3.2% for Easter lily amylose to 20.6% for potato amylose (Table II). Although the unfractionated amyloses are not homogeneous with respect to their molecular size, approximate molecular weights can be obtained from their intrinsic viscosity measurements.

TABLE II

COMPARISON OF THE DEGREE OF POLYMERIZATION OF AMYLOSES FROM VARIOUS PLANT SOURCES OBTAINED FROM OSMOTIC PRESSURE AND INTRINSIC VISCOSITY MEASUREMENTS

Plant source	*DP* from osmotic pressure	*DP* from intrinsic viscosity	Difference, %
Tapioca	1300	1360	4.6
Potato	970	1170	20.6
Wheat	860	930	8.1
Corn	800	740	7.5
Sago	740	680	8.1
Easter lily	620	640	3.2
Apple	560	600	7.1
Acid modified corn	390	340	12.8

Acknowledgment.—The authors are grateful to the Corn Industries Research Foundation for their support of this work and to Dr. T. J. Schoch for his generous contribution of the starch samples.

Summary

The molecular weights of potato and corn amylose subfractions were determined by osmotic pressure measurements and by estimation of their chain-lengths with the periodate oxidation end-group method. Comparison of the molecular weights by the two methods indicates that, like in the parent amylose, the molecules of the potato subfractions constitute single chains. However, the corn subfractions, like the unfractionated material, appear to be slightly branched, or may contain branched components, which cannot be removed by the usual procedures of separation.

The intrinsic viscosities and the molecular weights of the linear amylose subfractions show a relationship which can be expressed by the following formula: $[\eta] = 0.00166\ M$. Using this expression, the approximate molecular weight of an amylose from various plant sources can be determined from its intrinsic viscosity.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Degradation of Glycogen to Isomaltose[1]

BY M. L. WOLFROM, E. N. LASSETTRE AND A. N. O'NEILL[2]

Considerable evidence exists that glycogen is a two-dimensional polymer composed of α-D-glucopyranose units joined through the 1,4- and 1,6-positions in the frequency ratio of 12:1, respectively. This is based upon the establishment of D-glucose[3] and maltose[4] as acid hydrolytic products of glycogen; upon the isolation from the hydrolyzate of trimethylglycogen of the 2,3-, 2,3,6- and 2,3,4,6-methyl ethers of D-glucose[5]; and upon periodate assay.[6] It was desirable to place this structure on a more definitive basis through the isolation of the disaccharide containing the 1,6-α-D-glucopyranosyl linkage from an acid hydrolyzate of glycogen. This disaccharide, 6-α-D-glucopyranosyl-D-glucose or isomaltose, has been characterized as its crystalline β-D-octaacetate obtained from an acid-hydrolyzed dextran (from *Leuconostoc dextranicum*)[7] and from the hydrolysis of amylopectin with "Takadiastase"[8] (but not with malt diastase[7,9]) and with acid.[10]

Since preliminary experiments failed to yield isomaltose (as its β-octaacetate) from glycogen acetolyzates, it was deemed advisable to calculate the degree of hydrolysis required to give the maximum yield of isomaltose from glycogen. Some evidence existed that the maltose glycosidic linkage was more readily acid-hydrolyzable than that of isomaltose.[11] This was directly determined in our Laboratory and the data of Table I demonstrate that maltose hydrolyzes four times as fast as isomaltose in 2% concentration in 0.050 *N* sulfuric acid at 99.5°.

Information on the dependence of the nature of the depolymerization product upon the degree of hydrolysis of a polymer is obtainable from a statistical treatment of the depolymerization reaction and leads to equations representing the distribution of all possible chain lengths at different degrees of hydrolysis. From the results of a kinetic investigation of the hydrolytic degradation of starch, Meyer, Hopff and Mark[12] reported that such a degradation could be followed with a fair degree of accuracy by assuming that all hydrolyzable bonds in the large molecules were broken at approximately the same rate. Hence the total process could be calculated by a comparatively simple equation.

(1) A preliminary report of the experimental portion of this work appeared in THIS JOURNAL, **71**, 3857 (1949).

(2) Supported in part by a fellowship grant from the Corn Industries Research Foundation, New York, N. Y.

(3) W. N. Haworth, E. L. Hirst and J. I. Webb. *J. Chem. Soc.*, 2479 (1929).

(4) P. Karrer, C. Nägeli (and H. Hoffmann), *Helv. Chim. Acta*, **4**, 267 (1921); W. N. Haworth and E. G. V. Percival, *J. Chem. Soc.*, 1342 (1931).

(5) W. N. Haworth and E. G. V. Percival, *ibid.*, 2277 (1932); D. J. Bell, *Biochem. J.*, **29**, 2031 (1935); W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 577 (1937); W. N. Haworth, E. L. Hirst and F. Smith, *ibid.*, 1914 (1939).

(6) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *ibid.*, 1399 (1947).

(7) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **69**, 473 (1947); **71**, 125 (1949).

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

(9) M. L. Wolfrom, L. W. Georges, A. Thompson and I. L. Miller, *ibid.*, **71**, 2873 (1949).

(10) M. L. Wolfrom, J. T. Tyree, T. T. Galkowski and A. N. O'Neill, *ibid.*, **72**, 1427 (1950).

(11) Marjorie A. Swanson and C. F. Cori, *J. Biol. Chem.*, **172**, 797 (1948); K. Myrbäck, B. Örtenblad and K. Ahlborg, *Biochem. Z.*, **307**, 53 (1940); K. Ahlborg and K. Myrbäck, *ibid.*, **308**, 187 (1941).

(12) K. H. Meyer, H. Hopff and H. Mark, *Ber.*, **62**, 1103 (1929).

Freudenberg, Kuhn and co-workers[13] extended this investigation and presented a statistical treatment for the random degradation of polymers with infinite chain length. Assuming the original polymeric material to be homogeneous and that all linkages except those at the ends of the chain are broken with the same probability, Kuhn has developed expressions which describe quite accurately the distribution of the various chain lengths at different degrees of hydrolysis, as well as the time variation of the degree of depolymerization. On the basis of Kuhn's theory, Mark and Simha[14] have developed an expression which does not assume the existence of infinite chains. The acid hydrolysis of cellulose acetate yielded products whose distribution curves were in qualitative agreement with those derived from their statistical treatment. Later, Montroll and Simha[15] presented a more complete statistical analysis of the theory of depolymerization of long chain molecules with finite length. Assuming that all bonds have the same probability of being broken, expressions were derived giving the distribution of molecular sizes in the degraded system as a function of the initial chain length and the average number of bonds split per molecule. In a later publication[16] the case was considered in which those linkages at the ends of the chains were hydrolyzed at a rate different from that of the interior linkages.

TABLE I

RELATIVE RATES OF HYDROLYSIS OF MALTOSE AND ISOMALTOSE

Init. sugar concn., 2%; 0.05 N H_2SO_4; 99.5°

Maltose[a]			Isomaltose[a]		
Time, hr.	α, 2 dm.	k[b] (hr.$^{-1}$)	Time, hr.	α, 2 dm.	k[b] (hr.$^{-1}$)
0	4.92°		0	4.54°	
1	4.43	0.19	1	4.42	0.052
2	4.03	.19	3	4.22	.049
3	3.75	.18	5	4.07	.045
5	3.24	.18	7	3.85	.050
7	2.85	.19	10	3.65	.048
10	2.50	.20	14	3.37	.049
13	2.32	.20	21	3.09	.046
16.5	2.20	.21	27	2.83	.048
∞	2.12		35	2.64	.047
			∞	2.19	
	Av.	.195		Av.	.048

$k_{maltose}/k_{isomaltose} = 4.06$

[a] Prepared from its recrystallized, chromatographically pure octaacetate. [b] $k = 1/t\ 2.303 \log (\alpha_0 - \alpha_\infty)/(\alpha_t - \alpha_\infty)$.

The problem of the distribution of the products of polymer degradation at various degrees of hydrolysis was also considered by Sillén,[17] who from a slightly different statistical analysis has derived expressions which correspond with those of the previous investigators. In a more recent study of the acid hydrolysis of starch involving the application of Kuhn's theory, and making use of the Sillén equations, Myrbäck and Magnusson[18] noted that if the effect of the α-1,6 linkages be disregarded, such an influence being observed only in the later stages of hydrolysis, then the depolymerization could best be interpreted by the assumption that all glycosidic linkages are hydrolyzed with the same first order specific reaction constant k except those at the ends of the chain, and that these would be hydrolyzed with a velocity constant $k(1 + p)$ characteristic of maltose, in which p is positive. The over-all specific reaction constant for the hydrolysis was found to increase steadily in the early part of the reaction but to decrease toward the end of the reaction. Carlqvist[19] has studied the hydrolysis of glycogen in 0.05 N hydrochloric acid at a temperature of 100°. It was noted that, in contrast to starch, the distribution of products as determined by a copper reduction method was not in agreement with that predicted on the basis of Sillén's equations. The first order specific reaction constant was found to increase steadily from an extrapolated value of 0.120 hr.$^{-1}$ at zero time, to pass through a maximum of 0.241 at 84% hydrolysis, and then to decrease to 0.151 at 98% hydrolysis. Swanson and Cori,[11] however, followed the hydrolysis of maltose, amylose, amylopectin and glycogen by means of a reagent of the Somogyi–Shaffer high alkalinity type[20] whose oxidizing power was inversely proportional to the length of chain. They found that k was essentially constant throughout the hydrolysis and that maltose, amylose and amylopectin were all hydrolyzed at the same rate, while glycogen was hydrolyzed at a measurably slower rate, a fact which was attributed to the relatively large proportion of α-1,6 linkages which were hydrolyzed at a much slower rate than those of the α-1,4 type. Theoretically, both linkages exercise their effect immediately and simultaneously. In the case of glycogen, the initial effect of the more slowly hydrolyzable α-1,6 unions may become apparent initially due to their higher frequency ratio. Nevertheless, the first order reaction constant should decrease in the final stages of the reaction as the effect exercised by the α-1,6 linkages becomes greater.

In the present investigation a statistical analysis of the hydrolytic degradation of branched chain molecules was made whereby the yields of those oligosaccharides which contain one α-1,6 glycosidic linkage could be calculated for any desired degree of hydrolysis. This treatment is presented for the case of the disaccharide isomaltose from glycogen. The results of these calculations indicate that the maximum yield of isomaltose obtainable from the hydrolysis of glycogen is 6.8% and that this maximum will occur when 89% of the glycogen is hydrolyzed.

It is assumed: (1) that the original chains are sufficiently long that the effect of terminal units may be neglected; (2) that all α-1,4 and all α-1,6 linkages are hydrolyzed at different but uniform rates, regardless of their position in the chain and the length of chain in which they are found; and (3) that the two types of linkage are hydrolyzed independently.

(13) K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz and G. Steinbrunn, *Ber.*, **63**, 1510 (1930); W. Kuhn, *ibid.*, **63**, 1503 (1930); K. Freudenberg and W. Kuhn, *ibid.*, **65**, 484 (1932); K. Freudenberg and G. Blomqvist, *ibid.*, **68**, 2070 (1935).

(14) H. Mark and R. Simha, *Trans. Faraday Soc.*, **36**, 611 (1940).

(15) E. W. Montroll and R. Simha, *J. Chem. Phys.*, **8**, 721 (1940).

(16) R. Simha, *J. Applied Phys.*, **12**, 569 (1941).

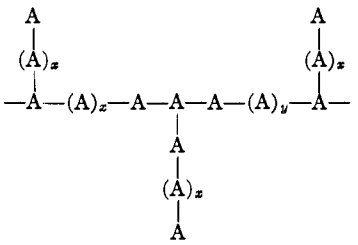
(17) L. G. Sillén, *Svensk Kem. Tid.*, **55**, 221, 266 (1943).

(18) K. Myrbäck and B. Magnusson, *Arkiv Kemi, Mineral. Geol.*, **20A**, No. 14 (1945).

(19) B. Carlqvist, *Acta Chem. Scand.*, **2**, 759 (1948).

(20) P. A. Shaffer and M. Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

Consider a section of a branched chain polymer such as that shown below.



At each branch a D-glucose unit A is attached through three linkages to adjacent units. Of these, two are the same as the linkages in a chain but the third is different. Those of the first type, the α-1,4 linkages, will be referred to as chain linkages and the second, the α-1,6, as branch linkages. Let N_t be the number of branch linkages remaining at time t and N_t' the number of chain linkages remaining at time t. Let the rate constants for the hydrolysis of branch and chain linkages be k and $4k$, respectively. Then

$$N_t = N_0 e^{-kt} \quad (1)$$

$$N_t' = N_0' e^{-4kt} \quad (2)$$

N_0 and N_0' represent the numbers of branch and chain linkages, respectively, at $t = 0$. The fraction of linkages unhydrolyzed at time t is

$$\frac{N_t}{N_0} = e^{-kt} = p_1 \quad (3)$$

$$\frac{N'_t}{N_0'} = e^{-4kt} = p_2 \quad (4)$$

These fractions, p_1 and p_2, are also the probabilities that linkages of the two types are unhydrolyzed. The probabilities that they are hydrolyzed would be $(1 - p_1)$ and $(1 - p_2)$, respectively. In order that an oligosaccharide with n D-glucose units containing one α-1,6-glycosidic linkage and $(n - 2)$ α-1,4 linkages be produced, one linkage of the branched type and $(n - 2)$ linkages of the chain type must be unhydrolyzed while three of the latter must be hydrolyzed. The total probability of obtaining such an oligosaccharide then is

$$p_1 p_2^{n-2} (1 - p_2)^3 \quad (5)$$

Since $p_2 = p_1^4$ this would become

$$p_1^{4n-7} (1 - p_1^4)^3 \quad (6)$$

In the case of isomaltose, a disaccharide derived from starch or glycogen, this probability reduces to

$$p_1 (1 - p_1^4)^3 \quad (7)$$

There are in all N_0 linkages of the branched type and of these a fraction, $p_1(1 - p_1^4)^3$, has three adjacent chain linkages hydrolyzed. Hence the number of disaccharide molecules produced at time t is

$$N = N_0 p_1 (1 - p_1^4)^3 \quad (8)$$

N becomes a maximum when

$$\frac{dN}{dp_1} = N_0 (1 - p_1^4)^3 - 12 N_0 p_1^4 (1 - p_1^4)^2 = 0 \quad (9)$$

Solution of this equation gives

$$p_1^4 = p_2 = 0.077$$
$$p_1 = 0.525$$

These values are therefore the fractions of each type of linkage remaining unhydrolyzed at which the yield of isomaltose is a maximum. It follows from (3) and (4) that at the time t_{max} at which the yield of isomaltose is a maximum

$$N_{t\ max} = N_0 (0.525) \quad (10)$$

$$N_t'_{\ max} = N_0' (0.077) \quad (11)$$

The total fraction unhydrolyzed is therefore

$$\frac{N_{t\ max} + N_t'_{\ max}}{N_0 + N_0'} = \frac{N_0 (0.525) + N_0' (0.077)}{N_0 + N_0'} = \frac{(N_0/N_0')(0.525) + (0.077)}{1 + (N_0/N_0')} \quad (12)$$

For the glycogen molecule

$$N_0/N_0' = 1/12$$

Thus for the glycogen molecule, the fraction unhydrolyzed at t_{max} is

$$\frac{0.525/12 + 0.077}{1 + 1/12} = 0.111$$

The maximum yield of isomaltose, therefore, occurs when 100 − 11.1 or 88.9% of the glycogen is hydrolyzed.

If there are N_0 branch linkages, the maximum number of isomaltose molecules theoretically possible is N_0 and the fraction actually obtained at maximum yield is

$$N/N_0 = p_1(1 - p_1^4)^3 = 0.525\ (0.923)^3 = 0.42$$

The actual yield of isomaltose will therefore be 42% of the theoretically possible maximum.

The yields of isomaltose at various degrees of hydrolysis now remain to be determined. Consider a glycogen hydrolysis carried to 75% (degree of hydrolysis). That is

$$(N_t + N_t')/(N_0 + N_0') = 0.25$$

It follows from (3) and (4) that

$$(p_1 N_0 + p_2 N_0')/(N_0 + N_0') = 0.25$$

or

$$(p_1 N_0/N_0' + p_1^4)/(1 + N_0/N_0') = 0.25$$

Since $N_0/N_0' = {}^1/_{12}$, this equation becomes

$$\frac{p_1(1/12) + p_1^4}{1 + 1/12} = 0.25$$

Therefore

$$12 p_1^4 + p_1 - 3.25 = 0$$

and on solution of this equation

$$p_1 = 0.681$$
$$p_1^4 = p_2 = 0.214$$

The yield then at 75% hydrolysis is

$$N/N_0 = p_1(1 - p_1^4)^3 = 0.331$$

Therefore at 75% hydrolysis, 33.1% of the maximum amount of isomaltose theoretically possible would be obtained. Similar calculations for degrees of hydrolysis of 50% and 25% lead to the values 12.1% and 1.74%, respectively. Since $N_0/N_0' = {}^1/_{12}$, the theoretical yield of isomaltose based on the original glycogen is 16.2%. Thus at 75% hydrolysis, the maximum amount of isomaltose obtainable would be $0.331 \times 16.2 = 5.36\%$ of the original glycogen. A curve based on such calculations is depicted in Fig. 1.

If the above calculations are made for a glycogen hydrolysis carried out at 30°, at which temperature the ratio of the hydrolysis constants of α-1,4- and α-1,6-glucosidic linkages is equal to 7, according to

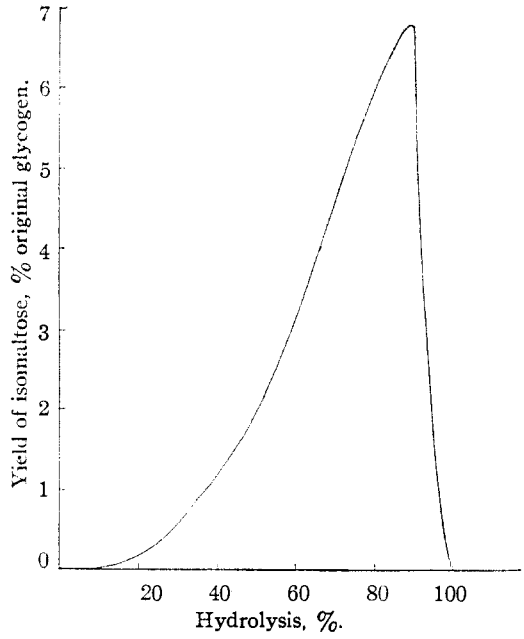


Fig. 1.—Calculated yield of isomaltose as a function of the degree of hydrolysis of glycogen.

the work of Swanson and Cori,[11] it is found that such conditions should be more favorable for the formation of isomaltose.

Experimentally, rabbit liver glycogen in 2% concentration was hydrolyzed with 0.05 *N* hydrochloric acid at a temperature of 100°. The reaction was followed up to 60% hydrolysis by measuring the reducing power by the method of Swanson and Cori.[11] The results are shown in Table II and demonstrate that the first order specific reaction constants are essentially constant during the portion (60%) of the reaction followed, in agreement with the work of Swanson and Cori.

TABLE II

HYDROLYSIS OF RABBIT LIVER GLYCOGEN

Init. glycogen concn., 2%; 0.05 *N* HCl; 100°

Time, hr.	D-Glucose, % (α)	Hydrolysis, % (α/c 100)	k[a] (hr.$^{-1}$)
0.5	0.110	4.97	0.102
1.5	.335	15.1	.110
2.5	.538	24.2	.111
3.5	.765	34.4	.120
4.5	.935	42.1	.121
5.5	1.10	49.5	.124
6.5	1.22	55.0	.123
7.5	1.34	60.3	.123
∞	2.22[b]	100	

[a] $k = 1/t\ 2.303 \log c/(c - \alpha)$ in which c is the % D-glucose obtained on complete hydrolysis and α is the reducing power at time t expressed as % D-glucose. [b] Calcd. value.

The crude solids obtained from such an hydrolysis, carried to approximately two-thirds of completeness and with sulfuric acid instead of hydrochloric acid, were acetylated with hot acetic anhydride and sodium acetate and the mixture of β-D-acetates was subjected to chromatographic resolution by methods developed in this Laboratory.[7,9,21] The main products were β-D-glucopyranose pentaacetate and β-maltose octaacetate. From the more highly adsorbed material two additional crystalline compounds were isolated and were adequately identified as β-isomaltose octaacetate and β-maltotriose hendecaacetate. The latter compound has been previously isolated from an enzymic hydrolysis of amylopectin[9] and identified as the hendecaacetate of a trisaccharide with two α-D-1,4 linkages.[22]

An appropriate blank experiment on amylose yielded β-D-glucopyranose pentaacetate, β-maltose octaacetate and β-maltotriose hendecaacetate. No β-isomaltose octaacetate was found and since the chromatographic technique employed would have revealed this compound if it had been present, its absence is cited as evidence that the compound isolated from a glycogen hydrolyzate was not formed by a reverse synthesis.

Experimental

Acid Hydrolysis of Glycogen.—A suspension of 25 g. of rabbit liver glycogen, $[\alpha]^{25}D$ +200° (*c* 0.9, water), in 625 ml. of 0.05 *N* sulfuric acid was added with continuous stirring to an equal volume of boiling acid solution of the same concentration. The mixture was refluxed for 8 hours, a time calculated from the rate constant to give a degree of hydrolysis of approximately 66%. The clear solution was neutralized with barium carbonate and filtered. Inorganic ions were removed by passage of the solution successively through Amberlite exchange resins IR-100 and IR-4,[23] and the solution was concentrated under reduced pressure to a thick sirup. Any water remaining was removed as the ethanol-water azeotrope by distillation under reduced pressure. The material was finally frothed and dried in a vacuum desiccator to an amorphous white powder; yield 24.3 g.

Acetylation of the Hydrolytic Products.—The above hydrolyzate (24.3 g.) was acetylated with 15 g. of fused sodium acetate and 150 ml. of acetic anhydride by initiating the reaction at a bath temperature of 120° and allowing it to proceed for 1 hour at 100°, after which time it was poured into 1 liter of ice and water. After the acetic anhydride had been hydrolyzed, the sirupy acetate mixture was extracted with five 200-ml. portions of chloroform and the extract was washed with water until neutral, dried over anhydrous calcium sulfate and finally concentrated under reduced pressure to a thick sirup which was frothed and dried to constant weight in a vacuum desiccator; yield 45.2 g.

Chromatographic Resolution of the Acetate Mixture.—An amount of 6.0 g. of the above acetylated hydrolyzate was dissolved in 180 ml. of benzene and chromatographed on a 265 mm. × 74 mm. (diam.)[24] column of Magnesol[25]–Celite[26] (5:1 by wt.) by development with 3000 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). The material in the effluent was recovered by solvent removal under reduced pressure and was recrystallized from absolute ethanol. It was identified as β-D-glucopyranose pentaacetate; yield 1.8 g., m.p. 134–135° unchanged on admixture with an authentic specimen; $[\alpha]^{25}D$ +3.9° (*c* 2.5, chloroform).

An alkaline permanganate streak (1% solution of potassium permanganate in 2.5 *N* sodium hydroxide) on the extruded column indicated two zones, one located on the lower half of the column and the other at the top. The column was sectioned and the individual zones were eluted with acetone. Solvent was removed from the eluents by distillation under reduced pressure. The sirup obtained from the lower zone crystallized from ethanol and was recrystallized from the same solvent. This product was identified as β-maltose octaacetate; yield 1.2 g., m.p. 159–160° unchanged on admixture with an authentic sample; $[\alpha]^{25}D$ +62.3° (*c* 1.5, chloroform).

The material from the zone at the column top was dissolved in 85 ml. of benzene and rechromatographed on a

(21) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(22) J. M. Sugihara and M. L. Wolfrom, *ibid.*, **71**, 3357 (1949).

(23) Products of the Resinous Products and Chemical Co., Philadelphia, Pennsylvania.

(24) Adsorbent dimensions.

(25) A product of Westvaco Chlorine Products Corp., South Charleston, West Virginia.

(26) No. 535, a product of Johns-Manville Corp., New York, N. Y.

240 × 54 mm. (diam.) column of Magnesol–Celite (5:1 by wt.) by development with 2750 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). Three zones were located on the extruded column. The material from the bottom zone on rechromatography in the same manner yielded a small amount (15 mg.) of β-maltose octaacetate (identified by melting point, 158–159°).

The sirup obtained from the zone located near the middle of the column was dissolved in 50 ml. of benzene and rechromatographed on a 215 mm. × 44 mm. (diam.) column of Magnesol–Celite (5:1 by wt.) by development with 1500 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). Four zones were located on the extruded column. The zone nearest the bottom yielded an unidentified sirup which has resisted crystallization. The third zone from the column top yielded a sirup which crystallized from absolute ethanol and was identified as β-isomaltose octaacetate; yield 55 mg. (1.3% as isomaltose), m.p. 144–145° unchanged on admixture with an authentic specimen of like melting point; $[\alpha]^{25}D$ +97° (*c* 1.1, chloroform). Wolfrom, Georges and Miller[7] cite for β-isomaltose octaacetate: m.p. 143–144°; $[\alpha]^{25}D$ +97° (*c* 2.7, chloroform). The eluent from the next higher zone was crystallized from absolute ethanol and was identified as β-maltotriose hendecaacetate; yield 62 mg., m.p. 134–135° unchanged on admixture with an authentic specimen of like melting point; $[\alpha]^{25}D$ +86° (*c* 1.5, chloroform). Wolfrom and co-workers[9] cite for β-maltotriose hendecaacetate: m.p. 134–136°, $[\alpha]^{25}D$ +86° (*c* 1.5, chloroform).

Another hydrolysis investigated in the same manner but wherein the hydrolysis was carried to 75% completion yielded 2.2% (92 mg.) of isomaltose as the crystalline β-octaacetate.

Acid Hydrolysis of Amylose.—An amount of 5.0 g. of corn amylose, prepared from defatted corn starch according to the procedure of Schoch,[27] was hydrolyzed in 2% concentration in 0.050 *N* sulfuric acid at 100° for 9 hours (degree of hydrolysis *ca.* 80%). The resulting amorphous solid (3.8 g.) was subjected to hot acetylation with 25 ml. of acetic anhydride and 2.8 g. of fused sodium acetate.

The acetylated hydrolyzate (5.7 g.) was chromatographed in the above-described manner on Magnesol–Celite (5:1 by wt.) and the following compounds were isolated and identified: β-D-glucopyranose pentaacetate (2.03 g.), β-maltose octaacetate (1.20 g.) and β-maltotriose hendecaacetate (0.59 g.). No isomaltose octaacetate was detectable. The remaining more highly adsorbed material (1.79 g.) on further resolution has yielded only sirups which have resisted crystallization.

(27) T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941); THIS JOURNAL, **64**, 2957 (1942).

Summary

1. The first order specific reaction constants for the hydrolysis of glycogen in 2% concentration in 0.05 *N* hydrochloric acid at 100° were found to be essentially constant throughout the range investigated (0 to 60% hydrolysis). Under these conditions the ratio of the rates of hydrolysis of maltose to isomaltose was found to be 4:1.

2. A statistical treatment of the random hydrolysis of glycogen was made whereby the yields of those oligosaccharides containing one α-1,6-glycosidic linkage may be calculated for any desired degree of hydrolysis. An analysis is presented for the case of isomaltose from glycogen which demonstrates that under the chosen hydrolytic conditions the maximum yield of isomaltose obtainable from the acid hydrolysis of glycogen is 6.8% and that this maximum will occur when 89% of the glycogen is hydrolyzed.

3. By utilizing chromatographic techniques, an acetylated hydrolyzate of glycogen was resolved into β-D-glucopyranose pentaacetate, β-maltose octaacetate, β-isomaltose octaacetate and β-maltotriose hendecaacetate, all isolated in crystalline condition.

4. An appropriate blank experiment with amylose indicated that the isomaltose was not formed by reversion; β-D-glucopyranose pentaacetate, β-maltose octaacetate and β-maltotriose hendecaacetate were the only crystalline products isolated from the acetylated hydrolyzate of this substance.

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The Preparation of 4,6-Ethylidene-D-glucopyranose from Sucrose and its Hydrogenation to 4,6-Ethylidene-D-sorbitol[1]

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Reports of only a few attempts to allow sucrose to react with carbonyl compounds can be found in the literature. Only one of these can be said to have been successful in the sense that crystalline substances were isolated. Ohle, Wolter and Wohinz[3] allowed sucrose to react with acetone and obtained "β-diacetonefructose" and 1,2-isopropylidene-D-glucofuranose in the crystalline condition. Sutra[4] allowed sucrose to react with a large excess of paraldehyde in the presence of sulfuric acid and obtained a sirup which he said apparently contained diethylidenesucrose which was not isolated in a pure condition. Sorgato[5] condensed sucrose with formaldehyde at 80–120° and obtained a solid polymerized material insoluble in water.

We have found that a crystalline reaction product is obtained from sucrose and a reasonable quantity of paraldehyde in the presence of the minimum catalytic concentration of sulfuric acid. This substance has been identified as 4,6-ethylidene-D-glucopyranose which was first obtained by Helferich and Appel[6] as the reaction product of D-glucose and paraldehyde in the presence of sulfuric acid. We obtained it in about 60% yield based on the D-glucose content of sucrose, and have also improved its method of preparation from D-glucose. The crystalline product obtained from sucrose appears to be identical with that obtained

(1) The material in this paper is taken in part from a thesis submitted to the Department of Chemistry of the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy by David V. Collins in June 1948. A preliminary report of some of this work was given at the 113th meeting of the American Chemical Society, Chicago, Ill., April, 1948.

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(3) Ohle, Wolter and Wohinz, *Ber.*, **63**, 843 (1930).

(4) Sutra, *Bull. soc. chim.*, **9**, 794 (1942).

(5) Sorgato, *Ann. chim. applicata*, **33**, 113 (1943).

(6) Helferich and Appel, *Ber.*, **64**, 1841 (1939).