

LXX.—*Action of Hydrogen Peroxide on Carbohydrates in the Presence of Ferrous Sulphate. III.*

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THE specific action of hydrogen peroxide in the presence of ferrous sulphate was first demonstrated by Fenton in the oxidation of tartaric acid to dihydroxymaleic acid (Trans., 1894, 65, 899), and later in the oxidation of polyhydric alcohols to aldoses (Fenton and Jackson, Trans., 1899, 75, 1). Cross, Bevan, and Smith (Trans., 1898, 73, 500) have investigated the action of these two reagents on dextrose, and found that in addition to tartronic acid a substance was formed which gave an im-

mediate precipitate with phenylhydrazine acetate, but they were unable to decide the nature of the substance. In communication with these authors, we have continued the investigation, and have shown that dextrose, lævulose, arabinose, and rhamnose are transformed by this peculiar action of hydrogen peroxide in the presence of ferrous sulphate into osones, which were recognised by their power to react with substituted hydrazines at the ordinary temperature, yielding osazones (Trans., 1899, '75, 787; 1900, '77, 1219).

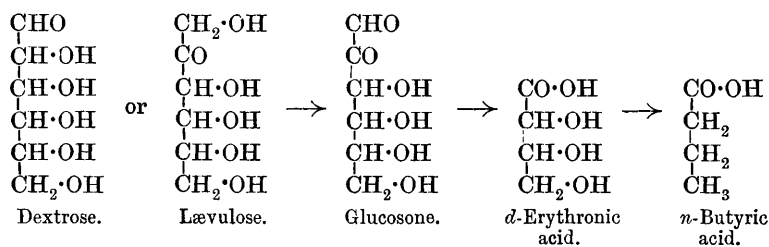
Mannose and galactose on oxidation would be expected to yield osones. In the case of mannose, an osone is formed giving, with phenylhydrazine, phenylglucosazone, but galactose behaves differently, and we are unable as yet to determine to what extent this sugar is oxidised by hydrogen peroxide in the presence of ferrous sulphate.

Fischer (*Ber.*, 1894, 27, 2031) states that galactose and *d*-talose are acted on by ferments with greater difficulty than dextrose and mannose, and in explanation of this difference he refers to the arrangement of the CH·OH groups, which in galactose and *d*-talose is very unlike that in dextrose and mannose. We consider that the internal compensating positions of the hydrogen atoms and hydroxyl groups may account for galactose behaving differently from other hexoses when oxidised by hydrogen peroxide in the presence of ferrous sulphate. We have attempted the preparation of glucosone from dextrose and lævulose, and have obtained a white, amorphous solid which gave analytical numbers agreeing with those required for the formula  $C_6H_{12}O_6$  or  $C_6H_{10}O_6$ ; it is not easy to decide between the two formulæ from the percentages of carbon and hydrogen. The white solid reacted immediately with phenylhydrazine acetate at the ordinary temperature and gave a good yield of phenylglucosazone. From its optical activity, we are led to the conclusion that it was contaminated with a small quantity of the parent carbohydrate, since the osone obtained from dextrose was slightly dextrorotatory, whilst the osone from lævulose had a lævorotatory power less than that of lævulose. Glucosone, obtained from phenylglucosazone by E. Fischer, is feebly lævorotatory (*Ber.*, 1889, 22, 89).

We have fermented solutions of glucosone from dextrose and lævulose and found that at the end of the fermentation the solution was slightly lævorotatory and still reacted immediately with phenylhydrazine acetate.

In order to test further the truth of the statement that osones are formed when carbohydrates are oxidised under the conditions given above, we have tried the action of bromine on aqueous solutions of glucosone obtained from dextrose and lævulose and have obtained good yields of salts of an acid which is not gluconic acid, but trihydroxybutyric acid identical with that obtained from erythritol by oxidation

with nitric acid (Lamparter, *Annalen*, 1865, 134, 260), or from *d*-erythrose by the action of bromine (Ruff, *Ber.*, 1899, 32, 3678). The barium and calcium salts of *d*-erythronic acid, obtained from dextrose or lævulose, have been reduced with hydriodic acid and phosphorus, and gave *n*-butyric acid which was easily identified by means of its silver salt. It has not been shown, so far as we know, that trihydroxybutyric acid is an oxidation product of dextrose, although Iwig and Hecht (*Ber.*, 1886, 19, 169) have obtained a trihydroxybutyric acid in small quantities from mannitol by oxidation with potassium permanganate. Trihydroxybutyric acid has also been obtained from lævulose by the action of mercuric oxide and baryta, or by the action of bromine (Börnstein and Herzfeld, *Ber.*, 1886, 18, 3354; Herzfeld, *Annalen*, 1888, 244, 291; Ruff, *Ber.*, 1899, 32, 3680). Ruff states that the yield of trihydroxybutyric acid obtained from lævulose is exceedingly small, but he was able to prepare a brucine salt of this acid which was identical with the brucine salt of *d*-erythronic acid. We have found that the amount of calcium trihydroxybutyrate obtained from lævulose by the direct action of bromine was not more than 1 per cent. of the weight of the lævulose taken. The transformation of dextrose and lævulose into glucosone, trihydroxybutyric acid (*d*-erythronic acid), and into *n*-butyric acid can be expressed in the following manner:



We are engaged on the further investigation of the properties of osones obtained by the action of hydrogen peroxide on carbohydrates in the presence of ferrous salts, and hope to communicate at an early date the results of the action of bromine on a solution of rhamnosone which would point to the formation of a methylglyceric acid—a result which may have an important bearing on the constitution of rhamnose.

#### EXPERIMENTAL.

##### *Oxidation of Mannose.*

Forty grams of seminose syrup were transformed into mannose hydrazone by treatment with phenylhydrazine acetate. The hydrazone was decomposed by benzaldehyde and the purified mannose obtained as a syrup, which was found to contain about 8 grams of the

sugar. One hundred and fifty c.c. of a 5 per cent. solution of mannose were treated with hydrogen peroxide of 20-volume strength in the presence of 1 gram of ferrous sulphate. The amount of hydrogen peroxide used was such as to yield 0.66 gram of oxygen, which was the calculated quantity required for the transformation of 7.5 grams of mannose into its osone. The oxidiser was added slowly in tenths of the required quantity, as in the oxidation of dextrose and lævulose. An aqueous solution of the osone was obtained in the manner described in a former paper (Trans., 1900, 77, 1219). On treatment of the solution of the osone with phenylhydrazine at the ordinary temperature, a yellow precipitate formed immediately. After being allowed to stand for a few hours, the precipitate was filtered, washed with alcohol and ether, and dried in a vacuum. The yield of glucosazone was 5 grams. On recrystallisation from alcohol, the glucosazone melted at 204° with decomposition and gave the following numbers on analysis :

0.1670 gave 0.3789 CO<sub>2</sub> and 0.1017 H<sub>2</sub>O. C = 60.1 ; H = 6.6.

0.0678 „ 9.1 c.c. moist nitrogen at 12° and 750 mm. N = 15.68.

C<sub>18</sub>H<sub>22</sub>O<sub>4</sub>N<sub>4</sub> requires C = 60.3 ; H = 6.2 ; N = 15.65 per cent.

Twenty grams of seminose, which had not been purified by the method given above, yielded, after oxidation with hydrogen peroxide in the presence of ferrous sulphate, 4 grams of glucosazone, which, on recrystallisation, melted at 199° with decomposition and contained 15.98 per cent. of nitrogen.

#### *Preparation of Glucosone from Dextrose or Lævulose.*

An aqueous solution of glucosone, prepared from lævulose (Trans., 1900, 77, 1219), was concentrated to a syrup in a vacuum at 50°. The syrup was poured into warm absolute alcohol and the alcohol solution filtered and concentrated in a vacuum on the water-bath and finally poured into dry ether. A white, amorphous solid separated, which was dissolved in hot absolute alcohol and the solution, after filtration and concentration, poured into dry ether. The yield of the white, amorphous solid from 60 grams of lævulose amounted to 3 grams. An analysis of the substance dried in a vacuum gave the following numbers :

0.1782 gave 0.2608 CO<sub>2</sub> and 0.0949 H<sub>2</sub>O. C = 39.92 ; H = 5.87.

C<sub>6</sub>H<sub>10</sub>O<sub>6</sub> requires C = 40.40 ; H = 5.61 per cent.

The glucosone, which was practically free from ash, reduced Fehling's solution without being warmed.

Glucosone from dextrose was prepared in the same way, except that

hydrogen peroxide of 10-volume strength was used instead of 20-volume strength. An analysis of the substance dried in a vacuum showed it to be less pure than the glucosone obtained from lævulose :

0.2002 gave 0.2905  $\text{CO}_2$  and 0.1162  $\text{H}_2\text{O}$ .  $\text{C} = 39.57$  ;  $\text{H} = 6.4$ .

It was not free from impurity, since 0.2 gram of substance, after ignition, was found to contain 0.9 mg. of ash. The glucosone reduced Fehling's solution at the ordinary temperature. Both the samples of glucosone reacted immediately with a cold solution of phenylhydrazine acetate. One gram of glucosone from dextrose dissolved in 10 c.c. of water was treated with 2 grams of phenylhydrazine dissolved in 2 c.c. of 50 per cent. acetic acid and diluted with 4 c.c. of water. A precipitate was formed immediately. After standing for some hours at the ordinary temperature, the glucosazone was filtered off, washed with water, and dried in a vacuum. The weight of the osazone amounted to 0.5 gram. Half a gram of glucosone from lævulose dissolved in 70 c.c. of water and treated with 1 gram of phenylhydrazine in 50 per cent. acetic acid gave 0.23 gram of glucosazone. The liquid was kept quite cold, and under these conditions both dextrose and lævulose do not give a precipitate with phenylhydrazine acetate unless the solutions are allowed to stand for several days. On recrystallisation from alcohol, the osazone melted at  $203^\circ$  with decomposition, and its identity was further established by a nitrogen determination :

0.0653 gave 8.4 c.c. moist nitrogen at  $12^\circ$  and 760 mm.  $\text{N} = 15.2$ .

$\text{C}_{18}\text{H}_{22}\text{O}_4\text{N}_4$  requires  $\text{N} = 15.65$  per cent.

The determination of the optical activity of the glucosone from dextrose and lævulose gave results which were unsatisfactory, and we concluded that small quantities of the parent sugar were present as impurity, especially in the case of the glucosone from dextrose, for the preparation of which 10-volume hydrogen peroxide was used. The glucosone in each case was dissolved in water :

0.504 gram of glucosone from lævulose had  $[\alpha]_D - 54^\circ$  ;  
 0.5247 " " dextrose "  $[\alpha]_D + 13.5^\circ$  ; whereas  
 0.5154 " lævulose dissolved in water had  $[\alpha]_D - 89^\circ$ .

E. Fischer states that glucosone prepared from phenylglucosazone is feebly lævorotatory (*Ber.*, 1889, 22, 89).

We have fermented solutions of glucosone from dextrose and lævulose with yeast in order to remove these carbohydrates. A solution of glucosone from dextrose had a decided dextrorotatory power, but after two days' fermentation at  $30^\circ$  became feebly lævorotatory. The fermentation was continued for another day, but there was no change in the rotatory power. After the removal of the inorganic impurities by

concentration of the aqueous solution of the glucosone in a vacuum on the water-bath and treatment with absolute methyl alcohol, a syrup was obtained which did not crystallise, but reacted immediately with phenylhydrazine acetate at the ordinary temperature.

A solution of glucosone prepared from l  vulose was treated in exactly the same manner. The l  vorotatory power became less on fermentation and after two days the fermentation stopped. The solution was found to be slightly l  vorotatory and reacted immediately with a cold solution of phenylhydrazine acetate.

*Oxidation of an Aqueous Solution of Glucosone prepared from Dextrose.*

An aqueous solution of glucosone prepared from 30 grams of dextrose was heated with 12.5 grams of bromine at 40   for 12 hours (the volume of the solution was 350 c.c.). The excess of bromine was removed by a current of air and the yellow liquid was treated with an excess of lead carbonate. After standing for 24 hours, the liquid was filtered from lead carbonate and lead bromide, and sulphuretted hydrogen was passed in. The filtrate from the lead sulphide was concentrated to a small bulk in a vacuum at 60   on the water-bath. It was found to be impossible to remove the last traces of hydrobromic acid by silver carbonate as the solution contained a reducing substance. The residual liquid was boiled with calcium carbonate until it was neutral, decolorised with animal charcoal, filtered, evaporated in a vacuum on the water-bath to a small bulk, and poured into absolute alcohol. The calcium salt was obtained as a granular precipitate which was washed with absolute alcohol and ether and the ether removed in a vacuum. The yield of the salt varied between 25 and 30 per cent. of the weight of dextrose taken. It was purified by treatment with the calculated quantity of oxalic acid, and after boiling with calcium carbonate and decolorising with animal charcoal was reprecipitated by absolute alcohol. The yield of the purified salt was never less than 12–15 per cent. of the weight of the dextrose used. Analysis of the calcium salt gave the following numbers :

0.1995, air dried, gave 0.2072 CO<sub>2</sub> and 0.0872 H<sub>2</sub>O. C = 28.38 ; H = 4.85.

0.1958 dried at 110  , 0.223 CO<sub>2</sub> ,, 0.0735 H<sub>2</sub>O. C = 31.0 ; H = 4.2.

0.4170 ,, 110   gave 0.1785 CaSO<sub>4</sub>. Ca = 12.6.

0.315 ,, 110–130   ,, 0.1405 CaSO<sub>4</sub>. Ca = 13.1.

0.4253 of the air-dried salt ,, 0.1602 CaSO<sub>4</sub>. Ca = 11.08.

(C<sub>4</sub>H<sub>7</sub>O<sub>5</sub>)<sub>2</sub>Ca.2H<sub>2</sub>O requires Ca = 11.56 ; C = 27.74 ; H = 5.2 per cent.

(C<sub>4</sub>H<sub>7</sub>O<sub>5</sub>)<sub>2</sub>Ca ,, Ca = 12.9 ; C = 30.96 ; H = 4.5 ,,

These numbers show that the salt is most probably calcium trihydroxybutyrate.

*Lead Erythronate*,  $(C_4H_6O_5)Pb$ .—The lead salt was prepared by adding normal lead acetate to a solution of the purified calcium or barium salt. The white precipitate so obtained was well washed with hot water, and after being dried at  $130^\circ$  was analysed :

0.1628 gave 0.1448  $PbSO_4$ . Pb = 60.8.

0.1550 „ 0.1660  $PbSO_4$ . Pb = 60.8.

$(C_4H_6O_5)Pb$  requires Pb = 60.7 per cent.

This salt was obtained by Lamparter from erythronic acid (*loc. cit.*), and is insoluble in dilute acetic acid. It has been considered to be a characteristic salt of *n*-trihydroxybutyric acid (Fischer, *Ber.*, 1889, 22, 110 ; Fenton, *Trans.*, 1899, 75, 7). The lead salt obtained by us was insoluble in dilute acetic acid.

*Barium Erythronate*,  $Ba(C_4H_7O_5)_2 \cdot 2H_2O$ .—For the analysis, the barium salt obtained by boiling the solution containing erythronic acid with barium carbonate and precipitating with alcohol must be freed from iron compounds, which are present in small quantity. This was done by dissolving the barium salt in a small volume of water, adding alcohol in slight excess, filtering, and washing the undissolved residue, which was brown in colour, with a little cold water. The filtrate contained the barium erythronate, which was treated with dilute sulphuric acid, the liquid boiled with barium carbonate, and the pure barium salt reprecipitated by alcohol. On analysis :

0.1803 lost, at  $130^\circ$ , 0.01450  $H_2O$ .  $H_2O$  = 8.04.

0.1653, dried at  $130^\circ$ , gave 0.0938  $BaSO_4$ . Ba = 33.31.

0.1645, „ „ 0.0943  $BaSO_4$ . Ba = 33.76.

$Ba(C_4H_7O_5)_2 \cdot 2H_2O$  requires  $H_2O$  = 8.03 per cent.

$Ba(C_4H_7O_5)_2$  „ Ba = 33.66 „

*Reduction of Calcium Erythronate to Butyric Acid*.—Fifteen grams of calcium erythronate, obtained from dextrose, were heated for 8 hours with 130 c.c. of hydriodic acid (b. p.  $127^\circ$ ) and 5 grams of amorphous phosphorus in a flask fitted with a reflux condenser on a sand-bath. The brown liquid was diluted with an equal volume of water and shaken six times with ether. The ether was distilled off and a brown oil was left which possessed a strong odour of butyric acid. To complete the reduction, the oil was heated with dilute sulphuric acid and zinc for an hour in a flask fitted with a reflux condenser. After being allowed to stand for several hours, the excess of zinc was filtered off and a little more sulphuric acid was added to the filtrate. A current of steam was passed into the liquid and the distillate, which was strongly acid, was neutralised with calcium carbonate. After filtration and concentration on the water-bath, 1.2 grams of a salt crystallised out. This salt had the characteristic properties of

calcium butyrate, being less soluble in hot water than in cold, and having the peculiar odour of the acid. To confirm the formation of butyric acid, the silver salt was prepared from the calcium salt, dried in a vacuum, and analysed :

0.2345 gave 0.1345 Ag. Ag = 55.4.

0.1708 „ 0.0948 Ag. Ag = 55.5.

$C_4H_7O_2Ag$  requires Ag = 55.4 per cent.

The total yield of calcium butyrate from 15 grams of calcium erythronate was about 15 per cent. of the theoretical amount.

*Oxidation of an Aqueous Solution of Glucosone prepared from Lævulose.*

The action of bromine on an aqueous solution of glucosone prepared from lævulose was exactly the same as in the case of glucosone obtained from dextrose, and it is unnecessary to repeat the details of the separation and purification of the calcium or barium erythronate. From 30 grams of lævulose in each case, the yield of calcium salt amounted to 15 grams or, 50 per cent. of the theoretical, and of barium salt to 11 grams, or 30 per cent. of the theoretical.

The *calcium* salt, dried at  $110^\circ$ , was analysed :

0.2050 gave 0.2360  $CO_2$  and 0.0845  $H_2O$ . C = 31.3 ; H = 4.6.

0.2875 „ 0.1285  $CaSO_4$ . Ca = 13.15.

0.2875 „ 0.1280  $CaSO_4$ . Ca = 13.1.

$(C_4H_7O_5)_2Ca$  requires C = 31.0 ; H = 4.5 ; Ca = 12.9 per cent.

The *barium* salt, dried at  $130^\circ$ , was analysed, with the following results :

0.2255 lost 0.0190  $H_2O$ .  $H_2O$  = 8.4.

0.2065 gave 0.1195  $BaSO_4$ . Ba = 33.8.

$(C_4H_7O_5)_2Ba \cdot 2H_2O$  requires  $H_2O$  = 8.0 per cent.

$(C_4H_7O_5)_2Ba$  „ Ba = 33.66 „

The *lead* salt of erythronic acid was prepared from the barium salt. The yield amounted to 1 gram from 3 grams of barium salt, that is, about 25 per cent. of the calculated quantity :

0.1648, dried at  $130^\circ$ , gave 0.1465  $PbSO_4$ . Pb = 60.5.

$(C_4H_6O_5)Pb$  requires Pb = 60.77 per cent.

It is not necessary to dry the salt at so high a temperature as  $160^\circ$  (Lamparter, *loc. cit.*).

*Strychnine Salt.*—When the calcium salt was treated with the calculated quantity of oxalic acid and any excess of oxalic acid removed by lime water, a solution of the free acid was obtained, which, on concentration, yielded a syrup which did not crystallise. From the



## 674 ACTION OF HYDROGEN PEROXIDE ON CARBOHYDRATES. III.

solution of the acid, by boiling with strychnine, filtering and concentrating, and treating with absolute alcohol, the strychnine salt was precipitated, which, after recrystallising twice from a small quantity of water, gave, on analysis, the following numbers:

0.1777, dried at  $130^{\circ}$ , gave  $0.4137 \text{ CO}_2$  and  $0.0985 \text{ H}_2\text{O}$ .  $\text{C} = 63.6$ ;  $\text{H} = 6.1$ .  
 0.1818, dried in a desiccator, gave  $0.4083 \text{ CO}_2$  and  $0.0185 \text{ H}_2\text{O}$ .  $\text{C} = 61.24$ ;  
 $\text{H} = 6.57$ .

$\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2, \text{C}_4\text{H}_8\text{O}_5$  requires  $\text{C} = 63.8$ ;  $\text{H} = 6.15$  per cent.  
 $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2, \text{C}_4\text{H}_8\text{O}_5, \text{H}_2\text{O}$  „  $\text{C} = 61.47$ ;  $\text{H} = 6.55$  „

The strychnine salt crystallised in needles from water, and contained one mol. of water of crystallisation which was not expelled under  $130^{\circ}$ .

*Reduction of Erythronic Acid, obtained from Lævulose, to Butyric Acid.*—The details of this reduction are identical with those described under the reduction of calcium erythronate from dextrose. The yield of calcium or barium butyrate was about the same as in the reduction of the calcium salt from dextrose. The calcium or barium butyrate was transformed into the silver salt, which crystallised from water in white needles.

The *silver* salt was analysed:

0.1814 gave  $0.1003 \text{ Ag}$ .  $\text{Ag} = 55.3$ .  
 $\text{C}_4\text{H}_7\text{O}_2\text{Ag}$  requires  $\text{Ag} = 55.4$  per cent.

*Oxidation of Erythritol by Nitric Acid.*

Lamparter prepared erythronic acid from erythritol by the action of strong nitric acid on a hot concentrated aqueous solution of erythritol (*loc. cit.*). Przibyt (J. Russ. Phys. Chem. Soc., 1881, 12, 208) states that dilute nitric acid transforms erythritol into oxalic acid and mesotartaric acid.

Ten grams of erythritol were oxidised by 25 c.c. of nitric acid of sp. gr. 1.2 at  $40^{\circ}$  for 28 hours, the liquid diluted with water, and the nitric acid removed by distillation in a vacuum at  $50^{\circ}$ . A syrup was obtained which contained oxalic and erythronic acid. The calcium erythronate was prepared in the usual way and precipitated by means of alcohol. The salt was purified by treatment with oxalic acid and calcium carbonate and was precipitated from its aqueous solution with alcohol, and washed with ether. The yield of the purified salt was 7.5 grams:

0.2432, dried at  $110^{\circ}$ , gave  $0.1085 \text{ CaSO}_4$ .  $\text{Ca} = 13.1$ .  
 $(\text{C}_4\text{H}_7\text{O}_5)_2\text{Ca}$  requires  $\text{Ca} = 12.9$  per cent.

This salt is evidently identical with the calcium erythronate already

described, and from it a lead salt was obtained insoluble in dilute acetic acid.

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