CCLXVII. THE OXIDATION OF FRUCTOSE BY HYPOIODITE.

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THE oxidation of fructose by hypiodite, as employed in the method of Willstätter and Schudel [1918] for the estimation of aldoses, is known to be small in amount, but very variable results as to the iodine equivalent of fructose have been reported from time to time. Whereas over a fairly wide range of conditions glucose can be oxidised to the equivalent of 1.41 g. of iodine per g. of glucose, fructose has given various iodine figures under varying conditions, *e.g.* 0.1028 g. [Judd, 1920], 0.10 g. [Baker and Hulton, 1920], 0.0065–0.020 g. [Hinton and Macara, 1924], 0.018 g. at 1° [Archbold and Widdowson, 1931].

In the present communication are reported the results of an investigation of the oxidation of fructose by hypiodite under conditions similar to those used in the original Willstätter and Schudel method. The investigation has included the following: (1) effect of temperature; (2) effect of excess alkali; (3) effect of rate of addition of alkali; (4) effect of concentration of fructose; (5) control experiments; (6) identification of products of oxidation.

These investigations have shown that, if constant results are to be obtained with fructose, the most important condition to be standardised is that of the rate of addition of the alkali. Willstätter and Schudel stated that in the case of glucose the sodium hydroxide must be added slowly to the sugar-iodine solution, and it is well known that if the addition is made instantaneously, quantitative oxidation of glucose is not secured. Goebel [1927, 1] explained this on a stoichiometric basis as follows. Two reactions commence with the addition of alkali and the formation of hypiodite.

"A sufficiently high concentration of hypiodite is possibly not attained when alkali is added immediately to a glucose-iodine solution for the reason that reactions (1) and (2) start out simultaneously. Under these conditions reaction (1), though it has a greater *initial* speed than reaction (2), will be overtaken by the second as the two near completion with the result that at this point the concentration of hypiodite falls below the necessary optimum, and both reactions end with the hypiodite completely converted to iodate and iodide and the glucose in an incomplete state of oxidation. If, on the other hand, the alkali be added over an interval of 2 minutes, the major part of the hypiodite will enter into the first reaction as it is formed, . . . the concentration of hypiodite in the solution would be kept low, a condition unfavourable for the reaction hypiodite→iodate."

Goebel found that, using 0.09 g. glucose in 10 cc., 20 cc. of *N*/10 iodine, 45 cc. of *N*/10 NaOH, temp. 23°, two minutes should be taken to add the sodium hydroxide, in which case quantitative oxidation of the glucose was attained

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after 10 to 20 minutes' standing. If the alkali were added very rapidly, quantitative results were never attained, however long the subsequent standing.

The results obtained on the effect of rate of addition of alkali make it clear that the oxidation of fructose is not due to a Lobry de Bruyn-van Ekenstein transformation into oxidisable aldoses. At the same time the relatively slow oxidation of fructose by hypiodite as compared with that of aldoses renders it probable that only one component, and that a small one, of the fructose solution is actually oxidised.

EXPERIMENTAL.

The fructose employed was B.D.H. dextrose-free fructose recrystallised by Harding's method [1922]; $[\alpha]_D^{16.7} - 92.1^\circ$ ($c=5$). Calculated from Honig and Jesser's formula, $[\alpha]_D^{16.7} - 92.1^\circ$. The glucose was B.D.H., A.R., recrystallised from dilute alcohol (the first crop of crystals was discarded); $[\alpha]_D^{20} + 52.6^\circ$. Both sugars were dried *in vacuo* over P_2O_5 at 72° . All pipettes and graduated flasks were calibrated, and a standard drainage time of 30 seconds was adopted with the former. The thermostat was electrically controlled and temperature variations did not exceed $\pm 0.1^\circ$. The thiosulphate was standardised every 3 days by liberating a known amount of iodine from KI by acidified standard potassium dichromate solution.

(1) *Effect of temperature.*

A volume of solution containing 0.5 g. of fructose was pipetted into each of a series of 250 cc. conical flasks at room temperature and allowed to attain the requisite temperature in a thermostat. 20 cc. of $N/10$ iodine at the latter temperature were added. (In the series at 35° iodine was pipetted at room temperature, the flask closed, and the mixture allowed to attain the temperature of the thermostat before addition of the alkali. Iodine loss is rapid at 35° .) Lastly 5 cc. of $N/2$ NaOH were added over a period of exactly 20 seconds with rapid shaking. The flasks were closed, and after the appropriate time interval acidification was effected with 10 cc. of N H_2SO_4 and the liberated iodine titrated against $N/20$ thiosulphate using the Hagedorn and Jensen starch indicator. An appropriate iodine control was performed for each experiment.

Two series of experiments were performed, Series A using 10 cc. of sugar solution and a total volume of 35 cc., and Series B using 10 cc. of sugar solution + 40 cc. water, total volume 75 cc. The control experiments of Section (5) show that at room temperature the conditions of oxidation in Series A and B give the theoretical iodine value for glucose.

The oxidations were allowed to proceed at 1, 15, 25 and 35° . The extents of oxidation expressed in cc. $N/20$ iodine at various times and temperatures are given in Table I.

From these results it appears that, under the conditions adopted, the maximum oxidation was soon reached except at 1° , in which case 10 hours were required. In the last case some hypiodite was still functioning after 8 hours. In control experiments with 0.08 g. of glucose in place of the fructose, oxidation of the sugar at 1° was still proceeding after 8 hours. As will be shown (Section (3)) the extent of oxidation of fructose is very susceptible to the rate of addition of the alkali, but under the conditions of the above experiment, *viz.* a 20 second period for the addition of the alkali, the minimum oxidation of fructose which is accompanied by theoretical oxidation of glucose is effected at 15° in 20 minutes.

Table I.

Temperature ° C.	Series A		Series B	
	Time (hours)	Oxidation (cc. N/20 iodine)	Time (hours)	Oxidation (cc. N/20 iodine)
1	1.0	1.06	1.0	0.86
	3.0	2.29	3.0	1.81
	5.0	3.15	5.15	2.60
	8.0	3.75	8.0	3.07
	9.0	3.86	8.5	3.17
	10.0	4.13	10.0	3.48
	22.0	3.96	22.0	3.30
15	0.33	2.09	0.33	1.69
	0.75	3.33	0.75	2.74
	1.5	3.81	1.5	3.20
	3.6	3.73	3.5	3.20
	5.0	3.66	5.5	3.20
	9.0	3.71	9.0	3.25
	10.0	3.83	10.0	3.31
26	0.33	3.62	0.33	3.50
	1.0	3.94	1.0	3.40
	3.0	3.99	3.0	3.52
	5.0	3.99	5.0	3.52
	6.0	4.01	8.0	3.59
	7.0	4.09	10.0	3.67
	10.0	4.08		
35.5	0.33	4.16	0.33	3.94
	1.0	4.37	1.0	4.09
	3.0	4.57	2.0	4.09
	5.0	4.60	4.0	4.14
	8.5	4.77	6.0	4.11
	10.0	4.94	10.0	4.22

The final oxidation attained in both series was minimum at 15°. The extent of oxidation depends on

- (a) concentration and persistence of hypoiodite;
- (b) rate of reaction between hypoiodite and oxidisable sugar;
- (c) concentration of the oxidisable component of the fructose.

A rise in temperature favours (b), is unfavourable to (a), and is very possibly favourable to (c). Disregarding the effect of temperature on (c), effects (a) and (b) combine to produce minimum oxidation at 15°. That (a) is very favourable at 1° is shown by the continuance of oxidation after 8 hours at that temperature.

The rates of oxidation at 1° and 15° may be approximately arrived at from the first time readings (and inspection of the results in graphical form). From such observations the rates of oxidation in both series (A and B) are seen to be at least 6 times as great at 15° as at 1°. From such a result it would seem probable that factor (c) is also effective, and that there is a greater concentration of the oxidisable component at 15° than at 1°.

(2) Effect of excess of alkali.

In the previous experiments the oxidising system contained a small excess of alkali over that required to form hypoiodite. Series A was repeated using twice as much alkali, 10 cc. of N/2 NaOH (and equivalent increase of H₂SO₄), the total volume being thus 40 cc. The results are given in Table II.

The chief features of the effect of excess of alkali are:

- (a) a more rapid disappearance of hypoiodite at 1°, and in consequence, less total oxidation;

Table II.

1°		17°		26°		35°	
Time (hours)	Oxidation (cc. N/20 iodine)	Time (hours)	Oxidation (cc. N/20 iodine)	Time (hours)	Oxidation (cc. N/20 iodine)	Time (hours)	Oxidation (cc. N/20 iodine)
1.0	1.72	1.0	3.36	0.5	5.17	0.5	7.90
3.0	1.87	3.0	3.75	2.0	5.60	2.0	9.25
6.0	1.97	5.0	4.00	3.0	6.19	4.5	11.10
8.0	2.02	7.0	4.00	5.25	6.60	6.0	12.00
10.0	1.97	10.0	4.15	8.75	7.40	8.0	13.20
				10.00	7.97	10.0	14.75

(b) progressive oxidation at 17, 26 and 35° long after oxidation in the corresponding members of series A (Table I) had ceased and hypoiodite is presumed to have disappeared.

Excess alkali has two effects, that of accelerating the formation of iodate from hypoiodite, and that of modifying the fructose rendering it susceptible to oxidation by iodate. Low results in the determination of glucose by iodimetric titration in cases where too large an excess of alkali has been added have probably been due to the first of these causes rather than the Lobry de Bruyn transformation to which they have been ascribed.

(3) *Effect of rate of addition of alkali.*

The experimental procedure adopted for Series A and B (Table I) was repeated at room temperature only, with the difference that the 5 cc. of *N*/2 NaOH were added in 0.5 cc. portions at intervals of 30 or 60 minutes. Certain flasks were withdrawn at certain stages and the titration completed. The results are given in Table III.

Table III. *Effect of rate of addition of alkali.*

0.5 g. fructose, 20 cc. *N*/10 iodine, *N*/2 NaOH 0.5 cc. at a time, total volume (without NaOH), Series A 30 cc., Series B 70 cc. Temp. 16°.

Rate of addition of NaOH	Duration of reaction from first NaOH addition (hours)	Number of additions of NaOH	Series A. Oxidation (cc. <i>N</i> /20 iodine)	Series B. Oxidation (cc. <i>N</i> /20 iodine)
0.5 cc. per half hour	1.0	2	2.18	1.51
	3.0	6	7.97	5.50
	4.0	8	12.22	8.60
	5.0	10	16.76	12.24
	6.0	10	16.96	—
	8.0	10	—	12.23
	10.0	10	16.90	12.34
			(=15.3% oxidation)	(=11% oxidation)
0.5 cc. per hour	5.0	5	10.15	7.39
	10.0	10	20.20	15.70
	24.0	10	20.20	15.90
			(=18.2% oxidation)	(=14.3% oxidation)

Before discussing the results of this experiment, the following control experiments should be considered. Series A and B were repeated at 16°. Firstly, 0.5 cc. of *N*/2 NaOH was added to the fructose-iodine mixture, and, at definite intervals of time, acidification was effected with 1 cc. of *N* H₂SO₄.

Table IV.

Time (minutes)	Oxidation (cc. <i>N</i> /20 iodine)	
	Series A	Series B
20	0.90	0.90
30	1.01	0.91
40	1.16	0.96
60	1.15	1.10

Oxidation appears to have ceased within 30 minutes.

Secondly, to the fructose-iodine mixture, 0.5 cc. of *N*/2 NaOH was added every half hour until 3.5 cc. had been added. After further time intervals (as stated in Table V) acidification was effected with 5 cc. of *N* H₂SO₄.

Table V.

Time (minutes)	Oxidation (cc. <i>N</i> /20 iodine)	
	Series A	Series B
20	9.05	6.20
30	9.60	6.82
45	9.60	6.70
60	(9.80)	6.70

Here again oxidation had ceased entirely within 30 minutes.

It is evident from Table III that an abnormally high oxidation can be effected if the alkali is added slowly enough, and from Tables IV and V that this is not due to a very slow decomposition of hypiodite. The best explanation seems to be that one component only of the fructose solution undergoes oxidation and that:

(a) each small increment of hypiodite produces an oxidation comparable with that obtained with excess of hypiodite;

(b) in neutral medium the removal of one component by oxidation induces the "inactive" components to establish a new equilibrium thus restoring the oxidisable component.

In this case the amount of oxidation should increase as the rate of addition of alkali decreases, which is the case (Tables I, III, VI). Comparing Tables I and III (Series A) 5 cc. of NaOH added in 20 seconds produced oxidation equivalent to 3.7 cc. of *N*/20 iodine after 5 hours, whereas 5 cc. of NaOH added in 0.5 cc. half hourly increments produced 16.76 cc. oxidation after 5 hours in all, and 5 cc., added in hourly increments, 20.20 cc. Further, 3.0 cc. of alkali after 3 hours (Tables III, Series A) had produced 7.97 cc. oxidation, whereas 5 cc. added in 20 seconds (Tables I, Series A) had produced only 3.7 cc. oxidation in 5 hours. If the whole of the fructose were susceptible to direct oxidation, then, since the quantity used was many times the molecular equivalent of the 20 cc. of *N*/10 iodine added, one would expect more to be oxidised by the full dose of hypiodite (5 cc. alkali) than by 3.0 cc. If only one component is oxidised, the small increments may be comparatively saturated with this component and each effect oxidation with rapidity comparable with that effected by the full dose.

Lastly, it should be noted that the high oxidation effected in this way without excess of alkali ever being present disproves the hypothesis that the oxidation of fructose in the iodimetric titration is due to a Lobry de Bruyn transformation.

Oxidation could be carried much beyond the 18 % recorded in Table III. To 0.081 g. fructose in 50 cc. were added 20 cc. of *N*/10 iodine and 0.5 cc. of

$N/2$ NaOH at varying time intervals as shown in Table VI. When 4 cc. of alkali had been added, *i.e.* just sufficient to absorb free iodine, to some flasks another addition of 20 cc. iodine was made and the process continued. The same procedure was repeated after 8 and 12 cc. of alkali had been added. Other flasks were treated with no more iodine after certain stages. In every case, after the last addition of iodine had been absorbed by alkali, two further increments of alkali were added at intervals to complete the reaction and then titration was performed in the usual way. The results are shown in Table VI. The percentage

Table VI.

0.5 cc. alkali/10 minutes		0.5 cc. alkali/30 minutes		0.5 cc. alkali/60 minutes	
Number of additions of 20 cc. $N/10$ iodine	% oxidation	Number of additions of 20 cc. $N/10$ iodine	% oxidation	Number of additions of 20 cc. $N/10$ iodine	% oxidation
1	10.4	1	19.5	1	23.6
2	20.6	2	40.6	2	61.1
3	40.1	3	77.3	3	98.9
		4	98.3		

fructose oxidised is calculated on the assumption that one atom of oxygen is absorbed by one fructose molecule. It is to be expected that, especially in the cases where the alkali additions were more frequent, a certain amount of unchanged hypiodite might accumulate. This would account for the rate of oxidation of fructose increasing as the additions of NaOH and iodine proceed.

These experiments were repeated, using 0.081 g. of fructose in 10 cc. instead of 50 cc.

Table VII.

0.5 cc. alkali/30 minutes		0.5 cc. alkali/60 minutes	
Number of additions of 20 cc. $N/10$ iodine	% oxidation	Number of additions of 20 cc. $N/10$ iodine	% oxidation
1	26.4	1	33.5
2	60.1	2	77.2
3	94.7	3	125.2
4	128.4		

It is evident that the oxidation proceeds beyond the insertion of one oxygen atom.

(4) Effect of fructose concentration.

If the hypothesis put forward in the preceding section is correct, then certain conclusions can be drawn. For example, in a dilute solution of fructose, varying within the limits of concentration 0.08–2 %, the amount of oxidisable component will be approximately proportional to the total fructose concentration. Under an appropriate set of oxidising conditions, the percentage fructose oxidised should be independent of the fructose concentration. To test this deduction, 20 cc. $N/10$ iodine were pipetted into 50 cc. of fructose solution containing 0.08–0.5 g. of sugar. 5 cc. of 0.5 N NaOH were added at the rate of 0.5 cc./30 mins. The mixture was acidified 1 hour after the last alkali addition, thus allowing a total oxidation period of 5.5 hours. The results obtained at 20° are given in Table VIII.

A second experiment designed for another purpose (*vide* Section (6)) gave similar results. Here the materials used were 0.081–0.5 g. fructose in 10 cc.

Table VIII.

Final fructose concentration (%)	Weight of fructose (g.)	Oxidation	
		cc. <i>N</i> /20 iodine	%
0.108	0.081	4.44	24.7
0.333	0.250	10.06	18.1
0.500	0.375	12.90	15.5
0.666	0.500	15.76	14.2

water, 6.66 cc. of 0.3 *N* iodine in BaI₂, 6.25 cc. of 0.4 *N* baryta added at a rate of 0.5 cc. per 30 mins. (total volume 22.91 cc.). Acidification was effected after oxidation had ceased with 10 cc. *N* HCl.

Table IX.

Final fructose concentration (%)	Weight of fructose (g.)	Oxidation	
		cc. <i>N</i> /20 iodine	%
0.354	0.081	6.18	34.3
1.092	0.250	14.01	25.3
1.747	0.400	19.83	22.3
2.184	0.500	23.64	21.3

The quantity of hypiodite maintained in these experiments (Tables VIII and IX) was of the order of the equivalent of 0.5 cc. *N*/2, which is equivalent to 0.0225 g. of glucose. If the whole of the fructose could react with hypiodite, there would be an overwhelming excess of fructose in all cases, and one would not expect much difference in the actual amounts oxidised in any one series. The whole or most of the hypiodite should be used up in oxidation and comparatively little would form iodate. However, the results in Tables VIII and IX support the hypothesis that only one component, a small one, of the fructose solution is oxidised. Some of the hypiodite is lost as iodate, the amount used for oxidation is largely a function of concentration of the oxidisable component. It is seen that, although the maximum fructose concentration is approximately 6 times as large as the minimum, the percentage oxidation varies by only 10–13 %. Indeed, if the minimum fructose concentrations of 0.108 in Table VIII, and of 0.354 in Table IX be disregarded, since here the excess of oxidising agent over percentage fructose oxidised is very large, the variation amounts to only 4 %.

(5) Control experiments.

In addition to such control experiments as have been mentioned it was shown that:

(a) there is only a negligible loss of oxygen from the hypiodite solutions as used in these experiments when maintained at temperatures from 1 to 35° for 24 hours;

(b) addition of the alkali at the rate of 0.5 cc. of *N*/2 per half hour made no appreciable difference in the oxidation of glucose, an increase from 100 to 103 % being the maximum observed;

(c) iodate exerted a negligible oxidising effect on fructose, and a slightly larger but small one on glucose;

(d) free iodine, as used in these experiments, but without addition of any alkali, did not oxidise fructose to any appreciable extent;

(e) excess of alkali such as was used in any experiment other than that of Table II had no effect on the optical rotation of fructose at 17° in 20 hours: when NaOH was present at *N*/14 concentration as in Exp. 2 (Table II) the observed rotation fell from –8.1° to –7.9° in a few minutes, to –7.45° in 1½ hours and to –6.55° after 20 hours (the oxidation results of Table II them-

selves pointed to the same conclusion, *viz.* that some change such as enolisation was taking place);

(f) oxidation of 0.072 g. of glucose under the conditions of Table I (Series A and B) showed complete oxidation (1 g. glucose = 1.41 g. iodine) almost attained in 8 hours at 1°, fully attained in 20 mins. at 15° and 35°.

(6) *Identification of the products of oxidation of fructose.*

For this purpose, oxidation of fructose was effected by 0.3*N* iodine in BaI₂ and 0.4*N* baryta added in small increments. (Compare Goebel's procedure [1927, 2] for the preparation of hexonic acids.) Preliminary experiments (results in Table IX) had shown that the conditions adopted would be most favourable. 666 cc. of 0.3*N* iodine were added to 40 g. of fructose dissolved in 1000 cc. of water. 625 cc. of 0.4*N* baryta were added at the rate of 50 cc./30 mins. The temperature of working was 21–22°. 1 hour after the last alkali addition, the solution was filtered free of barium iodate and the exact amount of Ba in 10 cc. of the solution accurately determined gravimetrically. The whole of the barium in the residual solution was precipitated by the theoretical amount of 5*N* sulphuric acid. 150 g. of pure lead carbonate were stirred in to decompose free hydriodic acid. After filtering, the clear solution was evaporated at 55°/15 mm. to 350 cc., the whole of the residual free iodine passing over in the first few minutes. The liquid during concentration remained slightly on the acid side of neutrality. After cooling, the precipitate of lead iodide which usually appeared during evaporation was filtered off and the clear filtrate made neutral to phenolphthalein by addition of clear baryta. 2 vols. of 95 % alcohol were now added and the flocculum so obtained was allowed to stand overnight and was then centrifuged off, washed with alcohol and dried *in vacuo* and at 100° for 3 hours. The white product thus obtained was not very soluble in water, reduced warm acid KMnO₄ but gave no reduction with Fehling's solution. The Ba content was determined and the bulk of the salt decomposed with the exact amount of *N*/10 sulphuric acid. After evaporation to a syrup *in vacuo*, a little acetone was added and after standing, crystals of oxalic acid appeared, melting at 97–98°. These were dissolved in alcohol, phenylhydrazine in alcohol was added, and on scratching the sides of the tube crystals of oxalic acid phenylhydrazide appeared in a few moments. These were filtered off, washed with hot 95 % alcohol, and after drying, melted at 176.5°. When mixed with an authentic specimen of the hydrazide (m.p. 179°), the mixed m.p. was 178°. The highest yield of crude barium oxalate obtained in several repeated experiments was 2.4 g., whilst the theoretical yield, calculated from Table IX, is 2.5 g.

A possible criticism of the above method is that, at the temperature used in the removal by evaporation of the free iodine, oxidation of unoxidised fructose by the latter may give rise to the oxalate eventually isolated. To eliminate any error on this account, another method was employed. Instead of precipitating the barium of the oxidation mixture with sulphuric acid, acidification was effected with *N* HCl, and free iodine removed by addition of concentrated thiosulphate. The solution was brought back to neutrality with 0.4*N* baryta, and alcohol added until a flocculum appeared. This was again identified as barium oxalate in the manner previously described.

Oxidation of fructose by hypiodite produces, therefore, oxalic acid, and, presumably, *d*-erythronic acid. An attempt at separation of the latter acid from unoxidised fructose was not successful.

Omitting the ring structure,

This oxidation is extremely remarkable, in that the small amount of oxidation which is induced by hypiodite proceeds as far as the insertion of 4 oxygen atoms and the oxidation of a terminal alcoholic group. This fact strongly supports the hypothesis that only one component—an easily oxidisable component—undergoes oxidation.

DISCUSSION.

Whilst it is probable that one component only of fructose solution is oxidised by hypiodite, the nature of this component is an open question. It may be the ketoform or it may be the labile (2, 5) form. In the previous paper (Bailey and Hopkins) the latter form, as split off from the sucrose molecule on hydrolysis by fructosaccharase, was shown to change to equilibrium fructose unimolecularly, the velocity constant being 0.3 (*t* in mins., base *e*, temperature 17°, *p*_H 4.6–6.1). The change is not so rapid as had been supposed. This observation supports the view that equilibrium fructose contains a small proportion of the (2, 5) form. The striking change in optical rotation with temperature can be best explained by a disturbance of the equilibrium between the (2, 6) and (2, 5) forms. Further evidence for the existence of the (2, 5) and open chain forms in equilibrium fructose solutions is summarised below:

(1) Oparin and Kurssanov [1931] have shown that fructosaccharase exerts a synthesising action on glucose and fructose in aqueous solution if phosphatase and potassium dihydrogen phosphate are present. They represent the reaction as (2, 6)-fructose $\rightleftharpoons$ (2, 5)-fructose $\rightleftharpoons$ potassium (2, 5)-fructosephosphate;

It is difficult to see how such a synthesis is to be achieved without the pre-existence in solution of (2, 5)-fructose, which on removal is replaced by the dynamic transformation of one or more remaining isomerides.

(2) According to Ohle [1927], the condensation of β -fructose with acetone in presence of mineral acid leads mainly to the production of α -di-isopropylidene fructose, *i.e.* without change of configuration or structure, whilst the liberated water converts a portion of the fructose into the butylene oxide form which is transformed into the corresponding isopropylidene ether. If fructose in neutral aqueous solution is changed by water in the same manner as during acetonisation, it follows, when the magnitude of specific rotation is taken into account, that the main chemical change during mutarotation is the transformation of the amylen oxide β -form into the butylene oxide β -form. This cannot occur without temporarily opening the oxygen bridge, with the production of the keto-form or its hydrate. The carbonyl absorption band is shown by concentrated fructose solutions.

(3) Pure methyl alcohol, freed from acetone, when mixed with less than 5 % fructose, exhibits a change in the sign of rotation 20 mins. after the addition of HCl. The syrup isolated is feebly dextrorotatory. Extraction with ethyl acetate leads to the separation of γ -methylfructoside mixed with small quantities of α - and β -(2, 6)-methylfructosides [Menzies, 1922].

Onslow, Kidd and West [1931], in a biochemical study of the senescence of apples, advanced tentatively the hypothesis that active fructose (presumably (2, 5)-fructose) is preferentially respired. Such a process is quite feasible, and could, moreover, proceed without the concomitant inversion of sucrose, *i.e.* it could proceed without a supply of freshly liberated or active fructose, if equilibrated fructose solutions contain this form of fructose. Whether the component

oxidised by hypiodite is the (2, 5) form or an active keto-form, is as yet impossible to decide. According to Isbell and Hudson [1932], ring forms can be directly oxidised. On the other hand, Lippich [1932] finds that the HCN-binding power of fructose is greater than that of glucose and is not wholly inhibited by strong alkali. Since HCN-binding power is a measure of CO groups, the active keto-form of fructose may thus be the form oxidised by hypiodite even at alkaline reactions. There is, however, a striking parallel between the *in vitro* experiments of Section (3), and the process which Onslow *et al.* presume to occur in the respiration of senescent apples. The fundamental nature of both processes may well be the oxidation (or respiration) of one component, accompanied by dynamic mutation of other components to produce more of the oxidisable isomeride.

SUMMARY.

1. The rate of oxidation of fructose by hypiodite under the conditions of Willstätter and Schudel's method for the estimation of aldoses increases with temperature to the point at which oxidation ceases. The extent of oxidation at this point, between the temperatures 1 and 35°, is minimum at 15°.
2. In presence of excess alkali, enolisation of the fructose occurs between 17 and 37° and renders it capable of oxidation by iodate. The oxidation thus becomes a progressive reaction which has no limit. At 1° enolisation is not induced, and oxidation rapidly reaches a limit lower than that obtained by the use of only a slight alkali excess.
3. A four to fivefold increase in the oxidation of fructose by hypiodite is obtained by adding the necessary alkali in small increments at definite time intervals. The extent of oxidation is an inverse function of the rate of alkali addition. Under specified conditions, the oxidation of fructose (using the iodine value 1.41 as for glucose) may exceed 100 %, on the basis of one atom of oxygen per molecule oxidised.
4. The percentage oxidation under arbitrary conditions of oxidation does not vary greatly with the fructose concentration.
5. The products of oxidation are oxalic and, presumably, *d*-erythronic acids.
6. A consideration of the factors influencing the kinetics of the oxidation of fructose by hypiodite leads to the hypothesis that only one component undergoes oxidation.

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