

XLIX.—*Reactions of the Carbohydrates with Hydrogen Peroxide.*

By C. F. CROSS, E. J. BEVAN, and CLAUDE SMITH.

HOWEVER obvious it may appear that the simpler carbohydrates would react with hydrogen peroxide under certain conditions, the matter has

been but little investigated. The only reference we can find dealing specifically with the subject is contained in a paper by C. Wurster (*Ber.*, 1887, 20, 2631), where the question of reaction is dismissed with the negative remark that glucose and cane-sugar are comparatively resistant to the action of the peroxide.

The recent series of researches of H. J. H. Fenton on the oxidation of tartaric acid by hydrogen peroxide has disclosed several points of especial interest in regard to the mode of action of the latter, more particularly the oxidation of the residue $-\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})-$ to $-\text{C}(\text{OH})\text{:C}(\text{OH})-$, and the dependence of this oxidation on the presence of soluble iron compounds (*Trans.*, 1894, 65, 899; 1895, 67, 48; 1896, 69, 546), and the results certainly suggest fruitful extension of the method to other hydroxy-compounds. In correspondence with Mr. Fenton, we undertook the preliminary investigation of the behaviour of typical carbohydrates with the peroxide under similar conditions of treatment, and the results of our observations are here briefly recorded.

It is recognised by chemists on general grounds, as it is more positively held by physiologists, that the carbohydrates are oxidised in the living cell by processes of varying intensity; but it must be admitted that very little direct light has yet been thrown on the actual mechanism of such processes. Our laboratory processes of oxidation are of two kinds, namely, 'constructive' oxidations of the hexoses to monocarboxylic acids (by bromine) and dicarboxylic acids (by nitric acid), and destructive oxidations to products of lower molecular weight, for instance, by alkaline cupric oxide, permanganates, and hypochlorites. The latter are so complex in character that they at present occupy only a subordinate place in the systematic investigation of constitutional problems, neither are such methods calculated to directly elucidate physiological problems.

Oxidations of the hexoses by hydrogen peroxide, on the other hand, such as we are about to describe, are of the kind which may be fairly presumed to take place in the plant; for although the existence of this particular 'kathoxide' in the plant cell may be difficult of proof by application of the ordinary reagents, it is generally admitted that the 'active oxygen' of the cell must in part at least take this form (*A. Bach, Compt. rend.*, 1894, 119, 286).

The dependence of such oxidations on a particular third substance, acting simultaneously, has not been assumed, nor, so far as we know, surmised by physiologists. As we are now able to show that iron salts have a specific influence in exciting or determining these oxidations, these investigations involve direct inquiry into the functions of the inorganic or ash constituents of plants. It must be admitted that the enormous accumulation of facts in this province, in the form

of analytical results, has as yet but little other than a general significance to the physiologist. It would appear that the inorganic salts must directly affect the many processes of hydrolysis and condensation going on in the plant; but their influence as determining or modifying oxidising actions, in which from their nature they can only play the subordinate part of "catalysts," is hardly to be predicted on such definite grounds as "the nature of things." There may, perhaps, be some suggestions, in the nature of the iron salts, of the mechanism of their action as the *tertium quid* in effects of oxidation; and this matter is dealt with by Mr. Fenton in a note communicated simultaneously with this paper. But similar reasons could not obtain for a compound such, for example, as phosphoric acid. And yet it has been already observed, as an "extraordinary circumstance," that in the oxidation of acetone by hydrogen peroxide (to the 'cycloacetone peroxide'), a small quantity of phosphoric acid determines the reaction, which does not take place with the pure reagents (Wolffenstein, *Ber.*, 1895, 28, 2267). A reaction of this kind had no obvious bearing on physiological problems; but in connection with the subject of this paper, it is a significant extension of the probabilities under discussion.

One other consideration we have to note by way of prelude to a brief account of the experimental results which we have obtained. These investigations arise out of our continuous search for a solution of the problems of the origin in the plant of (1) the lignocelluloses, and (2) the carbohydrates yielding furfuraldehyde. As a working hypothesis, it has been assumed that both groups of tissue constituents are formed from the normal carbohydrates. This hypothesis, taken together with the assumption that "the plant can do everything," has been, and is, a very unsatisfying resting place. It must be admitted that the systematic study of the carbohydrates, notwithstanding its rapid advance, has not afforded any suggestion as to the actual mechanism of such transformations. Fenton's researches coming at a time when we had exhausted the more obvious directions of indirect inquiry, imported entirely new suggestions; the first of which is that, as in the dicarboxylic hydroxy-acids, so in the carbohydrates, there is a direct passage to unsaturated compounds by way of oxidising processes. To prove this suggestion, and to open out these physiological problems from the more positive chemical standpoint, has been our first and main purpose.

This being merely a preliminary communication, we shall limit it to a brief explanation of the purpose and aims of the investigation, and an equally brief account of the experimental results so far obtained.

In some respects, the most striking result is the verification of Wurster's statement as to the comparative resistance of the hexoses (and cane-sugar) to the action of hydrogen peroxide, with the exten-

sion of Fenton's observation that the equilibrium is upset by the addition of ferrous salts in such small proportion as 1/10000 (Fe'') of the weight of the solution. This has been repeatedly verified. The obvious methods of noting equilibrium under the former condition are (1) absence of any change of temperature; (2) no change of rotation; (3) survival of the peroxide after prolonged contact. The addition of a ferrous salt—we have usually employed the sulphate—is followed by changes in all three directions. Unfortunately, owing to the turbidity occasioned by the addition of the iron salt, the intermediate stages of the reaction cannot be followed by polarimetric observation. The rise of temperature and disappearance of the peroxide are proportional in rate and degree to the concentration of the solutions.

With 4 per cent. solutions of the carbohydrates, and the peroxide sufficient to supply 1 atom of oxidising O per molecule ($\text{C}_6\text{H}_{12}\text{O}_6$), a rise of temperature from 15° to 30° takes place in a period of 1—2 hours; with 10 per cent. solutions, on the other hand, the proportions of the reagents otherwise remaining the same, the temperature will rise to 50 — 60° ; in the latter case, there is considerable evolution of gas. By comparing these effects with those obtained with the peroxide and iron salt alone, that is, in absence of the carbohydrate, allowance can be made for the relatively small evolution of heat due to the exothermic resolution of the peroxide itself; moreover, the latter is rapidly completed, and is accompanied by evolution of oxygen, whereas in presence of the carbohydrate, some considerable time always elapses before exothermic reaction sets in.

These effects were indirectly verified by taking advantage of the observation that by using only fractional proportions of the peroxide, products were formed which, whilst they have most of the properties of the glucoses (see below) resisted fermentation.

In the subjoined series of experiments, this residue was determined in terms of cupric-reducing power, and calculated to the original carbohydrate (dextrose).

To 25 c.c. of a 5 per cent. solution of dextrose was added in each case 20 milligrams of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. To each of three such quantities, hydrogen peroxide was added representing 1/40 to 1/10 atom of oxygen per mol. of $\text{C}_6\text{H}_{12}\text{O}_6$; a fourth (blank) received none. After reaction, they were fermented by yeast, and the residual 'sugar' determined as indicated.

	Proportion of peroxide O : $\text{C}_6\text{H}_{12}\text{O}_6$.	Residual sugar (Calc. from CuO reduction).
1.	None	None
2.	1/40	9.2 per cent.
3.	3/40	14.0 ,,
4.	4/40	20.0 ,,

These results will be referred to subsequently, as they have a bearing on the theoretical aspect of the reactions.

We shall not further discuss this side of the reaction. It is sufficiently proved that some third substance is necessary to determine reaction, and that iron salts have this function is a point which may be emphasised from its obvious physiological significance. Other compounds will no doubt be found which exert a similar influence, and it is quite probable that, corresponding with differences in chemical function, we may be able to establish specific differences in regard to the products formed in the reactions; this will be more clearly seen when the nature of the products and of the reaction has been more closely described. Operating in dilute solutions (4 per cent. of the hexoses), we may premise that there is very little effervescence or other indication of destructive oxidation, and after making comparative experiments at 0—4°, we find the ordinary atmospheric temperature to be the most favourable for the purpose. The proportion of iron (Fe) taken is usually 1/10000 the weight of the solution and in the form of ferrous sulphate. The peroxide is added gradually, and in proportion calculated to, say, 1 atom of 'oxidising oxygen' to each mol. of $C_6H_{12}O_6$, that is, about 9 per cent. by weight.

After the reaction is complete and the peroxide has entirely disappeared, the yellowish-brown solution is acid to the taste. The following numerical results obtained with a dextrose solution (4 per cent.), treated under the conditions above specified, will give a general idea of the products of the reaction.

Total 'organic' solids (dried at 105°), 87.7 per cent. of original.

Acidity, equal to 1/5 normal acid.

CuO reduction (calc. to organic solids), 96.5 (dextrose = 100).

Sp. rotation, $[\alpha]_D = 33.8^\circ$.

Furfuraldehyde, 3.75 per cent.

Reaction with phenylhydrazine acetate takes place in the cold, the products having all the characteristics of the osazones. The yield of insoluble osazone in this case was 14 per cent. of the weight of the original dextrose. After recrystallisation from dilute alcohol, it melted at 194°, and on combustion yielded N = 19.8 per cent. The filtrate from the osazone thus formed in the cold, and containing a calculated excess of phenylhydrazine, gave only a slight further precipitate on heating at 100°.

It would appear, therefore, that the original solution contained but little residual dextrose. After boiling with hydrochloric acid (added to the proportion of 15 per cent. HCl), the cupric reduction was in effect lowered from 96 to 5.7 (dextrose = 100). We will deal at once with a suggestion which obviously arises from these numbers, namely, that

the characteristic products which react with the phenylhydrazine in the cold are of the type of glucosone (or oxyglucose).

The solution was treated with zinc and acetic acid, and the reducing action allowed to continue some hours; the cupric reduction was thereby but little affected, falling from 96.5 to 95.4. The solution still gave the osazone in the cold, but the yield was reduced from 14 to 6.4. After hydrolysing, however, by boiling with sulphuric acid (2 per cent., H_2SO_4), the original yield of 14 per cent. was obtained, and the compound formed had the same properties. The products are by these properties differentiated from the 'osones.'

The above constants are sufficiently definite as a basis for a preliminary investigation of the nature of the reactions. Thus it might be presumed that, in the event of oxidation occurring in the case of an aldose, the first point to be attacked would be the terminal aldehydic group. The numbers above cited are sufficient to show that, although profound changes have been determined, they are not at all of the order of such simple oxidation. These numbers, representing the effects due to 1 atom of oxygen, serving as a basis of comparison, experiments were made with 2, 3, and 4 atoms of oxygen, and on comparing the constants it was found that the effects produced were by no means proportional to the amount of oxygen. Thus the following constants were determined in the solution obtained by treating the aldose with 2 atoms O (as H_2O_2), all other conditions remaining the same.

Total organic solids, 85.5 of original.

Acidity equal to 1/5 normal acid.

CuO reduction (calc. to organic solids), 86.5 (dextrose = 100).

Sp. rotation, $[\alpha]_D = 16.5^\circ$.

Furfuraldehyde, 3.75 per cent.

Osazone (in the cold), yield 22.6 per cent.

The additional quantities of oxygen failing to produce proportional effects, it was concluded that the characteristic products which react with phenylhydrazine in the cold, and also reduce Fehling's solution powerfully in the cold, although highly reactive substances, will resist the action of certain oxidising agents. We found, in fact, that they were not oxidised even by prolonged treatment with bromine, although some bromine is taken up and hydrogen bromide produced; calculating this to oxygen, it amounts on the average to 5—7 per cent. of the weight of the original carbohydrate, that is, about $\frac{1}{2}$ an atom of oxygen, but the cupric reduction is lowered not more than 20—25 per cent. and the solutions still give the characteristic reactions with phenylhydrazine. Even on warming with bromine, the oxidation is not carried much farther.

As regards the most characteristic of the products, therefore, the results bear no direct proportion to the amount of the oxidising agent employed, and that is, to a certain extent, explained by the points of divergence in properties between these products and the normal carbohydrates.

When proportions less than the 1 atom of oxygen were used, the results still failed to show any quantitative sequence downwards.

Our friend, Dr. R. S. Morrell, was kind enough to carry out an experiment for us in which 40 grams of dextrose were treated with the peroxide in the proportion of 1/10 atom of 'oxidising oxygen' per mol. of $C_6H_{12}O_6$. We were anxious to have our results confirmed by an independent observer, and Dr. Morrell's experience in Professor Fischer's laboratory adds value to the confirmation. He obtained from the products of the reaction 8 grams of the osazone formed in the cold, that is, a yield similar to that which we obtained when using twenty times this proportion of oxidising oxygen. In fractionating the osazone, he also confirmed our results in regard to its being a mixture of two compounds or groups of compounds.

(a) Soluble in hot water, m. p. 130° .

(b) Relatively insoluble, m. p. 185° .

If, in place of regarding these effects as an oxidation of 40 grams of the hexose by the peroxide in the proportion of 1/10 atom of oxygen, we interpret it as resulting from the attack of 4 grams by 1 atom of oxygen per 1 mol. of $C_6H_{12}O_6$, the effects are not accounted for; but such an interpretation is excluded by an examination of the whole series of results.

We have now to show how far the reaction is elucidated by the other products, which have, so far, only appeared in statistical results.

The Furfural-yielding Compounds

have not been isolated. It is probable that the furfuraldehyde obtained on boiling with hydrochloric acid results from a complex decomposition of the characteristic ketonic products, for, like these, the parent 'furfuroid,' is not oxidised by bromine, neither is it separated amongst the acid products about to be described. We have, therefore, nothing to identify it with the better known furfuroids, that is, with the pentoses, on the one hand, and glycuronic acid on the other. The formation of a furfural-yielding group is of some physiological significance, for the reasons given in the earlier part of this paper.

Acid Products.

The most characteristic of these is a substance which is precipitated as a lead salt in presence of acetic acid. The salt thus obtained has

been analysed several times after recrystallisation from highly dilute nitric acid, and the numbers obtained correspond with those for the lead salt of tartaric acid. The yield has also been several times estimated, the numbers indicating from 7 to 10 per cent. of the acid calculated to the original carbohydrate.

In addition to this dicarboxylic acid, monobasic acids are separated in some quantity on distilling the solutions. Estimated in terms of normal alkali by comparison with the acidity of the original solutions, the volatile acids amount to about one-half.

By redistillation after oxidation with neutral permanganate, these were resolved into acetic acid, resisting the oxidation, and formic acid oxidised. The quantities calculated to the original carbohydrates vary from 3—7 per cent. of acetic and 13—18 per cent. of formic acid.

The loss of volatile acids on evaporating the solution appears to correspond with the deficiency in the 'total solids' after reaction; but this point need not be pressed too closely, as it has not been ascertained what dehydration the ketonic products undergo by 'drying' at 105°. These statistical numbers, however, generally confirm the observation that the reactions, under the usual conditions, are not marked by destructive oxidation (to CO_2).

It is, of course, to the acid products that we first look in summing up the results to a quantitative account of the reactions. The acidity of the oxidised solutions, as originally obtained and determined by direct titration, is approximately accounted for in terms of the two groups of acids estimated as stated; there appears to be no subsequent production of volatile acids by changes in the products during the distillation. On the first obvious assumption that the COOH -groups are produced from the COH -groups of the aldose, we find in many cases that, although this accounts for all the oxygen added as H_2O_2 , it leaves us with 60—70 per cent. of the carbonyl oxygen of the original hexose, measured in terms of cupric reduction, and confirmed by the reactions with phenylhydrazine.

It appears, therefore, from this preliminary survey of the products that the reactions are not of such 'obvious' simplicity, but are complicated by internal rearrangement of the hexose molecule; a conclusion which appears necessary to explain the results of the fermentation experiments described in the earlier part of this paper. This conclusion is confirmed by the observation that the solutions give a pronounced iodoform reaction, as do also the acid distillates. We have not as yet further identified the methylated derivatives thus shown to be formed; their production is a sufficient index of the factor of rearrangement which has to be taken into account.

Before dealing finally with the issues raised by the empirical study

of the 'constants' of the reactions, we have to contrast with the above *the results obtained with levulose and cane-sugar*.

The solutions obtained from the ketose have generally the same characteristics as those from the aldose, but with the following specific and noteworthy difference: *furfuroids are not formed*, the solutions giving traces only of furfuraldehyde, the *total acidity* of the solutions is less, and the dicarboxylic acid is proportionally small in amount and appears to be an acid of higher molecular weight. On the other hand, the cupric reduction is relatively high, and the yield of osazone in the cold is also high, exceeding, in many cases, 50 per cent. of the weight of the solids in solution.

The following observations on the osazone obtained from the hexose, treated with 1 atom of oxygen, as H_2O_2 , are of interest. As obtained from the original solution, the osazone melted at 195° . After prolonged digestion with excess of bromine and removal of the bromine, the solution gave the same osazone in the cold. On combustion, it gave $N = 16.5$ per cent.

Treatment of the original solution with zinc and acetic acid (examination for 'osones') affected the cupric reduction somewhat (10 per cent.), and lowered the yield of the characteristic osazone from 30 per cent. to 10 per cent.; but after hydrolysing by boiling with sulphuric acid (2 per cent. H_2SO_4), the yield of the osazone was doubled. These numbers indicate, again, that the reactions are complicated by tendencies to condensation.

In general terms, the results with the ketose sufficiently resemble those with the aldose to lead to the conclusion that the position of the typical CO-group does not exert an influence such as it does in ordinary processes of oxidation; and this confirms the previous conclusion that the reactions of hydrogen peroxide are far removed from those of simple oxidation.

Cane-sugar gives us a third variation in regard to the typical CO-groups of the original carbohydrate, and as the results obtained with the peroxide under similar conditions of treatment are in all respects closely similar to those with the constituent hexoses, we may conclude that hydrolysis is rapidly determined. As regards the products of the reaction, the results may be taken as intermediate between those for the constituent hexoses in those directions in which differences have been established.

As this is only a preliminary communication, we limit ourselves to the statement of typical quantitative results and general indications of the scope of the investigation. It will be seen that the main interest of the inquiry centres in the identification of the characteristic ketonic products of the reaction. Were these products of a simple nature, we should have made more progress in this direction; so far,

we have been unable to crystallise them ; we have also been unable to obtain crystalline acetates or benzoates. On the other hand, we have noted the evidence of the tendency of the products to condensation, which, so far, accounts for the difficulty of obtaining them in their simplest form.

If we are dealing with groups of the form $-C(OH):C(OH)-$, the anticipation with which, on the basis of Fenton's researches, we set out, many of our results, both positive and negative, are generally explained. It is clear that in the carbohydrates such groups can be formed in two ways : either from a $-CH(OH)\cdot CH(OH)-$ residue by oxidation, or from the $-CO\cdot CH(OH)-$ residue by a simple transposition. We may also note that, in the condensation of the pentoses to furfuraldehyde, the formation of the aldehyde is usually formulated as prepared for by a similar grouping.

The only objection in the way of these views as a working hypothesis is that it conflicts somewhat with the present view of the osazone reaction. This objection will, of course, have weight in the further prosecution of the investigation, which will involve a critical examination of the evidence in this direction ; the more so as the "osazone" reactions which we have described are both novel and characteristic.
