LXXV.—*Action of Hydrogen Peroxide on Carbohydrates in the Presence of Ferrous Salts.*

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FENTON has shown, in the oxidation of tartaric acid by hydrogen peroxide in the presence of ferrous salts, that the $\text{CH}\cdot\text{OH}$ groups become oxidised to $\text{C}\cdot\text{OH}$ groups with the formation of dihydroxymaleic acid (Trans., 1894, 65, 899; 1895, 66, 774; 1896, 69, 546). Cross, Bevan, and Claude Smith (Trans., 1898, 73, 463) applied this method of oxidation to the carbohydrates glucose, lævulose, and cane sugar,

and found that substances were formed which reacted with phenylhydrazine at the ordinary temperature, and also reduced Fehling's solution in the cold. They were, however, unable to decide the nature of these substances, and, with their permission, we have examined the products of the reaction. In September, 1898, we communicated some of our results to the British Association, and showed that the first product belonged to the class of osones. We had not then isolated the osones, but their presence was recognised by the formation of phenylglucosazone when the solution of the oxidised glucose was treated with phenylhydrazine acetate at the ordinary temperature, and by the production of a methylphenylglucosazone by treatment of the oxidised glucose in the cold with methylphenylhydrazine. The formation of the latter is considered by Emil Fischer (*Ber.*, 1889, 22, 87) to be one of the characteristic tests for the presence of glucosone. The solution of the glucosone reduced Fehling's solution strongly when slightly warmed, and formed precipitates with diamines, hydrazine hydrate, or benzoylhydrazine in the cold, and with aniline at a slightly higher temperature.

The action of hydrogen peroxide in the presence of ferrous iron is seldom such as to completely oxidise these sugars to osones; some of the carbohydrate is always left over, and if it is fermentable by yeast, it may be removed in that way from the glucosone which is not attacked by yeast. The osone freed from fermentable sugar can be isolated by dissolving it in absolute alcohol and precipitating with ether, and is as active towards organic bases as the glucosone described by Emil Fischer (*loc. cit.*).

The behaviour of lævulose is almost identical with that of glucose under like conditions of treatment; glucosone is formed, and with this carbohydrate the action of hydrogen peroxide is more rapid than with glucose.

Arabinose, on oxidation, gives a substance resembling an osone, and the reaction is similar to that observed with the hexoses. The arabinosone obtained is identified by the formation both of phenylarabinosazone and of methylphenylarabinosazone at the ordinary temperature. Arabinose, however, even on warming with excess of methylphenylhydrazine, gives only the methylphenylhydrazone. Galactose, rhamnose, and even starch, under similar conditions of oxidation, give solutions which react with phenylhydrazine at the ordinary temperature, forming substances which are still under investigation. The results of the action of bromine water and of hydrogen cyanide on the oxidation products of glucose and lævulose will be communicated in a subsequent paper.

It would seem that the $\text{CH}\cdot\text{OH}$ group contiguous to the aldehyde group in the carbohydrates is oxidised to CO , and that the aldehyde

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group in the presence of this oxidising agent is not affected. In the case of lævulose, the primary alcohol group adjacent to the CO group undergoes oxidation to the CHO radicle, a transformation which Fenton (Trans., 1899, 75, 1) has shown to occur with glycerol, mannitol, and sorbitol, yielding glycerose, mannose, and sorbose respectively.

EXPERIMENTAL.

Oxidation of Glucose by Hydrogen Peroxide.

A 10 per cent. aqueous solution of glucose containing 0.3 per cent. of crystallised ferrous sulphate is oxidised by hydrogen peroxide of 20 volume strength. The neutralised peroxide is added in quantities corresponding to 1/10 atom of oxygen per gram-molecule of glucose until an atomic proportion of oxygen has been allowed to act on a molecular proportion of glucose. At first, the action is slow, and several hours elapse before all the ferric iron formed is reduced to ferrous iron again. After the reaction with the first 1/10 atom of oxygen is over, a little lead acetate is added; the addition of hydrogen peroxide is then continued, care being taken that the ferric iron has passed back again to ferrous before more of the oxidising agent is employed. The rise in temperature during the oxidation is never great, being about 2—6°. When one atomic proportion of oxygen has been allowed to react, the solution is treated with basic lead acetate, avoiding an excess; the precipitate, which is never large in quantity, is filtered off, the lead completely removed by just sufficient sulphuric acid for precipitation, the filtrate neutralised by caustic soda, and the remaining glucose destroyed by fermentation for several days at 30° with well-washed brewers' yeast until a small quantity, on dilution and treatment with a fresh quantity of yeast, gives no further evolution of carbon dioxide. The dark solution, after filtration from the yeast, is treated with phenylhydrazine acetate at the ordinary temperature, and, after standing for half an hour, the orange precipitate is collected, washed with water, dilute alcohol, and ether, and dried in a vacuum. Thirty grams of glucose, under these conditions of oxidation, give 9 grams of osazone.

0.182, dried at 105°, gave 0.4027 CO₂ and 0.1021 H₂O. C = 60.3; H = 6.2.

C₁₈H₂₂N₄O₄ requires C = 60.3. H = 6.1 per cent.

The osazone melts at 205° with decomposition, and is undoubtedly phenylglucosazone derived from glucosone, produced by the oxidation of glucose by hydrogen peroxide in the presence of iron.

Methylphenylglucosazone.—An aqueous solution of glucosone prepared as described above is warmed to 50° and treated with methylphenyl-

hydrazine dissolved in dilute acetic acid; an immediate turbidity ensues, and, on standing, a brownish-yellow precipitate separates. After being washed with water, dilute alcohol and ether, and recrystallised from benzene, methylphenylglucosazone forms slightly yellow, needle-shaped crystals, melting at 152° with decomposition, and giving the following numbers on analysis:

0.1505 gave 0.3413 CO_2 and 0.095 H_2O . $\text{C} = 61.85$; $\text{H} = 6.95$.

0.1095 „ 13.6 c.c. of moist nitrogen at 23° and 766 mm. $\text{N} = 14.15$.

$\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_4$ requires $\text{C} = 62.1$; $\text{H} = 6.7$; $\text{N} = 14.5$ per cent.

Separation of the Glucosone.—The aqueous solution of the glucosone is concentrated to a syrup at 50° in a vacuum, the syrup treated with absolute alcohol, filtered, the alcoholic solution concentrated in a vacuum at 50° , and the last traces of alcohol removed by dry ether. The residue, on treatment with absolute alcohol, dissolves, and after the addition of dry ether, a small quantity of a white, hygroscopic solid is obtained, which soon changes to a syrup, is hardly sweet to the taste, reduces Fehling's solution when slightly warmed, and reacts readily with phenylhydrazine at the ordinary temperature. 1.3 grams of the white solid gave a relatively large precipitate with phenylhydrazine in the cold, and, after warming *slightly*, the yield amounted to 0.5 gram of phenylglucosazone. A control experiment with 1.3 grams of glucose showed that no precipitate was formed with phenylhydrazine acetate at the ordinary temperature, and that 0.7 gram of osazone was obtained on warming for about a quarter of an hour. The identity of the phenylglucosazone from glucosone was proved by the melting point, 205° , and by a nitrogen determination.

0.1091 gave 14.8 c.c. of moist nitrogen at 23° and 761 mm. $\text{N} = 15.3$

$\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_4$ requires $\text{N} = 15.6$ per cent.

The aqueous solution of the glucosone gives precipitates with orthophenylenediamine or benzoylhydrazine at the ordinary temperature, and with aniline acetate on slightly warming. The oxime has not as yet been obtained crystalline.

Oxidation of Lævulose by Hydrogen Peroxide.

Under conditions similar to those described in the case of glucose, lævulose gives an osazone with phenylhydrazine acetate at the ordinary temperature, which after recrystallisation from dilute alcohol, melts at 205° and is phenylglucosazone.

0.1858 gave, after drying at 100° , 0.4085 CO_2 and 0.1050 H_2O .

$\text{C} = 59.96$; $\text{H} = 6.3$.

$\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_4$ requires $\text{C} = 60.3$; $\text{H} = 6.1$ per cent.

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The oxidation product from 30 grams of lævulose yields 10·5 grams of the osazone after standing for $\frac{3}{4}$ hour in the cold with phenylhydrazine. Lævulose seems to be more easily oxidised than glucose by hydrogen peroxide, as the reaction is completed easily in a day, whereas the addition of hydrogen peroxide has to be continued during two days in the case of the latter.

Oxidation of Galactose by Hydrogen Peroxide.

Galactose, when oxidised under like conditions with one atomic proportion of oxygen per gram-molecule of sugar, gives, with phenylhydrazine acetate, a red precipitate which is soluble in ether. As we could not recrystallise this satisfactorily, we used methylphenylhydrazine instead, and purified the oxidation product by concentration in a vacuum, treatment with absolute alcohol, subsequent concentration in a vacuum, and removal of the last traces of absolute alcohol by ether. The syrup so obtained contained galactose, as it yielded mucic acid (m. p. 213°) on oxidation with nitric acid, and with methylphenylhydrazine in the cold gave an immediate orange precipitate consisting of galactose methylphenylhydrazone readily soluble in hot water, and another substance insoluble in water but soluble in hot benzene. Galactose methylphenylhydrazone crystallises in colourless needles from hot water and melts at 187° with decomposition.

0·1725, dried at 100°, gave 0·3460 CO₂ and 0·1102 H₂O. C=55·25 ;
H=7·1.

0·1955, dried at 100°, gave 16·2 c.c. moist nitrogen at 18° and 773 mm.
N=9·7.

C₁₃H₂₀N₂O₅ requires C=54·93 ; H=7·0 ; N=9·8 per cent.

The substance insoluble in hot water is under investigation.

Oxidation of Arabinose by Hydrogen Peroxide.

A 5 per cent. aqueous solution of arabinose containing 0·5 per cent. of crystallised ferrous sulphate is oxidised by the careful addition of neutralised hydrogen peroxide of 20 volume strength in quantities corresponding with 1/10 atom of oxygen per gram-molecule of sugar, until one atomic proportion of oxygen has been added. The action is at first slow, but after half the requisite quantity of oxygen has been added there is a decided colour change on addition of the oxidising agent, the solution becoming greenish-blue ; the colour, however, is only temporary, and disappears when the hydrogen peroxide has been used up. It is advisable to keep the solution cool, for there is often a decided rise of temperature during the addition of the oxidising

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agent. When one atomic proportion of oxygen has been added, the solution is treated with normal and basic lead acetate, avoiding an excess of the latter, the solution filtered, and the lead in solution removed completely by just sufficient sulphuric acid for precipitation. The clear solution reacts readily with phenylhydrazine in the cold, giving an osazone which is easily soluble in alcohol or ether; the yield amounts to 17 per cent. of the weight of sugar taken, if the mixture is left for 2 hours at the ordinary temperature before filtration. After recrystallisation three or four times from dilute alcohol, the osazone melted at 158° , and on analysis gave the following numbers.

0.17, dried in a vacuum, gave 0.3885 CO_2 and 0.0905 H_2O . $\text{C} = 62.3$;
 $\text{H} = 5.8$.

0.111, dried at 100° , gave 16 c.c. moist nitrogen at 19° and 770 mm.
 $\text{N} = 16.75$.

$\text{C}_{17}\text{H}_{20}\text{O}_3\text{N}_4$ requires $\text{C} = 62.2$; $\text{H} = 5.5$; $\text{N} = 17.0$ per cent.

The substance therefore is arabinosazone, derived from the osone obtained by the oxidation of arabinose with hydrogen peroxide.

Methylphenylarabinosazone.—A solution of the arabinosone reacts with methylphenylhydrazine at the ordinary temperature; it is advisable, however, to warm it to 40° , and then to add not quite the calculated quantity of methylphenylhydrazine acetate. When cold, the precipitate is collected, washed with water and ether, and dried in a vacuum; a brownish-yellow solid is thus obtained which, when recrystallised from benzene, gives yellowish, needle-shaped plates melting at 168 — 170° with decomposition. The yield of crude osazone is 25 per cent. of the weight of the arabinose taken.

0.175, dried in a vacuum, gave 0.4104 CO_2 and 0.1055 H_2O .
 $\text{C} = 63.9$; $\text{H} = 6.7$.

0.173, dried at 105° until constant, gave 0.4024 CO_2 and 0.106 H_2O .
 $\text{C} = 63.4$; $\text{H} = 6.8$.

0.1258, dried at 105° , gave 17.2 c.c. moist nitrogen at 17° and 753 mm.
 $\text{N} = 15.4$.

$\text{C}_{19}\text{H}_{24}\text{N}_4\text{O}_3$ requires $\text{C} = 64.0$; $\text{H} = 6.7$; $\text{N} = 15.7$ per cent.

Arabinose with methylphenylhydrazine gives only the hydrazone even when warmed with an excess on the water-bath. An aqueous solution of 3 grams of arabinose, when warmed for a short time on the water-bath with 4.5 grams of methylphenylhydrazine dissolved in dilute acetic acid, gives a white, crystalline precipitate of the hydrazone, which, after recrystallisation from dilute alcohol, melts at 163° ; it is only sparingly soluble in benzene.

0.1812, dried at 100° , gave 0.3784 CO_2 and 0.1213 H_2O . $\text{C} = 56.9$;
 $\text{H} = 7.4$.

0.1687, dried at 100° , gave 17.0 c.c. moist nitrogen at 15° and
753 mm. $\text{N} = 11.5$.

$\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_4$ requires $\text{C} = 56.7$; $\text{H} = 7.1$; $\text{N} = 11.7$ per cent.

The solution of arabinosone obtained by oxidation with hydrogen peroxide in the presence of ferrous sulphate reacts easily with aniline acetate, giving a dark brown, flocculent precipitate on warming on the water-bath ; it reacts also easily with parabromophenylhydrazine in the cold, giving what is probably an osazone. Arabinose is perhaps more completely oxidised than glucose, and its disappearance can be noticed by the solution failing to give the colour reaction with phloroglucinol towards the end of the oxidation.

The ease with which methylphenylhydrazine gives easily crystallisable hydrazone when warmed with arabinose and galactose for a short time on the water-bath, may be of use in the separation of these two sugars from glucose and laevulose, which, as Emil Fischer has shown, do not react with it. The sugar so separated might be regenerated from the hydrazone, as is the case with mannose,

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