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NOTES ON THE HYDROLYSIS OF STARCH BY ACIDS.

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SOME years ago, 1896, Rolfe and Defren[1] published analytical data of acid-hydrolyzed starch products from which they concluded that the evidence was strong that a constant relation existed between the optical rotation and copper reduction of such products, independent of the conditions of hydrolysis; in other words, that hydrolyzed products of the same rotation had the same reducing power, and consequently were identical in composition, providing, of course, that they were normal, unmixed products of acid hydrolysis. Obviously, absolute confirmation of such a far-reaching law could only be arrived at by elaborate and lengthy research.

In 1897, Brown and Morris[2] published the results of some 500 analyses of *diastase*-converted starch products, showing, in confirmation of their earlier conclusions, that a law of relation did exist, not only in such products themselves, but in their fractions obtained by alcohol.

Perhaps the most important paper, bearing directly on the acid hydrolysis of starch, is that of Johnson, which appeared in 1898.[3]

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[1] This Journal, **18**, 869; revised: *Tech. Quart.*, **10**, 153

[2] *J. Chem. Soc.*, **71**, 115.

[3] *Ibid.*, **73**, 490.

Johnson found a constant relation between optical rotation and cupric reduction, but obtained results at variance with those of Rolfe and Defren.

Johnson concluded that maltose, or its molecular combinations, was not found in acid-hydrolyzed products, but that these bodies could be considered to be combinations of dextrose and a non-reducing dextrin, thus agreeing with earlier work, notably that of Salomon[1] and Pellet,[2] who had already come to the same conclusion.

Johnson ascribes the differences shown in Rolfe and Defren's data to errors introduced into the work of the latter through lime salts formed in neutralizing the acidified solutions. He publishes but little of his experimental data or the details of his work, but states that he has followed the analytical procedure of Brown and Morris, and that his conversions were made on a water-bath in open beakers. Apparently the hydrolyzing acid was sulphuric. originally, of about half-normal strength. We understand that Krieger has also published a paper confirming the work of Johnson, but we have not been able to find reference to it.

Morris[3] stated that the results of his investigations were quite at variance with those of Johnson; that his data indicated the presence of maltose, which he proved to exist in the solutions by obtaining crystals of undoubted maltosazone; furthermore, his results confirmed those of Rolfe and Defren, although he was inclined to doubt the universal application of their law of relation to all acid-hydrolyzed starch products. The untimely death of this brilliant investigator has doubtless prevented publication of his complete work.

Many circumstances have prevented the resumption of these investigations in this laboratory. Only quite recently has this research been taken up again with the following objects: (1) To obtain more data on the "law of relation"; (2) to see whether this law applied to alcoholic fractions; (3) to find out proofs of the existence of maltose in acid-hydrolyzed starch products.

The methods of the English chemists, adopted by us, have been much criticized, owing to the use of an arbitrary specific gravity factor in the calculations. As the use of this factor, its influence

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[1] *J. prakt. Chem.* (2), **28**, 82.

[2] *Compt. Rend.*, **2**, 450.

[3] *Proc. Brit. Assoc.*, Sept., 1899.

on the results, and their relation to the absolute values, etc., have been fully explained by Brown and Morris,[1] it is not necessary to discuss it in detail here. In the present work we have expressed the results in absolute values. It should be noted, however, that the use of the factor 0.00386, as detailed by Brown and Morris, simplifies the calculations, gives correct *proportional* values (per cents.) of the primary carbohydrates, and likewise optical and reducing figures, readily convertible into absolute values by use of known factors. The practical starch chemist, therefore, will probably continue the use of it, as a path of least resistance in attaining his desired information, just as the analyst will use the Mohr cubic centimeter, and the physical chemist the Briggs logarithm instead of the Naperian, when by such means he arrives at his objective point more quickly and easily. Results obtained by such expedients of calculation and experiment are permanently valuable, as they admit of standardization at will in terms of the accepted scientific constants of the day.

Analytical Methods.—In the earlier work, densities were obtained by a Westphal balance, carefully and frequently adjusted, and were made at ordinary laboratory temperatures, and corrected for 15.5°. More recently, we have made pyknometer determinations at 15.5°. Corrections were made for the dissolved mineral salts present. Ash determinations of carbohydrate solutions containing known amounts of mineral salts confirmed O'Sullivan's factor, 0.008, for the density correction of each gram of mineral matter, determined as ash, in 100 cc. of solution, when the amount of mineral matter did not exceed a few per cent. of the carbohydrate present. A more exact correction seems necessary when the mineral content is large. Ash determinations were made in a "low temperature" muffle, vaseline being used to prevent excessive swelling of the coal.

This influence of the mineral salts in solution was investigated with particular care, owing to its possible importance as a source of error, as suggested by Johnson. Evidently the most important error introduced, that of the density, lowers the cupric-reducing power and optical rotation by the same percentage, the error in reduction appearing greater in the higher converted products, that of the rotation in the lower. Hence, the effect of the ash correction on the reduction values of those products containing

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[1] *J. Chem. Soc.*, 71, 72.

the most sugars would be diametrically opposite to that suggested by Johnson's criticism. As a matter of fact, the correction for the concentration of acid used rarely exceeds 2 per cent., and in the majority of cases is much smaller. This would tend to increase the discrepancy of more than 10 per cent. between Johnson's and Rolfe and Defren's figures in those products where the presence of maltose is in dispute; that is, at the conversion point where maltose is supposed to be present in maximum amount.

We have made a careful study also of the original data of this earlier work and have recalculated all the results where the data admitted. This was made necessary from the revision of factors used in calculation. We have tabulated all these values figured in absolute constants. It will be seen that little change is made, many of the corrections being compensatory.

Another possible source of error caused by lime salts might be their precipitation with the copper oxide in the Fehling test. This seemed improbable, as the mineral matter rarely exceeded 0.100 gram in 100 cc. in the original solutions, which were diluted in most cases to $^1/_{80}$ of their original concentration. Blank tests made on solutions containing lime salts in the proportion present in the usual tests, and in fact, made by neutralizing an acidified solution in the same manner as the hydrolyzed products, showed no increase in weight due to precipitated lime.

The optical rotation constants have been determined by a Schmidt and Haensch saccharimeter, but the light factor used in converting readings to angular degrees of rotation of the "D" ray has been increased 0.2 per cent. to 0.3450. The original factor, 0.3443, which was worked out experimentally,[1] expresses the value of our saccharimeter in terms of yellow-light rotation, measured on a Laurent polariscope, the light being that of a sodium chloride flame, filtered through a section of potassium bichromate crystal. The Landolt polariscope uses sodium chloride, light filtered through a potassium bichromate solution, and also one of uranium sulphate. This seems to have caused a change in light standard which results in readings which are 0.2 per cent. higher. The wave length of the Landolt illuminating apparatus has been found to be 0.00058932,[2] corresponding to a position in the spectrum exactly midway between the two "D" lines. As the

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[1] This Journal, 18, 873.

[2] Landolt's "Das Optische Drehungsvermögen," p. 264.

Landolt values seem to be generally accepted as the standard for the "D" ray, we have made this trifling change in our own figures. The subject of the "light factor," that is, the equivalent value of one saccharimetric division in angular degrees of yellow-light rotation, is treated by recent text-books in a misleading way. Values are given some tenths of a per cent. higher than those of the earlier works, leading the reader to infer that the earlier figures are incorrect, whereas, as a matter of fact, these earlier factors are practically exact for by far the majority of saccharimeters in actual use to-day—those graduated in "Mohr" cubic centimeters at 17.5°, the only correction being for any change in the light standard of the rather indefinite "D" ray. The recent factors are obviously applicable only to those instruments recently made which are graduated for solutions made up in true cubic centimeters. Unquestionably, the use of volumetric apparatus graduated in true cubic centimeters is most commendable, but just as unquestionably, the starch chemist must use those factors which are applicable to the actual graduation of his saccharimeter. It is to be hoped that the makers of saccharimeters henceforth will mark on the instruments just how they are graduated.

As far as possible, saccharimeter readings have been made at 20° C., but, with the present laboratory facilities at our command, it has been impossible to control the temperature within several degrees. As soon as it is feasible to carry out such work, we intend to make a correction table for readings taken at temperatures other than 20°. Our determinations of cupric-reducing power have been made by Defren's method,[1] with slight modification. We have preferred this method to that of Brown and Morris,[2] which it closely resembles, merely because we obviate the correction due to the spontaneous reduction of the solution. In the past six years we have made blank tests on every lot of Fehling solution used, carrying out every detail of the regular determination. With the exception of two lots of solution—in which we traced the spontaneous reduction of 0.0017 gram to a bottle of Rochelle salts of inferior quality—we have never found either visible evidence of spontaneous reduction, or increase in weight of crucibles, these checking within 0.0002 gram. Therefore, we state with confidence that there is no spontaneous reduction of

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[1] This Journal, **18**, 749.

[2] *J. Chem. Soc.*, (London), **71**, 94.

the Fehling solution during the heating process of the test carried out by Defren's method. This seems to be the result of the greater dilution of the Fehling solution, and possibly the smaller amount of alkali. We have varied the method, however, in one detail. Instead of delivering the sugar solution as quickly as possible from a burette into the Erlenmeyer flask containing the hot, diluted Fehling solution, we have added the sugar all at once, without removing the Fehling liquor from the bath, by quickly inverting a 25 cc. flask which is left upside down in the mouth of the Erlenmeyer during the reduction. One short rotary shake is given the Erlenmeyer immediately after the solution is added to insure, at once, perfect mixture. This modification has increased the reducing power of the sugar solutions, and necessitated calculating a new set of equivalents, from tests made with pure dextrose. As we hope to modify the process somewhat more in the near future, we do not deem it advisable to take valuable space to publish our present equivalent table. The copper reduction, in general, both on dextrose and maltose, is about 5 per cent. greater than Defren's original figures, the dextrose equivalent, therefore, being correspondingly less. In the earlier work some correction has been necessary, owing to the more accurate density values for dextrose and maltose, which are now known.[1] These density corrections lower Defren's original equivalents about 0.6 per cent. and need only be considered in the most accurate work.

As the density factors corresponding to the actual weights of acid-hydrolyzed starch products, in solutions of a density approximating 1.04, have already been established experimentally,[2] we have been enabled to convert our results into absolute values by the following table (A), which gives the logarithms for convert-

Table A.—Density Factors for Reference to Actual Weights of Acid-Hydrolyzed Starch Products in 100 True Cubic Centimeters of Solutions.

$[\alpha]_{386}$.	Density factors $\left(d\frac{15.5^\circ}{15.5^\circ}\right)$.	Logarithms of conversion factors.[3]
55°	0.003837	9.9965
60°	0.003844	9.9973
65°	0.003850	9.9980
70°	0.003857	9.9988
75°	0.003864	9.9996
80°	0.003870	0.0002

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[1] *J. Chem. Soc.* (London), **71**, 79.

[2] This Journal, **19**, 698.

[3] Log. $\frac{F}{0.00386}$ 0.99802.

$[\alpha]_{386}$.	Density factors $\left(d\frac{15.5^\circ}{15.5^\circ}\right)$	Logarithms of conversion factors.[1]
85°	0.003877	0.0010
90°	0.003884	0.0018
95°	0.003890	0.0024
100°	0.003897	0.0032
105°	0.003904	0.0040
110°	0.003911	0.0048
115°	0.003918	0.0056
120°	0.003925	0.0063
125°	0.003931	0.0070
130°	0.003938	0.0078
135°	0.003945	0.0085
140°	0.003951	0.0092
145°	0.003958	0.0100
150°	0.003965	0.0107
155°	0.003971	0.0114
160°	0.003978	0.0121
165°	0.003985	0.0129
170°	0.003991	0.0136
175°	0.003998	0.0144
180°	0.004005	0.0151
185°	0.004011	0.0157
190°	0.004017	0.0164
195°	0.004023	0.0170

NOTE.—As the cupric-reducing power of pure dextrose, taken as 1.00 for the factor 0.00386 is 0.9915 in absolute value, it is necessary to add the co-logarithm of this number, or 0.0037, to the logarithm of the conversion factor in calculating the reducing power in terms of that of the equivalent weight of dextrose as unity.

ing results calculated by the factor, 386, into values referred to grams of hydrolyzed product in 100 true cubic centimeters of solution. The newer work published in this paper covers a comparatively limited field, being an investigation of hydrolyzed products of several commercial starches of different origin, formed by the action of hydrochloric acid at two concentrations, and at pressures between 2 and 3 atmospheres, unless otherwise stated. No attempt has been made to purify these starches, as we have been led to believe not only that purification is unnecessary, but may actually change the nature of the starch, since, in our experience, all purification methods produce a certain amount of hydrolytic change and formation of products which are diffusible through the starch grains, and subsequently removed by washing. That

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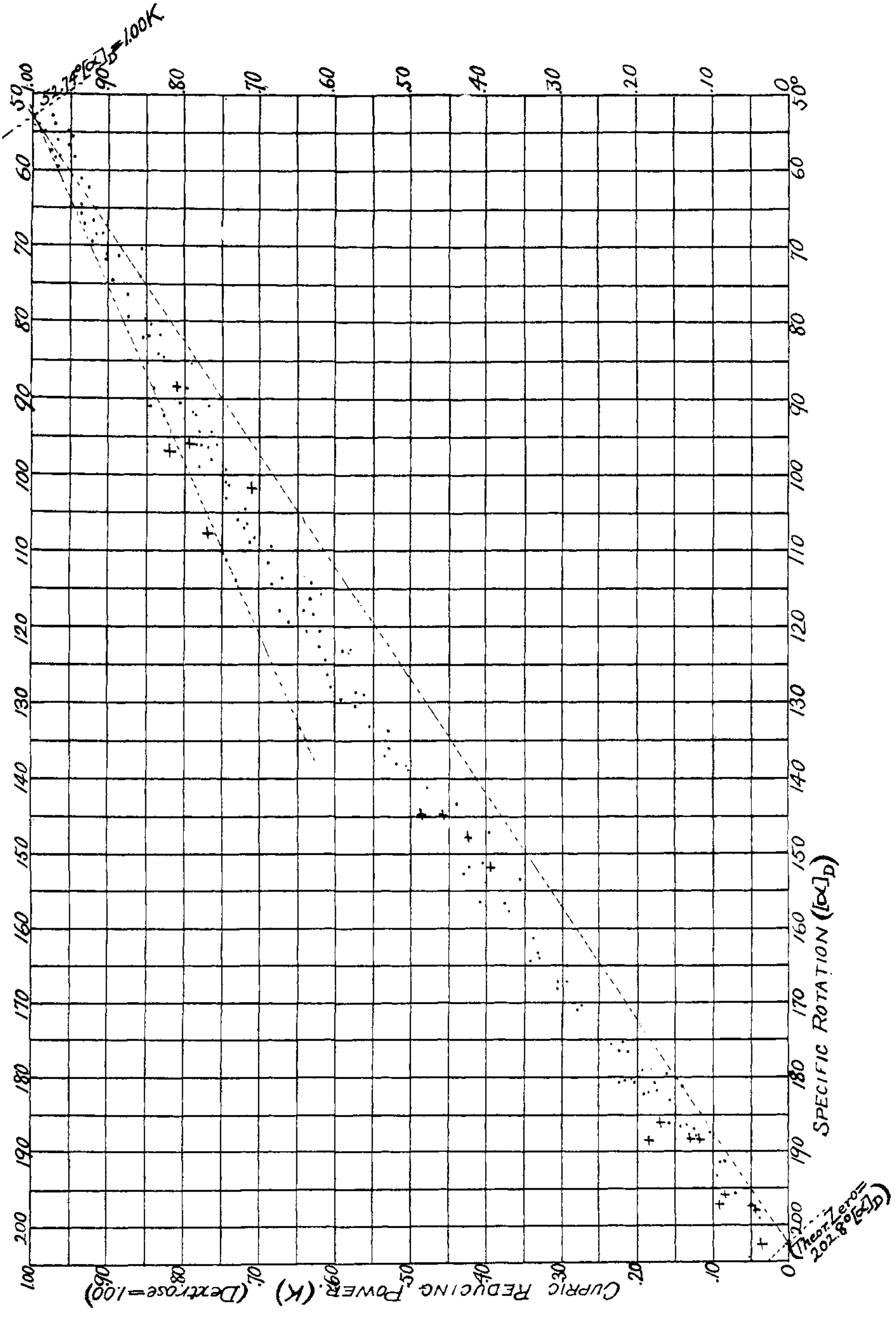
[1] Log. $\frac{F}{0.00386}$ 0.99802


52.74° [α]D = 1.00K
Theor. Zero = 202.8° [α]D
Specific Rotation ([α]D)
Cupric Reducing Power (K) (Dextrose = 1.00)
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
50°
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

such diffusion actually does take place, can be proved by testing the wash-waters used in the purification of starch with iodine, or shaking up the starch itself with cold water and testing the water. The colors obtained are not the deep blue of the unchanged and indiffusible starch, but the violet of hydrolyzed starch, providing, of course, that the grains are unruptured. Probably this slight loss from hydrolytic change has no influence on the results, when the purification method is not too drastic, but it is questionable whether the "purified" product obtained is any nearer pure *starch* than the original.

The following table (B) gives the corrected values of Rolfe and Defren's original work, together with those recently obtained by us. The plotted results[1] make it clear that the reducing values do not lie on a straight line as they should, if dextrose alone were present. On the contrary, the results are in substantial agree-

TABLE B.

Starch.	Acid.	Concentration.	$[\alpha]_D$.	κ.
Corn	HCl	N/100	147.0	0.489
			100.9	0.750
			73.8	0.855
			199.2	0.043
			179.6	0.161
			156.6	0.375
			129.2	0.563
			149.2	0.451
			88.8	0.799
			65.6	0.937
			58.2	0.974
			53.6	0.977
			180.5	0.206
			133.4	0.555
			108.7	0.712
			68.1	0.921
			59.0	0.949
			151.4	0.403
			118.0	6.637
			90.8	0.768
			70.3	0.855
			61.8	0.933
			164.4	0.346
			135.9	0.529
			92.4	0.786
			168.5	0.305

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[1] The plotted values marked by a cross are those of alcoholic fractions, to be discussed in a subsequent paper.

Starch.	Acid.	Concentration.	$[\alpha]_D$.	κ.
			141.7	0.510
			126.3	0.610
			101.2	0.745
Corn	HCl	N/200	195.6	0.067
			186.3	0.143
			180.6	0.225
			167.3	0.306
			160.3	0.349
Corn	HCl	N/100	108.1	0.705
			94.4	0.778
			81.9	0.854
			76.3	0.877
			187.7	0.122
			159.9	0.382
			120.5	0.640
			74.5	0.899
			71.1	0.902
Corn	$H_2C_2O_4$	N/100	181.8	0.172
			160.1	0.360
			138.7	0.504
Corn	$H_2C_2O_4$	N/50	140.9	0.485
			134.0	0.529
			120.4	0.623
			58.1	0.970
			136.9	0.538
			87.1	0.896
Corn	HCl	N/100	182.3	0.192
			186.9	0.128
			193.3	0.097
			186.0	0.158
			182.0	0.186
			180.3	0.217
			167.2	0.298
Potato	HCl	N/50	164.9	0.296
			153.9	0.358
			143.5	0.444
			144.9	0.457
			137.6	0.523
			127.8	0.604
			119.6	0.660
			163.9	0.333
			124.6	0.614
			116.2	0.639
			111.5	0.694
			103.2	0.750
Corn	HCl	N/50	130.4	0.574
			125.5	0.617

Starch.	Acid.	Concentration.	$[\alpha]_D$.	κ.
			117.7	0.645
			114.2	0.673
			107.3	0.723
Corn	HCl	N/50	109.7	0.680
			104.7	0.722
			99.3	0.750
			95.9	0.760
			91.9	0.790
			90.4	0.808
Cassava	HCl	N/50	79.8	0.852
			78.6	0.875
			74.0	0.884
			71.7	0.903
			96.2	0.773
			90.6	0.850
			84.3	0.835
"Thin boiling" corn-starch	HCl	N/50	81.8	0.851
			66.7	0.933
			60.8	0.941
			59.5	0.973
			57.5	0.980
			122.5	0.623
			114.6	0.631
			106.5	0.720
			97.6	0.769
			179.2	0.195
			52.5	100.4
Corn	HCl	N/50	68.1	0.910
			59.9	0.977
			56.7	0.953
Corn	$H_2C_2O_4$	N/25	163.6	0.333
			129.0	0.569
			98.9	0.783
			80.1	0.849
			69.3	0.925
Corn	H_2SO_4	N/50	170.9	0.281
			130.0	0.562
			105.9	0.728
			84.8	0.832
			71.3	0.889
Corn	HCl	N/100	152.6	0.427
			95.7	0.788
			66.5	0.924
			54.8	0.954
			54.2	0.998
Corn	HCl	N/50	95.8	0.783
			64.6	0.939

Starch.	Acid.	Concentration.	$[\alpha]_D$.	κ.
			55.5	0.972
			52.9	0.979
			88.7	0.843
			55.3	0.951
Corn	HCl	N/25	157.5	0.372
			114.7	0.686
Corn	HCl	N/50	82.0	0.886
			54.9	0.993
Potato	HCl	N/50	175.5	0.234
			151.3	0.404
			180.1	0.208
			156.6	0.406
			191.3	0.094
			176.8	0.213
			158.8	0.331
			123.2	0.592
			123.0	0.583
Cassava	HCl	N/50	151.6	0.427
			182.2	0.184
			118.5	0.668
			183.4	0.157
			94.6	0.783
			85.1	0.795
Potato	HCl	N/50	187.3	0.104
			175.3	0.220
			161.1	0.340
			191.2	0.087
			186.1	0.135
			180.7	0.176
			176.7	0.221
			170.4	0.275

ment with the original ones of Rolfe and Defren. While the tabulated values of the original paper, calculated on the factor 0.00386, are not exact, they have proved to be sufficiently approximate to be valuable aids in predicting practical laboratory results. It is intended to use more refined methods of analysis, if possible, and to locate this curve more definitely by the method of least squares. We have published the present figures, however, as conclusive of the presence of another reducing body than dextrose in acid-hydrolyzed starch products, formed under the conditions of conversion which we have defined.

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