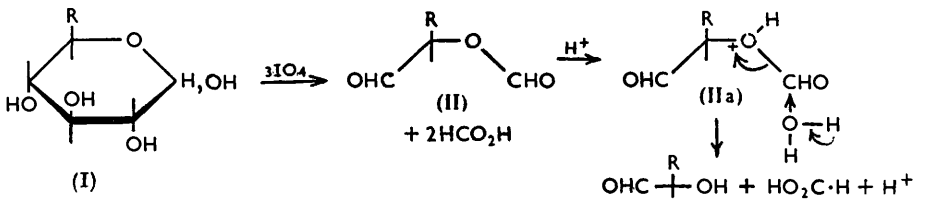
239. *The Oxidation of Monosaccharides by Periodate with Reference to the Formation of Intermediary Esters.*

By L. Hough, T. J. Taylor, G. H. S. Thomas, and B. M. Woods.

A study of the reactions of various monosaccharides with sodium metaperiodate at pH 3·6 at room temperature and in the dark has provided further evidence that the sugars are oxidised in their cyclic forms with the formation of intermediary esters. The different rates of hydrolysis of these esters have been related to the inductive effects of electrophilic groups in the alcohol component (CO_2H > CHO > CH_2·OH > Me, H). The behaviour of various related compounds possibly arising during the oxidation of carbohydrates has been examined.

Various *O*-substituted aldohexoses are oxidised by periodate in their pyranose forms to give formyl esters which are sensitive to pH. Alkaline or strongly acid conditions cause rapid hydrolysis of these esters, but they are relatively stable in weak acid (pH 3—5).[1] Thus, at about pH 4 the consumption of oxidant by many reducing disaccharides, methylated aldoses, and aldose phosphates is considerably reduced owing to the intervention of formyl esters. This knowledge had made possible the preparation, *via* formyl esters, of 2-deoxy-D-ribose from 3-deoxy-D-glucose,[2] of 4-*O*-formyl-2-*O*-methylsulphonyl-D-arabinose from 3-*O*-methylsulphonyl-D-glucose,[3] of D-glyceraldehyde 3-phosphate from D-glucose 6-phosphate,[4] and of various *O*-methylaldopentoses from *O*-methylaldohexoses.[5] However, there is little information concerning the mode of oxidation of unsubstituted monosaccharides and few quantitative studies have been carried out. Schöpf and Wild [6] treated D-glucose (I; R = CH_2·OH) with sodium metaperiodate (3 mol.) and obtained formic acid (2 mol.) and α-*O*-formyl-D-glyceraldehyde (II; R = CH_2·OH; 1 mol.), providing



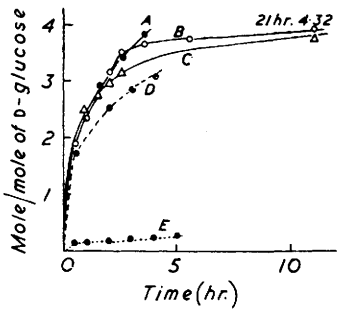
evidence that D-glucose is oxidised almost entirely, if not all, in the pyranose form. This conclusion is substantiated by Hughes and Nevell's observations [7] who noted that the theoretical 5 mol. of oxidant were rapidly reduced in neutral solution, whereas at pH 4·6, 3 mol. reacted rapidly and then 2 mol. much more slowly. Significantly determinations [8] of formaldehyde formed on oxidation of monosaccharides and, in particular, their *O*-methyl derivatives are invariably less than 1 mol. when carried out at below pH 7, as would be expected if the sugars were oxidised in their pyranose forms.

Oxidations have been followed by adding various crystalline monosaccharides to buffered (pH 3·6) periodate solution and, at intervals, determining the consumption of periodate and the formation of formaldehyde; the release of formic acid was also studied,

[1] Neumüller and Vasseur, *Arkiv Kemi*, 1953, **5**, 235.
[2] Gorin and Jones, *Nature*, 1953, **172**, 1057.
[3] Smith, D. C. C., *Chem. and Ind.*, 1955, 92; *J.*, 1957, 2690.
[4] Morrison, Rouser, and Stotz, *J. Amer. Chem. Soc.*, 1955, **77**, 5156.
[5] Barker and Smith, D.C.C., *Chem. and Ind.*, 1952, **30**, 1035; Hemming and Ollis, *ibid.*, 1953, 85; Fried and Walz, *J. Amer. Chem. Soc.*, 1952, **74**, 5468; Huffman, Lewis, Smith, F., and Spriestersbach, *ibid.*, 1955, **77**, 4346.
[6] Schöpf and Wild, *Chem. Ber.*, 1954, **87**, 1571.
[7] Hughes and Nevell, *Trans. Faraday Soc.*, 1948, **44**, 941.
[8] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476; Bell, *J.*, 1948, 992; Bell and Greville, *J.*, 1950, 1902; Greville and Northcote, *J.*, 1952, 1945.

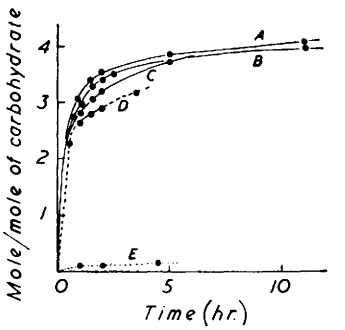
but in unbuffered solution (pH 2—4). Anomalies can arise in the determination of periodate uptake if during the estimation the reaction solution is made alkaline as in the Fleury–Lange method [9] or strongly acid as in the acid–thiosulphate method,[7] when rapid hydrolysis of the formyl ester will occur. For this reason, Neumuller and Vasseur's method,[1] whereby periodate is determined at pH 6·98, was selected and proved satisfactory. Similar errors can arise in estimates of formaldehyde, but were obviated by using the

FIG. 1.



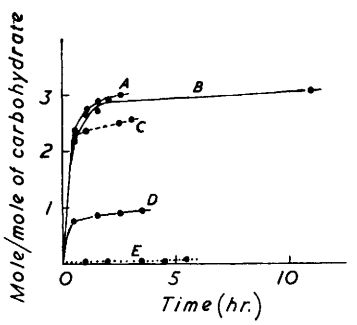
A, β-D-Glucose, periodate uptake. B, αβ-D-Glucose (mutarotated), periodate uptake. C, α-D-Glucose, periodate uptake. D, α-D-Glucose, formic acid released. E, α-D-Glucose, formaldehyde released.

FIG. 2.



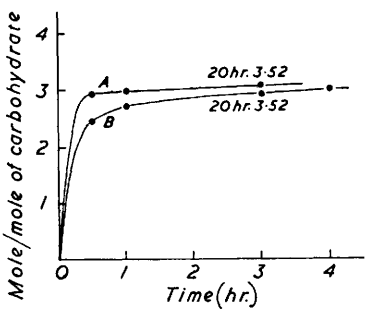
A, α-D-Galactose, periodate uptake. B, αβ-D-Galactose (mutarotated), periodate uptake. C, β-D-Mannose, periodate uptake. D, α-D-Galactose, formic acid released. E, α-D-Galactose and β-D-mannose, formaldehyde released.

FIG. 3.



A, D-Ribose (mutarotated), periodate uptake. B, D-Ribose, α-D-xylose and β-L-arabinose, periodate uptake. C, D-Ribose, formic acid released. D, 3-O-Methyl-D-glucose, periodate uptake. E, D-Ribose, formaldehyde released.

FIG. 4.



A, α-L-Fucose, periodate uptake. B, α-L-Rhamnose, periodate uptake.

chromotropic acid method [10] after complete removal of excess of periodate from the reaction mixture by precipitation with lead dithionate at pH 3·6.

The results (Fig. 1) obtained for α-D-glucose (I; R = CH_2·OH) are consistent with oxidation of the majority in the pyranose form with the rapid consumption of 3 mol. of periodate and the formation of D-glyceraldehyde formate (II; R = CH_2·OH). The rapid release of *ca.* 0·15 mol. of formaldehyde suggests that some 15% of the aldohexose was oxidised in either the furanose or the *aldehydo*-form. Oxidations of β-D-glucose and of αβ-D-glucose (a solution of α-D-glucose which had been allowed to mutarotate to equilibrium), although showing evidence of formyl ester intermediates, revealed differences

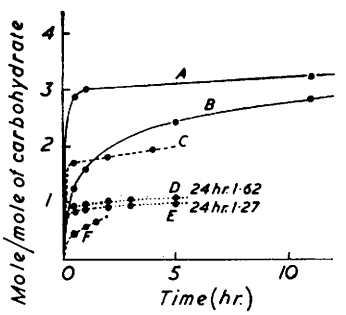
[9] Fleury and Lange, *J. Pharm. Chim.*, 1933, **17**, 196.
[10] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.

in the initial rapid reactions (Fig. 1), confirming the view that more than one form of the monosaccharide was concerned in the reaction.

α-D-Galactose and β-D-mannose gave similar results (Fig. 2) to those for D-glucose and the rapid release of formaldehyde (0·06—0·07 mol.) again indicated that a small amount was not oxidised in the pyranose form.

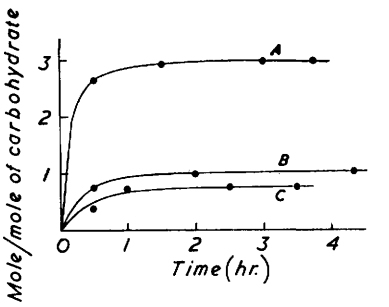
Oxidation in the pyranose form and subsequent formyl ester formation was even more apparent in the case of the aldopentoses since α-D-xylose (I; R = H), D-ribose, and β-L-arabinose showed very little reaction after the initial rapid consumption of 3 mol. of periodate (Fig. 3). Stabilisation was also apparent in the case of 3-*O*-methyl-D-glucose, which was oxidised in the pyranose form by 1 mol. of periodate (Fig. 3) to give 4-*O*-formyl-2-*O*-methyl-D-arabinose.[5] The stability of this ester is due to the absence of an electrophilic aldehyde group on the carbon atom adjacent to the ester linkage, and this explanation also accounts for the relatively stable esters formed on oxidation of other 3-*O*- and 4-*O*-substituted aldohexoses, as for example, the disaccharides, cellobiose, maltose, and lactose, and 1 : 3-linked oligoglucosaccharides.[1,11] These and the subsequent results are consistent with the view that the acid hydrolysis of the formyl ester (II) is influenced by the inductive effect of electrophilic substituents (II; R = CO_2H > CHO > CH_2·OH > Me, H) in the alcohol, the dissociation of the oxonium ion (IIa) being rate-determining.

FIG. 5.



A, β-D-Fructose, periodate uptake. B, L-Sorbose, periodate uptake. C, β-D-Fructose, acid released. D, L-Sorbose, formaldehyde released. E, β-D-Fructose, formaldehyde released. F, L-Sorbose, acid released.

FIG. 6.



A, Tartaric acid, periodate uptake. B, Glyoxylic acid, periodate uptake. C, Dimethyl tartrate, periodate uptake.

The 6-deoxyaldohexoses, α-L-fucose and α-L-rhamnose, were also oxidised in their pyranose modifications as shown by the rapid consumption of 3 mol. of oxidant, further reaction being very slow (Fig. 4). The greater stability of the formyl esters of lactaldehyde (II; R = Me) and of glycollaldehyde (II; R = H) than of α-*O*-formylglyceraldehyde (II; R = CH_2·OH) must therefore be attributed to the absence of a neighbouring hydroxyl group.

Oxidations of hexuloses are more complex since they could theoretically be oxidised in the acyclic, furanose, or pyranose form by two possible routes in each case. Arni and Percival,[12] and Khouvine and Arragon,[13] have interpreted their results in terms of the acyclic forms, but, in agreement with Sprinson and Chargaff [14] our results at pH 3·6 favour oxidation in a cyclic form with the formation of ester intermediates (*e.g.*, III, V, VI, VIII). In the initial rapid reaction, β-D-fructose (IV) consumed 3 mol. of periodate with the formation of 1 mol. of formaldehyde and 1·5 equivalents of acid (Fig. 5) which, considered

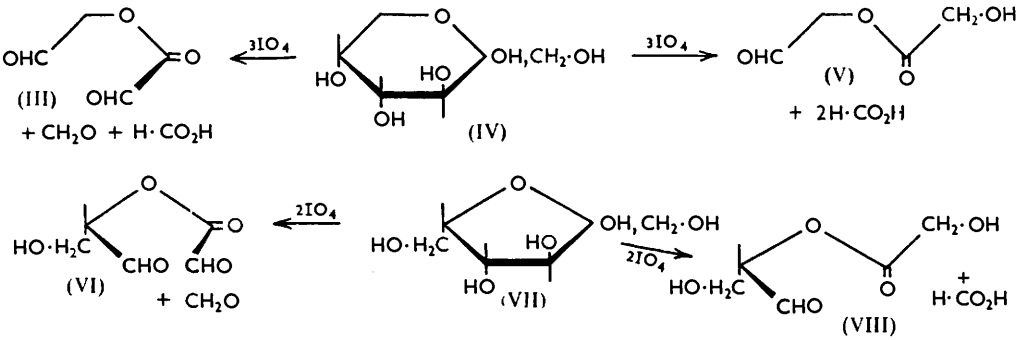
[11] Meyer and Rathgeb, *Helv. Chim. Acta*, 1948, **31**, 1540, 1545; Morrison, Kuyper, and Orten, *J. Amer. Chem. Soc.*, 1953, **75**, 1502; Head and Hughes, *J.*, 1954, 603; Manners and Archibald, *J.*, 1957, 2206.

[12] Arni and Percival, *J.*, 1951, 1822.

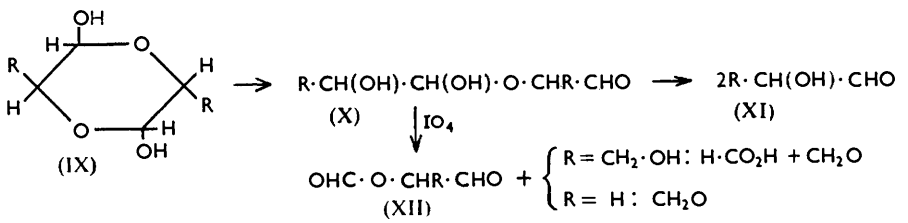
[13] Khouvine and Arragon, *Bull. Soc. chim. France*, 1941, **8**, 676.

[14] Sprinson and Chargaff, *J. Biol. Chem.*, 1946, **164**, 433.

with the slow over-oxidation typified by reaction of aldopentoses, suggests that the predominant pathway involves oxidation in the pyranose form with the formation of a glyoxylic ester (III) of glycollaldehyde. On the other hand, L-sorbose (VII) gave quite different results (Fig. 5) compatible with oxidation in the furanose form with the rapid formation of 1 mol. of formaldehyde and α-*O*-glyoxylylglyceraldehyde (VI). By comparison



with the oxidations of aldohexoses, this ester would be susceptible to hydrolysis and further oxidation. When dimethyl tartrate was oxidised with periodate at pH 3·6 only 1 mol. of oxidant reacted, confirming the view that glyoxylic esters can be stabilised at this pH; under the same conditions glyoxylic acid and tartaric acid consumed 1 and 3 mol. of oxidant respectively (Fig. 6).



Also of interest were the oxidations of DL-glyceraldehyde and glycollaldehyde which exist in their crystalline states as cyclic dimers (IX; R = CH_2·OH and H respectively).[15] The results (Fig. 7) show that unless they are left in solution for some time to revert to monomers (XI), they give rise to formyl esters (XII) as a result of oxidation of their acyclic dimers (X) which are presumably formed as intermediates between the monomeric (XI) and the cyclic dimeric state (IX).

At pH 3·6, methyl (methyl α-D-galactopyranosid)uronate (XIII; R = Me) consumed 3 mol. of periodate after 10 hr. and a further mol. after 50 hr. (Fig. 8), these resembling the results obtained at pH 4·5 by Edington, Hirst, and Percival;[16] this is compatible with previous suggestions[14] that the oxidation is arrested by the oxalyl ester (XVI; R = Me). This ester (XVI; R = Me) arises from the methyl ester methyl glycoside (XIII) by normal oxidative cleavage to the dialdehyde (XIV) followed by oxidation of the activated hydrogen atom[14,17] to the hydroxy-dialdehyde (XV) and subsequent oxidative cleavage. Such a mechanism was not operative in the case of sodium D-glucuronate at pH 3·6 since it reacted with 3 mol. of periodate in 0·5 hr., then with an extra 2 mol. in 6 hr. during which 1 mol. of carbon dioxide was liberated (Fig. 8); under these conditions oxalic acid was stable to periodate. Similar results were obtained by Sprinson

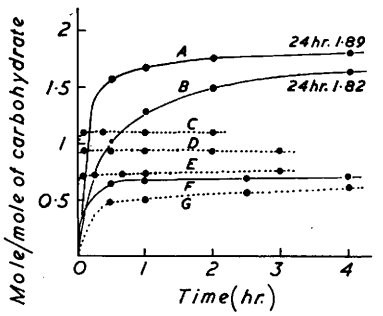
[15] Bergman and Miekeley, *Ber.*, 1929, **62**, 2297; 1931, **64**, 802; Summerbell and Rochen, *J. Amer. Chem. Soc.*, 1941, **63**, 3241; Baer and Fischer, *J. Biol. Chem.*, 1943, **150**, 213; Wohl and Neuberg, *Ber.*, 1900, **33**, 3095.

[16] Edington, Hirst, and Percival, *J.*, 1955, 2281.

[17] Huebner, Ames, and Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621.

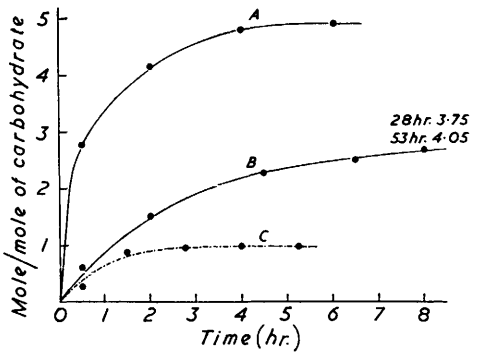
and Chargaff.[14] These observations can be accommodated by reaction of D-gluco-pyranuronic acid with 3 mol. of oxidant to give a formyl ester of tartronaldehydic acid (XVII), the hydrolysis of which would be facilitated by the carboxyl group next to the

FIG. 7.



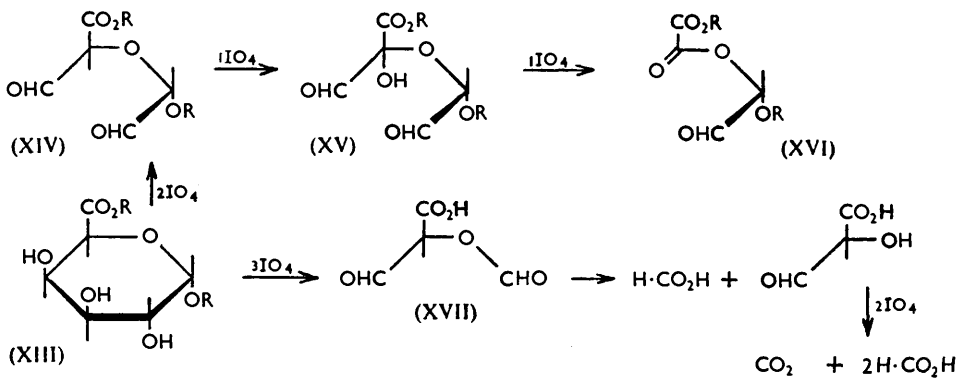
A, DL-*Glyceraldehyde* (*kept in solution for* 15 *hr.*), *periodate uptake. B*, DL-*Glyceraldehyde, periodate uptake. C*, DL-*Glyceraldehyde, formaldehyde released on oxidation at* pH 7·5 (*bicarbonate buffer*). *D*, *Glycollaldehyde* (*kept in solution for* 24 *hr.*), *formaldehyde released. E, Glycollaldehyde, formaldehyde released. F, Glycollaldehyde, periodate uptake. G*, DL-*Glyceraldehyde, formaldehyde released.*

FIG. 8.



A, Sodium D-*glucuronate, periodate uptake. B, Methyl* (*methyl* α-D-*galactopyranosid*)*uronate, periodate uptake. C, Sodium* D-*glucuronate, carbon dioxide released.*

ester linkage and would lead to the further reaction of 2 mol. of periodate with the formation of 1 mol. of carbon dioxide.



EXPERIMENTAL

The crystalline monosaccharides were dried at 60° under reduced pressure for 2 hr. Unless stated otherwise, the monosaccharide (*ca.* 30 mg., accurately weighed) was washed into a mixture of sodium acetate buffer solution (pH 3·6; 25 ml.) and sodium metaperiodate solution (0·3M; 5 ml.) and then made up to 100 ml. with water. The mixture was then quickly transferred to an amber bottle and kept in the dark at room temperature (*ca.* 18°).

The periodate uptake was determined on serial samples (10 ml.) by Neumüller and Vasseur's method,[1] the sample being pipetted into a *mixture* of phosphate buffer (pH 6·98; 25 ml.) and 20% potassium iodide solution (5 ml.) and the liberated iodine titrated with 0·01N-sodium thiosulphate solution (starch).[18]

[18] In collaboration with Mr. A. O. Pittet we have confirmed the results for D-glucose (Fig. 1) by using the ultraviolet spectrophotometric method (Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1219) for the estimation of periodate at pH 3·6.

Formic acid was determined on serial samples (10 ml.) from a reaction mixture similar to that described above except that the acetate buffer was omitted. Ethylene glycol (2 ml.) was added to the sample and after *ca.* 15 min. the solution was titrated potentiometrically with 0·01N-sodium hydroxide; pH 6·25 was taken as the end-point.[19]

Formaldehyde was determined by O'Dea and Gibbons's method.[10] Samples (1 ml.) were withdrawn from a buffered solution and after the removal of excess of periodate with 20% w/v lead dithionate solution (1 ml.) the clear solution (1 ml.) was treated with the chromotropic acid reagent. The absorption was read on a colorimeter (E.E.L. Filter 626). The formaldehyde liberated from known amounts of erythritol was used for the preparation of the standard curve.

The evolution of carbon dioxide was followed in the Warburg apparatus in the dark by adding the carbohydrate (*ca.* 1 mg., accurately weighed) in water (1 ml.) to sodium acetate buffer (pH 3·6; 3 ml.) and allowing the manometer to equilibrate to the bath-temperature (18°) before closing the tap and tipping 0·3M-sodium metaperiodate (1 ml.) from the side-arm.[20]

THE UNIVERSITY, BRISTOL. [*Received, October 29th,* 1957.]

[19] Anderson, Greenwood, and Hirst, *J.*, 1955, 225.
[20] Hough and Woods, *Chem. and Ind.*, 1957, 1421.