clarified liquid, +3.54°V. in a 2-dm. tube, together with its reduction of 0.608% after a Herzfeld hydrolysis, corresponded to a specific rotation of +93.8° in water.

The author wishes to record his appreciation of the interest which Professor C. S. Hudson has shown in the work and to thank the Chemical Foundation of New York for a Research Associateship.

Summary

1. In the presence of methyl alcoholic hydrogen chloride, the gamma-methylfructoside, $[\alpha]_D^{20}$ +93° in water and stable to invertase, very rapidly gave rise to an equal amount of the gamma-fructose derivative hydrolyzed by the enzyme and with a specific rotation of $[\alpha]_D^{20}$ $-52 \pm 2°$. The reaction was unimolecular and apparently involved no third substance.

2. The above change occurred more slowly when the acid reagent contained 1% of water and in addition a partial hydrolysis to fructose took place.

3. No side reactions were observed during the hydrolysis of the dextrorotatory gamma-methylfructoside by aqueous hydrochloric acid. This unimolecular reaction had a velocity constant 8.1 times as large as that determined for the hydrolysis of sucrose under similar conditions.

WASHINGTON, D. C. RECEIVED JUNE 4, 1934

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Analysis of Gamma-Methylfructoside Mixtures by Means of Invertase. IV. Behavior of Sucrose in Methyl Alcohol Containing Hydrogen Chloride[1]

BY C. B. PURVES AND C. S. HUDSON

The third paper in this series[2] described the equilibrium that was very rapidly established between equal amounts of a dextro-rotatory, crystalline gamma-methylfructoside denoted by (c) and a levorotatory, non-reducing gamma derivative (a) of the ketose when the former was dissolved in dry methyl alcohol containing hydrogen chloride. Although it was desirable to complete the investigation by submitting the latter derivative to the action of the same reagent and so approach the equilibrium from the opposite side, the levorotatory glycoside (a) was not isolated in a condition sufficiently pure to justify its examination. It resembled sucrose, however, in being readily hydrolyzed by invertase and this fact, considered in conjunction with the selective action of the enzyme, made it very probable that the fructose residue in the molecule of both compounds was the same in structure and configuration. Sucrose was therefore regarded in the present instance as the best available substitute for the inaccessible gamma-fructose derivative and the research was continued by examining the reaction occurring between this disaccharide and acid methyl alcohol.

The methyl alcoholic solution, 0.0263 molar with regard to sucrose and 0.0263 normal with respect to hydrogen chloride, corresponded exactly to that previously used in the examination of the crystalline gamma-methylfructoside. When kept at 20° the copper reduction of the sucrose solution rapidly increased from an initial value of zero to a maximum which was due to the liberation in an uncombined state of 94–95% of the theoretical amount of glucose. Thereafter, the reduction slowly diminished (Fig. 1) over a period of days as the aldose recondensed with the acid alcohol to give a non-reducing mixture of gamma-methylglucosides. The correctness of this interpretation of the sucrose reduction curve was confirmed (1) by arresting the reaction near the point of maximum reduction and by isolating from the product 81% of the possible amount of glucose in a pure, crystalline condition, (2) by the fact that the sucrose reduction curve, from the maximum onward for many hours, coincided within the experimental error with the graph depicting the condensation of glucose with acid methyl alcohol in the identical conditions, and (3) by the consideration that any fructose set free would immediately have been recondensed to a non-reducing mixture of gamma-methylfructosides. The latter reaction was 50% complete in six minutes (Fig. 1) while approximately nine minutes were required for a half hydrolysis of the sucrose. These rates of re-

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[1] Publication authorized by the Surgeon General, U. S. Public Health Service.

[2] Purves, THIS JOURNAL, **56**, 1969 (1934).

action may be compared to the half time period of 10,000 minutes determined for the hydrolysis of sucrose to invert sugar by aqueous hydrochloric acid at the same temperature and concentration.

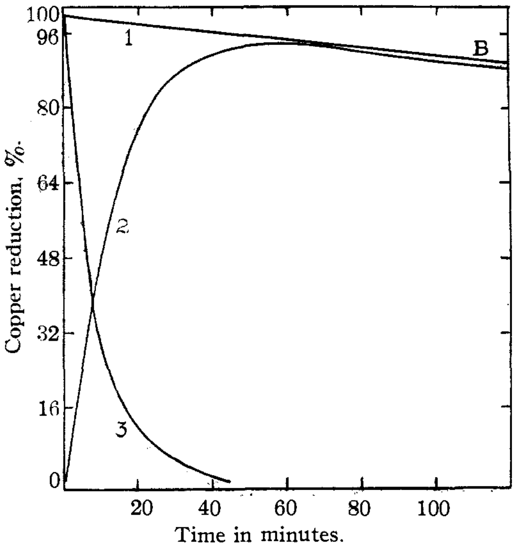

Fig. 1.—Copper reduction of 0.0263 molar glucose, fructose and sucrose in 0.0263 *N* methyl alcoholic hydrogen chloride at 20°: curve 1, glucose; curve 2, sucrose; curve 3, fructose.

The isolation of crystalline glucose in the above experiments suggested the possibility that the fructose also was liberated as such from the sucrose molecule but immediately recondensed with the solvent. If this was the case, the mixture of non-reducing gamma-fructose derivatives, formed from sucrose, would be identical in composition with that prepared from the pure ketose. The gamma-methylfructosides from this source have already been shown[3] to contain 45% as a maximum of the derivative (a), hydrolyzed by invertase and possessing a specific rotation of −52 ± 2° in water. In order to use these data as a test for the presence or absence of this particular fructoside mixture, the solution of sucrose in the acid methyl alcohol was neutralized at the point of maximum reduction (*i. e.*, when the rupture of the disaccharide linking was complete) and the amount of the glycoside (a) in the product was estimated by means of invertase. Three independent experiments indicated that no less than 58, 54 and 60% of the total available ketose was present as the glycoside (a) which had a calculated specific rotation on the same occasions of −51.5, −51.0 and −52.0°, respectively. While the sign and magnitude of the rotation confirmed the absence of sucrose and the presence of the fructoside (a) in the product, the high percentage of the latter was incompatible with its formation from the free reducing sugar. Moreover, as the reagent was acid methyl alcohol, the amount of this fructose derivative was continuously being diminished by the formation from it of the crystalline gamma-methylfructoside (c).[2] Glucose and the gamma fructose derivative (a) were therefore considered to be the initial products recognized in the sucrose scission and while the first was slowly changed to gamma-methylglucoside, the second rapidly established an equilibrium with the glycoside (c). These inferences were confirmed by neutralizing the sucrose solution one hour after it had attained its maximum reduction. The amount of the fructoside (a) had then attained the correct equilibrium value of 50% and the crystalline gamma-methylfructoside was isolated from the product in 20% of the possible yield. A description is given in the experimental portion of a large scale preparation of the crystalline gamma-methylfructoside from sucrose, which is a less expensive initial material than fructose.

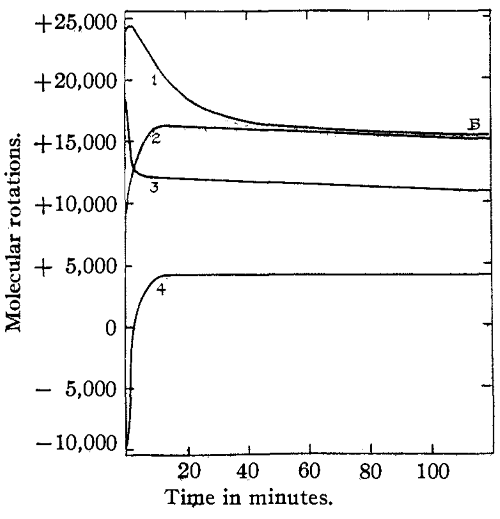

Fig. 2.—Molecular rotations in 0.0263 *N* methyl alcoholic hydrogen chloride at 20°: curve 1, sucrose; curve 2, algebraic sum of glucose and the derivative (a); curve 3, glucose; curve 4, the fructose derivative (a).

The optical data of the sucrose scission summarized in Fig. 2, were obtained simultaneously and from the same acid methyl alcoholic solutions as those used in the estimations of copper reduction. After a small initial rise, the molecular

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[3] Purves and Hudson, This Journal, **56**, 702 (1934).

rotation of the sucrose solution rapidly diminished until after sixty minutes, coincident with the point of maximum reduction, a break occurred in the optical curve and the subsequent decrease suddenly became very slow. The estimations of rotation and reduction in the initial period were readily confirmed by carrying out the reaction more slowly in 0.008 normal methyl alcoholic hydrogen chloride and the sharp decrease in the rate of optical change at sixty minutes was very marked when the acidity was raised to 0.5 normal. From the point of maximum reduction onward (sixty minutes), the molecular rotation of the sucrose solution was the sum of those due to the glucose as it recondensed with the solvent and to the equimolecular equilibrium mixture of the gamma-fructose glycosides (a) and (c). The agreement with theory remained within the observational error for many hours before the sucrose solution became somewhat more levorotatory than the control. This slight divergence was probably due to the influence of varying small amounts of moisture on the optical rotation of the gamma-methylfructoside mixtures. The slow decrease in the equilibrium rotation of the glycosides (a) and (c) was experimentally determined with the same conditions in the preceding paper[2] (Table II), which also provided the data here used as a guide in sketching the approximate form of the fructoside (a) curve before it attained equilibrium with (c). An initial rapid change in the molecular rotation of the glucose was due to the mutarotation of the alpha form examined.

It is interesting to note that Berner[4] has recently brought about a partial cleavage of the disaccharide linking in sucrose by heating a methyl alcoholic solution for eight to ten hours at 150–155° in an atmosphere of nitrogen and considers that the fructose residue in sucrose possesses the alpha configuration. The present research, on the other hand, indicates that the levorotatory glycoside (a) is the first gamma-fructose derivative to be formed from sucrose when hydrogen chloride is present in the methyl alcohol. A decision between these divergent results is made difficult by the possibility, not overlooked by Berner, of a Walden inversion taking place during the scission of the sucrose molecule. Perhaps the most reliable evidence available in favor of the conclusion that sucrose is more closely related structurally to the levorotatory glycoside (a) than to the dextrorotatory, crystalline gamma-methylfructoside (c) is that the two former, in contrast to the latter, are hydrolyzed by the enzyme invertase.

Experimental

The Shaffer-Hartmann reagents used in the estimation of copper reduction were calibrated against standard solutions of pure glucose, fructose and invert sugar and the reducing powers of the three were found to be in the ratio 1:0.92:0.96, respectively. All optical rotations were carried out at 20° and with sodium light on a saccharimeter with a conversion factor of 1°V. = 0.3462° circular. The other details of the analytical and preparative methods have already been published.[3]

Changes in the Reduction and Optical Rotation of Sucrose Dissolved in Methyl Alcohol Containing Hydrogen Chloride.—A 1.000% solution of the sugar in pure, dry, acetone-free methyl alcohol had an observed rotation of 8.23°V. in a 4-dm. tube, corresponding to a specific rotation of +71.25°. At 20° and zero time, 90 ml. was quickly mixed with 10 ml. of 0.263 N dry methyl alcoholic hydrogen chloride to give an 0.9% or 0.0263 molar sucrose solution which was 0.0263 normal with respect to the gas. One portion of the solution was then observed in a 4-dm. tube on the saccharimeter while the change in reducing power was followed in the remainder by discharging 1-cc. samples at intervals into 10-ml. volumes of 0.0027 N aqueous caustic soda. These faintly alkaline aqueous alcoholic mixtures were subsequently estimated for glucose in the usual way. The specific and molecular rotations quoted in Table I were based throughout on the concentration of sucrose initially present and the theoretical amount of glucose, 0.4734%, was assumed in calculating the percentage yield from the glucose content.

At the end of forty-one hours an Herzfeld hydrolysis increased the reduction of the solution from 0.080% as glucose to 0.888% as invert sugar instead of the theoretical amount of 0.947%. The difference was due to the presence of glycosides resistant to aqueous acid hydrolysis with gamma conditions and which accounted for 6.2% of the sucrose used.

Analysis of Product Isolated near Point of Maximum Reduction.—A solution (48 ml.) identical in composition to the above (0.9% sucrose, 0.0263 N dry methyl alcoholic hydrogen chloride) was made faintly alkaline with $N/10$ aqueous caustic soda (13.5 ml.) after it had stood at 20° for thirty-four minutes and its specific rotation had diminished to +49°. After estimating the reduction of the aqueous alcoholic mixture, 0.373% as fructose, the alcohol was replaced by an equal volume of water at pH 4.5 and the solution was analyzed in the usual way with the aid of invertase. The enzyme caused a decrease from 3.30 to 1.97°V. in the dextrorotation of the solution, observed in a 4-dm. tube, and increased the reduction from 0.370 to 0.594% as fructose. The constituent hydrolyzed therefore had a specific rotation of −51.5° in water, calculated as a methylfructoside and the concordance of this value with that previously found for the gamma-fructoside (a), −52 ± 2°, showed that no sucrose, +66.5°, remained

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[4] Berner, *Ber.*, **66**, 1076 (1933).

TABLE I

OPTICAL ROTATION AND COPPER REDUCTION OF 0.0263 M SUCROSE IN 0.0263 N METHYL ALCOHOLIC HYDROGEN CHLORIDE AT 20° [a]

Min.	V.°(L = 4)	$[\alpha]^{20}_D$	$[M]^{20}_D$	% Glucose	% of calcd.
0.0	7.41° (calcd.)	71.3	24370	0.0	0.0
.75				.017	3.6
1	7.55	72.6	24830		
1.25				.027	5.8
1.75				.049	10.4
2	7.55	72.6	24830		
3				.084	17.6
3.5	7.45	71.6	24500		
4				.111	23.4
4.5	7.40	71.2	24330		
5				.151	32.8
5.25	7.25	69.7	23840		
6	7.12	68.5	23410		
7	7.00	67.3	23020		
8	6.85	65.9	22520		
9	6.72	64.6	22100		
10	6.60	63.5	21700	.243	51.2
11	6.45	62.0	21210		
12	6.38	61.3	20980		
13	6.23	59.9	20490		
14	6.11	58.7	20090		
15				.313	65.8
15.25	6.03	58.0	19830		
16	5.95	57.2	19550		
17	5.89	56.6	19370		
18	5.80	55.8	19070		
20	5.70	54.8	18740	.361	76.0
22	5.57	53.6	18320		
24	5.45	52.4	17920		
25				.401	84.4
26	5.40	51.9	17760		
28	5.35	51.4	17590		
30				.410	86.4
32	5.20	50.0	17100		
40				.437	92.0
42	5.02	48.3	16510		
60				.450	94.8
62	4.87	46.8	16010		
120				.425	89.4
122	4.65	44.7	15290		
Hours					
23				.184	39.0
24	1.65	15.9	5430		
41	0.60	5.8	1970	.080	16.8

[a] See Fig. 1, curve 2, and Fig. 2, curve 1.

in the product. As the calculated amount of fructose was 0.3735%, the glycoside (a) was present to the extent of 100 (0.594 − 0.370)/0.3735 or 60% of the theoretical yield. In addition, the initial reduction of 0.370% as fructose was equivalent to (0.370 × 0.92) or 0.340% of glucose, the amount of which was therefore (100 × 0.340)/0.3735 or 91% of theory.

Analysis of Product Isolated One Hour after Maximum Reduction.—The 0.9% sucrose acid–methyl alcoholic solution (98 ml.) was neutralized after having stood at 20° for two hours and when its specific rotation was +45°. An aqueous solution (36 ml.) of the product had a dextrorotation of 5.09° in a 2-dm. tube and a reduction, as fructose, of 1.250%, changed to 3.75°V. and 1.904%, respectively, by invertase. Aqueous acid hydrolyzed the product to invert sugar (2.606%) with an observed levorotation of −3.32° corresponding to a specific rotation of

TABLE II

OPTICAL ROTATION AND COPPER REDUCING POWER OF 0.0263 M GLUCOSE DISSOLVED IN 0.0263 N METHYL ALCOHOLIC HYDROGEN CHLORIDE AT 20° [a]

Min.	V.° (L = 4)	$[\alpha]^{20}_D$	$[M]^{20}_D$	% Glucose	% of calcd.
0	5.75° (calcd.)	105.1	18910	0.483	100
2.2	4.25	77.7	13980		
3				.476	98.5
4	3.80	69.4	12500		
5				.467	96.7
6	3.68	67.2	12100		
8.25	3.65	66.7	12000		
10	3.69	67.4	12140		
10.5				.473	98.0
12	3.65	66.7	12000		
15				.473	98.0
19	3.64	66.5	11970		
26	3.63	66.3	11940		
30.5				.473	98.0
40.5	3.60	65.8	11840		
46				.470	97.3
63	3.54	64.7	11640		
65				.458	94.8
102	3.42	62.5	11250		
104				.443	91.7
151				.429	88.8
155	3.28	59.9	10810		
205	3.14	57.4	10330	.410	84.9
Hours					
20.5	1.31	23.9	4310	.217	44.9
73	0.0	0.0	0	.077	15.9

[a] See Fig. 1, curve 1, and Fig. 2, curve 3.

TABLE III

COPPER REDUCING POWER OF 0.0263 M FRUCTOSE DISSOLVED IN 0.0263 N METHYL ALCOHOLIC HYDROGEN CHLORIDE AT 20° [a]

Min.	% Fructose	% of calcd.
0.0	0.465	100
0.66	.440	94.6
1.25	.414	89.0
3	.310	66.6
4.5	.285	61.3
6	.229	49.2
8	.185	39.8
11	.125	26.9
14	.093	20.0
18.5	.056	12.0
24	.033	7.1
30	.021	4.5
45	.0	0
60	.0	0

[a] See Fig. 1, curve 3.

−22° in 0.25 *N* hydrochloric acid. Found, glycoside (a), 50.2% of the available fructose and with a calculated specific rotation of −52.7° in water; initial reduction as glucose, 88.2%.

Condensation of Glucose and Fructose with Dry Methyl Alcoholic Hydrogen Chloride.—Solutions containing either pure α-glucose or β-fructose were made up to be 0.4737% or 0.0263 molar with regard to the hexose and 0.0263 normal with respect to hydrogen chloride. The copper reduction determinations and the optical rotations were made as before on portions of the same solution.

Crystallization of Glucose from Sucrose and Acid Methyl Alcohol.—Anhydrous, very finely divided sucrose (110 g.) was shaken vigorously and continuously at 22–25° with 1100 ml. of 0.0263 *N* methyl alcoholic hydrogen chloride. The solution was neutralized with silver carbonate at the end of one hundred fifty minutes whether or not the sugar was entirely dissolved and the filtrate was made faintly alkaline with 3 ml. of normal caustic soda. Another filtration with absorbent carbon left a water-clear, methyl alcoholic solution which was found by analysis to contain glucose 49.2 g., gamma-methylglucosides 5.7 g., the gamma-methylfructoside (c) 29.3 g. and the fructose derivative (a) 28.6 g. The calculated specific rotation of the latter, −57°, was sufficiently accurate to confirm the absence of sucrose while the extent (10%) to which the glucose had recondensed with the solvent indicated that the alcoholysis had been stopped in the region marked B on the curves in Figs. 1 and 2.

After evaporating the solution under diminished pressure to a total weight of 250 g., neutral ethyl acetate (400 ml.) was gradually added at 0° to precipitate the glucose as a thick sirup which crystallized completely overnight in the presence of added nuclei. A mixture of methyl alcohol (100 ml.) and ethyl acetate (250 ml.) at 0° was used to wash the crystals which when dried weighed 47.5 g. and had a constant specific rotation in water of +49.8°, which rose to 51.7° (yield 40 g. or 81%) after recrystallization, showing the sugar to be glucose.

Preparation of the Crystalline Gamma-Methylfructoside from Sucrose.—The product obtained, as described above, from sucrose (110 g.) and acid methyl alcohol, was dissolved in 1 liter of water at *p*H 4.5 and fermented for three days with 10 g. of starch-free yeast. Glucose, the fructoside (a) and any trace of sucrose were entirely removed and after clarification there remained a colorless, nonreducing solution containing approximately 5.7 g. of gamma-methylglucosides and 29.3 g. of the gamma-methylfructoside (c). After removing most of the solvent from this solution by cautious distillation in a vacuum at the lowest possible temperature and at *p*H 8–10, normal butyl alcohol (100 ml.) was added in two portions to assist in the volatilization of the water. The nearly anhydrous butyl alcoholic sirup was then diluted with methyl alcohol (200 ml.) to precipitate yeast protein before the glycosides were precipitated in their turn from the concentrated filtrate by an excess of petroleum ether. The thick sirup was dried at room temperature in a high vacuum, dissolved in 50 ml. of dry methyl alcohol and reprecipitated at 0° with 500 ml. of ethyl acetate. Crude gamma-methylfructoside (4–5.8 g.) crystallized when the clear supernatant liquor was slowly concentrated at a low temperature. The crystals were melted in an oven kept at 70° before being dissolved in 1.5 times their bulk of normal amyl alcohol at 70° and filtered while hot through cellite; yield of the pure gamma-methylfructoside, 3–4.5 g., or 10–15% of the possible; melting point 69° and mixed m. p. with an authentic specimen derived from fructose 69°. The crystals had the correct specific rotations of +91.6° in methyl alcohol and −92.2° (recalculated for fructose) after complete hydrolysis with aqueous acid.

One of the authors (C. B. P.) desires to thank the Chemical Foundation of New York for a Research Associateship.

Summary

1. Sucrose, when dissolved in methyl alcohol containing hydrogen chloride, was very rapidly changed to glucose and the non-reducing gamma-fructose derivative, unstable to invertase and with a specific rotation in water of $[\alpha]^{20}_{D}$ −52 ±2°.

2. The glucose slowly condensed with the acid solvent to give a mixture of gamma-methylglucosides.

3. The gamma fructose derivative initially formed, rapidly established an equilibrium with an equal amount of the crystalline gamma-methylfructoside, unaffected by invertase and with a specific rotation of $[\alpha]^{20}_{D}$ +93° in water.

4. The above reactions were utilized in the preparation of the crystalline gamma-methylfructoside from sucrose.

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