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

Acetobrominolysis of Di- and Polysaccharide Acetates

BY ALLENE JEANES, C. A. WILHAM AND G. E. HILBERT

RECEIVED FEBRUARY 9, 1953

A rapid chemical method was sought for detecting the non-1,4-glucopyranosidic linkages believed to constitute branch points in amylopectins, limit dextrins and related substances. A polarimetric method, based on the action of hydrogen bromide–acetic acid–acetyl bromide (acetobrominolysis) on the fully acetylated polysaccharides, has been shown to differentiate linkages, not only in amylaceous substances, but also in dextrans. In establishing this method, various types of glucopyranosidic linkages present in disaccharides or in preponderant amount in certain polysaccharides, were found to differ in manner, or extent, or rate of reaction with this reagent. However, absolute differentiation of linkages in all combinations could not be made. Acetates of maltose or of polysaccharides having only α-1,4-glucopyranosidic linkages produced almost quantitatively the normal, high-rotating product, 2,3,4,6-tetraacetyl-α-D-glucosyl bromide (acetobromoglucose). Acetates of 1,6-linked di- and polysaccharides apparently produced no acetobromoglucose, but anomalous substances of low rotation and undetermined structure. Triacetates of polysaccharides believed to have both α-1,4- and α-1,6-linkages (such as those of branched amylaceous substances and certain bacterial dextrans) gave end rotations consistent with other indications of the nature and proportion of these linkages. The α-1,3-linked units in a polyglucosan acetate reacted essentially like the α-1,4- to produce acetobromoglucose. Acetates containing the other types of glucopyranosidic linkages tested, namely, α,α-1,1-, β,β-1,1-, β-1,3- and β-1,4-, gave either practically no reaction or varying degrees of both the normal and the anomalous reactions.

For use in structural investigations on amylopectins and their products of acid or enzymic degradation, a chemical means was sought for differentiating the glucopyranosidic linkages presumably at the branch points from the predominant α-1,4-type. Previous reports on the action of acetolysis reagents containing hydrogen bromide on amylaceous polysaccharides[2-4] and certain sugars[5,6] indicated to us the possibility of adapting this type of reaction to our purpose.

Pursuant of this objective, investigation has been made of the action of improved acetolysis reagents containing hydrogen bromide on carbohydrate acetates having various types of glycosidic linkages. Our study was based primarily on measurement of 2,3,4,6-tetraacetyl-α-D-glucosyl bromide (acetobromoglucose), which is the stable end-product of the acetobrominolysis of acetylated carbohydrates having α-1,4-glucopyranosidic linkages. Different products may result when the linkages involve other positions. Fully acetylated carbohydrates have been treated in acetyl bromide solution at 25° with a ten- or twenty-fold excess of the amount of hydrogen bromide dissolved in acetic acid and acetyl bromide required by theory to produce acetobromoglucose.

Rotational Observations

Polysaccharide Acetates.—When treated with equal parts by volume of hydrogen bromide-in-acetic acid and acetyl bromide (Reagent I), the acetates of starch, starch fractions, and of some related carbohydrates varied in the extent of their conversion to acetobromoglucose, as is shown by the end values[7] of their optical rotations (Table I). Acetobromoglucose, which remained unchanged by this reagent, was produced in theoretical amount from pentaacetyl-α-D-glucose and also from triacetyl levoglucosan (triacetyl-D-glucosan<1,5>β<1,6>). Octaacetyl-β-maltose was converted almost quantitatively to acetobromoglucose. This was indicated by the close agreement between the end rotation of its reaction mixture and that of acetobromoglucose in the same reagent. The rotations of the test solutions of the triacetates of the amylaceous polysaccharides did not rise to the theoretical end value; in each case the end rotation was lower than that for octaacetyl maltose.

TABLE I

END VALUES OF OPTICAL ROTATIONS OF REACTION SOLUTIONS OF ACETATES OF AMYLACEOUS POLYSACCHARIDES, AND RELATED SUBSTANCES[a]

Substance	Optical rotation at 24 hours reaction time[b]
Acetobromoglucose	+17.4
Pentaacetyl-α-D-glucose	17.4
Triacetyl levoglucosan	17.4
Octaacetyl-β-maltose	17.2
Schardinger β-dextrin triacetate	17.1
Corn amylose triacetate[c]	17.1
Potato amylose triacetate	17.1
Potato starch triacetate	16.7
Wheat starch triacetate	16.6
Corn starch triacetate	16.6
Sweet potato starch triacetate	16.6
Potato amylopectin triacetate	16.6
Waxy corn starch triacetate	16.5
Corn amylopectin triacetate	16.5
Glycogen triacetate	16.2
β-Amylase limit dextrin triacetate	16.1

[a] The weight of each substance used was that required by theory to produce 7.576×10^{-4} mole of acetobromoglucose. This was dissolved in 10 ml. of equal parts by volume of hydrogen bromide-in-acetic acid and acetyl bromide (Reagent I). [b] These are saccharimeter readings, in °S, read in a 1-dm. tube, at 25°, using sodium light, and are accurate to ±0.05°. [c] The rotation of all the amylaceous polysaccharide acetates increased steadily from initial observed rotations of about +11.0° to between +15.0° and +16.0° during the first six hours of reaction. Rotations at 24 and 30 hours were identical.

The rotational behavior of acetobrominolysis reaction mixtures of nine previously described

---

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

[2] P. Karrer, C. Nägeli, O. Hurwitz and A. Wälti, *Helv. Chim. Acta*, **4**, 678 (1921).

[3] K. Freudenberg and K. Soff, *Ber.*, **69**, 1252 (1936).

[4] K. Myrbäck, *Svensk. Kem. Tidskr.*, **49**, 271 (1937).

[5] G. Zemplén, *Ber.*, **57B**, 698 (1924).

[6] F. Micheel, *Ann.*, **456**, 69 (1927).

[7] The end values of the rotations are those observed when the rotations became constant.

dextran triacetates[8] contrasted sharply with that of the amylaceous acetates in following courses of decreasing values, in the low rotations reached, and in the much wider spread of values obtained (Fig. 1).

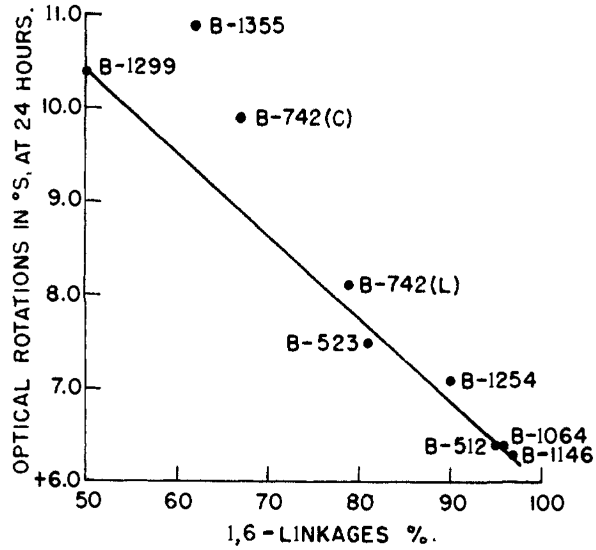

Fig. 1.—Relation between optical rotation of reaction mixtures and 1,6-linkage content of dextran triacetates. Reaction conditions are stated in Table I. For the triacetate of the dextran from NRRL strain B-512, the rotation of the reaction mixture decreased from the estimated initial value of +13.5 to +10.0, +8.1 and +6.4° after 2, 6, 18 and 24 hours, respectively, and then continued to decrease slowly. Triacetates of the other dextrans behaved similarly except those from fraction C of B-742 dextran and from B-1355 dextran, which showed little or no change after 8 hours.

The acetate of the polyglucosan (mycodextran) from *Aspergillus niger* strain 152[9] showed an increase in reagent I from the calculated initial rotation[10] of +9.9 to +16.7° in 24 hours, and continued to increase slowly.

Cellulose acetate, having a calculated initial rotation of −1.4°, reacted similarly to starch triacetates but reached the lower end-rotation, +12.2°. Laminarin triacetate, reacting much more slowly than cellulose triacetate, showed an increase from the calculated initial value of −3,8 to +1.4° in 24 hours and continued to increase slowly.

Disaccharide Acetates.—In experiments designed to follow clearly the course of acetobrominolysis of some 1,4- and 1,6-glycopyranosidically linked disaccharide acetates, the ratio by volume of hydrogen bromide-in-acetic acid to acetyl bromide in the reagent employed was decreased to 1:9 (Reagent II). The reaction curves are shown in Fig. 2. Under these conditions octaacetyl maltose appeared to be converted rapidly to heptaacetyl-α-maltosyl bromide, which was slowly and incompletely converted to acetobromoglucose. The observed rotations for the reaction mixture of octaacetyl gentiobiose revealed a rapid reaction that continued unabated past the rotation calculated for conversion to heptaacetyl-α-gentiobiosyl bromide (+7.7°) to a maximum of about +12.6°. The rotation then decreased. The curve for the reaction of the reagent with crystalline heptaacetyl-α-gentiobiosyl bromide was nearly superimposable on the curve for octaacetyl gentiobiose. The observed rotations indicate that octaacetates of isomaltose and melibiose were converted very rapidly to the corresponding α-forms of the acetobromo derivatives which underwent still another rapid reaction resulting in a pronounced decrease in optical rotation.

In order to obtain results strictly comparable with those from the polysaccharide acetates, reagent I was applied to a number of mono- and disaccharide acetates and to some of the corresponding acetobromo derivatives (Table II). The acetobromomonosaccharides employed were the products expected from the disaccharide derivatives.

If the 1,6-glycosidically linked octaacetates of isomaltose, gentiobiose and melibiose reacted as did the corresponding 1,4-linked octaacetates of maltose and lactose, the expected products would be two moles of acetobromoglucose from the first two acetates and an equimolar mixture of acetobromoglucose and 2,3,4,6-tetraacetyl-α-D-galactosyl bromide from the third. However, the end rotations differed markedly from those expected (Table II).

The end rotation for the reaction mixture of octaacetylcellobiose became still lower than that shown in Table II when a reagent containing a higher concentration of hydrogen bromide-in-acetic acid was used. Acetobromoglucose has been isolated from the acetolysis products of heptaacetyl-α-cellobiosyl bromide.[6]

The reactions of octaacetylmelibiose and of heptaacetyl-α-melibiosyl bromide with hydrogen bromide-in-acetic acid in chloroform at 0° gave the same rotational changes as reactions carried out at 25° with reagent II, except more slowly. When anhydrous glacial acetic acid was substituted for acetyl bromide in reagent II, the reactions of all acetates at 25° appeared to be like those obtained with reagent II, except that the rates were slower, and color, which developed after about 24 hours, increased rapidly to brown-black. With reagents I and II only a little color developed after two or three days. The use of acetyl bromide alone as a reagent at 25° resulted in a slow increase in rotation until a steady value was reached at +12.7° by octaacetylmaltose, at +11.3° by octaacetylmelibiose, and at about +12.7° by both gentiobiose and heptaacetyl-α-gentiobiosyl bromide.

Reaction Products

Two types of products have been isolated from the acetobrominolysis mixtures of the various carbohydrate acetates. These are crystalline acetobromoglucose or crystalline ethers or esters derived from it, and amorphous sirups or glassy solids from which no crystalline substance could be obtained. Part of the chemically bound bromine in the non-

---

[8] Allene Jeanes and C. A. Wilham, This Journal, **74**, 5339 (1952).

[9] Y. L. Yuill, *Chemistry and Industry*, 755 (1952).

[10] Calculated, as an approximate basis for interpretation, from the specific rotation of the substance in chloroform.

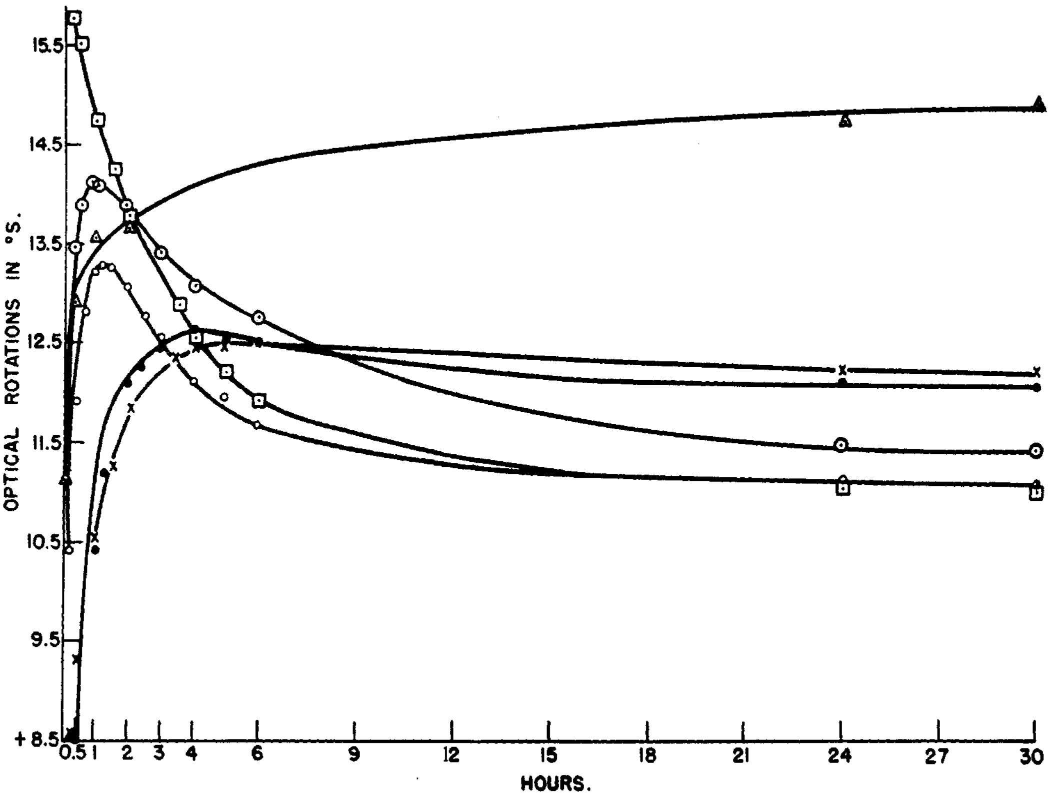

Fig. 2.—Optical rotations of disaccharide derivatives in Reagent II at 25° (see footnote *a*, Table II): ●, octaacetyl-β-gentiobiose; ×, heptaacetyl-α-gentiobiosyl bromide; ⊙, octaacetyl-β-isomaltose; △, octaacetyl-β-maltose; O, octaacetyl-β-melibiose; ⊡, heptaacetyl-α-melibiosyl bromide.

crystalline fractions was replaced as easily as the bromine in acetobromoglucose by methoxyl, hydroxyl, or acetoxyl groups. Under none of the conditions used, however, was all the bromine replaced from these non-crystalline fractions; some stable bromine always remained. After replacement of active bromine in the non-crystalline fractions by treatment with silver salts, the only crystalline reaction products isolated have been traces of glucose derivatives from α-1,4- and β,β,1,1-linked substances.

From Acetates of Glucose and of Saccharides Linked α-1,4 and α-1,3.—Acetobromoglucose in a practically pure state and in almost the theoretica-

TABLE II

REACTION OF SUGAR DERIVATIVES WITH REAGENT I AT 25°[a]

	Rotation, in °S, for 1-dm. tube						
	Initial					Expected end values[b]	
Substance	Calcd.[10]	Obsd.	Time, hr.	Maximum obsd.	Obsd. at 24 hr. reaction time	For acetobromomonosaccharide[c]	For acetobromodisaccharide[10]
2,3,4,6-Tetraacetyl-α-D-glucosyl bromide	+17.8			None	+17.4[d]		
2,3,4,6-Tetraacetyl-α-D-galactosyl bromide	+19.4			None	+18.7[d]		
1,2,3,4,6-Pentaacetyl-α-D-galactose	+ 9.1			None	+18.7[d]	+18.7	
Octaacetyl-β-maltose	+ 4.6	+14.0	1	None	+17.2	+17.4	+13.8
Octaacetyl-α-cellobiose	+ 3.0	+ 8.2	1/2	None	+12.8[e]	+17.4	+ 7.3
Octaacetyl-β-lactose	− 0.3	+ 9.8	1/2	None	+16.9	+18.0	+ 8.3
Octaacetyl-β-isomaltose	+ 7.1	+14.5	1/6	+14.6[f]	+11.8	+17.4	+15.4[g]
Octaacetyl-β-gentiobiose	− 0.4	+12.0	1/4	+12.6[h]	+11.8	+17.4	+ 7.7
Heptaacetyl-α-gentiobiosyl bromide	+ 7.7	+12.0[i]	1/4	+12.6[h]	+11.8	+17.4	
Octaacetyl-β-melibiose	+ 7.6	+14.6	1/4	+14.6[f]	+11.1	+18.0	+16.0
Octaacetyl-α-α-trehalose	+12.0	+11.4	1/3	None	+11.6[j]	+17.4	
Octaacetyl-β-β-trehalose	− 1.4	+ 0.6	1/3	None	+13.5[k]	+17.4	

[a] These measurements were made on 7.576 × 10^{-4} mole of monosaccharide derivatives and on 3.788 × 10^{-4} mole of disaccharide derivatives in 10 ml. of solution. See footnote,[a] Table I. [b] These are the constant rotations which would have been obtained if the disaccharide derivative had been converted to the corresponding 1-bromo-tetraacetyl-monosaccharides or to the corresponding 1-bromoheptaacetyl-disaccharide. [c] Obtained from the rotations observed for tetraacetyl-α-D-glucosyl bromide and for tetraacetyl-α-D-galactosyl bromide. [d] Rotation constant from 1/4 to 30 hours. [e] After 72 hours, rotation was constant at +14.5. [f] At 1/4 hour reaction time. [g] M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **71**, 125 (1949). [h] At one-half hour. [i] In acetyl bromide, in which reaction was slow, the initial rotation of this substance was observed to be +7.9°. [j] At 120 hours, rotation was +12.1. [k] At 96 hours, rotation was +14.7 and still increasing slowly.

yield, comprised the crude reaction product from pentaacetyl-β-D-glucose, as is shown in Table III. From the acetobrominolysis of octaacetyl-β-maltose a small amount of a non-crystalline residue was obtained in addition to acetobromoglucose. From the reaction products of corn starch triacetate, a larger amount of non-crystalline material was obtained. Some acetobromoglucose remained occluded in this amorphous product.

TABLE III

ACETOBROMOGLUCOSE FROM THE ACETOBROMINOLYSIS OF CARBOHYDRATE ACETATES AT 25°

				Recrystallized product		
	Optical rotation at 24 hr. reaction time,	Crude product		Acetobromoglucose		Non-crystalline fraction Yield,
Acetate	°S.	Yield, % of theory	M.p., °C.	Yield, % of theory	M.p., °C.	% of crude product
Pentaacetyl-β-D-glucose	+34.4	97.4	82–85	85.8[a]	88–89	0
Octaacetyl-β-maltose	+34.0	99.0	74–82	73.5[a]	84–87	6.5
Corn starch triacetate	+33.0	97.4	73–80	49.0	84–87	31.0

[a] An additional 9% of less pure acetobromoglucose was recovered from the mother liquors of recrystallization.

Acetobrominolysis of the acetate of a polyglucosan which has about equal proportions of α-1,4- and α-1,3-, and possibly a small amount of α-1,6-glucopyranosidic links,[11] appeared to produce acetobromoglucose as the main product. Replacement of active bromine by acetoxyl gave crystalline pentaacetyl-β-D-glucose and traces of a product containing stable bromine.

From 1,6-Linked Disaccharide Acetates.—From reaction mixtures of the octaacetates of gentiobiose and of melibiose, products were isolated which corresponded to various positions on the curves shown in Fig. 2. Acetobromomonosaccharides were not detected. Products corresponding to the maxima of the reaction curves for gentiobiose and melibiose octaacetates contained 4.7 and 2.5% of stable bromine, respectively. The total bromine content of these products was greater than the calculated bromine content of an acetobromodisaccharide by approximately the amount of the stable bromine. The amount of stable bromine increased with reaction time, even in the part of the curve where rotational changes were very small. This is an indication that introduction of stable bromine into these compounds caused only minor changes in rotation and was not the direct cause for the extreme changes immediately after formation of the acetobromo derivatives of gentiobiose and melibiose.

The material obtained by reaction of hydrogen bromide in acetic acid on heptaacetyl-α-melibiosyl bromide in chloroform at 0° contained stable bromine and had a relatively low specific rotation.

From Dextran Acetate.—The reaction products from the triacetate of NRRL B-512 dextran, which has about 95% α-1,6-glucopyranosidic linkages,[12] were examined intensively in the attempt to establish the nature of the anomalous reaction products from 1,6-glucosidically linked carbohydrates, and to gain information on the course of the reaction. Reaction products, isolated after 4, 18 and 40 hours of reaction, were fractionated. Data are shown in Table IV.

TABLE IV

DATA ON FRACTIONS FROM THE ACETOBROMINOLYSIS OF B-512 DEXTRAN TRIACETATE

			Bromine found, %		
				Remaining after AgOAc treatment	
Reaction time, hr.	Fraction	Yield, approximate % of total product	Total	At 25°	At 80°
4	Hexane-soluble	0.1	18.9		
18	Hexane-soluble	.4	25.0	9.5	
40	Hexane-soluble	.4	27.9	15.1	
4	Ether-soluble	47	11.7		3.2
18	Ether-soluble	50[a]	16.4	9.6[b]	6.4
40	Ether-soluble	98	24.1	16.9[c]	11.8
4	Ether-insoluble	47	5.5		4.2
18	Ether-insoluble	40	13.8	9.7	7.9

[a] By exhaustive extraction, which was undesirable because of the lability of the product to atmospheric moisture, this yield could have been increased to 90% with a corresponding decrease in the ether-insoluble material. [b] For this sample, found: degree of polymerization (D.P.) 2; mol. wt. 700–800. For the hypothetical main constituent of the original ether-soluble fraction, calcd.: $C_{12}H_{14}O_{10}(COCH_3)_8Br_2$; total Br, 19.4%; after replacement of one Br by $-CO_2CH_3$, Br, 9.9% and mol. wt. 801. [c] For this sample, found: D.P. 3; mol. wt. 700–800. For the hypothetical main constituent of the original ether-soluble fraction, calcd.: $C_{12}H_{14}O_{10}(COCH_3)_9Br_3$, total Br, 25.3%; after replacement of one Br by $-CO_2CH_3$, Br, 17.2% and mol. wt. 924.

Acetobromoglucose, which we have shown to be easily obtainable as a crystalline product of the acetobrominolysis of amylaceous triacetates, was not found in identifiable amount among the products from this dextran triacetate. The solubility of acetobromoglucose would permit it to be present in the hexane-soluble fractions (Table IV). But the stability of acetobromoglucose to the reagent would prevent its being the precursor of substances containing stable bromine such as were obtained in the hexane-soluble fractions.

Discussion

The nature of some of the reactions involved in this study baffles complete explanation at present. The literature provides little relevant information. Under conditions of reaction employed by previous investigators, acetobrominolysis of starch[2] and starch acetate[3] was reported to produce a maltose derivative (isolated as heptaacetyl-β-maltose) as the major product. Under similar reaction conditions, no substance of known identity was obtained from a limit dextrin of low molecular weight.[4] The role of anomalously combined bromine in these reactions was not recognized. The susceptibility of acetohalo derivatives of cellobiose,[6] maltose[13] and gentiobiose[14] to further attack by hydrogen bromide has been reported, and data show that certain preparations of heptaacetyl-α-melibiosyl bromide contained stable bromine.[15] In only one

---

(11) S. A. Barker, E. J. Bourne and M. Stacey, *Chemistry and Industry*, 756 (1952).

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

(13) D. H. Brauns, *ibid.*, **51**, 1820 (1929).

(14) D. H. Brauns, *ibid.*, **49**, 3170 (1927).

(15) P. A. Levene and E. Jorpes, *J. Biol. Chem.*, **86**, 403 (1930).

case was a fraction containing stable bromine investigated.[6]

Disaccharide Acetates.—The only α-linked disaccharides studied which gave a normal reaction to produce acetobromomonosaccharides were those linked 1,4-. Here the reaction was predominantly, but not exclusively, normal. Among the other types of linkages, the α,α-1,1- in α,α-trehalose octaacetate was attacked by the reagent only with very great difficulty. The β,β-1,1-linkage in β,β-trehalose octaacetate[16] underwent both the normal and the anomalous reaction. These octaacetyl trehaloses are unique in that they cannot react to form the acetobromodisaccharides, and acetobrominolysis must result, therefore, from the action of the reagent on the octaacetates themselves. The α-1,6-linked octaacetates of isomaltose and melibiose, as well as the β-1,6-linked gentiobiose octaacetate, gave anomalous reactions which resulted in very rapid and pronounced changes in rotation—an increase in rotation when the linkage was β-, and a decrease when it was α-. The magnitude of these changes indicates that either (both) isomerization at the 1,6-linkage or (and) a deep-seated molecular change, such as one involving the pyranose ring, must occur. In part, at least, these changes appear similar to the isomerization and transglycosidation produced by other acid catalysts.[17] The anomalous reaction produced by hydrogen bromide results in introduction of stable bromine into at least some of the molecules. There is no evidence that the derivatives of gentiobiose and melibiose underwent any of the normal reaction to form acetobromomonosaccharides. This might not prove to be true for all 1,6-disaccharides, for acetobromoglucose has been reported to be a product of the action of heptaacetylisoprimeverose (6-α-D-xylopyranosyl-D-glucose) with hydrogen bromide-in-acetic acid.[18]

Amylaceous Polysaccharide Acetates.—Polysaccharide acetates known to have only α-1,4-glucopyranosidic linkages, such as the Schardinger β-dextrin and the linear fraction of starch, approached most closely the behavior of octaacetylmaltose with our acetobrominolysis reagent (Table I). In rotational behavior, the other amylaceous triacetates diverged from octaacetylmaltose in direct proportion to the degree of branching in these molecules. Hydrogen bromide–acetic acid–acetyl bromide thus detects in a roughly quantitative way the amount of branching, or the diversity of linkages, in starch and related polysaccharides.

Comparison of the acetobrominolysis results on the acetates of the 1,6-linked disaccharides and of the branched amylaceous polysaccharides indicates, in agreement with other observations,[19] that 1,6-linkages are common at the points of branching in these polysaccharides. Likewise in agreement with other evidence[20] our data do not exclude the possibility of linkages such as α-1,3, or α,α-1,1, or the α-1,2 on which we have made no observations.

Acetates of small poly- or oligosaccharide products derivable from amylopectin by acid or enzymic degradation and having higher proportions of non-1,4-linkages than the amylopectin, are expected to give acetobrominolysis results amenable to stricter quantitative interpretation than have the high polymer acetates employed in our work reported here. Observations already made[21] on the hendecaacetate of 4-α-isomaltopyranosyl-D-glucose (panose) will facilitate these future applications.

Dextran Triacetates.—The optical rotations of reaction mixtures of dextran triacetates in the hydrogen bromide-containing reagent were dependent upon the point of attachment, proportions, and possibly the sequence of the glucosidic bonds. Triacetates of dextrans for which periodate oxidation indicates no more than about 2% of linkages other than 1,6- and 1,4-like[8,22] (that is, dextrans from the NRRL strains B-1146, B-1064, B-512, B-1254, B-523, B-1299, and fraction L of B-742 dextran), showed close approximation to inverse proportionality between the percentage of 1,6-linkages and the rotation of the reaction mixture (Fig. 1). The triacetates of dextrans having a high proportion of their non-1,6-linkages of the 1,3-like type (that is, B-1355 dextran and fraction C of B-742 dextran) also showed this inverse relationship, but their points fell far from the linear curve of all the other dextran triacetates. If α-1,4- and α-1,3-links were present in these two groups of dextran triacetates, no significant difference in their behavior would seem to be indicated by the results of acetobrominolysis of the polyglucosan (mycodextran) triacetate which is known to contain these links almost exclusively. However, in dextran triacetates, which contain 1,6-links, the sequence or position in the molecule of 1,4-like and 1,3-like linkages would determine whether acetobromoglucose was a product of their reaction.

The data (Fig. 1) show that dextrans in which chains of 1,6-linked units are interrupted least frequently by non-1,6-links appear to undergo the anomalous reaction most extensively. The divergence of values from the end rotation of octaacetylisomaltose (+11.8°) as the proportion of 1,6-linkages in the dextran triacetates increases, is in sharp contrast to the nearly identical values obtained from the acetates of maltose and amylose.

Data on the reaction products from the triacetate of B-512 dextran are shown in Table IV. At the time of practically complete reaction, the major products were no smaller than a disaccharide in molecular size. Such products, which are assumed to be of degradative rather than of synthetic

---

[16] C. M. McCloskey, R. E. Pyle and G. H. Coleman, THIS JOURNAL, **66**, 349 (1944).

[17] B. Lindberg, *Acta Chem. Scand.*, **3**, 1355 (1949).

[18] G. Zemplén and R. Bognár, *Ber.*, **72**, 1160 (1939).

[19] Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, THIS JOURNAL, **71**, 1682 (1949); M. L. Wolfrom, Y. T. Tyree, T. T. Galkowski and A. N. O'Neill, *ibid.*, **73**, 4927 (1951).

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

[21] Data from a forthcoming publication.

[22] The manner of linkage of the anhydroglucopyranose units of these dextrans is calculated from sodium metaperiodate oxidation data.[12] Units linked only through C_1 and/or C_6 are designated 1,6; units linked additionally through C_4 (or C_2), or C_3 (or both C_2 and C_4) are designated 1,4-like and 1,3-like, respectively. These types of units reduce 2, 1 and 0 moles of periodate, respectively, and produce 1, 0 and 0 moles of formic acid, respectively. Steric hindrance may give the same result as a C_3 bond, but seems unlikely here.[20] The calculated precision of our measurements of 1,4-like and 1,3-like linkages is 2%. We have reason to believe that the accuracy of our measurement of these two types of links may be off by as much as 5%.

origin, appear to indicate a lack of equivalence to this reagent of the 1,6-bonds in the dextran triacetate. However, probably not the original glucopyranose units, but structures greatly modified by the anomalous action of the reagent, determined the course of reaction of this dextran triacetate.

Hypothetical formulas for the presumed major products of reaction (Table IV) furnish a provisional basis for interpretation of the data obtained. These formulas agree well with the observed data on total bromine content, bromine content after treatment with silver acetate at 25° to replace active bromine by acetoxyl, and molecular weight after replacement of active bromine by acetoxyl. These hypothetical formulas are in agreement also with the data which show that the total increase in bromine in the ether-soluble fractions during the reaction period between 18 and 40 hours (7.7%) was nearly identical with the increase in the stable bromine content of these fractions, 7.3%. This increase was accompanied by little change in rotation. The obscure nature of these products is related to the presence of stable bromine, which is assumed to be on some carbon atom other than the anomeric.

Our application of the hydrogen bromide-containing reagent to the problem of dextran structure is being continued.

Acknowledgment.—The authors are deeply indebted to Cecil H. Van Etten and Mary B. Wiele for making the bromine and molecular weight determinations.

Experimental

Materials.—We are indebted to certain individuals for several of our materials.[23]

Pentaacetyl-β-D-glucose, octaacetyl-β-gentiobiose and cellulose acetate were commercial preparations of high purity. Other substances were prepared as indicated by references cited, as follows: the triacetates of amylaceous substances,[24] the triacetates of dextran,[8] octaacetyl-β-isomaltose,[25] Schardinger β-dextrin triacetate,[26] heptaacetyl-α-gentiobiosyl bromide[14] and octaacetyl-α,α-trehalose.[16] The triacetates of laminarin and of mycodextran were prepared as described for dextran triacetates. With the exceptions stated below, all other sugar derivatives were made by well-known procedures and so purified that their constants conformed to accepted values.

Heptaacetyl-α-melibiosyl bromide was prepared under milder reaction conditions and in much better yield than was reported by Brauns.[13] To 20 g. of octaacetyl-β-melibiose in 200 ml. of dry, alcohol-free chloroform at 0° was added 50 ml. of ice-cold hydrogen bromide-in-acetic acid. The solution was kept at 0° for 3.5 hours, when the optical rotation was at its maximum and near the value calculated for the theoretical amount of heptaacetyl-α-melibiosyl bromide. The chloroform solution was extracted with ice-water until free of acids, treated with anhydrous sodium sulfate and decolorizing carbon, filtered and concentrated *in vacuo* at 25°. From a solution of the resultant colorless sirup in anhydrous ether, crystallization occurred spontaneously. After one recrystallization from anhydrous ether, 10 g. was obtained; yield 50%, m.p. 112–114°, $[\alpha]^{25}D$ +209.0° (*c* 1, chloroform).

Analytical Methods and Physical Measurements.—A semi-micro Carius method of bromine analysis was used.

Molecular weight determinations on fractions from the action of hydrogen bromide–acetic acid–acetyl bromide on dextran triacetate were made by a modification[27] of Signer's method of isothermal distillation.[28] Molecular weights were obtained by extrapolation of a concentration curve to zero. Benzene and methyl acetate were used as solvents and octapropionyl-β-maltose[29] as standard.

The number of hexose units per reducing group (D.P.) was calculated from reducing determinations[30] made on deacetylated[31] ether-soluble fractions from degraded dextran triacetate (Table IV).

Preparation of Reaction Solutions for Optical Observation.—Air-dried samples of known moisture content[8] were used of the polysaccharide acetates (60 mesh) and of several hygroscopic oligosaccharide acetates.

The amount of substances used for rotational study is stated in Table I and Fig. 1. Samples were weighed into 10-ml. volumetric flasks. When reagent I was used, about 4 ml. of ice-cold acetyl bromide was added to the sample and then the ice-cold hydrogen bromide-in-acetic acid (30–32% HBr). When reagent II was used, the volume of acetyl bromide added initially was about 8 ml. Zero time for the reaction was the time of addition of hydrogen bromide-in-acetic acid. The mixture was shaken until all the acetate dissolved and the temperature of the solution reached 25°. The solution was then made up to volume with acetyl bromide and transferred to a 1-dm. polariscope tube with sealed-in glass end plates and provided with a tightly-fitting glass stopper. The tube and contents were kept at 25° in the dark for the remainder of the reaction time.

Isolation of Reaction Products. (a) From Acetates of Starch, Maltose and Glucose.—The data in Table III were obtained from 250 ml. of reaction mixtures containing reagent I and the weights of the acetates required by theory to produce 0.0379 mole of acetobromoglucose. After 24 hours reaction time the reagents were removed under anhydrous conditions by vacuum distillation with toluene. The temperature of the solution during distillation did not exceed 25°. The sirupy products were dissolved in anhydrous ether and treated with decolorizing carbon. The filtered solution was concentrated *in vacuo* and the residue was extracted with warm hexane to remove acetobromoglucose. The dense, hexane-insoluble fractions appeared to contain acetobromoglucose along with a small amount of low melting, amorphous material. The hexane-insoluble fraction from the acetolysis octaacetylmaltose was shaken in absolute methanol with excess silver carbonate (freshly prepared) for 18 hours at 25°. The product was fractionated into crystalline methyl tetraacetyl-β-D-glucopyranoside and a sirup. The sirup, doubtless retaining some methyl tetraacetyl-β-D-glucopyranoside, contained 4% bromine.

The hexane-insoluble fraction from the acetobrominolysis of 50 g. of starch triacetate was fractionally precipitated from dry ether by hexane until acetobromoglucose was no longer obtained. Portions of the residue (containing 17.6% bromine) were treated with three different reagents to replace the active bromine. The product from treatment with silver carbonate and absolute methanol for 18 hours at 25° was a sirup containing 7.3% bromine and from which no methyl tetraacetyl-β-D-glucopyranoside could be induced to crystallize. The products from treatment with silver acetate in glacial acetic acid, and from silver carbonate in moist acetone were also bromine-containing sirups. Under conditions identical to those employed in the silver acetate treatment, acetobromoglucose was completely and rapidly converted to pentaacetyl-β-D-glucopyranoside.

(b) From Disaccharide Derivatives at 25°.—In some cases reagents were removed from reaction mixtures by extraction of a chloroform solution with ice-water, and then the product was made free of water and chloroform. Such acid-free

---

[23] To Dr. W. A. P. Black of the Scottish Seaweed Research Institute for laminarin, to Dr. George H. Coleman for octaacetyl-β,β-trehalose, to Dr. T. J. Schoch for Schardinger β-dextrin, and to Dr. I. A. Wolff for triacetyllevoglucosan. Dr. V. L. Yuill made available the mold culture from which Dr. R. L. Lohmar prepared mycodextran.

[24] Allene Jeanes and R. W. Jones, THIS JOURNAL, **74**, 6116 (1952).

[25] Allene Jeanes, C. A. Wilham, R. W. Jones and C. E. Rist, Abstracts of Papers, 121st Meeting of the American Chemical Society, 1952, p. 4P.

[26] W. S. McClenahan, Evelyn B. Tilden and C. S. Hudson, THIS JOURNAL, **64**, 2139 (1942).

[27] Unpublished results of Cecil H. Van Etten.

[28] E. P. Clark, *Ind. Eng. Chem., Anal. Ed.*, **13**, 820 (1941).

[29] C. D. Hurd and K. M. Gordon, THIS JOURNAL, **63**, 2657 (1941).

[30] M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

[31] H. S. Isbell, *Bur. Standards J. Research*, **5**, 1179 (1930).

products remained stable for several months if stored *in vacuo* under anhydrous conditions at 4°.

The following is an example of the procedure employed in other cases. After octaacetyl-β,β-trehalose had reacted with reagent I for 4 days at 25°, the reaction mixture was poured into an ice-cold solution of anhydrous glacial acetic acid[32] which contained a 50% excess of silver acetate required to react with all the bromine present. After one hour at 0° and 18 hours of shaking at 25°, the silver salts were removed by filtration and hydrogen sulfide and the acetic acid by distillation *in vacuo* with toluene (bath temperature, less than 50°). Crystalline material appeared spontaneously in the residue. After removal from the sirupy residue by ether and recrystallization from ethanol, it did not depress the melting point of pentaacetyl-β-D-glucopyranoside. When no further fractions of this glucose derivative could be separated, the sirupy residue, which constituted about 25% of the total product, was found to contain 9.4% bromine.

(c) **From the Reaction of Heptaacetyl-α-melibiosyl Bromide at 0°.**—In a total volume of 25 ml., a solution of 2.5 g. of heptaacetyl-α-melibiosyl bromide in dry, alcohol-free chloroform was treated at 0° with 7 ml. of acetyl bromide and 3 ml. of hydrogen bromide-in-acetic acid. After 48 hours of reaction, when the rotation had decreased from its initial value of +60.5 to +48.2°, the solution was made free of reagents as described for the octaacetyl-β,β-trehalose reaction mixture. The product was separated into crystalline and non-crystalline fractions. The former, after recrystallization from ethanol, was found to be octaacetyl-β-melibiose; m.p. 173–175°, $[\alpha]^{25}\mathrm{D}$ +99.71° (*c* 1, chloroform), weight 0.6 g. The sirup was dried *in vacuo* to a frothy solid, weight 1.7 g., $[\alpha]^{25}\mathrm{D}$ +25.1° (*c* 1, chloroform), bromine content, 4.1%.

(d) **From Dextran Triacetate.**—Ten grams of the triacetate of dextran from NRRL B-512 was treated with 115 ml. of each of hydrogen bromide-in-acetic acid and acetyl bromide at 25°. Approximately 4 hours was required for the solid to dissolve. The observed rotations were +16.8°, +13.0°, +12.6°, +12.1° and +12.0° at 4, 18, 24, 40 and 48 hours, respectively. Reagents were removed by distillation *in vacuo* with toluene under anhydrous conditions (solution temperature, 25° or less). The products were fractionated roughly by extraction with anhydrous solvents at room temperature, as shown in Table IV. Active bromine was replaced by acetoxyl groups by treatment with excess silver acetate in anhydrous glacial acetic acid at 25°. The products were obtained in high yield as low-melting glassy solids. Bromine of apparently intermediate activity was replaced when some of these products were treated again with excess silver acetate in glacial acetic acid. This treatment was carried out at 80° for 4 hours. No crystalline substance was obtained from any fraction.

PEORIA, ILLINOIS

---

[32] W. C. Eichelberger and V. K. La Mer, THIS JOURNAL, **55**, 3633 (1933.)

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Derivatives of D-*manno*hexodialdose (6-*aldo*-D-Mannose)

BY CLINTON E. BALLOU[1] AND HERMANN O. L. FISCHER

RECEIVED MARCH 2, 1953

A crystalline substituted hexose dialdehyde with the D-*manno* configuration, 2,3-4,5-diisopropylidene-D-*manno*hexodialdose (II) has been obtained by the lead tetraacetate oxidation of 1,2-5,6-diisopropylidene-D-inositol. Deacetonation of II gave amorphous D-*manno*hexodialdose, characterized as the tetraethyl-1,2-3,4-mercaptal, while reduction of II gave 2,3-4,5-diisopropylidene-D-mannitol. Derivatives of the substituted dialdehyde prepared include the bisphenylhydrazone and the bis-*p*nitrophenylhydrazone.

With respect to the state of oxidation, hexose dialdehydes may be placed between the hexoses and hexuronic acids. That they have biological significance as intermediates in such a transformation has not been shown by experiment. From a chemical standpoint, however, the dialdehydes offer a wide field for study, including that of their behavior in the classical reactions of carbohydrate chemistry.

Although the literature contains several references to straight chain dialdehydes derived by chemical means from carbohydrate substances, the investigation of this class of compounds has not been extended beyond the preparation of a few simple derivatives. Micheel[2] mentions the preparation of 2,3-4,5-tetraacetyl-*galacto*hexodialdose[3,4] by the catalytic reduction of tetraacetylgalactaric acid dichloride. Mrs. Gerda Dangschat in 1942 obtained 2,3,4,5-tetraacetyl-D,L-*iodo*hexodialdose (not crystallized) from tetraacetyl myoinositol.[5] The substance gave a crystalline bisphenylhydrazone, bis-*p*-nitrophenylhydrazone and bis-*p*-dinitrophenylhydrazone. From tetraacetyl alloinositol, Dangschat and Fischer prepared 2,3,4,5-tetraacetyl-*allo*-hexodialdose.[6] The latter gave a crystalline bisphenylhydrazone, bis-*p*-nitrophenylhydrazone and a dioxime. The pentose dialdehyde, 1,2-isopropylidene-D-*xylo*pentodialdose,[7] prepared from 1,2-isopropylidene-D-glucose, has been used as an intermediate in the synthesis of hexoses, hexuronic acids and cyclitols.[8] Fischer and Appel obtained 2,3-isopropylidene-D-*threo*tetrodialdose from 3,4-isopropylidene-D-mannitol,[9] and prepared, among other derivatives of this dialdehyde, the bisphenylhydrazone and tetraethyl acetal.

Through the kindness of Dr. A. B. Anderson, we have obtained quantities of pinitol from which D-inositol is readily prepared. This paper describes a simplified procedure for the acetonation of D- or L-

---

[1] Public Health Service Research Fellow of the National Heart Institute.

[2] F. Micheel, "Chemie der Zucker und Polysaccharide," Akademische Verlagsgesellschaft, Leipzig, 1939, pp. 176–177.

[3] Uenzelmann, Dissertation, Gottingen (1931).

[4] Since a general system for the nomenclature of the sugar dialdehydes has not been devised, these substances have in the past been named as (a) polyhydroxy derivatives of aliphatic dialdehydes, (b) derivatives of saccharic acid dialdehydes, (c) 6-aldo-hexoses and 5-aldo-pentoses. In this paper the general name "dialdose" has been adopted, *i.e.*, hexodialdose, and the substances are treated as members of a distinct class rather than as derivatives of the hexoses.

[5] G. Dangschat, *Naturwissenschaften*, **30**, 146 (1942); *C. A.*, **37**, 3408 (1943).

[6] G. Dangschat and H. O. L. Fischer, *Naturwissenschaften*, **27**, 756 (1939).

[7] K. Iwadare, *Bull. Chem. Soc. Japan*, **16**, 40 (1941); *C. A.*, **35**, 4740 (1941).

[8] J. C. Sowden, THIS JOURNAL, **74**, 4377 (1952); J. M. Grosheintz and H. O. L. Fischer, *ibid.*, **70**, 1476, 1479 (1948).

[9] H. O. L. Fischer and H. Appel, *Helv. Chim. Acta*, **17**, 1574 (1934).