XLIX.—*Action of Diastase on Starch. Third Notice.*

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INTRODUCTORY.

THE present investigation forms a continuation of the work described in two previous papers (Trans., 1895, 67, 702 and 739). We have submitted to a careful examination all those products of the limited

action of diastase on starch at 70° which are soluble in alcohol, and have incidentally ascertained several other facts connected with the subject. Before describing our results, however, it will be necessary to briefly refer to some points in the literature bearing on the work.

The well-known experiments of C. O'Sullivan, and also those made by Horace T. Brown in conjunction with Heron and Morris, have indicated that a relation exists between the optical rotatory and the cupric-reducing powers of the products of the action of diastase on starch, and that this relation is such that the substances have optical rotatory and cupric-reducing powers corresponding with mixtures of maltose and a non-reducing dextrin. Moreover, evidence has been adduced by Rolfe and Defren (*J. Amer. Chem. Soc.*, 1896, 18, 869), which goes to show that a similar relation holds in the case of the products obtained by the action of acids on starch, which, however, contain three constituents, namely, glucose, maltose, and dextrin, all of which must be taken into account. This relation has been denied by several continental workers on the subject, but quite recently Brown, Morris, and Millar (this vol., p. 115) have brought forward fresh evidence in its support. These authors have shown (*loc. cit.*, 105) that the table most generally used on the continent for the calculation of the percentage of maltose from the amount of copper which the sugar reduces under definite conditions gives values which are low to the extent of about 5 per cent. We had independently arrived at a similar conclusion, having found that Wein's table gives 95.5 per cent. of anhydrous maltose instead of 100 per cent.* The optical rotatory and cupric-reducing powers of most of our substances agree approximately with the rule above mentioned.

In the year 1885, Brown and Morris (*Trans.*, 1885, 47, 465) described a product of the limited action of diastase on starch under the name of "maltodextrin," to which they assigned the formula

They found it to have a specific rotatory and cupric-reducing power of $[\alpha]_{\text{D}386} = 193.6^\circ$ and $\kappa_{386} = 20.7$ respectively, corresponding with $[\alpha]_{\text{D}386} = 174.2^\circ$, and R (per cent. of maltose) = 33.9. Subsequently (*Trans.*, 1889, 55, 465), they showed that the molecular weight of maltodextrin, as determined by the cryoscopic method, was in harmony with their formula. Some years later, Lintner and Düll (*Zeit. ges. Brauw.*, 1894, 17, 339) described a compound, "achroodextrin II," which had the

* When the reducing powers of the substances described in our first paper (*loc. cit.*) are corrected according to this observation, their properties conform fairly well to the rule of definite relation of optical rotatory power and cupric-reducing power. It is specially noteworthy that our preparation of "isomaltose" (*loc. cit.*), after treatment with diastase, had a reducing power which, when corrected, gives approximately R = 100, indicating that its conversion into maltose is complete.

same molecular weight as "maltodextrin"; the optical rotatory and cupric-reducing powers differed, however, from those of maltodextrin, and they were disposed, therefore, to regard the latter compound as impure. In our previous work (*loc. cit.*), we obtained evidence of the existence of a substance having approximately the properties of "maltodextrin," but we failed to recognise one having the properties of "achroodextrin II."

Quite recently, Prior (*Bayerisches Brauer. J.*, 1896, 6, 157) claims to have isolated a substance analogous to "achroodextrin II," which he proposes to call "achroodextrin III," and later (*ibid.*, 1896, 6, 385), he asserts that the molecular weight of this substance agrees with that required by the formula $(C_{12}H_{20}O_{10})_2 + H_2O$; its optical rotatory and cupric-reducing powers are said to be $[\alpha]_D = 171.1^\circ$ and $R = 42.5$.

We have isolated two substances, in what we consider to be a pure condition, that is to say, free from extraneous matters; these agree approximately in their properties with Brown and Morris's "maltodextrin" and with Prior's "achroodextrin III" respectively. The two substances, as we shall show, obviously belong to the same class of compounds, and we therefore propose to speak of them provisionally as *maltodextrin- α* and *maltodextrin- β* respectively. We have analysed both substances and are able to assign the following formulæ to them.

Assuming that the substance which we call *maltodextrin- α* is identical with the maltodextrin described by Brown and Morris, we are unable to confirm the statement of these chemists that it is rapidly and completely converted into maltose when digested with diastase at $50-60^\circ$. Undoubtedly, the conversion is complete when sufficient time is allowed to elapse, but when the action takes place at $50-60^\circ$ for 2 hours, or at the ordinary temperature for 48 hours, the reducing power of the product falls short of that of maltose by about 10 per cent. When *maltodextrin- β* is similarly treated, the cupric-reducing power of the product is $R = 91.5$. Brown and Morris state that the tendency of starch transformations, when subsequently acted on by malt-extract at $50-60^\circ$, is rapidly to attain a state of equilibrium corresponding apparently to the production of 81 per cent. of maltose. This we have also observed, but we do not feel disposed to accept the view that a stable dextrin is present as one of the constituents of such transformations, until its existence is supported by some other evidence.

Our remaining experiments are concerned with the so-called "iso-maltose" of Lintner and Düll. One of the main facts established by our previous work (*loc. cit.*) was that their product is not a specific compound. We were, in fact, led to regard it as consisting largely

of maltose together with what we styled a simple dextrin of the formula $C_{12}H_{20}O_{10} + H_2O$. Brown and Morris, in a paper on the same subject, likewise arrived at the conclusion that Lintner's isomaltose is a mixture of maltose and another substance or substances the nature of which is not absolutely defined. Closely following these papers, others appeared by Ost (*Chem. Zeit.*, 1895, 19, 150), Ulrich (*ibid.*, 1895, 19, 1523), and Jalowetz (*ibid.*, 1895, 19, 2003). We shall do no more than mention these communications, as the net result deducible from them, in so far as "isomaltose" is concerned, is the same as that to be derived from our own work and that of Brown and Morris. We would, however, in passing, draw attention to the fact which is brought out by the recent work of E. Fischer (*Ber.*, 1896, 28, 3024) and of Ost (*Chem. Zeit.*, 1896, 20, 761), namely, that the substance termed "isomaltose" by E. Fischer and obtained by him by the action of concentrated acids on glucose, is distinct from the so-called "isomaltose" obtained by Lintner by the action of diastase on starch.

Our former statement, that Lintner's isomaltose remains unaltered in properties when treated with brewery yeast, is only true when fermentation is allowed to proceed for a short time. Brown and Morris have shown that when "isomaltose" is fermented with brewery yeast until fermentation is at an end, an unfermentable portion remains having the properties $[\alpha]_{D^{38}} = 173.5^\circ$, and $\kappa_{38} = 38$, corresponding with $[\alpha]_{D^{38}} = 156.1^\circ$ and $R = 62.3$. This substance, according to the authors, did not yield an osazone; but they did not further investigate it. We have fermented several specimens of "isomaltose," and have isolated the unfermentable residues. After purification, they gave values for the optical rotatory and cupric-reducing powers agreeing approximately with the values obtained by Brown and Morris with their product. The point of greatest interest, however, attaching to our preparations is that they were in each case found to have a molecular weight corresponding with the formula $C_{12}H_{22}O_{11}$. This fact accords, therefore, with our view as to the nature of the constituent of "isomaltose" other than maltose (*loc. cit.*, 1895, 707).

We are able to confirm Prior's statement (*loc. cit.*) that a mixture of *maltodextrin*- β with maltose yields an osazone indistinguishable from Lintner's so-called "isomaltosazone"; this would, therefore, seem to indicate that "isomaltose" is a mixture of maltose and *maltodextrin*- β , and this is undoubtedly true to some extent. Some products which might justly be regarded as Lintner's "isomaltose," namely, those of rotatory power as high as $[\alpha]_{D^{38}} = 146^\circ$ have furnished evidence of the presence of *maltodextrin*- β . The substances which we worked with in the above-mentioned fermentation experiments had, however, rotatory powers of about $[\alpha]_{D^{38}} = 140^\circ$, and these appear not to have contained *maltodextrin*- β .

We have no precise knowledge of the dextrins of higher molecular weight which are insoluble in alcohol, and there are at present no satisfactory means of elucidating their nature; still it seems to be pretty generally agreed that they are cupric-reducing substances, and as such we consider that they would be better named "maltodextrins."

EXPERIMENTAL.

Production of Glucose from Starch by the Action of Malt-extract.—In our previous papers (*loc. cit.*), we stated that glucose appeared to be produced by the action of diastase from kiln-dried malt on starch paste. We have now to correct this statement, as, although we have observed the formation of glucose when acting on starch paste with certain kiln-dried malts, we are now certain that its production is in no way connected with the kilning process, numerous experiments we have made leading us to believe that glucose cannot be produced by the action of the diastase from *normal* malt on starch. This fact had, indeed, been established by C. O'Sullivan (*Trans.*, 1876, ii, 127), and by Brown and Heron (*Trans.*, 1879, 35, 648). Kröber, however (*Zeit. ges. Brauw.*, 1895, 18, 324 and 334), is of opinion that a glucase, that is, an enzyme capable of producing glucose, exists in normal malt. We are at present investigating the matter, and hope soon to be in a position to explain the production of glucose by the malts which we previously dealt with.

Determination of the Cupric-reducing Power of Maltose by Wein's Method.—The method adopted for this determination is substantially that of Wein (*Quantitative Bestimmung der Zuckerarten*, Stuttgart, 1888). The Fehling's solution used by us contained 50 grams of sodium hydroxide per litre. Our *modus operandi* was as follows.

Into a lipped beaker 9.5 cm. in height and 9 cm. in diameter, are measured 50 c.c. of Fehling's solution; 25 c.c. of an approximately 1 per cent. solution of maltose, containing an exactly known amount of maltose, are run into the Fehling's solution; the beaker is covered with a clock glass, and the contents heated over a Fletcher's gas burner covered with fine wire gauze, on which rests a ring of asbestos cloth. The liquid is raised to the boiling point, which operation should occupy 3—4 minutes, and is maintained in ebullition for 4 minutes, after which the precipitated cuprous oxide is collected in a Soxhlet's tube packed with asbestos which has previously been repeatedly extracted, first with concentrated nitric acid and then with caustic alkali. The cuprous oxide is finally reduced in a current of hydrogen in the well-known way and weighed as metallic copper.

The amount of maltose used was determined in two ways. First, pure maltose, which had been dried in a vacuum over concentrated sulphuric acid for at least a week, and during that time had been frequently removed and ground up in a mortar, was weighed out exactly

in a quantity approximating to 0.25 gram of anhydrous maltose. This was dissolved in a little water, and the solution rinsed into the Fehling's solution with the complement of 25 c.c. of water. Second, pure maltose—not necessarily dry—was dissolved in water, so that an approximately 10 per cent. solution was formed; the latter was heated to expel any alcohol which might adhere, and its specific gravity accurately determined. From the latter, the concentration of the solution in terms of anhydrous maltose was calculated by the aid of the formula

$$C = \frac{(\text{sp. gr.} - 1) \times 100}{0.3924}, \text{ in which } C \text{ denotes the number of grams per}$$

100 c.c. of solution. The "divisor" 0.3924 is that recently determined by Brown, Morris, and Millar, and we are indebted to these authors for giving us this information in advance. A quantity of the approximately 10 per cent. solution of maltose containing about 0.25 gram of anhydrous maltose was accurately weighed out and the weight converted into the corresponding volume by dividing by the specific gravity; from the data just given, the amount of anhydrous maltose could then be calculated. This quantity of maltose solution was then rinsed into the Fehling's solution with the complement of 25 c.c. of water. The results obtained by both methods were practically identical.

As a mean of a large number of determinations with several specimens of maltose, many times recrystallised, we find that 1 gram of anhydrous maltose reduces 1.079 grams of copper, which corresponds, when calculated by Wein's table, with 95.5 per cent.

We are indebted to the courtesy of Mr. Horace T. Brown for a sample of maltose, prepared in his laboratory, with which we obtained practically the same results as with our own preparations.

The values recorded in this paper for the cupric-reducing powers, and denoted by the symbol R , have been duly corrected to *true* percentages of apparent maltose in regard to these observations. Those denoted by the symbol $R_{3.86}$ do not represent *true* percentages of maltose, but may be converted into such by multiplying them with the factor $M/0.386$, in which M is the correct divisor for the particular transformation product. The same applies to $R_{3.93}$, which we have used in many cases, as it gives very nearly absolute values for maltose itself.

Incidentally, we have determined the specific rotatory power of maltose in 10 per cent. solution, and find it to be $[\alpha]_D = 137.9^\circ$ calculated as anhydrous maltose, a value agreeing with the recent determination of Brown, Morris, and Millar (this vol., p. 112).

Action of Diastase on Starch.—A 3 per cent. solution of starch (200 c.c.) was treated at 60° for an hour with a solution (20 c.c.) of the diastase precipitated from 20 grams of active malt; the iodine reaction could then no longer be observed, and the constants were $[\alpha]_{D 3.86} = 146^\circ$ and

$R_{386}=81$. Substantially the same values were obtained after the solution had remained at the ordinary temperature for two days. This agrees with the observation of Brown and Morris (Trans., 1885, 47, 568), that when starch transformations are subsequently acted on by malt extract at 50–60°, there is a tendency to attain a state of equilibrium corresponding with the following constants: $[\alpha]_{D386}=162.6^\circ$ and $\kappa_{386}=49.3$. A transformation carried out at the ordinary temperature with an excess of diastase which had not been heated, gave, after 4 days' action, $R_{386}=88.8$, and after 18 days' action, $R_{386}=99.4$. When the filtered solution was evaporated, and the syrup poured into alcohol of appropriate strength, crystals of maltose separated out after some time.

Study of the Limited Action of Diastase on Starch at 70°.—Two separate quantities of potato starch were transformed at 70° in the following manner. The starch (1000 grams) was mixed with water (1500 c.c.) and malt flour (8 grams); boiling water (5000 c.c.) was then poured upon the sludge, which at the same time was vigorously stirred. Under these conditions, a limpid solution is obtained in a few minutes. The solution was cooled to 70°, malt flour (52 grams) added, and the temperature kept at 70° for 70 minutes. Iodine then produced a reddish-brown coloration; the specific rotatory power of the mixed products was $[\alpha]_{D386}=170^\circ$. The filtered solution was evaporated to a syrup. A second transformation was carried out under precisely similar conditions; the two products were then mixed and fractionated with alcohol, as shown in the accompanying table (p. 515).

The fractionations shown in this table need not be described in detail. Treatment with alcohol of the specified strengths was continued until no more, or but very little, substance appeared to pass into solution. The residue from the boiling 94 per cent. alcohol was, however, treated with about 3 litres of boiling 90 per cent. alcohol, and the residue from this treated with a similar quantity of 80 per cent. alcohol.

These fractionations were, however, only preliminary, and we shall now proceed to describe the further fractionation of the products marked in the table A, B, C, D respectively. Each fraction will be dealt with under separate heads, and it is convenient to commence with fraction D.

Maltodextrin-a.—One hundred grams of fraction D in the above table, which gave $[\alpha]_{D386}=174^\circ$ and $R_{386}=36.5$, and yielded a small amount of crystalline osazone, was dissolved in 50 c.c. of water, and the solution poured in a thin stream into 1500 c.c. of 86 per cent. alcohol. The substance which passed into solution gave a crystalline osazone melting at 150–152°; it was not further examined. The precipitated syrup, which still gave a trace of crystallisable osazone, was dissolved in 40 c.c. of water and the solution poured into 1000 c.c. of 86 per cent. alcohol.

This solution was found to contain *maltodextrin- β* , and will be dealt with under that head. The precipitate was kept in a desiccator for some weeks, after which it was powdered and dried at 100° ; it yielded no crystallisable osazone. The following analytical determinations indicate, as will be seen, that it consisted of almost pure *maltodextrin- α* (compare below). It gave $[\alpha]_{D^{3.92}} = 179.7^\circ$ and $R_{3.92} = 29.9$. 15 c.c. of a solution ($p = 8.489$) gave a depression of 0.175° . M.W. = 927.

The formula $C_{36}H_{62}O_{31}$ requires M. W. = 990.

A 6 per cent. solution was digested with diastase at 70° during 5 hours, when it gave $[\alpha]_{D_{3-93}} = 139.3^\circ$ and $R_{3-93} = 90.7$.

After treatment for a week with a solution prepared by triturating brewery yeast with powdered glass and water and subsequently filtering, it gave a trace of osazone insoluble in hot water together with a small amount of a soluble osazone, which when recrystallised melted at $150-160^\circ$. This experiment is typical of others we have carried out with maltodextrin- α , and indicates that this substance is extremely slowly hydrolysed by yeast enzymes, and is one of the products which Prior has designated "*Schwer vergährbare Kohlenhydrate*" (see *Bayerisches Brauer-Journal*, 1896, 6, 385). It should also be mentioned that Brown and Morris (Trans., 1885, 47, p. 570) state that, whilst "maltodextrin" is unfermentable by yeast (*S. cerevisiæ* of the high fermentation), it is converted into fermentable, crystallisable maltose by the action of certain forms of *Saccharomyces*—those accompanying secondary fermentation.

In order to prepare maltodextrin- α in larger amount and obtain it in a pure state, another starch transformation with 1000 grams of starch was carried out at 70° under conditions similar to those already described on p. 514. Making use of the knowledge gained up to this point, 200 grams of maltodextrin- α were isolated in a state of purity such that it gave no crystallisable osazone on being heated with phenylhydrazine acetate. This preparation was analysed; it was found to contain 0.25 per cent. of ash and to have a cupric-reducing power of $R = 36$.

The crude substance just described was further purified in small quantities as follows. Twenty grams were extracted with two portions of boiling 80 per cent. alcohol. To the cold alcoholic extracts, acetone was added until a precipitate was produced, the whole was then vigorously shaken until the precipitate became flocculent, when it was collected, washed with 96 per cent. alcohol, and dried at first in a vacuum desiccator and subsequently at 100° . It still contained matter volatile at a higher temperature, and gave the following results: $[\alpha]_{D_{3-93}} = 178.2^\circ$; $R_{3-93} = 32.7$.

The residue left undissolved by the two treatments with 80 per cent. alcohol was soluble in a sufficiency of alcohol of that strength, and appeared to consist of the same substance as the above. It gave $[\alpha]_{D_{3-93}} = 178.8^\circ$; $R_{3-93} = 32.6$.

A second method was also adopted to purify the crude substance. Twenty grams were extracted with 500 c.c. of boiling 85 per cent. alcohol. The residue was dissolved in 40 c.c. of water, and alcohol added in quantity insufficient to produce a permanent precipitate, about one litre of a mixture of alcohol and acetone (equal parts) was then added

as rapidly as possible and the mixture vigorously shaken. The white flocculent precipitate was collected, redissolved in water, and the treatment with acetone-alcohol repeated. The substance was filtered under diminished pressure, washed with 96 per cent. alcohol, and dried as in the previous case. The substance was then dried at 120° until it no longer lost weight, which required 8 hours, and subsequently kept in a vacuum over concentrated sulphuric acid for 12 hours. It gave the following mean values for the specific rotatory power and cupric-reducing power $[\alpha]_D = 180^{\circ}$; $R = 32.8$.

Elementary analysis gave the following results.

- I. 0.1768 gave 0.2820 CO_2 and 0.1005 H_2O . $C = 43.49$; $H = 6.33$.
 - II. 0.1168 „ 0.1869 CO_2 and 0.0670 H_2O . $C = 43.63$; $H = 6.37$.
- 15 c.c. of a solution ($p = 8.341$), gave a depression of 0.171° .

M.W. = 926.

The formula, $\text{C}_{96}\text{H}_{62}\text{O}_{31}$ requires $C = 43.63$; $H = 6.26$ per cent., and M.W. = 990.

Maltodextrin-a, thus obtained, is quite amorphous, but perfectly white. The compound is sparingly soluble in boiling alcohol of above 85 per cent. by volume; it is, however, readily taken up by boiling alcohol of 80 per cent. and less, but is almost insoluble in cold alcohol. It dissolves readily in cold water, and is miscible in all proportions with hot water (compare Brown and Morris, Trans., 1885, 47, 562).

Action of Diastase on Maltodextrin-a for a Limited Period.—In these and other experiments, to be described later, diastase solution, prepared in the following way, was employed. One hundred grams of active malt were extracted with 20 per cent. alcohol at the ordinary temperature; the alcoholic filtrate was precipitated by the addition of strong alcohol, the diastase filtered off, washed with 96 per cent. alcohol, redissolved in water, and the solution made up to 100 c.c. 1—2 c.c. of this solution were added to about 30 c.c. of the solution of the starch product having a concentration of about 5—10 per cent. The following results have been obtained.

At $50-60^{\circ}$ for 2 hours:— $[\alpha]_{D_{3-93}} = 142.8^{\circ}$; $R_{3-93} = 87.0$.

At ordinary temperatures for 18 hours:— $[\alpha]_{D_{3-93}} = 145.7^{\circ}$; $R_{3-93} = 89.8$.

At ordinary temperatures for 48 hours:— $[\alpha]_{D_{3-93}} = 141.3^{\circ}$; $R_{3-93} = 90.0$.

Maltodextrin-β.—It has been already mentioned (p. 515) that fraction D in the table (p. 515) consisted in part of maltodextrin-β, and that this fraction was treated twice with alcohol. After removing the alcohol from the second alcoholic solution by distillation, the residue was found to give $[\alpha]_{D_{3-93}} = 173.5^{\circ}$.

15 c.c. of a solution ($p = 7.015$), gave a depression of 0.185° . M.W. = 702.

The formula, $\text{C}_{24}\text{H}_{42}\text{O}_{21}$ requires M.W. = 666.

Fraction B in the table (p. 515) weighed under 10 grams, and apparently consisted, as might be expected, of a mixture of maltodextrin- α and maltodextrin- β .

It gave $[\alpha]_{D^{3-93}} = 172^\circ$; $R = 37.3$; and $M.W. = 804$.

In order to obtain maltodextrin- β in a purer state, fraction C in the table (p. 515) was submitted to further fractionation with alcohol of different strengths. The crude fraction, having the constants $[\alpha]_{D^{3-93}} = 146^\circ$, and $R_{3-93} = 68$, when heated with phenylhydrazine acetate, gave an osazone melting at 150° . It contained, as will be seen, extraneous substances, possibly derived from the malt used.

One hundred and fifty grams of the fraction were dissolved in 100 c.c. of boiling water, and the hot solution poured into 2000 c.c. of boiling 90 per cent. alcohol. The substance remaining dissolved in the alcoholic liquid, gave $[\alpha]_{D^{3-93}} = 140^\circ$, and $R_{3-93} = 89.5$; it also yielded an osazone, melting at $150-152^\circ$. It contained the chief portion of the extraneous matter above mentioned, and was made use of subsequently for the preparation of "isomaltose." The precipitated substance (about 100 grams) gave $[\alpha]_{D^{3-93}} = 167^\circ$; $R_{3-93} = 55$. It was dissolved in about 40 c.c. of water, and the solution poured into 1 litre of 90 per cent. alcohol (alcoholic solution, see below). The precipitate thus obtained gave no crystallisable osazone, and consisted of maltodextrin- α ; it gave $[\alpha]_{D^{3-93}} = 178^\circ$; $R_{3-93} = 31.5$, and $M.W. = 947$. The formula $C_{36}H_{62}O_{31}$ requires $M.W. = 990$.

The alcoholic solution (see above) was distilled, and the residue triturated with strong alcohol; it then gave $[\alpha]_{D^{3-93}} = 168^\circ$ and $R_{3-93} = 43$. In this state of purity, no trace of crystallisable osazone could be obtained on treating the substance with phenylhydrazine acetate in the usual manner.

The substance was dissolved in water, and precipitated by the addition of a mixture of acetone and alcohol in the manner adopted for the purification of maltodextrin- α (*vide ante*). This was done twice. The precipitated substance was finally dried at 120° as in the other cases. It gave $[\alpha]_D = 171.6^\circ$ and $R = 43.0$. It was not, however, obtained quite free from colour.

The following results were obtained on analysis of the substance.

0.2231 gave 0.3541 CO_2 and 0.1270 H_2O . $C = 43.28$; $H = 6.32$ per cent. 15 c.c. of a solution ($p = 6.320$) gave a depression of 0.179° . $M.W. = 670$.

$C_{24}H_{42}O_{21}$ requires $C = 43.24$; $H = 6.30$ per cent.; and $M.W. = 666$.

Maltodextrin- β is, as seen, more soluble in strong alcohol than maltodextrin- α . The former is also slightly soluble in a boiling mixture of alcohol and acetone, in which the latter appears to be insoluble.

When digested with an excess of diastase (see above), at the ordinary temperature, for 24 hours, the substance undergoes hydrolysis; the

following values were obtained with a 5 per cent. solution submitted to this treatment:— $[\alpha]_{D_{3-93}} = 140^\circ$ and $R_{3-95} = 91.5$.

It has been stated already that maltodextrin- β does not yield a crystallisable osazone. When, however, maltosazone is crystallised from a solution of maltodextrin- β which has been heated with phenylhydrazine acetate, an osazone is obtained agreeing in appearance with Lintner's so-called isomaltosazone. Moreover, we can confirm Prior's statement that when maltodextrin- β is mixed with an equal quantity of maltose, and the mixture is heated with phenylhydrazine acetate, "isomaltosazone" melting at 154° is obtained.

It must be apparent to everyone that the chief merit of such work as this rests with the recognition of the existence of such substances as maltodextrin- α and maltodextrin- β . We can at present adduce no proof that we have absolutely separated the two compounds, and a small quantity of one intermixed with the other would hardly be discovered by ultimate analysis or by cryoscopic determinations. Much remains to be done with these compounds, as, for example, the endeavour to prepare crystalline derivatives. It will also be important to submit the products of the restricted action of diastase on maltodextrin- α and maltodextrin- β to a critical examination. In the starch transformations dealt with up to the present, the chief product soluble in alcohol is maltodextrin- α , but if hydrolysis were carried a little further, it is possible that maltodextrin- β might be formed in the larger quantity.*

Further Examination of Lintner's "Is maltose."—The mixture to which Lintner has applied the name "is maltose" was obtained from fraction A of the table (p. 515) and from the portion of fraction C more soluble in alcohol. These were purified by treatment with methylic and ethylic alcohol as directed by Lintner, and also by subjecting them to limited fermentation for 24 hours at $20-30^\circ$, with brewery yeast. Being a mixture, the so-called "is maltose" varies in its properties. By "is maltose" we mean those purified fractions of rotatory power $[\alpha]_{D_{3-93}} = 140-146^\circ$ giving an osazone melting at 150° . We shall, however, bring forward evidence pointing to the presence of maltodextrin- β in the "is maltose" fraction of higher rotatory power.

A convenient method of preparing "is maltose" is to act on a solution of starch, at the ordinary temperature, with diastase solution or malt-extract which has been previously heated at 70° for an hour. The following experiment is typical of those carried out in this way.

Potato starch (300 grams) was mixed with water (500 c.c.) and malt flour (3 grams), and to the cold sludge, boiling water (1500 c.c.) was added, stirring constantly. The solution when cool was placed in a

* Experiments carried out since this paper was written fully justify this statement.

Winchester quart bottle together with the extract of 18 grams of active malt previously heated at 70° for an hour; after the lapse of 2 hours, the iodine reaction had vanished. The action, however, was allowed to continue for two days; * the filtrate then gave the values

$$[\alpha]_{D^{386}} = 158.2^{\circ} \text{ and } R_{386} = 69.$$

By treating the mixed products of the transformation with 80 per cent. alcohol, an extract was obtained which, on distillation, left a residue having the constants $[\alpha]_{D^{386}} = 136.4^{\circ}$; $R_{386} = 92$. A solution of this, after being fermented for 24 hours at $20-30^{\circ}$, was filtered from the yeast, and the filtrate evaporated to a syrup; this was again taken up with alcohol, and after the dissolved matter had been isolated as a syrup, the latter was treated by Lintner's method with methylic and ethylic alcohols. The precipitated substance had the constants $[\alpha]_{D^{393}} = 145^{\circ}$; $R_{393} = 87$. It gave "isomaltosazone" melting at 151° , and containing 10.27 per cent. of nitrogen, instead of 10.76 per cent., the theoretical amount for a hexabiosazone. Another preparation of "isomaltosazone" from a crude preparation of "isomaltose" was found to contain 9.22 per cent. of nitrogen.†

Fermentation of "Isomaltose."—Three specimens of "isomaltose" of rotatory power about $[\alpha]_{D^{393}} = 140^{\circ}$ were submitted to fermentation with brewery yeast at $20-30^{\circ}$. The first two may be regarded as preliminary, and will therefore not be described in detail. The constants of the purified products were,

$$\dagger \text{No. I. } [\alpha]_{D^{393}} = 159^{\circ}; \text{ M.W.} = 337.$$

$$,, \text{ II. } [\alpha]_{D^{393}} = 160^{\circ}; \text{ M.W.} = 376.$$

The formula $C_{12}H_{22}O_{11}$ requires $\text{M.W.} = 342$.

A 3 per cent. solution of the second preparation was treated with an excess of diastase at the ordinary temperature for 12 hours and subsequently for an hour at 50° , when it gave $[\alpha]_{D^{393}} = 140^{\circ}$; $R_{393} = 94$.

* In subsequent experiments, it has been found advisable to allow the action to proceed for a week or even longer; it is then necessary to add some chloroform to the solution at the commencement of the experiment.

† We previously analysed an osazone melting at 151° , and found it to have the composition of a hexatriosazone, and at the time we regarded it as probable that a hexatriose was one of the products of the action of diastase on starch. We subsequently withdrew that view, and furnished some experimental evidence of how the hexatriose might possibly be formed by the interaction of some of the products (Trans., 1895, **67**, 744). Whatever the nature of the osazone melting at 150° may be, the analyses of Brown and Morris as well as our own prove conclusively that it contains less nitrogen than that required for a hexabiosazone, and, moreover, that the percentage of nitrogen varies.

‡ The cupric-reducing powers of these two products were also determined, the determinations being made on weighed quantities of the substances after they had been heated at 100° , but as we subsequently discovered they still contained volatile matter, the results are not trustworthy.

We will now describe the third experiment in detail. About 70 grams of a specimen of "isomaltose" giving $[\alpha]_{D_{3-93}} = 140^\circ$; $R_{3-93} = 88$ were dissolved in sufficient water to make a solution of 6 per cent. concentration; to the solution were added, at a temperature of 30° , 3 grams of fresh brewery yeast previously washed. Fermentation was allowed to proceed at this temperature for 72 hours, when it had slackened considerably, but was not quite at an end; the solution was then filtered, the filtrate evaporated to a syrup, and the latter poured into 500 c.c. of 90 per cent. alcohol. The alcoholic solution, which contained 75—80 per cent. of alcohol, was distilled to a small volume, and, when cold, acetone was added, and the whole shaken vigorously. The flocculent precipitate which formed was collected, washed with 96 per cent. alcohol, and dried first in a vacuum desiccator, and subsequently at $100-110^\circ$. It was a white, amorphous substance, and was not hygroscopic when dry. It had the following constants $[\alpha]_{D_{3-93}} = 156^\circ$; $R_{3-93} = 62.5$.

An elementary analysis of a specimen dried at 120° gave the following result.

0.1345 gave 0.2090 CO_2 and 0.0788 H_2O . $\text{C} = 42.37$; $\text{H} = 6.50$ per cent. 15 c.c. of a solution ($p = 6.659$) gave a depression of 0.334° . M.W. = 379. The formula $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ requires $\text{C} = 42.10$; $\text{H} = 6.43$ p.c. and M.W. = 342.

The substance yielded a small amount of osazone melting at $130-140^\circ$.

It seems pretty evident that these substances consisted mainly of a compound of the formula $\text{C}_{12}\text{H}_{22}\text{O}_{11}$, but having the properties of the maltodextrins; we reserve all discussion as to its precise nature, however, until we have obtained more experimental evidence.

About 50 grams of a sample of "isomaltose" having the constants $[\alpha]_{D_{3-93}} = 148^\circ$; $R_{3-93} = 82$ were dissolved in 50 c.c. of water, alcohol was added to the solution in quantity insufficient to produce a permanent precipitate, and then a mixture of acetone and alcohol (equal parts) until a fair amount of syrup was precipitated. The supernatant liquid was decanted from the syrup, and this was boiled first with strong ethylic alcohol and then with methylic alcohol, after which it was dissolved in 25 c.c. of water, and alcohol-acetone added to the solution until a flocculent precipitate separated; this was collected, washed with 96 per cent. alcohol, and dried in the usual manner. When free from alcohol, it was not hygroscopic. It gave $[\alpha]_{D_{3-93}} = 170.7^\circ$; $R_{3-93} = 52.3$. 15 c.c. of a solution ($p = 7.818$) gave a depression of 0.304° . M.W. = 488.

A mixture of equal parts of maltodextrin- β , $\text{C}_{24}\text{H}_{42}\text{O}_{21}$, and a substance of the formula $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ would give indications of such a molecular weight as the one just quoted. The cupric-reducing power is also approximately the mean of that of maltodextrin- β and of the fermentation residue described above. The specific rotatory power does not, however, agree with that required for such a mixture.

An approximately 10 per cent. solution of the "isomaltose" $[\alpha]_{D^{33}} = 148^\circ$ was mixed with an excess of diastase. The specific gravity of the mixture was determined at once, and found to be 1.03979. After the mixture had remained at the ordinary temperature for 24 hours, the specific gravity had increased to 1.03989, or an increase of 0.25 per cent. on the total solids per 100 c.c. The specific rotatory power of the substance in solution was then $[\alpha]_D = 136.1^\circ$.

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