The Oxidation of Cellobiose by Periodate.

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The oxidation of cellobiose by sodium metaperiodate has been studied. Analysis of the results suggests that the sugar is oxidised rapidly to an intermediate product with the structure of a formic ester, and that the subsequent oxidation is slow because its rate is determined by the rate of the hydrolysis of this ester. The total reaction can be represented by the equation $C_{12}H_{22}O_{11} + 11IO_4^- = 9H{\cdot}CO_2H + 2H{\cdot}CHO + CO_2 + 11IO_3^-$. The results are of significance in connection with the side reactions which occur when periodate acts on cellulose.

CONSIDERABLE interest attaches to the action of periodate on the reducing terminal glucose units in the chain-molecules of cellulose; for example, it has been suggested (Head, *J. Text. Inst.*, 1953, **44**, T209) that the "over-oxidation" that accompanies the normal Malaprade oxidation of cellulose is partly due to progressive attack on the chain-molecules from their reducing ends. It was thought that this question might be illuminated by an investigation of the action of periodate on cellobiose, the molecule of which may be regarded as a combination of the terminal glucose units of a cellulose chain-molecule.

Cellobiose was oxidised with unbuffered sodium metaperiodate in the dark at 20°. The results given in Fig. 1 show that the reaction may be divided into three main stages: (1) a rapid initial stage occupying a few hours, (2) a relatively slow second stage lasting about three weeks, and (3) an exceedingly slow final stage. During the first two stages 1 mole of cellobiose reduces about 11 moles of periodate and yields about 9 moles of formic acid, 2 moles of formaldehyde, and 1 mole of carbon dioxide. Similar figures were obtained by Courtois and Ramet (*Bull. Soc. Chim. biol.*, 1947, **29**, 240) for the oxidation of lactose and maltose by periodate at pH 1·2—2·5; they proposed the equation

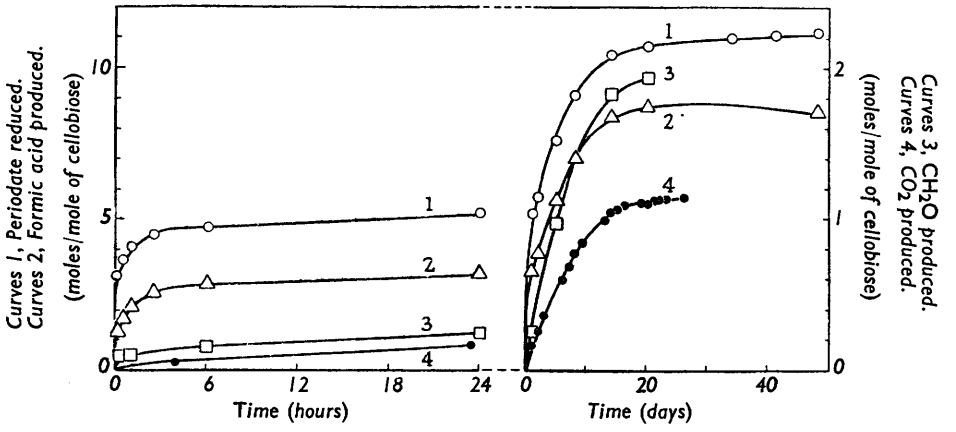
$$C_{12}H_{22}O_{11} + 11IO_4^- = 9H{\cdot}CO_2H + 2H{\cdot}CHO + CO_2 + 11IO_3^- \quad . \quad . \quad . \quad . \quad (1)$$

During the third stage formic acid and formaldehyde are very slowly oxidised to carbon dioxide (cf. Head and Hughes, *J.*, 1952, 2046). It is noteworthy that approximately 1 mole of carbon dioxide is produced before this final stage is reached.

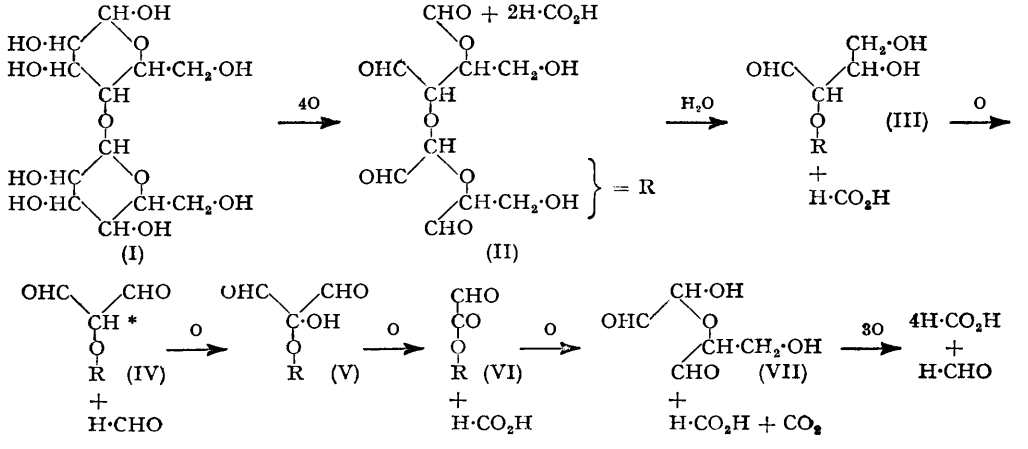
It has sometimes been supposed that under suitable conditions 1 mole of a reducing disaccharide consisting of two 1 : 4-linked hexopyranose units (I) should be oxidised quantitatively by 5 moles of periodate to 1 mole of a substance of type (IV), three of formic acid, and one of formaldehyde (Hirst *et al.*, *Nature*, 1945, **156**, 785; Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488). Reference to Fig. 1 shows that the composition of the system is never in agreement with exclusive oxidation to this definite stage; thus, although the reduction of 5 moles of periodate (in 18 hours) is accompanied by the formation of about 3 moles of formic acid, the formaldehyde produced amounts to only about 0·2 mole.

In Fig. 2 the yields of the various products formed during stages 1 and 2 are plotted against the amount of periodate reduced. Owing to the extreme rapidity of the initial reaction the earliest measurements of periodate consumption and yield of formic acid were made at a periodate consumption of 3·1 moles, and consequently the course of the lower part of the curve for formic acid is uncertain. Over the range covered by the observations, however, the relation between periodate consumption and yield of formic acid is virtually linear, and corresponds to a continuous production of 1 mole of formic acid per mole of periodate reduced. Extrapolation of the linear portion of the curve to the horizontal axis gives a value of almost exactly 2 moles for the periodate consumption at zero yield of formic acid, and this result suggests that the first step in the oxidation of the sugar entails the reduction of 2 moles of periodate without production of formic acid. Such a reaction

FIG. 1. *Oxidation of* 0·01M-*cellobiose with* 0·15M-*periodate in the dark at* 20°.



would correspond to the rupture of a carbon–carbon bond in each pyranose ring of the sugar molecule, but it would be less easily explicable if the reducing glucose unit reacted in the open-chain form. Moreover, oxidation of the open-chain form might be expected to produce a considerable proportion of formaldehyde from the outset, whereas the yields obtained during the early stages of the reaction are very low. It is therefore reasonable to



conclude that both halves of the cellobiose molecule react in the pyranose form. Fig. 1 shows that the rapid first stage of the reaction does not end with the rupture of a carbon–carbon bond in each glucose unit, but includes a further oxidation step. This is presumably

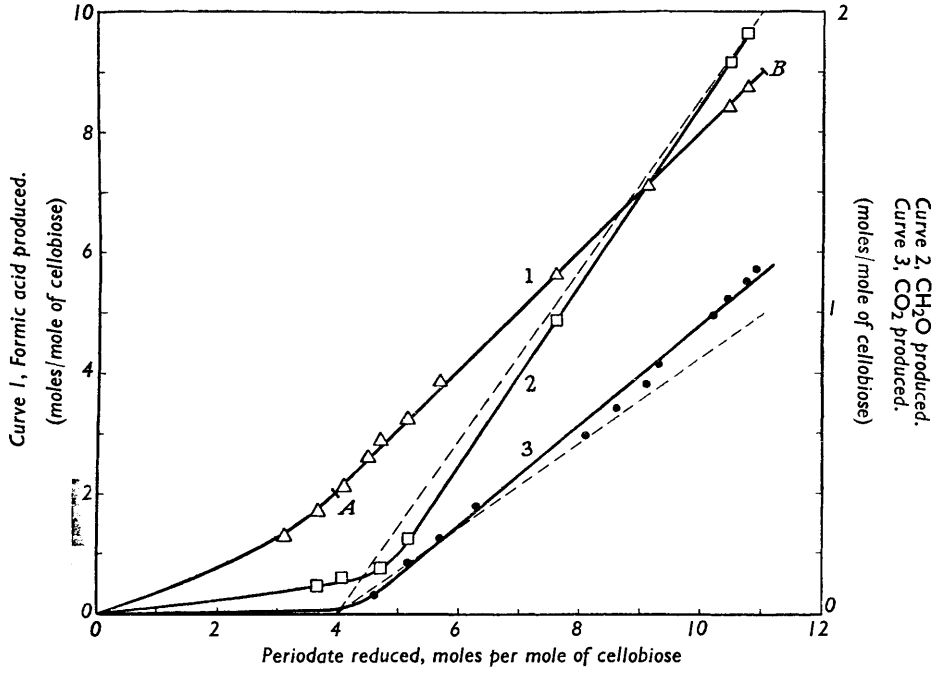
the formation of the formic ester (II), the production of which from cellobiose is represented by the equation :

$$C_{12}H_{22}O_{11} + 4IO_4^- = C_{10}H_{14}O_9 + 2H{\cdot}CO_2H + 2H_2O + 4IO_3^- \quad . \quad . \quad . \quad . \quad (2)$$

Meyer and Rathgeb (*Helv. Chim. Acta*, 1948, **31**, 1540, 1545), and Morrison, Kuyper, and Orten (*J. Amer. Chem. Soc.*, 1953, **75**, 1335), have obtained evidence of the formation of esters of this type in the periodate oxidation of lactose and maltose.

The curves for production of formaldehyde and carbon dioxide in Fig. 2 show that in stage 1 of the oxidation process the amounts of these products are very small in relation to the periodate consumption. When about 4 moles of periodate have been reduced, the slopes of the curves increase rather abruptly and then remain approximately constant as the periodate consumption increases from about 4·5 to 11 moles. The linear portions of

FIG. 2. *Oxidation of* 0·01M-*cellobiose with* 0·15M-*periodate in the dark at* 20°.



the curves correspond to zero yields of both products when about 4 moles of periodate have been reduced, *i.e.*, at the stage corresponding to quantitative formation of the ester (II).

These observations agree fairly well with what would be expected if the gross reaction in stage 2 were hydrolysis of the ester (II) and subsequent oxidation according to the equation :

$$C_{10}H_{14}O_9 + 7IO_4^- + 2H_2O = 7H{\cdot}CO_2H + 2H{\cdot}CHO + CO_2 + 7IO_3^- \quad . \quad . \quad . \quad (3)$$

The course of this reaction may be reasonably accounted for on the following basis (cf. Head, *loc. cit.*, and Neumüller and Vasseur, *Arkiv Kemi*, 1953, **5**, 235), though other possibilities cannot be excluded. The ester (II) is probably hydrolysed, and the product (III) is then oxidised to (IV), which contains an active hydrogen atom (marked *) and should therefore be readily oxidised to (V) (cf. Sprinson and Chargaff, *J. Biol. Chem.*, 1946, **164**, 433, and Huebner, Ames, and Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621; Potter and Hassid, *loc. cit.*, pointed out that this mechanism would account for the over-oxidation of maltose). The product (V) should be oxidised first to the glyoxylic ester (VI) and then to an unstable carbonic ester which would lose carbon dioxide and give (VII) [alternatively, the same net result would follow from the hydrolysis of (VI)]. Finally,

(VII), which is a possible intermediate product in the periodate oxidation of glucose, should be oxidised to 4 moles of formic acid and one of formaldehyde.

It is probable that the production of the ester (II) from cellobiose will be rapid, but that the hydrolysis of (II) will be relatively slow. If all the subsequent reactions were instantaneous, the yields of formaldehyde and carbon dioxide, and the additional yield of formic acid, at any given degree of oxidation could be calculated by proportion from equation (3). The results corresponding to stage 2 of the oxidation process are, in general, close to the figures obtained in this manner. The calculated results for formaldehyde and carbon dioxide are represented in Fig. 2 by broken lines; the calculated line (*A*—*B*) for formic acid agrees so closely with the experimental line that the two cannot be distinguished in the Figure. The results of this comparison thus support the view that the hydrolysis of (II) is the rate-controlling process and that the subsequent oxidation processes are, as a whole, much faster. The small but appreciable yields of formaldehyde before the end of stage 1 suggest that, as might be expected, some of the cellobiose is completely oxidised before all has been converted into the ester (II). The yield of carbon dioxide at this stage is less than would be expected, however.

The complete oxidation of cellobiose to simple products makes it seem probable that the effect of periodate on cellulose will include a step-wise degradation proceeding from the terminal reducing units.

Experimental

Reaction mixtures were kept in the dark in a thermostat at 20°. Formic acid was determined by titration with alkali after destruction of the excess of periodate with ethylene glycol and aeration to remove carbon dioxide. Formaldehyde was determined by the dimedone method after removal of the excess of periodate and iodate with arsenite. The detailed procedures for both methods were as given by Head and Hughes (*loc. cit.*).

Periodate was determined by the convenient Müller–Friedberger method (*Ber.*, **1902**, **35**, 2652), in which the periodate is reduced with iodide in weakly alkaline solution and the liberated iodine is titrated with arsenite. This method was selected with due regard to the work of Hughes and Nevell (*Trans. Faraday Soc.*, **1948**, **44**, 941) on the determination of periodate in the presence of partially oxidised glucose. Their results show that the Fleury–Lange method of analysis, in which the periodate is reduced by an excess of arsenite in the presence of potassium iodide and sodium hydrogen carbonate, is unsatisfactory under these conditions, but that reliable results are obtained by reduction of the oxidant with iodide in acid solution and titration of the liberated iodine with thiosulphate. Preliminary tests showed that the Müller–Friedberger method is unsatisfactory with solutions containing partially oxidised glucose, but gives the same result as the thiosulphate method with solutions containing partially oxidised cellobiose.

The carbon dioxide formed during the oxidation of cellobiose was determined as follows. A slow stream of nitrogen, freed from carbon dioxide, was passed through a periodate solution until the issuing gas was free from carbon dioxide. A weighed amount of cellobiose was then added to the solution, and the gas, after being dried with phosphoric oxide, was passed through a weighing tube containing "Carbosorb" backed with phosphoric oxide. A preliminary experiment showed that the tube did not increase in weight during 2 hr. when the reaction mixture was replaced by 0·2N-formic acid solution.

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