

V.—*Experimental Methods employed in the Examination of the Products of Starch-hydrolysis by Diastase.*

BY HORACE T. BROWN, F.R.S., G. HARRIS MORRIS, Ph.D., and J. H. MILLAR.

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As we hope to lay before the Society a series of papers embodying the results of our recent work on starch-hydrolysis, we have considered it desirable to preface these communications with a detailed and critical account of the methods we have employed in the examination of the products of change. A review of this branch of the subject is the more desirable as chemists are still not agreed on a common method of obtaining or expressing their results, and a considerable amount of

misunderstanding has at times arisen from individual workers not taking sufficient pains to master the systems of notation adopted by others.

In this preliminary paper, we have endeavoured as far as possible to remove some of the causes of misunderstanding, and to show the true relations existing between the different systems of notation, and the degree of accuracy which may be reasonably expected of the analytical methods. In such an enquiry, there are many pitfalls which can only be avoided by great care and circumspection.

I.—*Estimation of Solids from Solution-density.*

For a correct determination of the specific rotatory and reducing powers of the products of starch-hydrolysis, it is essential to know the *concentration* of the solution, that is, the weight of dry solids contained in a unit volume of the solution. To obtain the amount of solids by evaporation of the solution is a laborious operation, and a task of no small difficulty, as some of the carbohydrates retain water with great obstinacy and have to be submitted to very special processes in order to expel the last traces of moisture.

This was a difficulty which was fully recognised and appreciated by O'Sullivan more than 20 years ago, and he turned the position by using the *density of the solution* as a guide to the total solids present. This principle has been very much employed since that time, although it has met with an occasional protest on the score of want of accuracy. We have no hesitation, however, in stating that when proper precautions are taken the estimation of solids from solution-density is capable of as great, or in most cases of greater, accuracy than a determination based on evaporation and drying, unless the latter method is carried out with such refinements as are practically impossible when a large number of determinations have to be made.

In his earliest papers, O'Sullivan assumed that, within certain limits of dilution, a solution of starch-products at 15.5° increases in specific gravity by equal increments of 3.85 for every gram of dry solids in 100 c.c. of the solution, water being taken at 1000 at 15.5° . A solution of either maltose or dextrin containing 10 grams of dry substance in 100 c.c. at 15.5° is stated to have a sp. gr. of 1038.5, "hence the number of grams in 100 c.c. of a solution of any specific gravity can be determined by dividing the weight above water by 3.85. For example, a solution of sp. gr. 1003.85 contains one gram; one of sp. gr. 1007.7 two grams per 100 c.c., and so on." (O'Sullivan, *Trans.*, 1876, ii, 130).

O'Sullivan used this constant divisor of 3.85 throughout all his

researches prior to 1879, but he particularly states that he only regards it as approximately accurate.

In the paper by one of us and Heron of 1879 (Trans., 1879, 35, 596), the uniform divisor of 3.86 was used in the same manner, and we have retained this factor throughout our subsequent papers, except where specially stated to the contrary, and have indicated its use in the value denoting the specific rotation and reduction by appending the number 3.86 to the respective symbols; thus $[\alpha]_{3.86}^{162^\circ}$, $\kappa_{3.86} 48$.

This divisor 3.86 for the determination of solids from solution-density was adopted after a careful determination of the density of *cane-sugar* solutions of varying concentrations. In the above mentioned paper, the results for cane-sugar were plotted in the form of a curve in such a manner as to indicate the particular divisor to be employed in order to determine from the solution-density the grams of dry sugar per 100 c.c. This curve, along with those of other carbohydrates, is reproduced in Plate I. (p. 80). It will be seen how the divisor varies with the concentration, and that whilst it is exactly 3.86 at a solution-density of 1055, it rises to 3.868 for a density of 1020.

We were quite aware, even at that time, that the true divisors for maltose, and for mixtures of maltose and dextrin, by no means correspond with the divisors for cane-sugar. Thus in the case of perfectly anhydrous maltose, for a concentration of 5.0655 grams per 100 c.c. the divisor was found to be 3.9314 (Trans., 1879, 35, 602). Since, however, the solution-densities of the products of starch-hydrolysis had not at that time been determined for varying concentration, it was decided to use the constant divisor 3.86, with the full knowledge that when the true divisors were determined the values could readily be reduced. In a footnote to the paper of 1879, attention was drawn to the fact that the analytical results, so far as *percentages of constituents* go, were really not affected by the divisor employed differing from the true divisor. This is apparent when we consider that the values taken for the specific rotatory powers of maltose and of dextrin, into terms of which all the results were calculated, were based also on this same 3.86 divisor. Where mere *percentages* of a mixture of carbohydrates are required, we might, in fact, assume a divisor for the mixed solids which might depart considerably from the true divisor and yet give a correct percentage composition, *provided always the calculation is based on the specific rotatory and reducing values of the constituents corresponding to the particular divisor taken*. Thus in working out the apparent composition of the mixed products of a starch transformation, and expressing them as percentages of maltose and dextrin, we might take a divisor of 3.86, 3.95, or in fact any number we like, the only requisite condition being that we should use specific rotatory and cupric-reducing

values for maltose and dextrin, respectively, which correspond to the particular divisor we may decide to employ.

The truth of this is obvious if we bear in mind that the values representing specific rotation and reduction, whatever notation is used, vary directly as the specific gravity divisor employed. Thus if 150° be the specific rotation of maltose for $[\alpha]_{j8.86}$ (that is, on the basis of the 3.86 divisor), the specific rotation where a divisor of 3.93 is used must be taken as $\frac{150^\circ \times 3.93}{3.86} = 152.7^\circ$, and so on. Again, if a dextrin

on the basis of the 3.86 divisor has a specific rotation of 216° , on that of the 3.95 divisor it must be taken, for purposes of calculation, at 221° . All that we have to take care of is that we employ optical and reducing constants bearing their proper relation to the divisor used, and that we do not vary the divisor in the same experiment.*

It may be considered that in the above remarks we have dwelt unnecessarily on what is evident to any one who has paid any attention to the subject, but these elementary facts have unfortunately often been misunderstood and misinterpreted by recent workers on the Continent, who have failed to apprehend the true numerical relations of the constants we have employed. Quite recently, for instance, Ost (*Chem. Zeit.*, 1895, 19, 1501) raises serious objections to our expression of the law of relation of rotation and reduction on the ground that we have estimated the amount of substance indirectly from the solution-density by means of the constant factor 3.86. This he pronounces as "allowable for approximate determinations but not for the deduction of important laws." He overlooks the fact that the numerical relations of the two properties are not in any way influenced by the greater or less accuracy of the divisor, and that the law in question only deals with these relations.

Where density of the solution is used for the determination of the solids, it is much to be desired that chemists would append an indication of the divisor used to the symbols denoting specific rotation and reduction. Such a practice would enable us to readily reduce the results where required for purposes of comparison, and it would much

* For purposes of strict comparison, the analyses must all be made in solutions of approximately the same density, and the above remarks are only *strictly* correct if the constituents of the mixture possess identically the same divisor. The larger the difference between the divisors of the constituents, the greater will be the error introduced from this source. We now know that the divisor for the dextrin or amylin portion of starch-conversion products is sensibly greater than that of the maltose constituent (*see* later in this paper), but the error introduced from this cause is but small, and does not in the least vitiate our former work, or the conclusions based thereon.

diminish the risk of misunderstanding in a subject which is in itself sufficiently difficult and complex.

If, as frequently happens, a knowledge of the percentage composition of the solids is insufficient, and we require to know the *actual weight of these per unit of volume of solution*, this must be determined either by the evaporation and drying of the residue from a given amount of solution, or it must be deduced from the density of the solution after the density constants of each constituent have been accurately determined.

We have already referred to the great difficulty experienced in driving off the last traces of moisture from many of the carbohydrates. This is especially the case with those derived from the hydrolysis of starch, which are most difficult to obtain in a perfectly anhydrous state without decomposition. After many trials, we have finally adopted a form of apparatus essentially similar to the one recommended by Lobry de Bruyn and Van Leent (*Rec. Trav. Chim.*, 1894, 13, 218.)

Two small flasks are united by a tube furnished with a stop-cock and a side-tube leading to a good air pump. The substance to be dried is introduced into one of the flasks, the other being partially filled with phosphoric anhydride. After exhausting the apparatus, the flask containing the substance is immersed in a water, salt-water, or oil-bath, according to the final temperature required, and is slowly heated up to a point at which it ceases to lose weight.

Crystallised hydrated maltose may in this manner be rendered completely anhydrous in a few hours at 105–106° without any signs of fusing or of discolouration. Hydrated dextrose is still more easily rendered anhydrous in this manner, and crystallised levulose completely loses all traces of adherent moisture below its fusing point.

The dextrins, maltodextrins, and the mixed starch-transformation products, on the other hand, require much longer drying in this apparatus, and temperatures as high as 120–130°, before losing the whole of their water.

With the aid of this convenient apparatus we have determined, with every possible precaution, the density in solution, at varying concentrations, of maltose, dextrose, levulose, soluble-starch, and the mixed products of starch hydrolysis with diastase, carried to various points, and of definite and known composition.

In tabulating the results, we have put them in such a form as to indicate the divisor to be used at the various concentrations, in order to determine the grams per 100 c.c. of solution. For any given concentration, the proper divisor can be found either by inspection of the curves given in Plate I.(p. 80), or by employing the equation of

the curves given, in each case, in the text after the experimental numbers.

It will be seen that whilst the various carbohydrates examined differ considerably in solution-density at similar concentrations, they all show that the volume occupied in solution by a unit of weight of the substance is less at lower than at higher concentrations, hence dilution must in all cases be attended with a contraction of volume.

The specific gravities given were all taken at 15.5° , and are referred to water at the same temperature. Strictly speaking, the values given are not exactly grams per 100 c.c., but the weight of substance (weighed in air) contained in a volume of the solution equal to that occupied by 100 grams of water at 15.5° , weighed in air against brass weights.

In order to convert the results into true grams per 100 c.c., where great accuracy is required, as, for instance, in determining specific rotatory constants, they must be multiplied by the factor 0.99802, thus reducing them by about 0.2 per cent.*

In the following tabulated results,

Column (a) gives the weight of dry substance taken.

Column (b) gives the total weight of solution.

Column (c) gives the specific gravity of the solution at 15.5° , referred to water at 15.5° .

Column (d) gives grams of maltose per 100 c.c. (reputed).

Column (e) gives the divisor for the determination of grams per 100 c.c. (reputed) from the specific gravity.

The greatest possible care was taken to ensure the purity of the various substances.

TABLE I.—*Solution Density of Maltose (anhydrous).*

	a.	b.	c.	d.	e.
1	1.0071	39.6425	1010.11	2.566	3.939
2	1.9783	40.8331	1019.43	4.939	3.933
3	1.4515	29.8000	1019.55	4.966	3.936
4	1.9595	40.1240	1019.59	4.979	3.934
5	4.9596	52.1081	1038.83	9.886	3.927
6	3.7107	38.1672	1039.67	10.107	3.925
7	5.8320	41.8137	1057.77	14.753	3.915
8	6.3222	33.6245	1079.14	20.291	3.900
9	7.0812	33.9980	1088.23	22.666	3.893
10	9.3267	42.3482	1093.81	24.090	3.894
11	10.3720	38.7564	1115.84	29.865	3.879
12	10.7029	34.0471	1138.39	35.784	3.867

* This factor is arrived at in the following manner. The weight of the volume of water at 15.5° to which all the densities are referred is 100 grams, weighed in air. Reduced to a vacuum, this will weigh 100.106 grams, and as the density of water at

The above results are expressed in the maltose curve of Plate I. p. 80 and the equation* for calculating the divisor for any given gravity is,

$$D = 3.9435 - 0.00044 (G - 1000) - 0.000001 (G - 1000)^2,$$

in which D = the required divisor, and G = the specific gravity of the solution.

TABLE II.—*Solution Density of Dextrose (anhydrous).*

	a.	b.	c.	d.	e.
1	1.2988	49.8518	1010.12	2.6317	3.845
2	1.6324	30.5690	1020.95	5.4516	3.842
3	3.2659	51.2638	1025.07	6.5306	3.839
4	3.2751	31.1712	1041.94	10.9470	3.831
5	8.3380	53.7774	1062.93	16.4800	3.818
6	6.4414	36.5604	1072.03	18.8870	3.813
7	9.0925	39.9196	1094.66	24.9330	3.796

These results are shown in the dextrose curve (Plate I), the equation for which is,

$$D = 3.848 - 0.00028 (G - 1000) - 0.0000028 (G - 1000)^2$$

TABLE III.—*Solution Density of Soluble Starch.*

	a.	b.	c.	d.	e.
1	0.5606	50.7022	1004.44	1.1105	3.998
2	1.1380	50.5957	1009.08	2.2691	4.000
3	2.2063	56.5394	1015.95	3.9640	4.023
4	2.3166	50.5954	1018.75	4.6645	4.019
5	1.6118	56.0157	1011.68	2.9110	4.012

15.5° is 0.99908 as compared with that of water at 4°, the true volume of the "reputed" 100 c.c. at 15.5° will be $\frac{100 \cdot 106}{100 \times .99908} = 100.198$ c.c. This represents the volume in true cubic centimetres of 100 grams of water weighed in air. The reciprocal of this 0.99802 is consequently the factor to be employed for reducing the above experimental numbers to grams per 100 true cubic centimetres. We much require a convenient term to express the volume occupied at 15.5° by a definite number of grams of water, weighed in air. This value of the cubic centimetre, which is the one employed in the graduation of most of the flasks and pipettes used in the laboratories of this country, is manifestly not identical with the true cubic centimetre. We have ventured to append the term "reputed" to the value in question. 100 "reputed" cubic centimetres are equal to 100.198 true cubic centimetres, and the ratio of a true to a "reputed" cubic centimetre is 1.0.99802.

* We are indebted to Dr. A. Lapworth for kindly calculating this and the following equations.

Nos. 1 to 4 give the results obtained with a specimen of C. J. Lintner's soluble-starch prepared by the action of dilute acid, in the cold, on ungelatinised potato-starch.

No. 5 was a specimen of C. J. Lintner's "amyloextrin," prepared with malt-extract according to his directions. This is the first product of the liquefaction of starch-paste, and gives a deep blue reaction with iodine. It is the "soluble-starch" of O'Sullivan and others.

Owing to the comparative insolubility of these preparations of soluble-starch, the range of concentration in these experiments is necessarily small, and they do not admit, on this account, of quite the same degree of accuracy as the other carbohydrates.

Solution Densities of the Mixed Products of Starch-hydrolysis.

The conversions were in these cases made with as small an amount of diastase as possible, consistent with the proper stage of conversion being reached. The mixed products, after analysis, were in the first place concentrated on the water bath, and were then transferred to the vacuum-apparatus, the temperature being slowly raised as the last traces of moisture were expelled. A final temperature of 110° is generally sufficient, but when the solution contains much soluble-starch it may be necessary to finish at 130°.

Series A.—The solution-density and corresponding divisors here given are the experimental numbers obtained with the mixed products of hydrolysis when the conversion was arrested soon after complete limpidity of the starch-paste was attained. The products had the following optical and reducing properties.

$$\begin{array}{c} [\alpha]_{j3.86} \quad 203.6^\circ \{ [\alpha]_D \quad 188.6^\circ \} \\ \kappa_{3.86} \quad 11.8 \quad [R \quad 20.2] \end{array}$$

The letters at the heads of the columns have the same significance as in the foregoing tables.

TABLE IV.

	<i>a.</i>	<i>b.</i>	<i>c.</i>	<i>d.</i>	<i>e.</i>
1	1.2733	51.0694	1010.14	2.518	4.026
2	1.7032	30.3714	1023.05	5.737	4.017
3	5.6768	52.4770	1045.30	11.311	4.005
4	6.0568	41.4603	1061.96	15.514	3.993

The variation of the divisor with the concentration is shown in the curve of the "High Transformation" of Plate I., the equation for which is,

$$D = 4.032 - 0.0006 (G - 1000).$$

Series B.—In this case the solution-density of the mixed products of a somewhat more advanced conversion were determined, the products of hydrolysis having a specific rotation and reducing power of

$$\begin{array}{l} [\alpha]_{j3.86} \quad 186.6^\circ \{ [\alpha]_D 173.9^\circ \}, \\ \kappa_{3.86} \quad 26.6 \quad [R \ 45.4] \end{array}$$

TABLE V.

	<i>a.</i>	<i>b.</i>	<i>c.</i>	<i>d.</i>	<i>e.</i>
1	1.0552	44.9101	1009.51	2.3719	4.009
2	2.7575	53.3388	1021.31	5.2791	4.002
3	3.6031	51.9826	1028.51	7.1286	3.999
4	3.6317	34.1428	1044.32	11.1080	3.989
5	7.5073	54.0266	1058.62	14.7110	3.984
6	7.9600	43.4377	1078.58	19.7640	3.975

These results are denoted graphically by the curve of Plate I, for the "Medium Conversion." The equation for finding the divisor is—

$$D = 4.012 - 0.00044 (G - 1000) - 0.000001 (G - 1000)^2.$$

Series C.—Here are given the results obtained with a low starch-conversion, that is, one in which the hydrolysis was carried almost to the lowest point, and in which the maltose is in a readily fermentable and diffusible condition. The optical and reducing properties of the mixture were as follows.

$$\begin{array}{l} [\alpha]_{j3.86} \quad 162.2^\circ \{ [\alpha]_D 149.7^\circ \} \\ \kappa_{3.86} \quad 49.2 \quad [R \ 82.8.] \end{array}$$

TABLE VI.

	<i>a.</i>	<i>b.</i>	<i>c.</i>	<i>d.</i>	<i>e.</i>
1	0.8470	35.5237	1009.55	2.407	3.967
2	1.9545	39.1675	1020.23	5.091	3.973
3	2.2602	31.3523	1029.39	7.424	3.960
4	4.4225	45.3500	1040.16	10.143	3.959
5	4.1633	41.6143	1041.23	10.417	3.958
6	4.6694	33.9971	1057.35	14.522	3.949
7	7.6485	47.4471	1067.82	17.214	3.940
8	10.5212	51.3166	1087.67	22.300	3.931

The results are given graphically in the Curve for "Low Conversion," Plate I, the equation corresponding to which is,

$$D = 3.9742 - 0.000403 (G - 1000) - 0.0000014 (G - 1000)^2.$$

When we regard the curves denoting the variation in the divisors for the mixed products of the hydrolysis of starch proceeding from the different grades of transformation, the important fact comes out that for equal concentrations the divisor to be applied for the determination of dry solids per 100 c.c. increases with the specific rotatory power of the mixture, and decreases with the rise in cupric reduction ; in other words, the divisor varies in some inverse ratio to the apparent maltose present.

If we consider the mixed products of hydrolysis as composed of maltose and dextrin, and assume that the maltose constituent has the same solution-density per unit weight, and therefore the same divisor as ordinary maltose, then from the experimental data given we can determine the divisor for the dextrin or amylin constituent.

Let us, for example, take the medium transformation of Table V, in which the specific rotatory power is $[\alpha]_{D}^{20} 186.6^{\circ}$ $\{[\alpha]_D 173.9^{\circ}\}$, and the apparent maltose is 45.4 per cent. $[R = 45.4]$.

For the mixed products of hydrolysis, we find by the experimental curve that the divisor at a density of 1020 is 4.003. The divisor for maltose which goes to make up this density is 3.939, that is, the divisor corresponding to 45.4 per cent. of the density $1020 = 1009.1$. The divisor due to the dextrin or amylin constituent will consequently be

$$\frac{20 \times 4.003 - 9.1 \times 3.939}{20 - 9.1} = 4.056.$$

This, of course, is not the amylin divisor for a density of 1020, but for a density of 54.6 per cent. of this, *i.e.*, sp. gr. 1010.9.

Working in this manner with the results of the high transformation given in the curve, we find the following series of divisors for the amylin constituent at different densities.

Density.	Divisor.
1016.24	4.038
1032.48	4.023
1048.72	4.010
1064.96	3.996

These when plotted in the usual manner give the "Amylin" Curve of Plate I., shown by a dotted line. It will be noted that this curve is much steeper than any of the others.

It may be urged that we are attaching too much importance to this calculated amylin curve expressing the variation in solution-density of the non-maltose constituent of starch transformations, and that we can adduce no certain proof that the reducing constituent or constituents of different grades of transformation are comparable in this respect with pure maltose.

This is perfectly true, and, moreover, knowing as we do that the maltose constituents of low-grade transformations exist in a readily fermentable and readily diffusible condition, whilst those of higher grade transformations exist to a great extent in some sort of combination as maltodextrins, amyloins or reducing dextrins (according to the point of view from which we regard them), it certainly did seem improbable that the solution-densities of crystallisable maltose would be applicable to the reducing constituents throughout the whole range of starch-transformation products. As a matter of undoubted fact, however, we find that when a series of dextrin or amylin divisors is deduced from the products of a single starch-transformation in the way we have indicated, then these divisors are applicable to *all other starch-transformations* of very different grades. This goes very far to show that in starch-transformations of very different stages of hydrolysis the divisor for the reducing portion is identical for the same concentration; and it necessarily follows as a corollary that there is the same constancy in the divisor of the amylin or non-reducing portion of the products of hydrolysis.

Be that as it may, however, there can be no doubt that by making use of the calculated dextrin curve given, and that of maltose, we can at all times determine within certain limits of concentration, and with a close approximation to accuracy, the divisor (and of course if required the density in solution) of any mixed products of the hydrolysis of starch prepared with diastase, provided always we know the apparent percentage of maltose, either through the specific rotation or the cupric reducing power.

In proof of this proposition, we have given below the calculated and observed divisors respectively of the mixed products of starch-hydrolysis for three transformations determined by the evaporation method. These are transformations occupying very different positions in the range between soluble starch and maltose, and are those of which the curves are given in the Table. The calculations are made for four different specific gravities of solution.

TABLE VII.—*The observed and calculated divisors of three starch-transformations.*

[α] _D	R.	Sp. gr. 1020.			Sp. gr. 1040.			Sp. gr. 1060.			Sp. gr. 1080.		
		Calculated.	Observed.	Difference.	Calculated.	Observed.	Difference.	Calculated.	Observed.	Difference.	Calculated.	Observed.	Difference.
188.6°	20.2	4.020	4.020	.000	4.008	4.008	.000	3.997	3.997	.000	3.985	3.985	.000
173.9°	45.4	3.997	4.003	+.006	3.990	3.992	+.002	3.982	3.982	.000	3.974	3.971	-.003
149.7°	82.8	3.959	3.964	+.005	3.949	3.955	+.006	3.941	3.946	+.005	3.933	3.933	.000

The following general formula is applicable for the determination of the divisor for the mixed products of the hydrolysis of starch when the value of R (apparent percentage of maltose) is known.

$$D = \frac{\frac{RS}{100} d' + \left(S - \frac{RS}{100} \right) d''}{S}$$

in which

S = the specific gravity of the solution of the mixed products of the hydrolysis of starch at 15.5° after deducting 1000 (water at 15.5° = 1000).

R = the apparent percentage of maltose.

$$S \text{ is made up of } \frac{RS}{100} + \frac{S - RS}{100}$$

d' = the divisor for maltose at a concentration of $\frac{RS}{100}$. This value is obtained from the maltose curve on Plate I.

d'' = the divisor for the dextrin or amylin constituents at a concentration of $\frac{S - RS}{100}$. This value is obtained from the amylin or dextrin curve shown by the broken line on Plate I.

D = the required divisor for the mixed products of starch-hydrolysis.

For practical use in the examination of starch-transformations, we have calculated, by means of the above formula, the divisors of the mixed products of starch-hydrolysis for specific gravities of 1010, 1020, 1030, and 1040, and for increments of 5R between R=0 and R=100. If the specific gravity of any mixture of starch-transformation products has been determined within these limits of concentration and the approximate value of R (which may, if desired, be obtained by the 3.86 divisor), we can at once, by reference to this Table (p. 84), find the appropriate divisor for the determination of the grams of solid matter per 100 c.c.

TABLE VIII.—*The divisors for the mixed products of the hydrolysis of starch corresponding to R and $[\alpha]_D$.*

α_D	R.	Sp. gr. 1010.	Sp. gr. 1020.	Sp. gr. 1030.	Sp. gr. 1040.
198.8	5	4.038	4.031	4.023	4.015
195.6	10	4.033	4.027	4.020	4.013
192.4	15	4.029	4.023	4.017	4.010
189.2	20	4.024	4.019	4.013	4.008
186.0	25	4.019	4.014	4.010	4.005
182.8	30	4.014	4.011	4.006	4.001
179.6	35	4.009	4.005	4.002	3.997
176.4	40	4.004	4.001	3.997	3.993
173.2	45	3.999	3.996	3.992	3.989
170.0	50	3.994	3.991	3.987	3.985
166.8	55	3.989	3.986	3.983	3.979
163.6	60	3.984	3.980	3.977	3.974
160.4	65	3.979	3.975	3.972	3.969
157.2	70	3.973	3.970	3.966	3.963
154.0	75	3.968	3.964	3.961	3.957
150.8	80	3.962	3.958	3.955	3.951
147.6	85	3.957	3.952	3.948	3.945
144.4	90	3.951	3.946	3.942	3.938
141.2	95	3.945	3.940	3.935	3.931
138.0	100	3.939	3.934	3.929	3.924

II.—*The Determination of Specific Rotatory Power.*

The photogyric properties of a substance in solution, that is, the properties in virtue of which the substance is capable of rotating the plane of a linear polarised ray of light, are referred, for purposes of comparison, to a standard concentration and a standard length of column of the solution.

The specific rotatory power of an optically active substance in solution may be defined as the angle through which a linear polarised ray of light of definite refrangibility is rotated by a column, 1000 millimetres in length, of a solution containing 10 grams of the substance in 100 c.c.

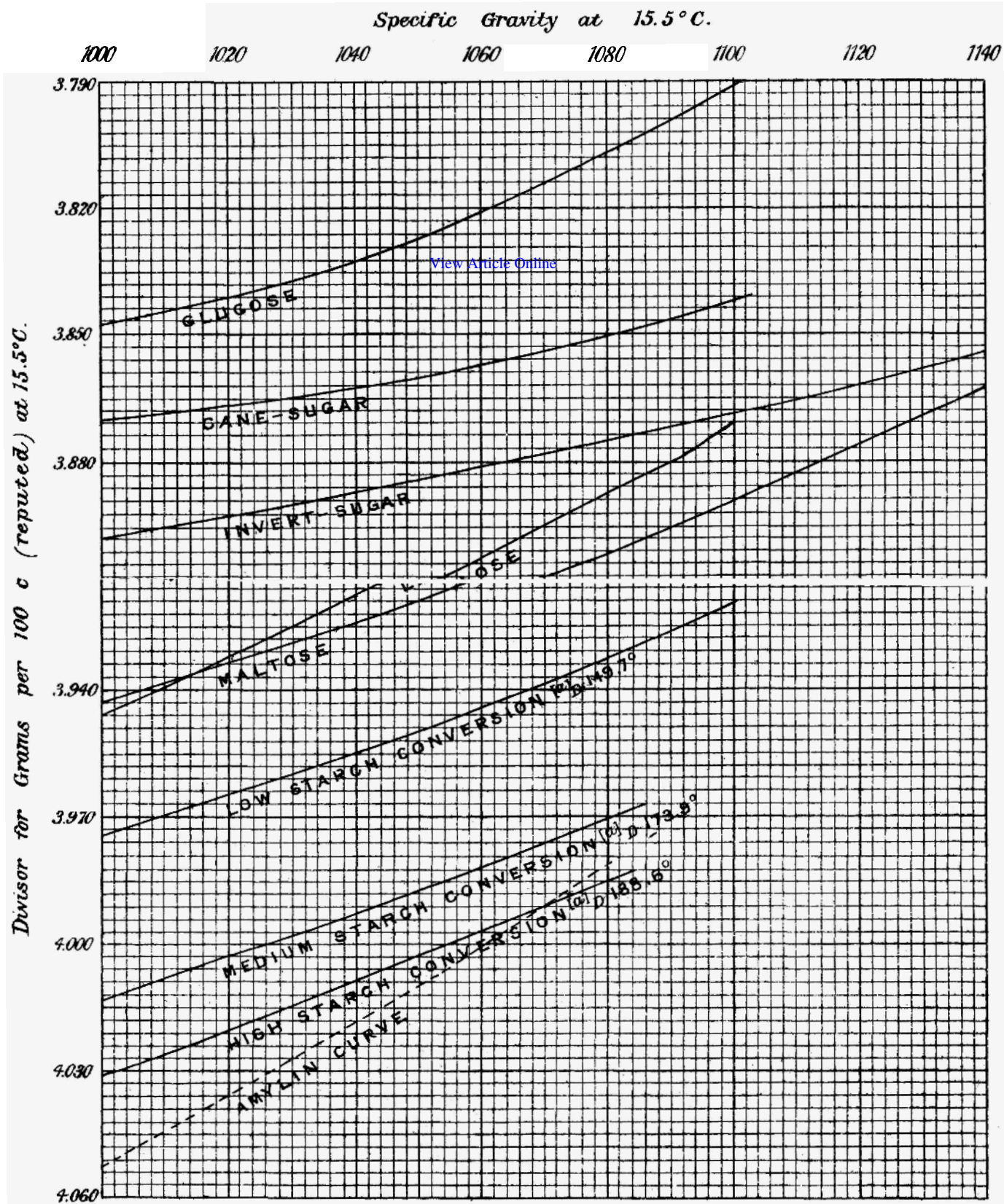
For the determination of specific rotatory power we must know,

- a. The observed angle of rotation for a ray of definite refrangibility ;
- c. the concentration of the solution expressed in grams per 100 c.c. ;
- L. the length of the column of the solution in millimetres.

The specific rotation $[\alpha]$ is then expressed by the formula

$$[\alpha] = \frac{10^4 a}{L \times c}.$$

DIVISORS FOR CARBOHYDRATES AT VARIOUS DENSITIES



It will be convenient in the first place to consider the value of c in the above formula. Where absolute accuracy is required, as, for instance, in the determination of the specific rotation of a pure substance and its variation under different degrees of concentration in a given solvent, we must substitute for c the number of grams of substance contained in 100 *true* c.c. of the solution. If we denote the density of the solution at 15.5° , referred to water at 15.5° , by d $15.5^\circ/15.5^\circ$, and the percentage weight of the substance in solution by p , then d $15.5^\circ/15.5^\circ \times p$ equals the number of grams of substance in 100 *reputed* c.c. at 15.5° : that is, in a volume of the solution equal to that occupied by 100 grams of water at 15.5° , weighed in air. This is, of course, as we have already seen, not exactly the same thing as grams per 100 *true* c.c. In order to convert "reputed" into true c.c. we must ascertain the density of the solution at 15.5° referred to water at 4° , with due correction for air displacement. If we express this by d $15.5^\circ/4^\circ$ the concentration will then be expressed by $c = d$ $15.5^\circ/4^\circ \times p$.

A near approach to accuracy will be attained, as we saw in the last section, by multiplying the grams in 100 reputed c.c. at 15.5° by the factor 0.99802.

The following formula, as given by Landolt, is of general application for determining the value of d $t^\circ/4^\circ$, where t° represents the temperature at which the density of the solution has been determined with reference to water at the same temperature.

$$d \ t^\circ/4^\circ = \frac{F}{W} (Q - \delta) + \delta$$

where

W = the weight of water ;

F = the weight of an equal volume of the solution ;

Q = the density of water at t° , that of water at 4° being taken as unity ;

δ = the weight of 1 c.c. of air in grams.

For δ the value of 0.00119 may be taken for temperatures between 10° and 25° , and at barometric pressures between 720 and 770 mm.

The value of α varies with the refrangibility of the linear polarised ray, and it is therefore necessary in expressing $[\alpha]$ to specify the position in the spectrum of the particular ray employed in the determination.

There are two systems of notation in use at the present time. In one the value of $[\alpha]$ is referred to the ray D of the solar spectrum, the specific rotation being then expressed by $[\alpha]_D$; whilst in the other

notation the photogyric properties of the substance are referred to the so-called "medium yellow ray," the complement of Biot's *teinte sensible* or "transition tint," in which case the specific rotation is expressed by $[\alpha]_j$. In the determinations of $[\alpha]_D$, the sodium light is used in a Mitscherlich, Wild, Jellett-Cornu, or Laurent instrument, and the angular rotation produced by the solution is determined in degrees of arc. The scale is consequently a natural one, and differences of rotatory dispersion in the substances examined do not interfere with the comparison of results.

The determinations of $[\alpha]_j$ are made by the aid of neutral-tint or half-shade polarimeters, such as the older instruments of Soleil, or their more recent modifications by Ventzke and Scheibler. The scale divisions of these instruments are of an arbitrary character, and have to be converted into angular values before specific rotations can be calculated. It cannot be too clearly borne in mind that the readings given by all such instruments are based on the rotation value of a quartz plate, cut perpendicular to its optic axis, and that no matter what the arbitrary scale employed may be, the readings must in the first place necessarily be in terms of the rotation of quartz for a plate of definite thickness. These instruments have also the disadvantage that, without proper reduction of the readings, they cannot be used for the comparison of the rotation values of different substances which happen to have a sensibly different power of rotatory dispersion from that of quartz. This fact was fully recognised in the early days of the original Soleil instruments, and is very clearly expressed in the writings of Biot, on whose discoveries the saccharimeter of Soleil was based.

In his earlier work, O'Sullivan used a Soleil-Duboscq instrument for the determination of $[\alpha]_j$, in which 100 divisions of the scale corresponded to 24° of arc, this being the angular rotation experienced by Biot's "medium yellow" or *jaune moyen* ray (as determined by the transition tint) in traversing a quartz plate of 1 mm. in thickness, cut perpendicular to the optic axis.

In the Soleil-Duboscq instrument as improved by Ventzke and Scheibler, the direct dependence of the graduation on quartz values was somewhat obscured, 100 scale-divisions being made to correspond to the amount of rotation experienced by the "medium yellow" in passing through a column of a solution of pure cane-sugar 200 mm. in length, containing 26.048 grams of cane-sugar per 100 c.c. at 17.5° . Such a solution has a specific gravity of almost exactly 1100 (water at $17.5^\circ = 1000$). The readings for cane-sugar in these instruments consequently correspond closely to the specific gravity of the solution less 1000. There is no special advantage for general purposes in this

change of graduation, and it has been rather an unfortunate one, as tending to obscure the fundamental fact underlying the construction of such instruments, that observations made with them are in reality all referred to the photogyric properties of a definite thickness of quartz. In the old Soleil-Duboscq instrument, as we have already said, each scale-division was equal to the rotation experienced by the "medium yellow" or *jaune moyen* of Biot in passing through $\frac{1}{100}$ th of a millimetre of quartz,* that is, to a rotation of 0.24° of arc. In the case of the later instruments which we have described as being standardised by a solution of cane-sugar of definite strength, the arbitrary scale-divisions, in order to be converted into degrees of arc, have also to be translated into quartz values, which can be effected by making use of the known relations of the rotatory power of quartz and cane-sugar established by Biot for his neutral or transition tint. When the millimetre-quartz value of 24° is taken for the transition tint, the specific rotatory power of cane-sugar for medium concentrations (5 to 15 grams per 100 c.c.) is found to be 73.8° , and from this number the value of each scale-division can of course be estimated. In the instrument we are now using, a half-shade Schmidt and Haensch, we find that 100 divisions of the scale correspond to 38.43° of arc, taking the photogyric value of 1 mm. of quartz for the transition tint at 24° .

A considerable amount of confusion has arisen of late years with regard to the use of the expression "medium yellow" as applied to the position in the spectrum of the particular ray used in the determination of $[a]_j$. As this has led to very contradictory statements on the relations existing between $[a]_j$ and $[a]_D$, we must refer somewhat in detail to the history of the subject in order to show how this serious misunderstanding arose.

When a quartz plate cut perpendicular to its optical axis is placed between two Nicol prisms the "transition tint" or *teinte sensible* is observed when the principal plane of the analysing Nicol is parallel to the plane of polarisation of the *jaune moyen* ray of Biot. In this relative position of the polariser and analyser, the *jaune moyen* ray is *extinguished*, the rays which collectively form the complementary transition tint being alone transmitted. This may be seen by passing the light, as it emerges from the analyser, through a spectroscope, when it will be found that the spectrum is crossed by a dark band corresponding to the missing ray. The rotation which this ray experiences in passing through a quartz plate was determined by Biot and found to be 90° for a thickness of plate of 3.75 mm. or 24° for

* In speaking of the rotatory values of quartz plates throughout this paper, it is understood that such plates are all cut perpendicular to the optic axis of the crystal.

a thickness of 1 mm. From this rotation, we can determine the refrangibility of the *jaune moyen* ray expressed in wave-lengths, by employing the following dispersion formula of Boltzmann, which is approximately accurate for rays of any refrangibility.

$$a = \frac{7.07018}{10^6 \times \lambda^2} + \frac{0.14983}{10^{12} \times \lambda^4}$$

where a = the angle of rotation which a given ray experiences in passing through a quartz-plate 1 mm. in thickness; whilst λ is the wave-length of the ray expressed in millimetres.

If we substitute 24 for a in the above formula, we find that the *jaune moyen* ray of Biot, as determined by its complement, the transition tint, has a wave-length of λ 0.0005608. The yellow of the solar spectrum extends according to Thalen from λ 0.000535 to λ 0.000586, so that the true mean yellow has a refrangibility corresponding with λ 0.000560, which is identical with that of Biot's *jaune moyen*.

Up to about the year 1874, amongst those chemists who had a clear idea of the meaning of $[\alpha]_j$, as distinguished from $[\alpha]_D$, there seems to have been no difference of opinion as to the particular ray to which $[\alpha]_j$ was referred; it was always the *jaune moyen* of Biot, which is rotated through 24° by 1 mm. of quartz, and which, as we have seen above, is identical in refrangibility with the true mean yellow of the solar spectrum.

In or about 1874, the innovation was made which has resulted in so much confusion, and which has practically introduced a totally different set of values for $[\alpha]_j$. It has not hitherto been recognised that we have at the present time two different ways of expressing specific rotation as $[\alpha]_j$, and that these two systems, in which exactly the same symbol is used, are not even referred to the same ray of the spectrum, and that consequently they bear quite different relations to $[\alpha]_D$, even for substances of exactly similar rotatory dispersion.

As far as we have been able to ascertain, it was Montgolfier who was in the first instance responsible for this serious confusion.

In a paper published in 1874 entitled "Pouvoirs Rotatoires du camphre, et de quelques autres corps" (*Bull. Chim., Soc.* 22, 487), after justly calling attention to the confusion which at that time still existed amongst certain authors as to the expressions $[\alpha]_j$ and $[\alpha]_D$, he proceeds to discuss the relative rotation, for the same substance, of the D ray on the one hand, and of the *jaune moyen* of Biot on the other. It is not at all clear from the description how the comparison was made, but for the determinations of the rotation of the D ray he appears to have used a Cornu instrument.

At the commencement of the table in which the results of his

experiments on sugar and camphor are given, he correctly states that the rotation of the D ray in passing through a quartz plate 1 mm. thick is 21.67° , but he gives the rotation of the *jaune moyen* of Biot, determined by the transition tint, as 24.5° for the same thickness of quartz, instead of 24° as determined by Biot himself. In the column headed "Rapport des deviations" he gives 1 : 1.048, with Broch's name attached to it, whilst the relations of the two rotations for a series of by no means concordant experiments with cane-sugar vary from 1.1167 to 1.1350. The important point, however, to bear in mind is that we here have for the first time a reference of the rotation of Biot's *jaune moyen*, not to the millimetre-quartz value of 24° , as originally determined, but to a ray of decidedly higher refrangibility with a millimetre-quartz rotation of 24.5° .

It is not difficult to see how this value of 24.5° was arrived at. It is the exact arithmetical mean of the rotation experienced by the D ray and the E ray of the solar spectrum in passing through 1 mm. of quartz; the rotation of D being 21.67° , and of E 27.4° under these conditions, and to this ray the term "medium yellow" was improperly applied as a synonym for Biot's *jaune moyen*, although it is a ray of quite a different refrangibility.

Unfortunately Landolt, in his book on the Polariscopes, adopted the same ray as Montgolfier had done for the "medium yellow," objecting to the "transition tint" as corresponding to no sharply-defined ray, but he fails to draw attention to the important fact that if values of $[a]_D$ are referred to this new ray they cannot be directly compared with determinations made on the basis of Biot's *jaune moyen*. For instance, the specific rotatory power of cane-sugar for a certain medium concentration is $[a]_D 73.8^\circ$ if we refer it to the true medium yellow of Biot; but if the specific rotation is to be referred to Montgolfier and Landolt's "medium yellow" the value becomes $\frac{24.5}{24} \times 73.8^\circ = 75.3^\circ$, a very serious difference.

The value of each scale division of the Ventzke-Scheibler or any similar instrument when expressed in degrees of arc will also be materially influenced by the particular "medium yellow" we chose to refer to. If one scale division, as on our instrument, is equal to 0.3843° for Biot's ray, it will correspond to $\frac{24.5}{24} \times 0.3843^\circ = 0.3923^\circ$ for the so-called "medium yellow" of Montgolfier and Landolt.* It is

* These facts must be carefully borne in mind when reading Landolt's book on the polariscopes, as he altogether omits calling attention to the important results which follow from a selection of a "medium yellow" of different refrangibility from Biot's original ray.

most important to remember these facts when the ratio of $[a]_j$ to $[v]_D$ is discussed, as we shall have occasion to see later on.

We have already stated that some objection has been made to the employment of Biot's *jaune moyen* ray on the ground that it is not a ray of very definite refrangibility. This is, however, by no means a valid objection, since the wave-length admits, as we have seen, of being calculated from the known rotation for a given thickness of quartz. This original ray of Biot has, in fact, as great a title to definite refrangibility as the substituted ray of Montgolfier, and has the extra advantage of really being what its name implies, *the true medium yellow of the solar spectrum*, that is, a ray with a wave-length of 0.0005608, which is almost exactly intermediate between the value of λ for the sodium and thallium lines. Montgolfier, on the other hand, and more recently Landolt and others, have, for $[a]_j$ determinations, made use of a ray having certain relations to the lines D and E, which bound a portion of the spectrum somewhat more extended than the yellow as defined by Thalen. They have not, however, chosen the ray intermediate in *wave-length* between D and E, but have taken the one which undergoes *an intermediate or about an intermediate amount of rotation on passing through 1 mm. of quartz*. The wave-length of this empirically chosen ray, which, unlike the D ray, or the *jaune moyen* of Biot, corresponds with no ray employed in any existing instrument, can of course be calculated with the aid of Boltzmann's dispersion formula from its millimetre-quartz rotation value of 24.5° . We find it to be λ 0.0005553, so that the Montgolfier ray for $[a]_j$ lies considerably on the green or more refrangible side of the true medium yellow, whilst the Biot ray, on the other hand, as determined by the transition tint, corresponds very closely with the true medium yellow.

III. *The Relation of $[a]_j$ to $[a]_D$.*

It will be evident from the remarks in the foregoing section, that in speaking of $[a]_j$ and the relations which exist between this and $[a]_D$, we shall in future have to specify to what particular ray in the yellow the $[a]_j$ value is referred, whether on the one hand to the true medium yellow or *jaune moyen* of Biot, with a wave-length of 0.0005608, and a millimetre-quartz rotation of 24° , or on the other to the so-called medium yellow of Montgolfier and Landolt, with a wave-length of 0.0005553, and a millimetre-quartz rotation of 24.5° . Where there is any chance of confusion we shall refer to the former as $[a]_j$ Biot, and to the latter as $[a]_j$ Montgolfier.

As the determinations of both values of $[a]_j$ are dependent either directly or indirectly on quartz values, it is manifest that there can

be no constant factor applicable to the reduction of $[\alpha]_j$ to $[\alpha]_D$ in the case of substances whose rotatory dispersion sensibly differs from that of quartz. There will be a constant factor for each separate substance under these circumstances, but it will not be applicable to a series of substances unless they are of equal rotatory dispersive power.

With the exception of cane-sugar, there are but few of the carbohydrates which have had their rotatory dispersive powers compared.

The specific rotation of cane-sugar for rays of different refrangibility was investigated by Arndsten (*Ann. Chim. Phys.*, 1858, [3], 54, 403) and by Stefan (*Sitz. Ber. d. Wiener Akad.*, 52, ii, 486), who used Broch's spectroscopic method. Their results show the almost absolute identity of the rotatory dispersion of cane-sugar and quartz throughout the whole visible spectrum. Quite recently the subject has been further investigated by Landolt (*Ber.*, 1894, 27, 2872), who employed a new method based on the direct determination of the angular deviation of rays of definite refrangibility obtained by passing white light through a series of coloured screens. Landolt has in this way been able to still further confirm the exact correspondence of quartz and aqueous solutions of cane-sugar as regards rotatory dispersion.

A comparative determination of the specific rotatory power of dextrose for different rays was made by Hoppe-Seyler in 1866 (*Zeits. anal. Chem.*, 1866, 412). His results indicate that the rotatory dispersion of dextrose, whilst closely approximating to that of quartz, and consequently of cane-sugar, is really very slightly greater.

Bearing in mind the identity of the rotatory dispersions of cane-sugar and quartz, it is evident that the factor necessary to convert $[\alpha]_j$ Biot into $[\alpha]_D$ in the case of cane-sugar will be identical with the ratio of the rotations experienced by the D ray and *jaune moyen* ray respectively in passing through quartz plates of equal thickness. The D ray in passing through a quartz plate 1 mm. thick is rotated, according to Broch, Stefan, and to Wild, through 21.67° , whilst the *jaune moyen*, according to Biot, is rotated through 24° under the same conditions. The ratio of these numbers is 1.1075 , and the latter number is the factor to be applied in converting values of $[\alpha]_j$ Biot into $[\alpha]_D$ in the case of all substances having an equal rotatory dispersion to that of quartz or cane-sugar.

The factor for the conversion of $[\alpha]_j$ Montgolfier into $[\alpha]_D$ with the same limitations as regards dispersion will of course be $24.5/21.67 = 1.130$.

It will be seen that there is sufficient difference in these two factors to cause grave errors in the conversion of one notation into the other if the facts we have stated are not kept constantly in mind. It is, for

instance, by no means an unusual thing to find papers published within the last ten or fifteen years in which the factor applicable only to $[\alpha]_j$ Montgolfier has been used for the conversion of $[\alpha]_j$ Biot into $[\alpha]_D$.

In considering how far the factors 1.1075 and 1.130 are applicable to the carbohydrates with which we have most to do in this particular investigation, we have in the first place to satisfy ourselves as to whether the carbohydrates produced during starch-hydrolysis differ sensibly from cane-sugar in *rotatory dispersion*. Unfortunately, we have very few published data from which any conclusions can be drawn. It occurred to us, however, that if the carbohydrates of starch-hydrolysis differ in rotatory dispersion from cane-sugar to a sufficient extent to be of any practical moment, this ought to become evident by a direct comparison of the various solutions in a Ventzke-Scheibler quartz instrument, and in a sodium-light instrument such as the Jellet-Cornu or Laurent.

If we are dealing with a series of substances which do not differ *inter se* in *rotatory dispersion*, then, no matter what the actual differences may be in the specific rotatory powers, the number of scale-divisions of the Ventzke-Scheibler instrument (in which we measure the rotation of the *jaune moyen* ray) corresponding to a degree of arc of the sodium-light instrument ought always to be the same. If there is not this correspondence, it can only be due to the fact that the *rotatory dispersions* of the different substances are not identical. This method of comparison will also give us the factor to be used for each substance in converting $[\alpha]_j$ into $[\alpha]_D$ or *vice versa*.

Through the kindness of Dr. Armstrong, who put his fine Jellet-Cornu polarimeter at our disposal, we have been able to make careful comparisons of this kind with solutions of (1) cane-sugar, (2) dextrose, (3) maltose, and (4) the mixed products of starch-hydrolysis apparently containing 80 per cent. of maltose and 20 per cent. of dextrin. The last-mentioned solution had a specific rotation of $[\alpha]_{j,86} 162^{\circ} \cdot 0$.

Numerous closely concordant readings of each solution were taken in each polarimeter successively, using a 200 mm. tube, which was interchangeable with the two instruments.

The circle-readings of the Jellet-Cornu were taken in two parts of the scale 180° apart, in order to correct for any irregularities in the calc-spar prisms, and all care was taken to make the comparisons as rigorous as possible. It is unnecessary to give the details of the observations, which are summarised below.

Column A gives for each separate solution the result of dividing the scale reading of the V.-S. instrument by the circle reading of the Jellet-Cornu polarimeter: it consequently represents the number of

V.-S. scale divisions corresponding to 1° of the sodium-light instrument.

Column B gives the value of a scale division of the V.-S. instrument expressed in terms of ray D.

Column C gives for each solution the ratio of $[\alpha]_j$ Biot to $[\alpha]_D$. As cane-sugar and quartz have exactly the same rotatory dispersion, the ratio in this case is $24/21.67 = 1.107$, and on this basis the other figures in this column are made proportional to those of column A. These ratios of column C can also be obtained by dividing the results of column B into 0.3843, which gives the angular value of each scale division of the V.-S. instrument expressed in terms of Biot's *jaune moyen*.

Column D gives the ratio of $[\alpha]_j$ Montgolfier to $[\alpha]_D$ in each case.

TABLE IX.

		A.	B.	C.	D.
Cane-sugar	10 p.c.	2.882	0.3469	1.107	1.130
Maltose.....	10 p.c.	2.899	0.3449	1.113	1.136
„ ..	5 p.c.	2.892	0.3457	1.111	1.134
Dextrose	10 p.c.	2.904	0.3442	1.115	1.138
„ ..	5 p.c.	2.894	0.3454	1.111	1.134
Starch-products	10 p.c.	2.891	0.3458	1.111	1.134
„ „ ..	5 p.c.	2.895	0.3454	1.111	1.134

It will be observed that there are slight but sensible differences between cane-sugar and the other carbohydrates examined, and these can only be explained by assuming that *cane-sugar is slightly less dispersive than the other substances*. The factor 1.107 for the conversion of $[\alpha]_j$ Biot into $[\alpha]_D$ is only strictly correct for cane-sugar, and is slightly too low for the other carbohydrates examined, for which the factor 1.111 is more nearly correct. This is the value we have adopted for the translation of $[\alpha]_j$ Biot of starch-products into $[\alpha]_D$, whilst 1.134 represents the ratio of $[\alpha]_j$ Montgolfier to $[\alpha]_D$, for starch-products somewhat more accurately than does the cane-sugar factor 1.130.

It was only after planning and completing the above experiments that our attention was directed to a paper by Landolt published in 1888 (*Ber.*, 21, 191) in which he mentions that he has applied the same process of comparison to cane-sugar, milk-sugar, dextrose, invert-sugar, cholesterin, and turpentine oil, using a half-shadow V.-S. quartz instrument by Schmidt and Haensch, and two polaristrometers of Laurent and Lippich. The results are given only in the

form of the values of one division of the V.-S. instrument in degrees of the sodium-light instruments, and are comparable with column B of the above table. He obtains for cane-sugar the value 0.3465, and for dextrose the value 0.3448, numbers remarkably close to our own, and indicative of the same want of exact correspondence of the rotatory dispersion of these two carbohydrates.

We have still to consider the relation of the $[\alpha]_j$ values to those of $[\alpha]_D$ in cases where the former value has been determined on the solids deduced from a divisor which differs sensibly from the true divisor. In order to convert values of $[\alpha]_{j \cdot 86}$, for instance, into $[\alpha]_D$, it is not sufficient merely to know the above-mentioned optical relations of $[\alpha]_j$ to $[\alpha]_D$, but we must know also the true divisor for the solids at the particular concentration at which the determination is made. The true value of $[\alpha]_j$ is then obtained by raising the $[\alpha]_{j \cdot 86}$ value in the proportion of 3.86 to the true divisor, and $[\alpha]_D$ is deducible directly from the result by applying the optical relations of $[\alpha]_j$ to $[\alpha]_D$ given above.

This will be evident from the following example.

The specific rotatory power of maltose, in a 5 per cent. solution, when calculated on the basis of the 3.86 divisor for the solids, is $[\alpha]_{j \cdot 86} 150.5^\circ$. On reference to the maltose curve of Plate I, we find the true divisor for maltose at this concentration is 3.934, and as the optical relations of $[\alpha]_j$ to $[\alpha]_D$ for a substance of the dispersive power of maltose are as 1.111:1 the value of $[\alpha]_D$ for maltose calculated in this manner will be

$$150.5 \times \frac{3.934}{3.86} \div 1.111 = 138.05$$

The following general formula is applicable to the products of starch-hydrolysis for the conversion of $[\alpha]_{j \cdot 86}$ Biot into $[\alpha]_D$.

$$[\alpha]_D = [\alpha]_{j \cdot 86} \times \frac{D}{3.86} \div 1.111$$

where D, which is obtained by inspection of Table VIII., p. 84, is the true divisor for the particular grade of transformation, and for the particular concentration of the solution.

IV. *The Determination of the Cupric-reducing Power.*

In determining the important factor of the cupric-reducing power of the products of starch-hydrolysis, we have hitherto adopted a modification of the gravimetric method, described by C. O'Sullivan in 1876 (Trans., 1876, ii, 130), and provided the conditions under which it is carried out are exactly defined and followed, it is one which is

capable of yielding very consistent and concordant results. Careful attention must, however, be paid to the following points.

- (1). The exact composition of the Fehling's solution, especially as regards the nature and amount of the alkali it contains.
- (2). The degree of dilution of the Fehling's solution.
- (3). The restriction of the amount of copper reduced within certain prescribed limits.
- (4). The mode of heating the solution and the time occupied in reduction.*

When all these conditions have been definitely fixed for any particular reducing sugar, and the amount of Cu or CuO corresponding to 1 gram of the sugar under these conditions has been determined, there must be no deviation from them in any analytical process. If for any reason a deviation becomes necessary, the reducing power of the sugar in question must be again determined under the altered conditions.

The following are the normal conditions under which all our determinations are made.

1. Composition of the Fehling's solution, in grams per litre.†

* An extremely important paper on the action of alkaline copper solutions on the sugars has recently been published by J. Kjeldahl (*Résumé du Compte-rendu des travaux du laboratoire de Carlsberg*, 4^{me} vol., 1^{re} livr. 1895), in which the influence of varying conditions on the amount of copper reduced is very fully discussed. It is shown that the amount of copper reduced by any given amount of sugar is appreciably decreased as the surface of liquid exposed to the air is increased; that within certain wide limits the amount of soda has little influence on the reducing power of dextrose, but with maltose and lactose a different proportion of soda exercises a considerable effect on the amount of copper reduced; that the longer the liquid is heated the greater the amount of reduction, this being apparently due to spontaneous reduction; that the extent to which spontaneous reduction takes place depends on the dilution of the Fehling's solution; that the amount of copper reduced by a given weight of sugar is greatly influenced by the state of dilution of the Fehling's solution: the greater the dilution, the smaller the reduction. Kjeldahl also gives an elaborate series of tables showing the amount of copper reduced by different weights of dextrose, levulose, invert-sugar, galactose, lactose and maltose under varying conditions. In making the determinations, Fehling's solution of the same composition as that used by us was taken, but the heating was continued for 20 minutes, and all the reductions were made in hydrogen, the result being that Kjeldahl's values for maltose are about 6 per cent. higher than those obtained by our method. We shall refer more fully to this work of Kjeldahl in a subsequent paper.

† As Fehling's solution is very prone to undergo change, it is essential that it should either be freshly prepared for each series of experiments, or be stored in two solutions and mixed when required. In the latter case, the above quantity of copper sulphate is dissolved in 500 c.c. of water, and the Rochelle salt and alkali in a similar volume; the two solutions are then mixed in equal volumes immediately before each determination is made.

Recrystallised copper sulphate.....	34·6 grams.
Rochelle salt	173·0 „
Anhydrous sodium hydroxide	65·0 „

As the oxidising power of the solution is very sensitive to small changes in the amount of alkali present, great care must be taken to ensure accuracy in the above weight of sodium hydroxide.* The influence of varying amounts of alkali will be more fully referred to later.

2. The degree of dilution of the copper solution, after taking into account the volume of the sugar solution added, is one part of Fehling's solution to one part of water; 50 c.c. of the undiluted Fehling being used in each experiment, and made up to 100 c.c.

3. An amount of the reducing sugar is taken which will give a weight of CuO lying within the limits of 0·15 to 0·35 gram.

4. The diluted Fehling's solution is heated in a small beaker,† in a boiling water bath until the temperature is constant, and then the weighed or accurately measured volume of liquid containing the reducing substance is added, and the heating in the water bath continued for exactly 12 minutes, the beaker being covered with a clock glass during the whole period of heating. The filtration is performed as rapidly as possible through a Soxhlet's tube under reduced pressure, and the cuprous oxide is either oxidised to CuO in a stream of oxygen, or is weighed as Cu after reduction in hydrogen.

As the most carefully prepared Fehling's solution always gives a very slight precipitate of cuprous oxide on heating, due to spontaneous reduction, it is necessary that this should be determined for every fresh batch of the solution, and be allowed for in the final result of each determination. This spontaneous reduction usually amounts to from 0·002 to 0·003 gram of CuO per 50 c.c. of Fehling's solution employed.

In order to conveniently compare the reducing power of various substances, it has been usual to refer the results to some definite standard, which is either that of dextrose or of maltose. C. O'Sullivan adopted *dextrose* as his standard, and referred the reducing powers of

* The nature of the alkali in the Fehling's solution is also important in dealing with the cupric-reduction of maltose and the starch-transformation products. This has recently been well shown by Glendinning (Trans., 1895, **67**, 999—1002), who obtained considerably higher results when potassium hydroxide was substituted for the sodium compound. It is necessary to bear this in mind, as in some analytical works the formula for Fehling's solution contains potash in place of soda.

† It is important that the beaker used should always be of the same size and shape. In all our experiments the surface of the liquid had an area of 44 square centimetres.

all substances to it. He took its specific reducing power as 100, and then a substance which had half the reducing power of dextrose, weight for weight, was said to have a specific reducing power of 50, whilst a substance having, say, one-tenth the reducing power of dextrose had a specific reduction of 10, and so on. O'Sullivan used the symbol K to express the specific reducing power so found, and in our previous papers we have used the symbol κ to express the same value. When the solid matter, on which the reductions were calculated, was found by the 3.86 divisor, we have expressed this fact, just as we have done in the case of the specific rotatory power, by appending the figures 3.86 to the symbol. Thus $\kappa_{3.86} = 25$ means that the reducing power of the substance is one-quarter that of dextrose when the solid matter is determined by the 3.86 divisor.

In referring the reducing power of the sugars to dextrose, O'Sullivan stated that, under the particular conditions he employed, one gram of dextrose reduced exactly 2.205 grams of CuO .^{*} We find, however, that this value is only obtained if we employ conditions of reduction differing materially from those laid down above as our standard conditions for the estimation of the reducing power of starch-transformation products. Thus, the commonly accepted value of 2.205 grams of CuO per gram of dextrose is only correct when the Fehling's solution is diluted with considerably more water than that given above, when nearly the whole of the copper present is reduced, and when the amount of alkali in the Fehling's solution is less than 65 grams per litre. Under our conditions, the amount of CuO reduced by one gram of dextrose varies between 2.558 and 2.321 grams, the exact amount depending on the extent to which reduction takes place. We shall refer more fully to this in a subsequent paper.

Notwithstanding the fact that the ratio of 2.205 : 1 does not represent the true relation of CuO reduced to dextrose oxidised when working under our standard conditions of reduction, yet this does not in the least stultify the values of K or κ , which have been given by O'Sullivan and ourselves in previous papers. It is only necessary to remember the basis to which the values are referred, and then there is no reason why this basis should not be a purely arbitrary one. As we shall presently show, one gram of maltose, estimated by the 3.86 factor, reduces under our standard conditions almost exactly 1.345 gram of CuO , and so long as this value is constant it is immaterial on what basis it is expressed, provided, of course, that all results are referred to the same basis, and that this is clearly stated. Thus, on the 2.205 basis the cupric-reducing power of maltose is

^{*} Since the above was written, C. O'Sullivan and A. L. Stern have shown (Trans., 1896, **69**, 1691) that under their standard conditions 1 gram of dextrose reduces about 2.306 grams of CuO .

$$\frac{1.345 \times 100}{2.205} = 61.0,$$

and we have, therefore, hitherto always taken the $\kappa_{3.86}$ of maltose as 61.0.

In order to convert the $\kappa_{3.86}$ values for maltose into absolute values, it is only necessary to raise the former in the relation of the true divisor for maltose for the solution employed (which may be obtained from the maltose curve of Plate I, p. 80) to 3.86. Thus if M be the true divisor, then $\frac{\kappa_{3.86} \times M}{3.86} = \kappa$ absolute.

Under the conditions which give us $\kappa_{3.86} = 61.0$, we find that the value of κ expressed on the true weight of anhydrous maltose is 62.1.

Another mode of stating the specific reducing power has been adopted, among others, by C. J. Lintner. It is one which is certainly very convenient in the case of starch-conversion products, although not applicable to all reducing substances. It consists in expressing the amount of Cu or CuO reduced by 1 gram of anhydrous maltose as 100, and calculating all other quantities of Cu or CuO as a percentage on this amount. The symbol R is adopted for this value, and the specific reducing power of pure maltose is then $R = 100$, whilst a substance 1 gram of which reduces half the amount of copper reduced by 1 gram of maltose has $R = 50$, and so on.

The conversion of K or κ into R is very simple when we have to do with the absolute value for the former, since by calculating the percentage of maltose we also get R , thus $\frac{\kappa \times 100}{62.1} = R$ (or the percentage of maltose).

When we have to deal with $\kappa_{3.86}$, however, the conversion is not quite so simple, since we have to take into account the true divisor for the strength of the solution used. The calculation is then expressed by the following equation, where M stands for the true divisor for maltose—

$$\frac{\kappa_{3.86} \times M \times 100}{3.86 \times 62.1} = R.$$

The foregoing considerations apply particularly to solutions of maltose, but when we have to deal with starch-conversion products the problem becomes more complicated, owing to the solution-densities of the individual constituents differing considerably from each other, as has been shown in a previous section. In this case, when we require to convert $\kappa_{3.86}$ into R , it is necessary to multiply the apparent percentage of maltose, deduced from $\kappa_{3.86}$, by the ratio of the true divisor for the starch-products to the true divisor for maltose. These factors can be obtained from the maltose curve and the table given on page 84,

and then, if S be the divisor for the mixed starch-products and M the true divisor for maltose, the equation becomes

$$\frac{\kappa_{3.86} \times 100 \times S}{61.0 \times M} = R.$$

In the following table, we give some results obtained by reducing Fehling's solution with maltose under our standard conditions. The determinations were made exactly as previously described: 50 c.c. of Fehling's solution were diluted to 100 c.c. with the sugar solution and water, and the heating was continued for 12 minutes. The quantity of sugar added was so arranged that the amount of copper weighed varied from 0.080 to 0.315 gram. The final results are expressed as $\kappa_{3.86}$, κ absolute, and R.

TABLE X.—*Cupric-reduction of Maltose.*

Maltose by 3.86 divisor.	Maltose absolute.	Cu. weighed.	CuO corresponding to 1 gram of 3.86 maltose.	CuO corresponding to 1 gram of absolute maltose.	$\kappa_{3.86}$.	κ_{absolute} .	R.
0.0728	0.0716	0.0787	1.354	1.377	61.4	62.4	99.3
0.0740	0.0728	0.0807	1.366	1.389	61.9	63.0	100.1
0.0733	0.0721	0.0812	1.389	1.412	63.0	64.0	101.8
0.0744	0.0732	0.0825	1.390	1.413	63.0	64.1	101.8
0.0762	0.0750	0.0806	1.325	1.347	60.0	61.1	97.1
0.0738	0.0726	0.0782	1.327	1.349	60.1	61.2	97.3
0.0721	0.0709	0.0773	1.344	1.366	60.9	62.0	98.5
0.0754	0.0741	0.0826	1.373	1.396	62.2	63.3	100.6
0.0720	0.0707	0.0770	1.341	1.365	60.8	61.9	98.4
0.0716	0.0703	0.0791	1.385	1.410	62.8	63.9	101.6
0.1458	0.1434	0.1581	1.359	1.381	61.6	62.6	100.4
0.1475	0.1451	0.1588	1.349	1.371	61.1	62.2	99.7
0.1456	0.1432	0.1573	1.354	1.376	61.4	62.4	100.1
0.1471	0.1447	0.1585	1.350	1.373	61.2	62.3	99.8
0.1451	0.1460	0.1606	1.356	1.378	61.5	62.5	100.3
0.1465	0.1441	0.1581	1.352	1.375	61.3	62.3	100.0
0.1524	0.1499	0.1647	1.355	1.377	61.4	62.4	100.1
0.1457	0.1433	0.1582	1.361	1.383	61.7	62.7	100.6
0.2262	0.2221	0.2428	1.348	1.371	61.1	62.2	99.9
0.2225	0.2185	0.2397	1.351	1.373	61.2	62.3	100.2
0.2259	0.2218	0.2411	1.341	1.365	60.7	61.9	99.4
0.2928	0.2880	0.3124	1.340	1.363	60.6	61.8	99.3
0.2913	0.2865	0.3116	1.343	1.367	60.8	62.0	99.5
0.2932	0.2884	0.3152	1.348	1.371	61.1	62.2	100.0
0.2931	0.2883	0.3125	1.340	1.363	60.6	61.8	99.3

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It will be seen that with an increasing weight of Cu, there is a slight decrease of CuO corresponding to 1 gram of maltose, but it may be taken that between the limits of 0.150 and 0.300 gram of copper the amount of CuO reduced by 1 gram of the sugar is practically the same, the mean values between these limits being $\kappa_{3.86} = 61.14$, κ absolute = 62.24, and $R = 99.9$.

From the above experimental data, we have constructed the following table for the reducing power of absolute maltose. The table commences with 70 mgms. of the sugar, and rises by increments of 5 mgms. to 300 mgms., giving the amount of Cu and CuO reduced by each weight, and the amounts of Cu and CuO respectively reduced by 1 gram of the sugar when the respective quantities of copper or copper oxide are weighed.

TABLE XI.—*Reducing values of varying quantities of Maltose under Standard Conditions.*

Maltose, mgms.	Cu weighed, grams.	CuO weighed, grams.	Cu corresponding to 1 gram maltose.	CuO corresponding to 1 gram maltose.	Maltose, mgms.	Cu weighed, grams.	CuO weighed, grams.	Cu corresponding to 1 gram maltose.	CuO corresponding to 1 gram maltose.
70	·0772	·0966	1·1029	1·3800	190	·2072	·2593	1·0953	1·3708
75	·0826	·1034	1·1026	1·3796	195	·2126	·2661	1·0949	1·3704
80	·0880	·1102	1·1023	1·3792	200	·2180	·2729	1·0946	1·3700
85	·0934	·1169	1·1020	1·3788	205	·2234	·2797	1·0943	1·3696
90	·0988	·1237	1·1017	1·3784	210	·2288	·2865	1·0940	1·3692
95	·1042	·1305	1·1013	1·3780	215	·2342	·2933	1·0937	1·3688
100	·1097	·1373	1·1010	1·3777	220	·2397	·3000	1·0933	1·3685
105	·1151	·1441	1·1007	1·3773	225	·2451	·3068	1·0930	1·3681
110	·1205	·1509	1·1004	1·3769	230	·2505	·3136	1·0927	1·3677
115	·1259	·1576	1·1001	1·3765	235	·2559	·3203	1·0924	1·3673
120	·1313	·1644	1·0997	1·3761	240	·2613	·3272	1·0921	1·3669
125	·1367	·1712	1·0994	1·3757	245	·2667	·3340	1·0917	1·3665
130	·1422	·1779	1·0991	1·3754	250	·2722	·3407	1·0914	1·3662
135	·1476	·1848	1·0988	1·3750	255	·2776	·3475	1·0911	1·3658
140	·1530	·1916	1·0985	1·3746	260	·2830	·3543	1·0908	1·3654
145	·1584	·1983	1·0981	1·3742	265	·2884	·3610	1·0905	1·3650
150	·1634	·2051	1·0978	1·3738	270	·2938	·3678	1·0901	1·3646
155	·1692	·2119	1·0975	1·3734	275	·2992	·3747	1·0898	1·3642
160	·1747	·2186	1·0972	1·3731	280	·3047	·3814	1·0895	1·3639
165	·1801	·2254	1·0969	1·3727	285	·3101	·3882	1·0892	1·3635
170	·1855	·2323	1·0965	1·3723	290	·3155	·3950	1·0889	1·3631
175	·1909	·2400	1·0962	1·3719	295	·3209	·4017	1·0885	1·3627
180	·1963	·2458	1·0959	1·3715	300	·3264	·4085	1·0882	1·3623
185	·2017	·2526	1·0956	1·3711	305	·3318	·4154	1·0879	1·3619

With the aid of the foregoing table we can easily calculate the reducing power, R, of any starch-conversion product. The necessary quantity of the solution of the substance is accurately measured or weighed, and the amount of solid matter corresponding to this measure or weight is ascertained from the specific gravity and true divisor for the solution. The latter is obtained from the table given on p. 84, section I. The amount of copper or copper oxide given by this amount of substance is converted into maltose by means of the above table, and this is then calculated as a percentage on the amount of solid matter taken. The result is the reducing power, expressed as R. An example will perhaps make this clearer.

11·846 c.c. of a solution of specific gravity 1020·37 gave 0·1934 gram of metallic copper. The true divisor for a solution of this sp. gr. is 4·01, therefore 100 c.c. (reputed) contains 5·0798 grams of solid matter, and 11·846 c.c., the quantity taken for the determination, 0·6016 gram. But on reference to the above table we find that 0·1934 gram of copper corresponds to 0·1765 gram of maltose, Therefore

$$\frac{0.1765}{0.6016} = 29.3 = R.$$

The reducing power may also be determined by calculating the weight of copper or copper oxide into grams per 100 c.c., and then using the gram equivalent, corresponding to the copper precipitated. Supposing in the above instance the copper had been weighed as CuO, the quantity per 100 c.c. would then be 2·047 grams. From the above table the amount of CuO corresponding to 1 gram of maltose, when the reduction is carried to the point of this experiment, is 1·3717. Then

$$\frac{2.047}{1.3717 \times 5.0798} = 29.3 = R.$$

We have already referred to the necessity, especially when dealing with maltose and starch-conversion products, of keeping the amount of sodium hydroxide constant in the Fehling's solution. In some formulæ for this solution, the amount of alkali used is 52 grams, and the following results show the influence of this decreased amount of sodium hydroxide. The solution was made up as before, with the exception that 52 grams, instead of 65 grams, of anhydrous soda were used. The determinations were carried out in exactly the same manner as those given in the previous table, and the results are expressed in the same way. The quantities of copper weighed fall practically within the same limits as in the previous series of experiments.

TABLE XII.—*Cupric-reduction of Maltose with Fehling's Solution containing 52 grams of Soda per litre.*

Maltose by 3·86 divisor.	Maltose absolute.	Cu weighed.	CuO corresponding to 1 gram of 3·86 maltose.	CuO corresponding to 1 gram of absolute maltose.	$\kappa_{3.86}$.	κ_{absolute} .	R.
0·0715	0·0701	0·0814	1·426	1·453	64·7	65·9	104·9
0·0682	0·0669	0·0770	1·414	1·441	64·1	65·4	104·0
0·0689	0·0676	0·0788	1·433	1·460	65·0	66·2	105·3
0·0700	0·0687	0·0798	1·427	1·455	64·8	66·0	104·9
0·1398	0·1375	0·1567	1·405	1·429	63·7	64·8	103·8
0·1407	0·1384	0·1568	1·397	1·420	63·3	64·4	103·2
0·1362	0·1339	0·1541	1·418	1·442	64·3	65·4	104·8
0·1375	0·1352	0·1541	1·405	1·428	63·7	64·8	103·8
0·1484	0·1456	0·1676	1·416	1·443	64·2	65·5	104·9
0·1434	0·1407	0·1618	1·415	1·441	64·2	65·4	104·8
0·2800	0·2753	0·3168	1·418	1·442	64·3	65·4	105·3
0·2766	0·2720	0·3105	1·407	1·431	63·8	64·9	104·5

It will be seen from the above that the specific reducing power of maltose throughout the series is appreciably higher than when the normal amount of alkali is used, and from this it also follows that the amount of CuO reduced by 1 gram of maltose is also greater. If we bear this in mind, however, results obtained with Fehling's solution of the composition used in the above experiments can be converted with equal ease into R (the percentage of maltose). All that is necessary is that the mean values from the above table be substituted for the values given in the preceding equations, which then become

$$\frac{\kappa_{3.86} \times M \times 100}{3.86 \times 65.4} = R. \quad \text{and} \quad \frac{\kappa_{3.86} \times 100 \times S}{64.0 \times M} = R.$$

In comparing our results with those of continental workers, there is still an important point to be considered, namely, the manner in which the determinations are made. We have already described the method adopted by us, and generally by English workers, but the majority of the continental chemists, who employ a gravimetric method, adopt an entirely different procedure for the determination of the cupric-reduction of starch-conversion products. This is known as Wein's method, and it appeared very desirable to make a compari-

son, using identical reducing solutions, between this method and the one in use by ourselves.

Wein's method is described in a little book published by him in 1888, entitled "Tabellen zur quantitativen Bestimmung der Zuckerarten." This contains tables for obtaining the sugar values of varying quantities of copper for the majority of the reducing carbohydrates, but it is only with maltose that we are concerned at present.

Wein makes up his Fehling's solution in two solutions, which are prepared as follows.

(a) Copper solution, 69.278 grams of carefully re-crystallised copper sulphate are dissolved in 1 litre of water.

(b) Soda solution, 173 grams of Rochelle salt are dissolved in 400 c.c. of water, and mixed with 100 c.c. of a solution containing 516 grams of sodium hydroxide per litre.

These two solutions are then mixed in equal volumes immediately before use. The undiluted Fehling's solution thus contains 51.6 grams of sodium hydroxide per litre.

In carrying out the determinations, the quantity of Fehling's solution required for the sugar under examination (usually 50 c.c. are taken) is placed in a porcelain dish and heated to boiling over a naked flame. The necessary amount of the sugar solution is then run into the vigorously boiling liquid from a pipette, and boiling continued for a certain number of minutes, the exact time depending on the sugar under examination. After boiling for the requisite time, the precipitated cuprous oxide is quickly filtered off through a Soxhlet tube, and after reduction in a current of hydrogen weighed as metallic copper.

For maltose the precise conditions are—

- 25 c.c. of copper solution,
- 25 c.c. of Rochelle salt-soda solution, and
- 25 c.c. of sugar solution, containing not more than 1 per cent. of maltose,

and the boiling is continued for 4 minutes after addition of the sugar solution.

In the following table we give the results obtained by us with the two methods when the same solution of maltose was employed. We also extended the comparison to solutions of starch-conversions of different types.

In column (1) is given the volume of reducing solution used; in (2) the weight of solid matter corresponding to this volume; in (3) the amount of copper weighed, (a) when the determination was made according to Wein's method, and (b) when made according to our method; in (4) the amount of maltose corresponding to (3), (a') calcu-

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lated from Wein's tables, (b') calculated from the table given on p. 100; and in (5) the value of R , calculated on (2) (a'') from Wein's results, (b'') from the results by our method.

TABLE XIII.

Pure Maltose.							
1	2	3		4		5	
Volume of solution taken.	Weight of substance x .	Cu obtained.		Maltose found.		R .	
		Wein's method. a .	Own method. b .	By Wein's tables. a' .	By own table. b' .	$\frac{a'}{x} = a''$.	$\frac{b'}{x} = b''$.
25 c.c.	0.2422	0.2591	0.2648	0.2290	0.2423	94.5	100.04
"	"	0.2600	0.2650	0.2298	0.2425	94.9	100.1
"	"	0.2612	—	0.2309	—	95.3	—
"	"	0.2600	—	0.2298	—	94.9	—
10 c.c.*	0.0969	0.1060	0.1081	0.0919	0.0980	94.9	101.1
"	"	0.1051	0.1076	0.0911	0.0976	94.0	100.7
"	"	0.1041	—	0.0902	—	93.1	—
"	"	0.1055	—	0.0914	—	94.3	—
Low Conversion, $[\alpha]_{D}^{20} 163.1$; $[\alpha]_D 149.7$.							
20 c.c.	0.3410	0.3047	0.3085	0.2697	0.2825	79.1	82.8
"	"	0.3058	0.3080	0.2707	0.2819	79.4	82.7
10 c.c.	0.1705	0.1542	0.1533	0.1352	0.1397	79.3	81.9
"	"	0.1538	—	0.1348	—	79.1	—
5 c.c.	0.0852	0.0781	0.0787	0.0672	0.0711	78.9	83.4
"	"	0.0792	0.0778	0.0682	0.0702	80.0	82.4
Medium Conversion, $[\alpha]_{D}^{20} 186.5$; $[\alpha]_D 173.9$.							
25 c.c.	0.5065	0.2472	0.2480	0.2183	0.2268	43.1	44.8
"	"	0.2474	0.2513	0.2185	0.2299	43.1	45.4
"	"	0.2492	—	0.2201	—	43.4	—
10 c.c.	0.2026	0.0996	0.0986	0.0863	0.0894	42.5	44.1
"	"	0.0997	0.0990	0.0864	0.0895	42.6	44.1
"	"	0.0994	—	0.0861	—	42.4	—
High Conversion, $[\alpha]_{D}^{20} 203.6$; $[\alpha]_D 188.6$.							
20 c.c.	1.0112	0.2231	0.2235	0.1967	0.2043	19.4	20.2
"	"	0.2209	0.2231	0.1948	0.2039	19.3	20.1
10 c.c.	0.5056	0.1107	0.1111	0.0962	0.1009	19.0	19.9
"	"	0.1110	0.1098	0.0964	0.0997	19.1	19.7
5 c.c.	0.2528	0.0550	0.0552	0.0470	0.0495	18.6	19.6
"	"	0.0567	0.0544	0.0483	0.0488	19.2	19.3

* Where less than 25 c.c. of the solution of reducing substance was taken, it was made up to this volume with distilled water, so that the dilution was the same in all the experiments.

TABLE XIII—(*continued*).

Fractionated product, $[\alpha]_{D^{20}} 194.0$; $[\alpha]_D 181.0$.							
1	2	3		4		5	
Volume of solution taken.	Weight of substance x .	Cu obtained.		Maltose found.		R.	
		Wein's method. a .	Own method. b .	By Wein's tables. a' .	By own table. b' .	$\frac{a'}{x} = a''$.	$\frac{b'}{x} = b''$.
10 c.c.	0.5900	0.2089	0.2120	0.1841	0.1937	31.2	32.8
"	"	0.2090	0.2101	0.1841	0.1920	31.2	32.5
"	"	0.2094	0.2119	0.1845	0.1936	31.2	32.8
5 c.c.	0.2950	0.1059	0.1072	0.0919	0.0973	31.2	33.0
"	"	0.1057	0.1061	0.0915	0.0963	31.0	32.6
"	"	0.1043	0.1038	0.0903	0.0943	30.6	31.9

From the above results, it is evident that any given weight of maltose or starch-conversion product, when oxidised under the conditions described in an earlier part of this section, reduces considerably more copper oxide than it does when oxidised under the conditions of Wein's method. This, however, would be immaterial in comparing the reducing power, R, of maltose or of a starch-conversion product, arrived at by the two methods, if the table drawn up by Wein truly represented the anhydrous maltose value of the copper reduced. Unfortunately, this does not appear to be the case, as when the maltose found by the Wein method and table is calculated as a percentage on the actual weight of maltose taken, the result, expressed as R, is almost exactly 5 per cent. lower than it should be; in other words, the R of pure maltose is 95 instead of 100 when determined according to Wein. This difference is also found in the reductions made with conversions of different types, in all of which the reducing power comes out approximately 5 per cent. of R lower when determined by Wein's method and table than it does by our method and table. Thus with the medium conversion, Wein's method gives a mean R of 42.8, whilst R by our method is 44.6, the difference between the two being somewhat above 4 per cent.; and the reductions with the other conversions show a corresponding percentage difference.

What the reason of this is, we are not prepared to say, as in the book above referred to, Wein does not give any explanation of the way in which he obtained the experimental data on which the table is founded, and we have been unable, up to the present, to obtain a copy of the original paper (*Allg. Brauer u. Hopfen Zeitung*, 1885) in which the method was first described. It is, however, very important to remember that the reducing powers, given by workers who use Wein's

table, are about 5 per cent. lower than the actual values, that is, they require to be raised by about 5 per cent. of R in order to bring them into line with R as determined by our method and table.

In considering the experimental errors in the determination of the cupric-reducing power of maltose and starch-conversion products, we have to regard them from two standpoints. In the first place, there are the errors introduced by varying the conditions laid down for any given method of determination. These have been already touched on, and, moreover, in the paper by Kjeldahl previously referred to, the influence of such variations are so exhaustively treated that it is unnecessary to further discuss them here. It suffices to say that the precise conditions laid down for any particular method, must be rigidly adhered to, and, in case any slight deviation from the conditions is made, each worker must determine for himself the effect of this deviation on the final result. In the second place, there are the experimental errors incidental to any particular method, when the conditions of that method are rigidly adhered to. Very extended experience has shown us that within the limits of CuO, mentioned on p. 96 (0.15 to 0.35 gram), the error amounts to 0.001 gram on the amount of copper weighed. This error is fairly constant within the above limits; it is therefore obvious that it represents a greater percentage error on the reducing power when the amount of copper weighed approaches the lower limit, than it does when the larger quantity of copper is reduced. Thus, with 0.15 gram the error amounts to 0.7 per cent. R, whilst with 0.35 gram the error is only 0.3 per cent. R. When smaller quantities of copper than 0.15 are weighed, the error appreciably increases, for not only is the percentage greater, but there also appears to be less stability in the reaction. We therefore consider that 0.15 gram is the lowest permissible amount of copper oxide which should be reduced, and we prefer that the amount should fall between 0.25 and 0.30 gram. When the experiment is so arranged that the amount of copper oxide reduced falls within the last-mentioned limits, we regard the maximum experimental error as falling within 0.5 per cent. of R.

V. *On the Limits of Accuracy of the Methods Employed.*

In the determination of specific rotatory power, the value of $[\alpha]$ in the formula $[\alpha] = \frac{10^4 a}{L \times c}$ will be most influenced by (1) actual errors of instrumental reading, which are included in a , and (2) by the greater or less exactness of the factor c , which represents the concentration or the weight of substance in a given volume of the solvent.

The error introduced by any want of accuracy in the length of the

column of liquid can, of course, always be allowed for if necessary, but in our case no correction was made for this as the 200 mm. and 100 mm. tubes used were found to have a length of 199.99 mm. and 99.99 mm. respectively, at a temperature of 18° .*

Provided the polarimetric readings are taken at approximately the same temperature as that at which the density is determined (in our case 15.5°), there is no appreciable error introduced in the readings of solutions of the products of starch-hydrolysis; where, however, it is a question of establishing constants for the pure carbohydrates, the readings must be made at an exactly known temperature.

As regards (1), the actual error of reading in our Ventzke-Scheibler half-shade instrument is certainly not more than ± 0.05 of a scale-division on any single observation, which represents ± 0.019 degree of arc for Biot's *jaune moyen* ray, or ± 0.017 degree for sodium light. On a series of readings, the error is much less, and we are over-stating it at ± 0.009 of a degree for the *jaune moyen*, and $\pm 0.008^{\circ}$ for sodium light.

This probable error is, of course, constant for large or small readings alike, and the extent to which it will affect the specific rotatory power depends on the magnitude of the total reading as conditioned by the concentration of the solution, the length of column, and the specific rotatory power of the substance or mixture of substances we are dealing with.

This is shown by the following concrete example. A solution of cane-sugar of sp. gr. 1.03654 (about 9.4 grams per 100 c.c.) gave a reading in the 200 mm. tube of 32.88 scale-divisions for sodium light.

The probable error on a series of observations, as we have already seen, does not exceed 0.025 scale-division, which is 0.076 per cent. of the total reading in this case. As the specific rotatory power of cane-sugar at the above concentration is $[\alpha]_D 66.45^{\circ}$, the error of reading will not affect this value more than $\pm 0.05^{\circ}$.

For a concentration of half the amount, that is, about 5 grams of sugar per 100 c.c., errors in readings in the 200 mm. tube will be liable to affect the specific rotatory power $\pm 0.10^{\circ}$.

In working on the products of starch-transformation, we generally have to deal with solutions varying in concentrations between 4 and 10 grams per 100 c.c., and with substances varying in specific rotatory power between $[\alpha]_D 202.0^{\circ}$ and $[\alpha]_D 138^{\circ}$. It can readily be shown, as in the case of the cane-sugar example given above, that a 0.025 error in the scale reading will influence the specific rotatory powers from $[\alpha]_D 0.04^{\circ}$ to $[\alpha]_D 0.10^{\circ}$.

With regard to (2), the possible error in the value of c , this is the product $d \times p$, that is, the density of the solution by the percentage

* We are indebted to Mr. W. Watson, of the Royal College of Science, for these measurements.

weight. If the weight of substance in a given volume is already accurately known, a small error in d will make very little difference in the value of $[\alpha]$. If, however, as is usually the case in experiments on the products of starch-hydrolysis, the percentage weight p has to be determined from the density of the solution, then this density has to be determined with the greatest accuracy attainable.

The error in our determinations of the specific gravity of the liquid by means of the pycnometer or the Sprengel-tube is not more than 0.01 when water is taken at 1000. The influence of this source of error has to be considered from two points of view, (A) as an error in the actual determination of the density of the solution under experiment, and (B) as an error in the previous determination of the divisor which has to be applied for the determination of the true weight of solids.

If we confine ourselves to the products of starch-hydrolysis of a concentration of from 4 to 10 per cent. or thereabouts, that is, to solutions having a density of from about 1015 to 1040, and varying in specific rotatory power from $[\alpha]_D 202^\circ$ to $[\alpha]_D 138^\circ$, it can readily be shown that the experimental errors due to A will fall between $[\alpha]_D 0.03^\circ$ and $[\alpha]_D 0.14^\circ$; and as the determinations of the divisors are subject to the same errors which will equally affect the specific rotations, we arrive at the conclusion that even if the errors of A and B are cumulative, that is, are both plus, or both minus, their sum must lie between $[\alpha]_D 0.06^\circ$, as a minimum, and $[\alpha]_D 0.28^\circ$ as a maximum, that is, a mean error of $[\alpha]_D 0.17^\circ$.

When the calculations of the specific rotatory powers are not referred to water at 4° , that is, to *true* cubic centimetres, and the correction for air displacement is not made, the values of $[\alpha]$, as we have seen in a previous section, will be about 0.2 per cent. below their true value.

The limits of accuracy of the cupric-reducing methods have already been referred to in Section IV, p. 106.

A word still remains to be said on the corrections to be applied to starch-transformation products when the hydrolysis has been conducted with malt-extract, or with a diastase solution where solids bear any sensible proportion to the amount of the products of starch-hydrolysis formed. The precautions to be taken in such cases have been so fully described in a previous paper (Brown and Heron, *Trans.*, 1879, 35, p. 601) that it is only necessary to allude to them very briefly. As the properties of malt-extract undergo slight changes under the action of heat, it is a matter of importance to determine the specific gravity, optical activity, and cupric-reducing power of the transforming agent after it has been heated up to the point at which the transformation is to be carried out, and for the same length of time as is occupied by the hydrolysis. An accurate correction can then be made for the amount of transforming agent used.