.

Anal. Calcd. for $C_{24}H_{30}O_7N_6$: N, 16.30. Found: N, 16.08.

NEWARK, DELAWARE

[CONTRIBUTION FROM THE STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY][1]

Isomaltose and Isomaltotriose from Enzymic Hydrolyzates of Dextran

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Isomaltose (6-[α-D-glucopyranosyl]-D-glucose), and a new sugar which appears to be isomaltotriose (6-α-isomaltopyranosyl-D-glucose), were prepared in yields of 50 and 20%, respectively, from enzymic hydrolyzates of the dextran from *Leuconostoc mesenteroides* NRRL B-512. The dextran was hydrolyzed by culture filtrates from *Penicillium funiculosum*, NRRL strains 1768 and 1132 which had been cultured on a medium containing B-512 dextran. Characterizing data are given for the amorphous sugars and their acetates. Reaction of the sugars with sodium metaperiodate showed that they were composed of aldopyranosidic units linked 1,6. Reaction of the sugar acetates with hydrogen bromide–acetic acid–acetyl bromide was typical of glucopyranosidic units linked α-1,6.

During the past eighty years the name isomaltose has been applied to numerous poorly defined substances or mixtures derived mainly from the action of strong acid on glucose and from enzymic action on starch.[2] In recent years isomaltose has been established definitely as 6-[α-D-glucopyranosyl]-D-glucose through study of the pure sugar and some of its crystalline derivatives. The pure sugar has been obtained in about 5% yield by the action of taka-amylase on amylopectin.[3] Crystalline octaacetyl-β-isomaltose has been obtained from acid hydrolyzates of a bacterial dextran in about 5% yield,[2,4] from acid hydrolyzates of glycogen[5] and of amylopectin[6] in yields of 1 to 2%, and from hydrol in 5% yield.[7] It has also been synthesized from octaacetyl-β-gentiobiose.[8]

Reported here is the direct isolation, for the first time in high yields, of isomaltose and in addition its homolog, the new sugar isomaltotriose.

The present report of the preparation of isomaltose and isomaltotriose in high yields from the dextran from *Leuconostoc mesenteroides* NRRL B-512, is the fruition of a previously stated objective.[9] The respective yields of 50 and 20% from dextran make these two sugars accessible on a preparative basis. The dextran used, having about 95% of its constituent glucopyranosidic units linked α-1,6-,[10,11] is peculiarly suited to the preparation of isomaltose and its homologs. It was degraded by mold enzyme preparations developed especially for this purpose,[12] for at the initiation of this work in 1945, enzymes capable of degrading dextran were not known.

Results and Discussion

Products of Enzyme Action.—Numerous species and strains of *Penicillium* and other molds, when cultured on B-512 dextran, were found to produce exocellular enzymes[12] which degraded this dextran with resulting high yields of isomaltose. Chosen from among these for our present work were the two strains of *Penicillium funiculosum* NRRL 1768 and 1132. Culture filtrates from these molds acted on B-512 dextran to give different distributions of mono-, di- and trisaccharides (Table I). In addition, each culture filtrate also produced a continuous series of higher homologs. As shown in Table I, the amounts of mono-, di- and trisaccharides isolated by carbon column chromatography were in close agreement with the amounts indicated by quantitative paper chromatography.

TABLE I

PROPORTION OF SUGARS IN DEXTRAN HYDROLYZATES

Saccharide	Isolated from columns, %[b] Strain[a] 1768	Strain 1132	By paper chromatography, % Strain 1768	Strain 1132
Mono	3.7	11	..	9
Di	45	53	42	52
Tri	22	7	25	7

[a] NRRL strains of *Penicillium funiculosum*. [b] Per cent. by weight of carbohydrate applied to column.

Identity and Characterization of the Di- and Trisaccharides.—The identity of the disaccharide as isomaltose has been established by the corre-

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) The history of isomaltose has been given by M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **71**, 125 (1949).

(3) (a) Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, *ibid.*, **69**, 2249 (1947); (b) **71**, 1682 (1949).

(4) L. W. Georges, I. L. Miller and M. L. Wolfrom, *ibid.*, **69**, 473 (1947).

(5) M. L. Wolfrom, E. N. Lassettre and A. N. O'Neill, *ibid.*, **73**, 595 (1951).

(6) M. L. Wolfrom, J. T. Tyree, T. T. Galkowski and A. N. O'Neill, *ibid.*, **73**, 4927 (1951).

(7) M. L. Wolfrom, A. Thompson, A. N. O'Neill and T. T. Galkowski, *ibid.*, **74**, 1062 (1952).

(8) B. Lindberg, *Acta Chem. Scand.*, **3**, 1355 (1949); *Nature*, **164**, 706 (1949).

(9) Allene Jeanes, C. A. Wilham and J. C. Miers, *J. Biol. Chem.*, **176**, 603 (1948).

(10) Allene Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(11) M. Abdel-Akher, J. K. Hamilton, R. Montgomery and F. Smith, *ibid.*, **74**, 4970 (1952).

(12) H. M. Tsuchiya, Allene Jeanes, Helen M. Bricker and C. A. Wilham, *J. Bact.*, **64**, 513 (1952).

spondence of the physical constants of the amorphous sugar and of its crystalline octaacetate with accepted values. The identity of the trisaccharide as isomaltotriose (6-α-isomaltopyranosyl-D-glucose) is based upon its physical constants and the results of acid hydrolysis and periodate oxidation. Neither the trisaccharide nor its acetate has been crystallized. These identities have been substantiated further by paper chromatography of the sugars and by reaction of the sugar acetates with hydrogen bromide–acetic acid–acetyl bromide (acetobrominolysis).

The correspondence between the purified di- and trisaccharides obtained from enzymic hydrolyzates, and the sugars of the same apparent degree of polymerization, respectively, from B-512 dextran partially hydrolyzed by acid,[13] is shown in the chromatogram, Fig. 1. For comparison, there is shown in Fig. 1 the trisaccharide, 4-α-isomaltopyranosyl-D-glucose (panose)[14] and also a homologous series of α-1,4-linked polymers obtained from amylose partially hydrolyzed by acid.[13] Glucose is the only sugar common to the homologous series from B-512 dextran and from amylose, and panose does not appear in either series.

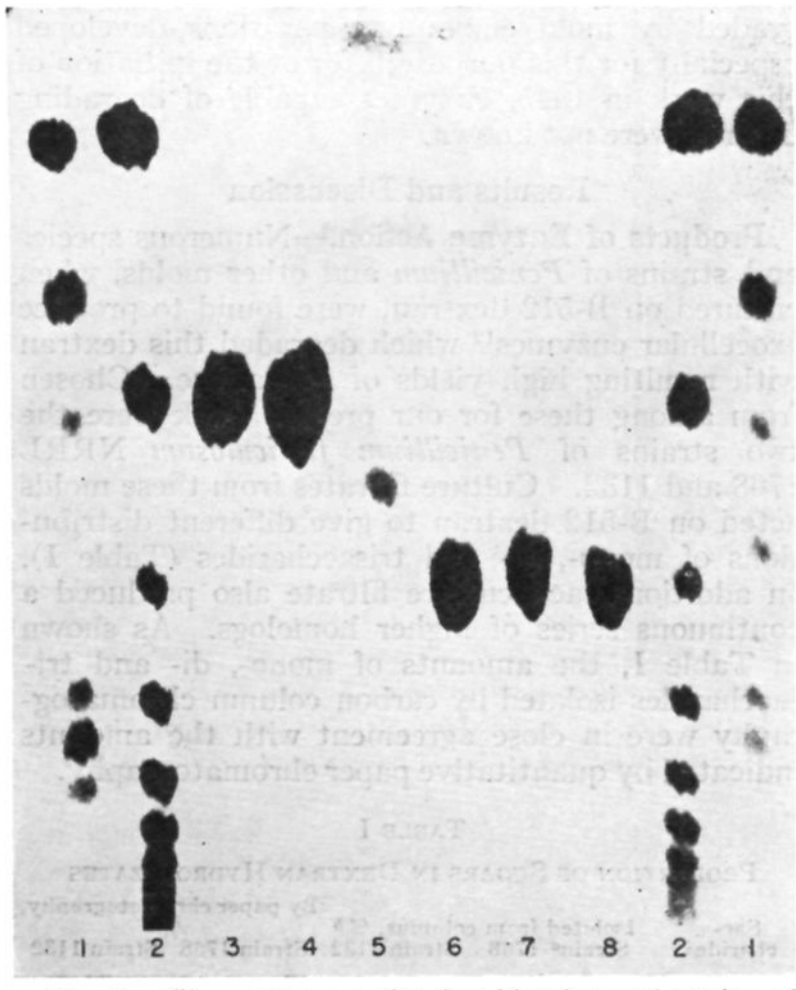

Fig. 1.—Chromatogram showing identity and purity of isomaltose and isomaltotriose preparations. Reading from the top downward: 1, glucose, maltose, maltotriose, and higher homologs from amylose partially hydrolyzed by acid; 2, glucose, isomaltose, isomaltotriose, and higher homologs from B-512 dextran partially hydrolyzed by acid; 3 and 4, isomaltose preparations; 5, panose; 6, 7 and 8, isomaltotriose preparations.

(13) Although all these products of partial acid hydrolysis have not yet been isolated and their structures proved, there is no reasonable basis for doubt of the interpretation used here.

(14) S. C. Pan, L. W. Nicholson and P. Kolachov, THIS JOURNAL, **73**, 2547 (1951); M. L. Wolfrom, A. Thompson and T. T. Galkowski, *ibid.*, **73**, 4093 (1951)

The absence of detectable amounts of other reducing sugars in our di- and trisaccharides is also demonstrated in Fig. 1, in which these two new sugars were spotted in high concentration.

Shown in Table II are some of the physical constants which serve to characterize the di- and trisaccharides obtained from enzymic hydrolyzates of B-512 dextran, and to identify them as isomaltose and isomaltotriose, respectively. The specific rotation of the isomaltose agrees with one reported value,[3] but is higher than another.[2] The specific rotation and the melting point of the octaacetyl-β-isomaltose agree with accepted values.[2,3] The data for isomaltotriose show it to be a reducing trisaccharide of α-glucosidic configuration. Its specific rotation distinguishes it from two other α-linked trimers of glucose, namely, maltotriose and panose (Table II).

TABLE II

PHYSICAL CONSTANTS FOR DI- AND TRISACCHARIDES

	Sugar			Sugar acetate	
Substance	$[\alpha]^{25}D$ (H_2O)	R_G[a,15]	D.P.[b]	$[\alpha]^{25}D$ ($CHCl_3$)	M.p., °C.
Isomaltose[c]	+122.0	0.66	1.97	+96.9	145.2–146.2
Isomaltotriose[c]	145	.43	2.90	....[e]	
Maltose hydrate	130.4	.79		62.6	
Maltotriose[c]	160[d]	.63		86[6]	133–135[6]
Panose	154[14]	.55			

[a] Measured from chromatogram shown in Fig. 1. [b] Degree of polymerization determined from reducing power. [c] This substance was amorphous. [d] J. M. Sugihara and M. L. Wolfrom, THIS JOURNAL, **71**, 3357 (1949).

The data in Table II permit several other significant comparisons. The specific rotations of the α-1,4-linked sugars are higher than those for α-1,6-linked sugars of corresponding size. Panose, which has both an α-1,4- and an α-1,6-linkage, has a rotation intermediate between isomaltotriose and maltotriose. The reverse order apparently applies in the sugar acetates, which are all believed to be the β-anomers because of their preparation by sodium acetate catalysis.

The effect of the point of attachment of the glucopyranosidic bond on the rate of chromatographic movement of the sugars is shown also in Table II (as well as in Fig. 1). Each oligosaccharide member of the α-1,6-linked series from B-512 dextran travelled more slowly on the paper chromatogram than did the member of apparently corresponding size in the series obtained from amylose. This is an extension of the previous observation that disaccharides linked 1,6- have lower R_G[15] values than those linked 1,4-.[16] Panose, having one of each of these types of linkages, had an R_G value intermediate between isomaltotriose and maltotriose.

Acid Hydrolysis and Periodate Oxidation of the Sugars.—The trisaccharide was found to have one reducing group for three anhydrohexose units (Table II). These were shown to be anhydro-

(15) Ratio of distance moved by sugar to distance moved by glucose, measured from the starting line to center of spot (Fig. 1). Terminology first used by F. Brown, E. L. Hirst, L. Hough, J. K. N. Jones and H. W. Wadman, *Nature*, **161**, 720 (1948).

(16) Allene Jeanes, C. S. Wise and R. J. Dimler, *Anal. Chem.*, **23**, 415 (1951).

glucose units held in α-configuration. During acid hydrolysis, the rotation decreased to a value near that calculated for three moles of glucose. Only glucose and isomaltose were found as hydrolytic products when aliquots removed at intervals during acid hydrolysis were spotted heavily on paper chromatograms.

The oxidation of this reducing trisaccharide with sodium metaperiodate at room temperature gave results in complete agreement with the structure proposed and cannot be accounted for by a trisaccharide having other points of glucosidic attachments or other ring forms. As was anticipated from the observations of Ahlborg on 1,6-linked disaccharides[17] and our own observations on a fraction of acid-hydrolyzed dextran,[10] over-oxidation did not occur. The reaction reached a definite end-point and stopped. The apparent mechanism for over-oxidation by periodate[10,18] indicates that such stability in a reducing oligosaccharide is dependent upon a glucosyl attachment at the C_6 position of at least the reducing hexose unit.

Beginning with the initial measurement (Table III), the moles periodate reduced indicate that one of the first two carbon atoms to be removed as formic acid required only one mole of periodate and was, therefore, the anomeric. At 1.5 hours, the anomeric carbon atom and C_3 from both the middle and non-reducing end units appear to have been split out as formic acid, with a total reduction of approximately 5 moles of periodate. The successive removal of three other carbon atoms as formic acid, each by the reduction of one mole of periodate, appeared to have been completed at 3, 9 and 46 hours of reaction, respectively. These, therefore, must have been C_2, C_3, and C_4 of the reducing end unit. Thus, it appears that the reducing end unit of this trisaccharide either reacted in the aldehydo form, or there was no tendency for the oxidized anomeric carbon atom to exist in a formyl ester structure as has been proposed for maltose and lactose.[19]

In order to determine whether this behavior of the anomeric carbon during oxidation with sodium metaperiodate was characteristic of 1,6-linked sugars in contrast to 1,4-linked, oxidation was carried out on isomaltose, β-melibiose dihydrate and β-maltose monohydrate. The data shown in Table III indicate that the isomaltose appeared to react in the same manner as isomaltotriose. The reaction of β-melibiose differed from that of isomaltose in the apparent order of oxidation of carbon atoms, since at one-half hour reaction time the values were 2 moles of formic acid and 4 of periodate, and at 1.5 hours they were 3 and 5 moles, respectively. The other two moles of formic acid (presumably from hydrolysis of the oxidized anomeric carbon atom and oxidation of C_4 of the reducing unit) were obtained slowly while the sixth mole of periodate was being reduced. This difference between melibiose and isomaltose must be due to the galactose unit of the melibiose or to the fact that our isomaltose, like our isomaltotriose, appeared to react in the aldehydo form.

(17) K. Ahlborg, *Svensk. Kem. Tid.*, **54**, 205 (1942); *C. A.*, **38**, 4254[9] (1944).

(18) Other evidence and references have been cited previously.[7]

(19) K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **31**, 1540, 1545 (1948).

TABLE III

PERIODATE OXIDATION OF ISOMALTOSE, ISOMALTOTRIOSE AND OTHER SUGARS

	Mole/mole sugar							
	Isomaltose		Isomaltotriose		Melibiose·2H₂O		Maltose·H₂O	
Time, hr.	HCOOH formed	IO_4^- reduced	HCOOH formed	IO_4^- reduced	HCOOH formed	IO_4^- reduced	HCOOH formed	IO_4^- reduced
0.17							0.23	1.45
0.5	2.15	3.35	2.21	3.48	1.97	4.16	0.56	1.93
1.5	3.15	4.36	3.32	5.19	2.99	4.98	1.08	2.70
3	3.81	5.03	4.11	6.15	3.72	5.44	1.43	3.14
5	4.16	5.27			4.14	5.64	1.61	3.55
9	4.54	5.55	5.08	7.04				
24	4.78	5.80	5.73	7.86	4.83	6.05	2.31	4.26
46	4.93	5.94	5.99	7.99	4.98	6.10	3.08	5.07
72	4.91	5.99	5.99	8.05	4.88[a]	6.15[a]	3.74	5.78
151	4.96	6.04	5.99[b]	7.99[b]				
Theory	5.00	6.00	6.00	8.00	5.00	6.00	3.00[c]	5.00

[a] At 96 hr. [b] At 102 hr. [c] One mole formaldehyde also is expected.

The behavior of β-maltose was more similar to that of isomaltose than to melibiose in that the anomeric carbon atom appeared to reduce periodate most rapidly. However, the formic acid was liberated very slowly. This manner of oxidation was indicated by the initial low titers and badly fading end-points for formic acid, and by the initial very rapid reduction of 1 mole of periodate. Formic acid from C_3 of the non-reducing unit appeared to come out much more slowly than from the corresponding position in isomaltose. At 72 hours, both periodate reduced and formic acid liberated exceeded theoretical values.

Acetobrominolysis of the Sugar Acetates.—A reagent containing hydrogen bromide-in-acetic acid and acetyl bromide has been shown to react in a striking manner with acetylated di- and polysaccharides composed of 1,6-linked anhydroglucopyranose units.[20] Curves for the reaction of this reagent with our di- and trisaccharide acetates are typical of α-1,6-linked substances (Fig. 2). For comparison, the reaction curves for acetylated maltose, panose and B-512 dextran are included in Fig. 2.

The course of reaction of isomaltotriose hendecaacetate paralleled that of octaacetyl-β-isomaltose, but at lower rotations. This provides further confirmation of the structure of this trisaccharide, since it has already been shown that the higher the proportion per molecule of units which are glucosidically bound through the C_6 position, the lower is the end rotation of the acetobrominolysis reaction mixture.[20] The end rotations of reaction mixtures of acetates of higher homologs of the isomaltose series would be expected to decrease toward that of B-512 dextran triacetate in direct proportion to the degree of polymerization of the saccharide.

A low concentration of hydrogen bromide was used for the reactions of the sugar acetates shown in Fig. 2 in order to observe the very rapid initial changes in octaacetyl-β-isomaltose and isomaltotriose hendecaacetate. This concentration was insufficient to change octaacetyl-β-maltose through

(20) Allene Jeanes, C. A. Wilham and G. E. Hilbert, THIS JOURNAL, **75**, 3667 (1953).

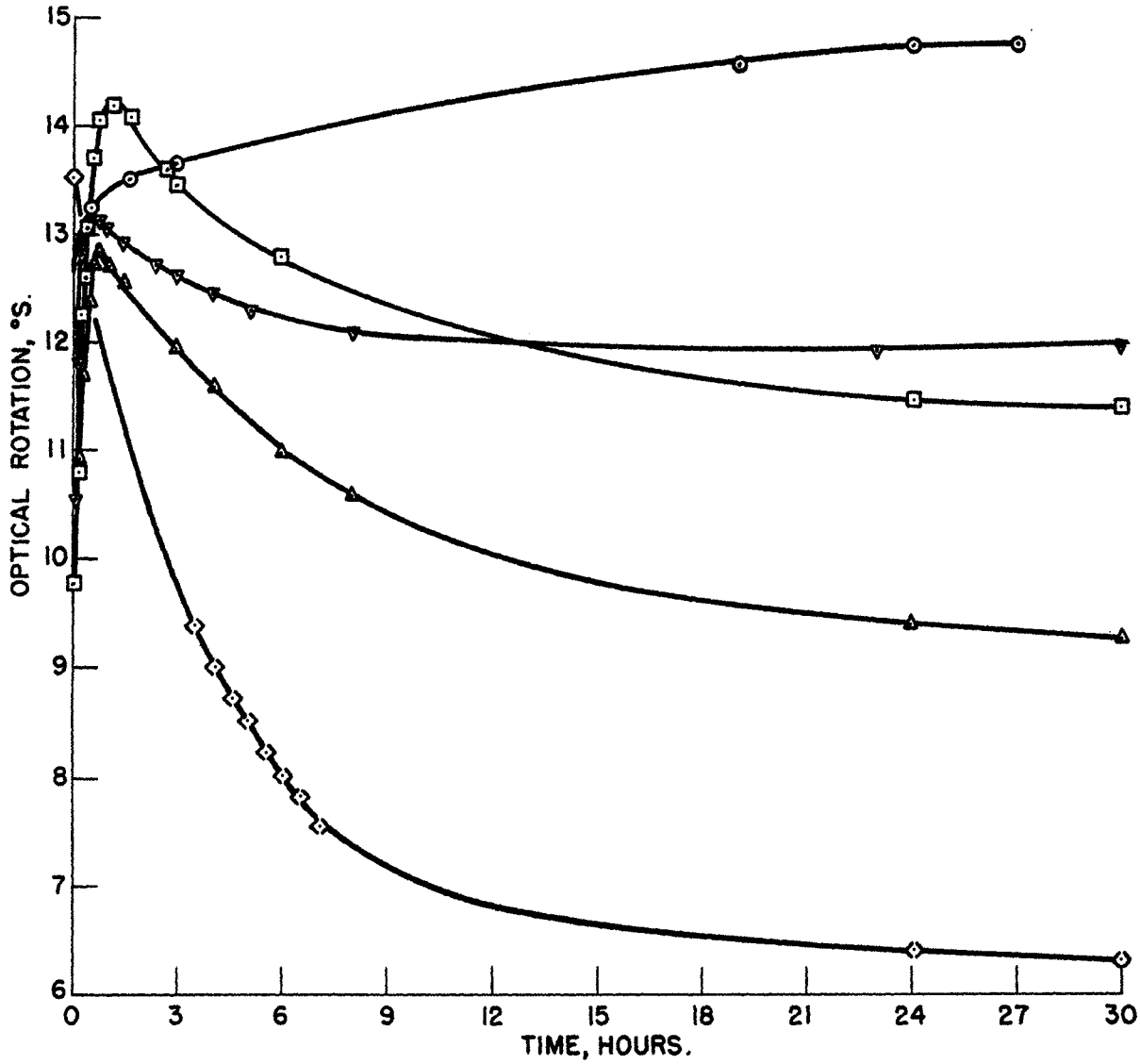

Fig. 2.—Action of hydrogen bromide–acetic acid–acetyl bromide on acetates of sugars and of B-512 dextran: ⊙, octaacetyl-β-maltose; ⊡, octaacetyl-β-isomaltose; △, isomaltotriose hendecaacetate; ▽, panose hendecaacetate (after 30 hours, the rotation of this reaction mixture increased steadily; at 120 hours it was +12.8°); ◇, triacetate of B-512 dextran.

the intermediate heptaacetyl-α-maltosyl bromide, completely to the end product, 2,3,4,6-tetraacetyl-α-D-glucosyl bromide.[20] Completion of this reaction would be indicated by an end rotation of +17.2°.[20] In the case of the hendecaacetate of 4-α-isomaltopyranosyl-D-glucose (panose), decreasing values following the rapidly attained maximum rotation resulted from the α-1,6-linkage present. The α-1,4-linkage manifested itself in the slow increase which first became apparent at 30 hours reaction time, and then continued through the 90 succeeding hours of observation. Here, as in the case of octaacetyl-β-maltose, the hydrogen bromide content of the reagent mixture apparently was insufficient for more rapid and complete cleavage of the α-1,4-linkage.

Acknowledgment.—We should like to acknowledge our indebtedness to Dr. R. J. Dimler for supervision of the paper chromatography and of the deionization of the sugars; to C. S. Wise for the paper chromatograms; and to C. H. Van Etten for the microanalyses. We are indebted to Dr. S. C. Pan for the sample of panose.

Experimental

Materials and Methods.—Descriptions are given in the literature references cited of the preparation of dextran from *Leuconostoc mesenteroides* NRRL B-512[9] and of crude culture filtrates containing dextranases produced by strains of *Penicillium funiculosum* NRRL 1132 and 1768 when cultured on B-512 dextran.[11]

Reducing power was determined by a method of Somogyi using a 20-minute heating period[21]; calculations were based on the maltose hydrate factor. The molar reducing powers toward this Somogyi reagent of isomaltose and isomaltotriose as compared with glucose and anhydrous maltose were found to be as follows.

Sugar	Mole[a]/mole Glucose	Maltose
Isomaltose	0.869	0.952
Isomaltotriose	.840	.925

[a] Mole of 1,6-linked sugar.

(21) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

Qualitative[16] and quantitative[22] paper chromatography were carried out as described in the references cited. For the qualitative paper chromatogram shown in Fig. 1, each spot of the isomaltose and isomaltotriose preparations represents about 1 mg. of the sugar; each spot was made by two applications of 20% aqueous solution in five contiguous positions. Development was by two descents with fusel oil:pyridine:water, 7:7:6.[16] The spray reagent was ammoniacal silver nitrate.[23] As little as 0.1% of other sugars in our di- and trisaccharide preparations would have been detectable.

Isomaltotriose was oxidized by sodium metaperiodate under the same conditions as are used for dextrans.[10] The sugar was dried *in vacuo* over phosphorus pentoxide at 78° for 3 hours. A 0.1984-g. sample was dissolved in freshly boiled water and 130 ml. of 0.03809 *M* sodium metaperiodate solution (a 50% excess) was added. The volume was made to 1000 ml. with boiled water. Determinations of periodate reduced and formic acid produced were made on 10-ml. aliquots, in duplicate. The dimedon test for formaldehyde, carried out as described by Reeves,[24] was negative at 102 hours of reaction. The oxidations of isomaltose and the other disaccharides were carried out similarly. Only in the case of maltose hydrate was fading of the formic acid end-point encountered. This was pronounced at first but became less after about 24 hours. The formic acid values shown for maltose hydrate in Table III correspond to the first end-point. When titration was continued to a more stable end-point, values for formic acid were 1.84, 2.02 and 3.43 at 2, 3 and 46 hours, respectively.

For acetobrominolysis,[20] the weight of acetate used was that required by theory to produce 7.576×10^{-4} mole of 2,3,4,6-tetraacetyl-α-D-glucosyl bromide. To the sugar acetates, contained in 10-ml. volumetric flasks, were added hydrogen bromide-in-acetic acid and acetyl bromide in a ratio of 1:9 by volume. For the dextran triacetate, a 1:1 ratio by volume of the components of the reagents was used. If the reagent mixture containing the lower concentration of hydrogen bromide had been used, the only differences would have been somewhat slower rates of dissolution and reaction of the dextran triacetate.

Enzymic Degradation of B-512 Dextran.—A solution containing 100 g. of B-512 dextran and 1570 ml. of acetate buffer (0.01125 *M*) at *p*H 5.2 in a total volume of 3962 ml. was brought to 40°. To this was added 1960 ml. of diluted culture filtrate from *Penicillium funiculosum* 1768, at *p*H 5.2 and 40°, and which had an activity of 20 dextranase units/ml.[12] The mixture of solutions, after being stirred for 4 hours at 40°, showed reducing power equivalent to 77% conversion to isomaltose, and a greatly diminished rate of change. The *p*H was adjusted to 6.2 and the solution autoclaved at 120° (15 lb./sq. in. gage) for 10 minutes to inactivate the enzyme.

Carried out in a similar manner was the degradation of dextran by a diluted culture filtrate of *P. funiculosum* 1132 which had dextranase activity of 30 units/ml. After 5 hours, the reducing power was equivalent to 94% conversion to isomaltose, and the reaction was stopped.

The protein contents of these hydrolyzates were decreased by use of chloroform and *n*-butanol as previously described.[25]

Fractionation of Enzymic Hydrolyzates.—Fractionation was carried out in individual chromatographic tubes (8.0 × 33 cm.) containing a mixture of 336 g. of Norite SG[26] and 249 g. of Celite 501. This is a modification of a previously described procedure.[3b] The mixture, in 1100 ml. of 2.5% aqueous ethanol, was packed into the tubes by tamping and applying regulated vacuum from a water aspirator. The column was then conditioned by passage of at least 5 liters of 2.5% aqueous ethanol at a rate regulated to about 1200–1300 ml./hour.

A portion of enzymic hydrolyzate (*p*H 6.0) containing 24 g. of isomaltose equivalents was applied to a column at a concentration of 20% total sugars and 2.5% ethanol. Monosaccharides were washed from the columns by 2.5% ethanol, the majority of the isomaltose was displaced by 5% ethanol, and the tailings by 7% ethanol. Isomaltotriose was displaced by 10% ethanol. This method of sugar separation is a modification of one previously described.[27] The course of fractionation was followed qualitatively by anthrone color test for carbohydrate and quantitatively by optical rotation, anthrone analysis[23] and paper chromatography.

Approximately 20 liters of aqueous ethanol was required to remove mono-, di- and trisaccharides from a column. In a typical case, 90% of the isomaltose (9.7 g.) was brought off in about 5 liters of 5% ethanol, and about 96% of the trisaccharide (5.1 g.) in 4.3 liters of 10% ethanol. The volumes of effluent which intervened between mono- and disaccharide fractions and showed a negative test for carbohydrate, was 2 to 2.5 liters. The corresponding volume between di- and trisaccharide fractions was 1 to 1.5 liters.

When rechromatographed on carbon–Celite, the main cuts of isomaltose, representing about 90% of the total yield of this sugar from the original fractionation, appeared to contain no other sugars. The trisaccharide fraction contained traces of isomaltose, which were removed by rechromatographing.

Considerable amounts of minerals were dissolved from the Celite component of the columns during fractionation. Analysis of concentrates showed that for 1 g. of isomaltose there was as high as 4.6 mg. ionic material (as sodium chloride), of which 0.5 mg. was silica. Silica and other minerals were removed from the di- and trisaccharides by a special adaptation of the ion-exchange procedure[28] to be described elsewhere.[29]

Isomaltose.—After deionization, the main isomaltose cuts from the carbon–Celite columns were obtained as a white, amorphous, sweet powder by concentration *in vacuo* and dehydration with ethanol. Drying to constant weight at 56° *in vacuo* over phosphorus pentoxide resulted in an alcoholate which had approximately the composition $C_{12}H_{22}O_{11} \cdot {}^1/_2C_2H_6O$.

Anal. Calcd. for C, 42.7; H, 6.90; C_2H_6O, 6.2. Found: C, 42.7, 42.9; H, 6.84, 6.92; C_2H_6O, 6.8.

On the anhydrous basis, found: $[\alpha]^{25}D$ +122.0° (*c* 2, water), after slight downward mutarotation.

Acetylation by sodium acetate catalysis gave octaacetyl β-isomaltose, which crystallized as brilliantly clear, elongated prisms from ethanol in about 80% yield.

Other data for both the sugar and its acetate are shown in Table II.

Isomaltotriose.—After being rechromatographed on carbon–Celite and deionizing, isomaltotriose was obtained as an amorphous powder by concentration *in vacuo* and dehydration with ethanol. Drying *in vacuo* at 60° over phosphorus pentoxide gave an anhydrous product which showed $[\alpha]^{25}D$ +145.0° (*c* 2, water) after very slight downward mutarotation.

Isomaltotriose was hydrolyzed in 0.25 *N* sulfuric acid at 100°. Aliquots removed at 0.5, 2 and 12 hours were neutralized with barium hydroxide solution, centrifuged, and the supernatant concentrated for paper chromatography. After 30 hours hydrolysis, an aliquot gave a reducing power equivalent to 95.6% conversion to glucose, and $[\alpha]^{25}D$ +50°.

Acetylation of isomaltotriose by sodium acetate catalysis gave an amorphous product. Found: 49.8% acetyl; theory for $C_{18}H_{21}O_{16}(COCH_3)_{11}$, 48.9%. This high acetyl content suggests that some of the trisaccharide was in the aldehydo form when acetylated.

PEORIA, ILLINOIS

(22) R. J. Dimler, W. C. Schaefer, C. S. Wise and C. E. Rist, *Anal. Chem.*, **24**, 1411 (1952).

(23) L. Hough, *Nature*, **165**, 400 (1950).

(24) R. E. Reeves, THIS JOURNAL, **63**, 1476 (1941).

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(26) Mention of trade names should not be construed as a recommendation or endorsement by the Department of Agriculture over those not mentioned.

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