XXXV.—*The Specific Rotatory and Cupric Reducing Power of Invert Sugar and of Dextrose obtained from Cane Sugar by means of Invertase.*

By JAMES O'SULLIVAN.

HITHERTO the numbers published for the specific rotatory and cupric reducing power of invert sugar and of the dextrose prepared from invert sugar have been determined from the products obtained from a solution of cane sugar by heating it with an acid.

Herzfeld, Bornstein, and Winter (*Ber.*, **18** and **19**) have thrown considerable doubt on Dubrunfaut's number $[\alpha]_j = -106°$ for levulose by the isolation of crystalline levulose prepared from inulin and cane sugar, which they say has only an $[\alpha]_j$ C 20 per cent. at $20° = -83$.

Dubrunfaut's well known numbers (*Compt. rend.*, **42**, 901) are—

$$[\alpha]_j \text{ T } 14° = -106°; \text{ T } 52° = -79{\cdot}5°; \text{ T } 90° = -53°.$$

If we take the first number $-106^{14°}$, and apply Montgolfier's ratio $[\alpha]_j : [\alpha]_D = 1{\cdot}129 : 1$, we get $[\alpha]_D = -93°$.

The following numbers are also given for levulose:—Neubauer (*Ber.*, 1877, 829), $[\alpha]_D^{14^\circ} = -100^\circ$; Jodin (*Compt. rend.*, **58**, 613), C = 5 per cent., $[\alpha]_D = -93^\circ$; Kiliani (*Ber.*, **14**, 2530), C = 1 to 4 per cent., $[\alpha]_D = -92$—93°; Rotondi and Zecchini (*Deutsche Zucker-indust.*, 1887, 1091), $[\alpha]_D = -100^\circ$; and, finally, M. Honig and J. Jesser (*Monatsh. Chem.*, **9**, 562—578) give for levulose, which they prepared by the same method as Herzfeld and Winter, $[\alpha]_D^{15\cdot4^\circ} = -93\cdot526^\circ$.

Tuchmid (*J. pr. Chem.*, **2**, 235) gives for a solution of invert sugar with C = 17·21 per cent. at 0° $[\alpha]_D = -27\cdot9^\circ$, and that this value decreases with increase of temperature according to the formula $[\alpha]_D^t = (-27\cdot9^\circ - 0\cdot32^\circ t)$.

Dubrunfaut found for invert sugar—strength not stated (*Compt. rend.*, **42**, 901), T 14° $[\alpha]_D = -23\cdot6^\circ$, which, by allowing for 14° of temperature, we obtain at 0° $(-23\cdot6^\circ - 0\cdot32^\circ \times 14) = -28^\circ$, a number which agrees with Tuchmid's.

Maumené (*Compt. rend.*, **80**, 1139) says that invert sugar possesses similar properties only when the proportions of water and acid, the temperature and duration of the action, and the mode of neutralisation employed have been strictly identical.

It is evident that if Herzfeld's number for levulose be correct, invert sugar cannot be composed of equal parts of levulose and dextrose possessing the rotatory powers usually ascribed to them.

I intend to record in this paper that invert sugar, obtained from cane sugar by means of invertase, has a specific rotatory power T 15·5° $[\alpha]_j = 24\cdot5^\circ$, and that it yields a dextrose with an $[\alpha]_j = 57$—58°, and that both have the same cupric reducing power.

As far back as 1878, it was found necessary, in quantitative analytical work, to discard the use of acid for the inversion of small quantities of cane sugar in mixtures containing substances which were acted on by acids, and to employ a method of inversion which would have no influence on these bodies. Invertase prepared from yeast presented itself as an inverting agent which was free from this disadvantage. Before invertase could be used it was necessary, first, to prove that cane sugar could be completely inverted by it, and also to determine what were the properties of the inverted product. As the numbers obtained have a bearing upon the rotatory power of levulose and upon the optical and cupric reducing methods of estimating cane sugar, I shall now give the method by which they were obtained.

The faces of a number of large and clear crystals of white sugar candy were carefully cleaned with a dry towel, and the crystals were then finely pulverised in an agate mortar. The pulverised sugar was kept in a stoppered bottle. At the same time some of the same sugar, which had been dissolved in water and crystallised from ethyl alcohol (0·80), was similarly treated. 3·292 grams of the first-mentioned sugar were taken, and it was found to lose nothing by drying, first, over sulphuric acid in a vacuum, and then in a current of dry air at 100°. It was dissolved in about 25 c.c. of cold water, which had been previously boiled. This solution weighed 30·463 grams, and its sp. gr., as determined in a Sprengel's tube, was found to be 1043·35 at 15·5°/15·5°. In the weighed solution there were 3·292 grams of sugar; therefore, in 100 c.c. of the solution there were 11·261 grams $\left[\frac{104 \cdot 335 \times 3 \cdot 292}{30 \cdot 463} = 11 \cdot 261\right]$. This gives a divisor of 3·85 for the 11·261 per cent. solution $\left[\frac{43 \cdot 35}{11 \cdot 261} = 3 \cdot 85\right]$.

The opticity of this solution in a 200 mm. tube of a Soleil-Ventzke-Scheibler polariscope, was found to be 43·3 divisions. As 0·384° of angular rotation is equal to 1 division of rotation on this instrument, and the 200 mm. tube was used, the specific rotatory power will be $[\alpha]_j = +73 \cdot 8°$ $\left[\frac{10 \times (43 \cdot 3 \times 0 \cdot 384)5}{11 \cdot 261} = 73 \cdot 8\right]$.

The divisor then for the cane sugar employed and its specific rotatory power were

$$C = 11 \cdot 261. \quad D = 3 \cdot 85. \quad [\alpha]_j = 73 \cdot 8°.$$

To 25·839 grams of this solution 0·03 gram of invertase (water deducted) was added, and the solution was digested for 17 hours at a temperature of 14—16°. The small thermometer used was washed with water, and the solution was again weighed. It weighed 29·261 grams, and its sp. gr., taken as before, was found to be 1040·71, and its opticity 200 mm. = −13, T 15·5°, which did not diminish after standing for 48 hours. In the 29·261 grams of solution there was 0·03 gram of invertase, therefore, in the 104·071 grams (100 c.c.) there was 0·106 gram.

The total rotation observed for the solution is −24·96°, (−13 × 0·384)5, and the total sp. gr. 1040·71.

The sp. gr. due to the invertase in the 100 c.c. of solution is 0·424 (1 gram of invertase in 100 c.c. = 1004); therefore, 1040·286 is the gravity due to invert sugar in the 100 c.c.

The specific rotatory power of the invertase was $[\alpha]_j = 31 \cdot 2°$; then the rotation due to 0·106 gram is 0·33°.

The gravity due to the invert sugar, 1040·286 ÷ 3·85 = 10·463 grams in 100 c.c., and the total rotation −24·96° + 0·33°, due to invertase, gives −25·29° for the 10·463 grams of invert sugar, or an $[\alpha]_j = -24 \cdot 1°$, $\left[\frac{25 \cdot 29}{10 \cdot 463}\right]$.

The cupric reducing power of the invert sugar was determined by O'Sullivan's modified gravimetric method (Trans., 1876, ii, 130), a weighed quantity of the solution being used, and it was found to be K = 101.

There are, in 100 c.c. of the solution of invert sugar, 10·463 grams, or in the 29·261 grams of the same solution there are 2·941 grams, which is the quantity of invert sugar produced by 2·788 grams of cane sugar employed $\left[\frac{25{\cdot}839 \times 11{\cdot}261}{104{\cdot}335} = 2{\cdot}788\right]$, or an increase of 0·153 gram.

10 grams of cane sugar on inversion, according to the equation

$$C_{12}H_{22}O_{11} + OH_2 = C_6H_{12}O_6 + C_6H_{12}O_6,$$

requires 5·26 per cent. of an increase, due to hydration, and the above increase amounts to 5·5 per cent.

In another experiment in which I employed the cane sugar crystallised from alcohol, and operated in a similar manner in every way to that I have just described, I obtained the following numbers:—

Specific rotatory power of the cane sugar, C = 11 per cent.; $[\alpha]_j =$ 73·7°.
Divisor for solution of gravity, 1043·35 = D 3·85.
Specific rotatory power of the invert sugar, C = 11·4 per cent.; $[\alpha]_j = -24{\cdot}8°$.
Cupric reducing power of the invert sugar, K = 99·0.
Hydration in per cent. on cane sugar, 5·5.

It will be observed that in order to obtain the amount of invert sugar present in the solution after inversion, that the total gravity, less the gravity due to the invertase present, is divided by 3·85. That this number, although slightly high, is practically correct, I have just recently proved.

An experiment was made, as before, with large crystals of cane sugar, and gave the following numbers:—

Specific rotatory power of the cane sugar, C = 15·481; $[\alpha]_j = 73{\cdot}2°$.
Divisor for solution of cane sugar of gravity 1059·5 = 3·84.
Specific rotatory power of the invert sugar, C = 14·07; $[\alpha]_j =$ −24·5 .
Cupric reducing power of the invert sugar, K, I = 99·2; II = 99·4.
Hydration in per cent. on cane sugar, 5·4.

A weighed uantity of the inverted solution of this experiment was evaporated in a strong glass test-tube provided with a rubber plug, through which passed two glass tubes, one of which connected the tube with a suction pump and the other with an air-drying apparatus. The tube was immersed in a salt-water bath kept boiling at 104° whilst the pump was working. In this manner the water was removed at a diminished pressure (100 to 120 mm.). When the syrup ceased to bubble in the tube an extremely gentle current of dry air was allowed to enter through the second tube, which was kept closed till then. This current of air was so slight that it did not materially influence the pressure. The tube and its contents were weighed from time to time until three consecutive weighings, made four hours apart, were found to be the same. The weight of the glass tube, after the experiment, was found to have diminished 0·004 gram.

4·62 grams of the inverted solution, which had a total gravity of 1054·26, were found to leave, when dried in this manner, 0·622 gram of dry substance, or 100 c.c. contains 14·193 grams, $\left[\frac{105·426 \times 0·622}{4·62}\right]$. In the 100 c.c. of solution there was 0·117 gram of dry invertase; therefore 14·193 − 0·117 = 14·076 grams of invert sugar. The total gravity 1054·26 − 0·462, due to invertase, is 1053·798 due to invert sugar. Now, 53·798 ÷ 14·076 = 3·82, the divisor for the invert sugar solution mentioned in the last experiment. The numbers given for the invert sugar in that experiment are worked out from this divisor, and it will be seen that they do not practically differ from the numbers given for the other experiments in which the divisor 3·85 was used.

Three inversions of cane sugar were made with very small quantities of invertase, in order to see if, by employing the number $[\alpha]_j = -24·5°$ for the cupric-reducing power of the inverted solution and $[\alpha]_j = 73·8°$ for the non-reducing substance, the observed $[\alpha]_j$ for the inverted product would agree with the calculated number. The following are the results:—

			Observed $[\alpha]_j$.	Calculated $[\alpha]_j$.
Exp.	I.	K = 95·73 per cent.	−21·1°	−20·3°
,,	II.	,, = 95·00 ,,	−21·6	−19·7
,,	III.	,, = 97·53 ,,	−21·6	−22·0

The experiments mentioned, together with these results, prove pretty conclusively that in the optical and cupric reducing method of estimating cane sugar, the numbers $[\alpha]_j = -24·5°$ and K 100 per cent. for the invert sugar are correct.

In a 200 mm. tube of a Soleil-Ventske-Scheibler polariscope, 1 gram of cane sugar in 100 c.c. of solution gives a rotation of 3·84 divisions, and 1 gram of invert sugar gives −1·27 divisions. Theoretically, 1 gram of cane sugar yields 1·0526 grams of invert sugar; therefore, if the volume of the solution is kept constant, the true optical loss in a 200 mm. tube, which 1 gram of cane sugar, after inversion, will show, is $3{\cdot}84 - (-1{\cdot}27 \times 1{\cdot}0526) = 5{\cdot}18$ divisions. From this it will be seen that the loss in opticity per 100 c.c. of a cane sugar solution inverted either partially or completely by invertase, divided by 5·18—the 200 mm. tube being used—will give, in grams, the amount of cane sugar and which has been inverted.

To prepare dextrose from cane sugar by means of invertase, 100 grams of pure white sugar-candy were dissolved in about 300 c.c. of boiling water; when cold, 0·3 gram of invertase was added to it, and after well agitating the solution it was laid aside for four days. On the fourth day, a portion of the solution was diluted, and, on analysis, the sugar in solution was found to have $[\alpha]_j = -23{\cdot}6$. On the sixth day the whole of the remaining portion of the solution was evaporated under a diminished pressure to about 80 c.c. This syrup was laid aside, and in about a week a large crystallisation had taken place. This crystalline mass was washed with alcohol of 0·82, and then dried upon filter paper. The crystals thus obtained appeared to have no syrupy matter mixed up with them, and, in fact, from their appearance, I fancied that I had a fairly pure dextrose. This, however, was not so, for on dissolving some in water and boiling off the alcohol I found, in a 5 per cent. solution, the $[\alpha]_j = 12°$. By repeated washing by decantation with alcohol, crystals were left which were much whiter than the original, and which, on analysis of a portion, gave C 3 per cent., $[\alpha]_j = 54°$. The whole of the remaining crystals was dissolved in about 20 c.c. of water and to this solution 20 c.c. of dry methyl alcohol was added. In 17 days, the crystals that had formed were removed from the mother liquor and were washed on a filter with methyl alcohol. Only 7 grams of crystals were obtained, as I aimed at the first crystallising portion. These crystals were white, clear, large, and well defined. After drying over sulphuric acid in a vacuum for 48 hours, the portion taken was weighed, and it amounted to 4·869 grams; on further drying in a current of dry air at 100° and finally in a vacuum, the weight was found to remain constant. It was dissolved in about 20 c.c. of water, and its opticity and divisor determined in the same manner as that employed in the case of cane sugar. The numbers yielded were :—

T 15·5°. C = 19·628. D = 3·8. $[\alpha]_j = 57{\cdot}1°$.
,, C = 10·095. D = 3·84. $[\alpha]_j = 57{\cdot}2°$.
K, I = 99·5° ; II = 100·5°.

I might mention here that I prepared dextrose from honey in a similar manner and obtained for two preparations, one crystallised from water, the other crystallised from alcohol, the following numbers :—

I. Crystallised from Water.

T 15·5°.	C = 17·061.	D = 3·8.	$[\alpha]_j$ = 59·0°.
,,	,, = 10·844.	,, = 3·79.	,, = 58·5.
,,	,, = 6·969.	,, = 3·83.	,, = 58·4.
,,	,, = 3·521.	,, = 3·85.	,, = 56·0.

II. Crystallised from Methyl Alcohol.

T 15·5°.	C = 14·000.	D = 3·8.	$[\alpha]_j$ = 58·5°.
,,	,, = 7·300.	,, = 3·83.	,, = 57·6.
,,	,, = 3·890.	,, = 3·85.	,, = 56·0.

As the specific rotatory power of invert sugar, obtained by means of invertase where levulose is not acted on, is $[\alpha]_j = -24{\cdot}5°$, and that of the dextrose prepared from it is $[\alpha]_j = 57°$, the apparent specific rotatory power of levulose calculated from these numbers must be $-24{\cdot}5° \times 2 + 57° = [\alpha]_j = -106°$, $[\alpha]_D = -93{\cdot}8°$; invert sugar being composed of equal portions of levulose and dextrose.