LXXIII. THE IODIMETRIC ESTIMATION OF SUGARS.

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In the course of an enquiry on the iodimetric estimation of sugars, Hilda M. Judd [1920] examined the methods of Romijn [1897], Colin and Liėvin [1918], and Willstätter and Schüdel [1918]. The principle involved in these processes is the oxidation of the sugar to the corresponding monobasic acid by means of iodine in alkaline solution. In the first two methods it was found by Miss Judd that the sugar was not oxidised quantitatively; the third method, however, was adopted by her as it afforded good results. We also found it accurate and rapid.

Briefly the method consists in adding 20 cc. of *N*/10 iodine and 30 cc. of *N*/10 soda to 0·1 g. of the sugar dissolved in 10 cc. of water. The mixture is left at the air temperature for 15 to 20 minutes, then acidified with *N*/1 H_2SO_4 and the excess of iodine titrated with *N*/20 thiosulphate using starch solution as indicator. The reaction in its simplest form may be represented in the case of a hexose as proceeding according to the equation

$$C_6H_{12}O_6 + I_2 + 3NaOH = C_5H_{11}O_5COONa + 2NaI + 2H_2O$$

from which it will be found that 1 g. of the anhydrous hexose reacts with 1·411 g. of iodine, or 1 g. of anhydrous biose, such as lactose or maltose, with 0·743 g. of iodine. How closely this theoretical iodine gram-equivalent is reached in practice will be seen from the figures given in Table I.

In practice an excess of iodine and alkali is employed beyond that required by the equation; for 0·1 g. of sugar, 20 cc. of *N*/10 iodine and 30 cc. of *N*/10 NaOH are taken, although theoretically the necessary iodine for the reaction is present in 11 cc. of the decinormal solution when the sugar is a hexose or in 6 cc. for a biose sugar. Attention may be drawn to the following points.

(1) Our experiments show that it is essential that the reacting solutions should be mixed in the order of sugar, iodine, alkali. If the iodine and alkali are mixed prior to the addition of the sugar a lower iodine value is obtained, due probably to the loss of oxygen from the sodium hypoiodite.

(2) The time required for the oxidation mentioned by Willstätter and Schüdel and adopted by Miss Judd is 15 to 20 minutes; we find, however, that 3 to 5 minutes suffice for the completion of the reaction.

Working under the conditions described we obtained the following results:

Table I.

Sugar	Gram-equivalent Found	Gram-equivalent Calculated	H. M. Judd's results
Dextrose ($C_6H_{12}O_6$) ...	1·41	1·411	1·315
Levulose ($C_6H_{12}O_6$) ...	0·10	—	0·103
Galactose ($C_6H_{12}O_6$) ...	1·37	1·411	1·418
Sucrose ($C_{12}H_{22}O_{11}$) ...	0·02	—	0·01
Maltose ($C_{12}H_{22}O_{11}$) ...	0·746	0·743	0·7456
Lactose ($C_{12}H_{22}O_{11}$) ...	0·762	0·743	1·502
Raffinose ($C_{18}H_{32}O_{16}5H_2O$)	0·05	—	—
Soluble starch	0·06	—	—

The anhydrous dextrose used had a specific rotatory power of $[\alpha]_D + 53{\cdot}21^\circ$ after crystallisation from alcohol. The galactose had $[\alpha]_D + 80^\circ$ and levulose recrystallised from alcohol had $[\alpha]_D - 88{\cdot}4^\circ$ and a reducing power with Fehling's solution, calculated as levulose, of 99 %. The maltose and sucrose we employed were chemically pure; the former sugar as used for the determination contained 1 mol. of water which has been allowed for in the iodine equivalent recorded in the table. It will be noted that the gram-equivalent of iodine found by us for lactose differs very markedly from the value recorded by Miss Judd. We worked with four separate specimens of carefully purified lactose hydrate and in over a dozen determinations found figures similar to those recorded in the table. We believe, therefore, we are justified in concluding that lactose behaves with iodine in the same way as maltose and gives practically a quantitative yield of the monobasic acid. Consequently, there is no reason to suppose, as suggested by Miss Judd [1920], that lactose is hydrolysed and that both the glucose and galactose molecules are oxidised.

The citation by Miss Judd of Lobry de Bruyn and van Ekenstein's results [1896] in this connection would only be justified if the complete hydrolysis of lactose under the influence of cold alkali were effected in a few minutes, a transformation which was never obtained by these chemists. To settle the point we added alkali to solutions of lactose, maltose and levulose for a period of 20 minutes before the addition of iodine which was then allowed to act for the usual time. Under these conditions we should have obtained even more evidence of the Lobry de Bruyn transformation had it occurred, in the form of a higher gram-equivalent of iodine, but the values obtained were identical with those found when working under normal conditions.

In the course of this work we attempted to ascertain something of the nature of the mechanism of the iodimetric oxidation of carbohydrates and to this end made a series of determinations of the rate at which oxidation took place. Fifty per cent. of the total iodine we found was used up in the first five seconds and the reaction is in all cases complete within five minutes in the cases of dextrose, maltose and lactose. On investigating the values thus obtained by the customary formulae we found that the reaction is not mono-, di- or tri-molecular.

The fact that the time necessary for the reaction as employed by Willstätter and Schüdel [1918] can be reduced from 20 to 5 minutes considerably enhances the usefulness of the method and makes it as convenient as the volumetric Fehling reduction, while an accuracy of 1 % in the case of maltose and lactose and 0·5 % with dextrose and galactose is obtainable.

We applied the method to the analysis of the products of the action of diastase on starch and found that iodine does not differentiate between free maltose and that supposed to exist as "maltodextrin" in a state of combination with dextrin. When precipitated malt diastase was allowed to act on soluble starch for three hours at 50°, the conversion products had the constants $[\alpha]_D + 156{\cdot}5°$ and R, by Fehling's solution, 85·8 % of maltose. A mean of six iodimetric oxidations showed 85·4 % of maltose. From this it is evident that dextrin is unattacked by hypoiodite and that the whole of the maltose present both free and combined is quantitatively oxidised. When starch conversion products are fractionated with alcohol the iodine values agree with the apparent maltose content as found by Fehling's solution. Soluble starch and α-amylodextrin [J. L. Baker, 1902]—the dextrin obtained from starch when hydrolysed with barley diastase—are unacted on by an alkaline solution of iodine.

In the results recorded above solutions of pure sugars were employed. It should, however, be borne in mind that proteins are oxidised by iodine, thus egg albumin and Witte peptone have iodine gram-equivalents of 0·53 and 0·58 respectively. If any intention be entertained of employing the method for the analysis of solutions other than those of the pure sugars, the possible disturbing effects of protein and impurities will have to be considered.

Note added November 15th.

Since writing the above paper our attention has been drawn to a communication by N. Bland and L. L. Lloyd [1914]. These authors have examined (*inter alia*) the behaviour of the sugars towards *neutral* sodium hypoiodite and find that the oxidation of maltose, dextrose and lactose proceeds quantitatively to the corresponding monobasic acids in five minutes at ordinary temperature; sucrose and levulose were unaffected. The neutral hypoiodite used was obtained by mixing equal volumes of $N/10$ iodine and $N/10$ caustic soda.

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