

VII.—*The Relation of the Specific Rotatory and Cupric-reducing Powers of the Products of Starch-hydrolysis by Diastase.*

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

IT is a fact familiar to all who have given even the most passing attention to the phenomena of starch-hydrolysis by diastase, that in the progressive stages of the reaction the power of the solution to rotate a ray of plane polarised light gradually diminishes, whilst there is a corresponding increase in its capacity to reduce cupric salts. That the two functions of the hydrolysed products, namely, specific rotatory power and cupric-reducing power, stand in some very intimate relation to each other, followed, as a matter of course, from the conclusions drawn by O'Sullivan in 1876, and described in his epoch-making paper, "On the Action of Malt-extract on Starch" (Trans. 1876, ii, 125).

Four years prior to this, O'Sullivan had shown that the end-product of the action of malt-extract on starch-paste was the sugar *maltose*, and that if the action were arrested before the conversion of the starch into sugar were complete, it was possible to isolate two dextrans, α and β , which have the same action on polarised light as soluble-starch. Although he never succeeded in obtaining either of these dextrans absolutely devoid of reducing power, yet such a close approximation to this was attained that he felt justified in concluding that, in a perfectly pure state, they would exert no action on cupric salts. In the section of his 1876 paper devoted to the proof that maltose and dextrin are the only products of the reaction, O'Sullivan, for the first time, clearly showed that there is a definite relation between the rotatory power and reducing power of the products of transformation. He found this relation to be of such a nature that if he calculated the reducing power of the mixed products as *maltose*, and assumed the difference in weight between this and the total solids to be *dextrin* with optical properties identical with those of the dextrans he had previously described, then, on re-calculating the specific rotatory power of such a mixture of maltose and dextrin, the value he obtained was in very close agreement with the *observed* specific rotatory power.

O'Sullivan's subsequent work (Trans., 1879, 35, 770) fully confirmed this relation, as also did the work of one of us and Heron, published in the same year (Trans. 1879, 35, 596).

In 1885 (Brown and Morris, *Trans.*, 1885, 47, 527), we subjected the non-crystallisable products of starch-hydrolysis to a more rigorous examination, and although we were able to show that there exist substances of definite composition intermediate between soluble-starch, on the one hand, and its penultimate products of hydrolysis, the dextrin of the so-called "No. 8 equation" and maltose, on the other, yet the previously-expressed relations of rotatory and reducing power still held good, even for these intermediate products: that, in fact, the composition of the mixed starch-transformation products, *at any stage of the reaction*, or the composition of *any fractionated portion of these products*, could always be consistently expressed in terms of *maltose*, and of a non-reducing *dextrin* having a rotatory power of $[\alpha]_{D^{20}}^{216^{\circ}}$. From this, it naturally followed that if one of the two functions, rotation or reducing power, were known, the other could always be calculated.

In view of the large amount of experimental proof of this relation which has been accumulated by workers in this country during the last twenty years, it is remarkable that this most fundamental principle has not been recognised by some Continental chemists, whose work on starch during the last five years has attracted much attention.

C. J. Lintner, for instance, in a recent contribution to the subject (*Zeitschr. f. d. ges. Brauwesen*, 1895, 255), sums up his opinion by asserting that the law of definite relation of the optical and reducing powers of starch-products is an artificially constructed one, which in no way corresponds to the actual conditions, and that it depends on the unproved and unprovable assumption of the existence of a non-reducing achroo-dextrin.

Still more recently, we find Ost stating (*Chem. Zeit.*, 1895, 19, 1510) that he has been unable to substantiate the relation between opticity and reducing power of starch-products which is said to exist by English chemists.

It is evident that both these observers have entirely failed to understand the terms in which this law of relation has hitherto been expressed, and, so far from their own observations disproving the existence of such a relation, they are strikingly confirmatory of it, as we shall see later on.

The evidence so far accumulated that, at a certain stage of the reaction, the products of hydrolysis consist of maltose and a comparatively stable dextrin, which has no reducing power and is very resistant to further hydrolysis, appears to us to be unmistakable, and, hitherto, for convenience' sake, we have preferred to express the law of relation in terms of these two products. The expression of the relation is, however, capable of being put in a much more abstract form,

and that without pre-judging in the slightest degree the true nature of the products of hydrolysis; in fact, if we knew absolutely nothing of the true chemical changes attending starch-transformation, the relation between rotation and reduction would be just as readily expressed, and with one property known, it would be possible to predict the other.

In the following table, we have given, in columns I, II, V and VI, the experimental numbers obtained in the examination of a large number of starch-transformations, the reaction having been arrested at various stages of the hydrolysis. Besides the analyses of the total mixed products of starch-hydrolysis, we have included a considerable number of examinations of *fractionated* products, obtained by precipitating the mixed products with alcohol of various strengths. These are distinguished in the table by the letter F being appended to them.

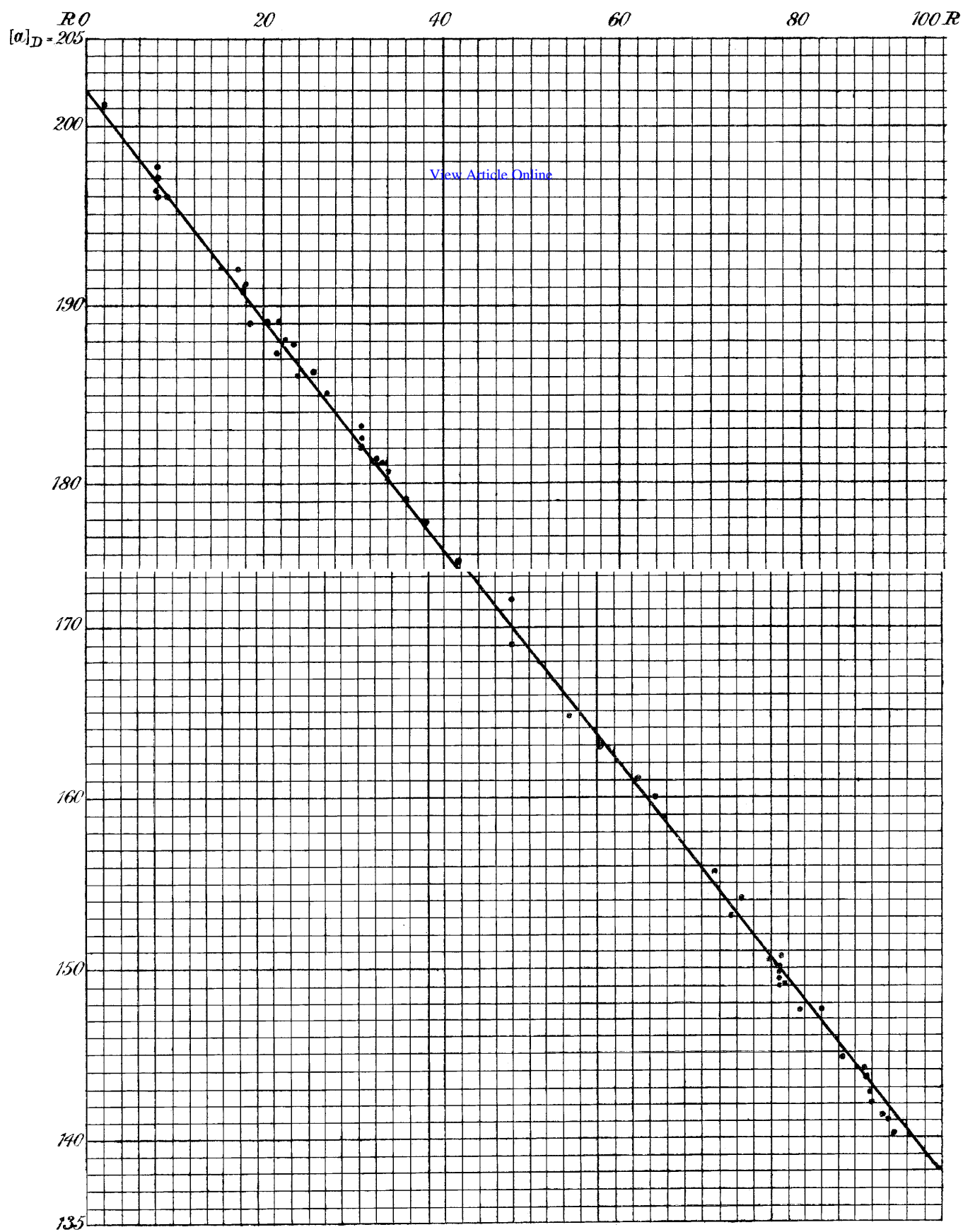
The reducing powers and specific rotatory values have been worked out according to the two methods most generally in use. Columns I and II give the values, deduced from the 3.86 divisor, of κ and $[\alpha]_D$, the reducing power κ being referred to an empirical dextrose reduction, as explained in our previous paper. Columns V and VI, on the other hand, give the absolute values for R (maltose = 100) and $[\alpha]_D$, on actual weights of starch-products, as deduced from their proper divisor according to Table VIII of a previous paper (p. 84).

TABLE I.—*The optical and reducing properties of starch-transformations of various grades.*

	I	II	III	IV	V	VI	VII	VIII
	$\kappa_{3.86}$ (Experimental).	$[\alpha]_{3.86}$ (Experimental).	$[\alpha]_{3.86}$ calculated from $\kappa_{3.86}$.	Difference	R (Experimental).	$[\alpha]_D$ (Experimental).	$[\alpha]_D$ calculated from R.	Difference
	1.2	215.1	214.7	+0.4	2.0	201.4	200.7	+0.7
F.	4.8	211.2	210.8	+0.4	7.9	197.6	196.9	+0.7
	4.8	209.9	210.8	-0.9	7.9	196.4	196.9	-0.5
F.	5.5	209.3	210.0	-0.7	9.1	195.9	196.1	-0.2
F.	5.0	209.3	210.6	-1.3	8.3	195.9	196.6	-0.7
F.	5.5	209.3	210.0	-0.7	9.1	195.9	196.1	-0.2
	4.9	209.1	210.7	-1.6	8.1	195.7	196.8	-1.1
F.	4.9	209.1	210.7	-1.6	8.1	195.7	196.8	-1.1
	10.1	205.6	205.1	+0.5	16.7	192.2	191.3	+0.9
F.	10.1	204.6	205.1	-0.5	16.7	191.3	191.3	0.0
F.	10.1	204.6	205.1	-0.5	16.7	191.3	191.3	0.0
	10.5	204.3	204.6	-0.3	17.4	191.0	190.8	+0.2
F.	10.5	204.3	204.6	-0.3	17.4	191.0	190.8	+0.2
	13.2	202.4	201.7	+0.7	21.9	189.1	188.0	+1.1
F.	13.2	202.4	201.7	+0.7	21.9	189.1	188.0	+1.1
	12.4	202.2	202.6	-0.4	20.5	188.9	188.8	+0.1
F.	11.7	202.2	203.3	-1.1	19.4	188.9	189.5	-0.6
F.	13.6	201.5	201.3	+0.2	22.5	188.2	187.6	+0.6

TABLE I. (continued)—*The optical and reducing properties of starch-transformations of various grades.*

	I	II	III	IV	V	VI	VII	VIII
	$\kappa_{3.86}$ (Experimental).	$[\alpha]_{3.86}$ (Experimental).	$[\alpha]_{3.86}$ calculated from $\kappa_{3.86}$.	Difference	R (Experimental).	$[\alpha]_b$ (Experimental.)	$[\alpha]_b$ calculated from R.	Difference
F.	13.9	201.2	201.0	+0.2	23.0	187.9	187.3	+0.6
	13.4	201.0	201.5	-0.5	22.2	187.8	187.7	+0.1
	13.0	200.6	201.9	-1.3	21.5	187.4	188.2	-0.8
	14.1	200.4	200.7	-0.3	23.3	187.2	187.0	+0.2
F.	15.5	199.5	199.2	+0.3	25.7	186.3	185.5	+0.8
F.	14.5	199.1	200.3	-1.2	24.0	185.9	186.6	-0.7
	14.4	198.7	200.4	-1.7	23.9	185.5	186.7	-1.2
F.	15.9	198.2	198.8	-0.6	26.3	185.0	185.1	-0.1
F.	18.6	196.4	195.9	+0.5	30.8	183.2	182.3	+0.9
	18.7	195.4	195.8	-0.4	30.9	182.3	182.2	+0.1
	18.8	195.1	195.7	-0.6	31.0	182.0	182.1	-0.1
F.	18.9	195.0	195.6	-0.6	31.1	181.9	182.0	-0.1
	19.6	194.4	194.8	-0.4	32.4	181.3	181.2	-0.1
F.	20.1	194.2	194.3	-0.1	33.3	181.0	180.7	+0.3
F.	20.7	193.6	193.6	0.0	34.2	180.4	180.1	+0.3
F.	22.0	192.4	192.1	+0.3	36.4	179.2	178.7	+0.5
F.	23.2	190.3	190.9	-0.6	38.4	177.4	177.4	0.0
F.	25.2	188.7	188.7	0.0	41.7	175.6	175.3	+0.3
	24.8	188.1	189.2	-1.1	41.0	175.1	175.7	+0.6
F.	26.6	187.6	187.2	+0.4	44.0	174.5	173.8	+0.7
F.	26.4	186.7	187.4	-0.7	43.6	173.7	174.1	-0.4
	30.4	184.6	183.1	+1.5	50.2	171.5	169.8	+1.7
F.	30.5	182.1	183.0	-0.9	50.3	169.2	169.8	-0.6
	34.4	177.7	178.8	-1.1	56.7	164.8	165.7	-0.9
F.	36.6	176.1	176.4	-0.3	60.3	163.1	163.4	-0.3
F.	39.2	173.9	173.6	+0.3	64.5	160.9	160.7	+0.2
	40.7	173.0	172.0	+1.0	67.0	160.0	159.1	+0.9
F.	44.5	168.6	167.8	+0.8	73.1	155.6	155.2	+0.4
F.	46.6	167.2	165.6	+1.6	76.5	154.2	153.0	+1.2
	45.8	165.8	165.6	+0.2	75.2	152.9	153.7	-0.8
	49.5	163.6	162.4	+1.2	81.2	150.6	150.0	+0.6
	48.8	163.3	163.2	+0.1	80.0	150.4	150.8	-0.4
	49.5	162.9	162.4	+0.5	81.2	150.0	150.0	0.0
F.	49.4	162.7	162.5	+0.2	81.1	149.8	150.1	-0.3
	49.3	162.4	162.7	-0.3	81.0	149.6	150.1	-0.5
	49.6	162.3	162.3	0.0	81.3	149.5	149.9	-0.4
	49.3	162.3	162.7	-0.4	81.0	149.5	150.1	-0.6
	49.1	162.1	162.9	-0.8	80.8	149.3	150.2	-0.9
	49.7	162.1	162.2	-0.1	81.4	149.3	149.8	-0.5
	49.2	162.0	162.8	-0.8	80.9	149.2	150.2	-1.0
	51.1	160.3	160.7	-0.4	83.7	147.5	148.4	-0.9
F.	52.5	160.3	159.2	+1.1	86.0	147.5	146.9	+0.6
F.	54.4	157.6	157.1	+0.5	89.0	144.7	145.0	-0.3
	55.7	156.9	155.7	+1.2	91.1	144.0	143.7	+0.3
	55.9	156.4	155.5	+0.9	91.4	143.5	143.5	0.0
F.	56.0	156.2	155.4	+0.8	91.5	143.4	143.4	0.0
	56.2	155.5	155.2	+0.3	91.9	142.7	143.1	-0.4
	56.2	155.5	155.2	+0.3	91.9	142.7	143.1	-0.4
F.	56.5	154.7	154.9	-0.2	92.4	141.9	142.8	-0.9
	56.7	154.2	154.6	-0.4	92.7	141.5	142.6	-1.1
F.	57.6	154.1	153.7	+0.4	94.1	141.2	141.7	-0.5
F.	57.8	153.0	153.3	-0.3	94.4	140.2	141.5	-1.3

SPECIFIC ROTATION & CUPRIC-REDUCING POWER OF
STARCH CONVERSION PRODUCTS OF TABLE I

If we plot the experimental numbers of columns I and II, or of V and VI, of the above table on a system of rectangular co-ordinates, the degrees of specific rotation between soluble-starch and maltose being represented on the line of ordinates, and the cupric-reducing powers from soluble-starch to maltose on the line of abscissæ, we at once see that the experimental values *all fall practically on a straight line, joining the points of intersection of the co-ordinates corresponding to the optical and reducing properties of soluble-starch and of maltose respectively.*

This is shown on the Plate, for R and $[\alpha]_D$ of columns V and VI, and exactly the same result would, of course, be obtained by plotting the values of $\kappa_{3.86}$ and $[\alpha]_{j3.86}$ of columns I and II.*

The fall of specific rotation is strictly proportional to the rise in cupric-reduction at all stages of the transformation between soluble-starch and maltose and for all fractionated products. If the properties of soluble-starch and of maltose are taken as given in the preceding paper (p. 114), namely

$$\begin{array}{ll} \text{Soluble-Starch} & R=0 \quad [\alpha]_D=202.0^\circ \\ \text{Maltose} & R=100 \quad [\alpha]_D=138.0^\circ \end{array}$$

then the relation of specific rotation $[\alpha]_D$, and cupric-reduction R for any mixture or fractionation of the products of starch-hydrolysis will be expressed by the equation

$$[\alpha]_D = 202 - \frac{202 - 138}{100}R$$

$$\therefore [\alpha]_D = 202 - 0.64R.$$

If the experimental numbers have been expressed in terms of $\kappa_{3.86}$ and $[\alpha]_{j3.86}$ then the equation becomes

$$[\alpha]_{j3.86} = 216 - \frac{216 - 150}{100}\kappa_{3.86}.$$

$$\therefore [\alpha]_{j3.86} = 216 - 1.082\kappa_{3.86}.$$

By the aid of one or other of these formulæ, or by mere inspection where the graphic method is employed, we are able to determine, within reasonable limits of error, either the specific rotation of starch-conversion products from their cupric-reduction, or, conversely, the cupric-reduction from the optical properties.

In columns III and VII of Table I, we have given the specific rotatory powers calculated in this manner from the cupric-reducing powers, and in columns IV and VIII, respectively, are shown the differences

* The whole of the tabulated results cannot be shown on the Plate, as many of the values coincide.

between these calculated and experimental results ; it will be noted that the differences are for the most part very small, and this is still further shown on the Plate (see p. 118) by the close approximation of the experimental results, shown by the dots, to the theoretical straight line.

It cannot be too strongly emphasised that this relation of optical to reducing properties is, in the first place, *solely based on experiment*, and is quite independent of any theoretical views which may be held on the nature and constitution of the products of hydrolysis, but this empirical law is on such a sound experimental basis that, when we do not find the proper numerical relation holding good, we are justified in assuming either that some analytical error has been made, or that the products of the hydrolysis of starch are contaminated with some other substance or substances. In the few cases of discrepancy which have occasionally arisen, further investigation has always confirmed this. We have, in this "law of relation," the most valuable and useful criterion of purity which can be applied to starch-transformation products.

Both C. J. Lintner and Ost (*loc. cit.*) have strenuously denied the existence of any relation of optical and reducing properties, and we have now to enquire how far their own results justify them in assuming this position. In the earlier work of C. J. Lintner and Düll (*Zeit. f. angewandte Chem.*, 1892, 263), the large number of fractions of starch-transformation products were characterised only by their optical, and not by their reducing, properties. In the paper of 1893 by the same authors (*Ber.*, 1893, 26, 2533), the reducing powers of the various dextrans are given as well as their specific rotations, and in a later paper still (*Ber.*, 1895, 38, 1522), they have given both properties of a considerable number of fractions precipitated with alcohol from the products of the limited action of dilute oxalic acid on starch. As the whole of the *dextrose* had been carefully eliminated from the fractions before analysis, it might be expected that their properties would fall under the same law of relation as those of the products of the hydrolysis of starch prepared with diastase. In their examination of the substances, Lintner and Düll deduced the amount of solids in solution from the specific gravity, the weight-constants being determined by drying 2 to 3 grams of a 10 per cent. solution at 100°, or sometimes only at 60°, until the weight of the residue was constant.

We have already seen that it is impossible to expel the last traces of moisture from the products of starch-hydrolysis in this manner, as nothing short of heating in a vacuum over phosphoric anhydride and considerably above 100° is effective, and it follows that both the values of $[\alpha]_D$ and R as given by Lintner and Düll must be somewhat too low for the perfectly dry substance. If we had before us the densities of the solutions analysed, and the actual weight of solids found on

drying at 100°, we could make the necessary correction of the results. We shall not be far wrong, however, in assuming that Lintner and Düll's fractions for the most part contained 2 per cent. of water. There is some internal evidence of this in the numbers given, and this is also about the difference we find between the products of starch-hydrolysis dried at 100°, and the same products dried completely in a vacuum over phosphoric anhydride as previously described.

In Table II, we have brought together all the available analyses given by Lintner and Düll in their papers. In columns I and II are the values of R and $[\alpha]_D$ as given by the authors. Columns III and IV show the same values corrected for dry weight, on the assumption that Lintner and Düll's products contained 2 per cent. of moisture. Column V gives the value of $[\alpha]_D$ as calculated from R by the formula $[\alpha]_D = 202 - 0.64 R$, and column VI gives the difference between the observed and calculated specific rotatory powers.

TABLE II.—*Lintner and Düll's Fractions.*

No.	I R .	II $[\alpha]_D$.	III R (Cor- rected.)	IV $[\alpha]_D$. (Cor- rected.)	V $[\alpha]_D$. Calc.	VI Differ- ence.	
1	0	196.3°	0	200.1°	202.0°	+1.9°	"Amylodextrin" (Lintner).
2	1.0	196.0	1.0	200.0	201.3	+1.3	Erythrodextrin "
3	10.1	192.0	10.3	195.8	195.4	-0.4	Achroodextrin I "
4	26.6	183.0	27.1	186.6	184.7	-1.9	Achroodextrin II "
5	30.0	178.0	30.6	181.4	182.4	+1.0	
6	12.8	190.0	13.0	193.8	193.7	-0.1	
7	13.2	190.0	13.4	193.8	193.5	-0.3	
8	8.5	194.0	8.6	197.8	196.7	-1.1	Fractions from conversions with dilute oxalic acid after separation of the dextrose.
9	3.0	196.0	3.0	199.8	200.1	+0.3	
10	8.0	194.0	8.1	197.8	196.8	-1.0	
11	30.0	181.0	30.6	184.6	182.4	-2.2	
12	25.0	184.0	25.5	187.6	185.7	-1.9	
13	20.0	188.0	20.4	191.6	189.0	-2.6	
14	82.4	140.0	84.0	142.8	148.3	+5.5	"Isomaltose" (Lintner).

We see that in Lintner and Düll's fractionated starch-products there is for the most part a very fair accord in the relation between the calculated and observed values of $[\alpha]_D$, and doubtless this accord would have been even closer if we had possessed the requisite data for exactly correcting in each case for the retained moisture. There is one considerable exception, however, in No. 14 of the series, which is the so-called "isomaltose." As we have recently dealt very fully with

this substance (see Trans., 1895, 67, 709), and have shown what its true nature is, it will be unnecessary to say more about it here. Even if it could be shown that such a substance as isomaltose existed amongst the products of starch-hydrolysis, it is impossible that it should possess the optical and reducing properties assigned to it by C. J. Lintner. The substance described cannot be either a pure product of the hydrolysis of starch or a mixture of such products, its wide departure from the "law of relation" clearly indicating either the presence of some impurity, or errors in the determination of its optical and reducing properties.

We have still to consider the properties of the fractionated products of starch-transformations described by Ost in his *Studien über die Stärke* (*loc. cit.*). As all his fractions were finally dried at 130°, it may be assumed that they were quite free from moisture when examined, and the general agreement of their optical and reducing properties indicates that such was the case. We have given the results in Table III, and in column III have appended the specific rotation as calculated from R, by the usual equation $[\alpha]_D = 202 - 0.64 R$. The differences between the calculated and observed values are given in column IV. The reference numbers are those given by Ost.

TABLE III.—Ost's Fractions.

	I	II	III	IV
	R.	$[\alpha]_D$ Observed.	$[\alpha]_D$ Calculated	Difference
I	30.6	183.5°	182.4°	-1.1
Ia	27.4	182.4	184.5	+2.1
II	31.5	181.1	181.9	+0.8
IIa	28.2	183.0	184.0	+1.0
(1 and 2) b	37.7	180.5	180.0	-0.5
III	41.5	178.0	175.7	-2.3
IIIa	32.5	180.1	181.2	+1.1
IV	38.0	176.7	177.7	+1.0

Out of these eight fractionated products obtained by Ost, six conform closely to the "law of relation," whilst the other two do not depart widely from it.

We see, therefore, that even the results of C. J. Lintner and of Ost, who have both been active in denying the existence of any relation between the optical activity and reducing power of the products of

starch-hydrolysis are, for the most part, strongly confirmatory of there being such a relation.

The small amount of real advance made in our knowledge of starch-hydrolysis of late years, an advance altogether disproportionate to the amount of work done, is in no small measure due to the neglect of this important principle, which lies at the root of the whole question.

Any serious attempt to explain the complicated changes which starch undergoes when acted on by diastase must take into account first of all this "law of relation"; the crux of the whole question, in fact, lies in it, and when the true meaning of the law is understood we shall know all about starch-hydrolysis.

Addendum.—Since this paper was written, there has appeared an important communication bearing on the subject: "An Analytical Investigation of the Hydrolysis of Starch by Acid," by Rolfe and Defren (*Journ. Amer. Chem. Soc.*, 1896, 18, 869). The authors find, when starch is hydrolysed by dilute mineral acids, that, notwithstanding the fact that dextrose is produced along with the other products of hydrolysis similar to those produced by the action of diastase, the cupric-reduction of the total products at any stage of the reaction bears a constant relation to the specific rotatory power, even when the starch is hydrolysed under very varying conditions; that, in fact, the specific rotatory power during the reaction is an exact guide to the composition of the products of hydrolysis, and that there is a "law of relation" for acid just as there is for diastase conversions. It is manifest, however, that the law cannot be as simple in the former case as in the latter, as maltose is, of course, being converted into dextrose simultaneously with the breaking down of the starch and higher dextrins. This law of relation for acid conversions laid down by the authors does not apply to mixtures of different transformations as it does in the simpler case of the transformations by diastase; and it would also follow from the authors' statements, although they do not draw the conclusion themselves, that the relation they find in the case of acid conversions will not hold good for fractionations by alcohol or by dialysis.
