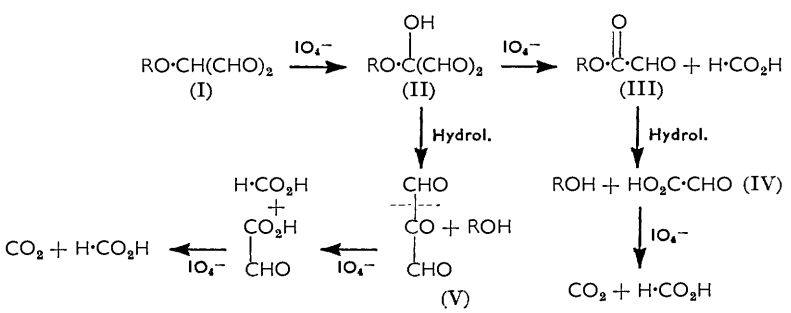
465. *The Non-Malapradian Oxidation of Carbohydrates and Related Compounds by Periodate.*

By M. CANTLEY, L. HOUGH, and A. O. PITTET.

Oxidation of benzyloxymalonaldehyde, malonic acid, glyoxylic acid, methyl α-D-galactopyranosiduronic acid and its methyl ester, 2-*O*-benzyl-D-arabinose, 3-*O*-methyl-D-glucose, 3-*O*-methyl-D-glucitol, and cellobi-itol have been studied at various pH values by following the uptake of periodate and the liberation of carbon dioxide and formaldehyde. Details of the reaction pathways are discussed.

PERIODATE oxidation of carbohydrates can be profoundly affected by changes in pH, particularly when formic and glyoxylic esters[1] and malonaldehydes,[2] which impose a rate-determining step on the overall reaction sequence, are produced as intermediates. The intervention of formic esters has been satisfactorily explained by their maximum stability against hydrolysis at pH 3—4 and by the influence of electronic factors induced by neighbouring groups on their rate of hydrolysis.[1] These studies have now been extended to other intermediates—malonaldehydes in particular—in an attempt to establish conditions under which " over-oxidation " can be promoted or arrested, thus affording useful structural information for work on oligo- and poly-saccharides.[2] The rates of oxidation of various malonaldehyde derivatives and hydroxy-acids, and their carbohydrate precursors, have been examined at pH 1 and pH 3·6, and in sodium hydrogen carbonate (*ca.* pH 7·6) at 25° in the dark. Oxidation of D-glucose and erythritol proceeded normally above pH 7, consumptions being, respectively, 5 mol. of periodate in 100 min. and 3 mol. in 20 min.

Schwarz and MacDougall[3] have shown that benzyloxymalonaldehyde (I; R = Ph·CH_2) is oxidised by sodium metaperiodate to benzyl glyoxylate (III; R = Ph·CH_2) in about 70% yield, which after hydrolysis afforded carbon dioxide and formic acid by oxidation of the glyoxylic acid (IV). In agreement with this reaction sequence, the malon-



aldehyde (I; R = Ph·CH_2) reacted at pH 3·6 with 2 mol. of periodate within 1 hr. and thereafter very slowly (Fig. 1, curve C); only a further 0·5 mol. of periodate had reacted after 100 hr. owing to the resistance of the glyoxylic ester (III; R = Ph·CH_2) to hydrolysis at this pH. (In these conditions, dimethyl tartrate reacted rapidly with only one mol. of periodate.[1]) However, during the first 2 hr. of this reaction about 0·2 mol. of carbon dioxide was liberated, slowly rising to 0·3 mol. after 90 hr., suggesting that a minor but competitive reaction was in operation, the most feasible being hydrolysis of 20% of the hemiacetal (II; R = Ph·CH_2) to benzyl alcohol and mesoxalaldehyde (V) which would

[1] Hough, Taylor, Thomas, and Woods, *J.*, 1958, 1212.

[2] (*a*) Hough and Perry, *Chem. and Ind.*, 1956, **34**, 768; (*b*) Hough, Woods, and Perry, *ibid.*, 1957, **35**, 1100; (*c*) Hough and Woods, *ibid.*, 1957, **35**, 1421; (*d*) Cantley, Hough, and Pittet, *ibid.*, 1959, **37**, 1126, 1253.

[3] Schwarz and MacDougall, *J.*, 1956, 3065.

then be further oxidised to formic acid and carbon dioxide. Head [4] drew attention to the fact that this pathway had been overlooked in previous studies.

When glyoxylic acid (IV) was oxidised at pH 3·6 and in sodium hydrogen carbonate, 1 mol. of carbon dioxide was released in 2—5 hr., whereas at pH 1 the reaction was very slow, being incomplete after 22 hr. (Fig. 2). Using very dilute periodate (0·0004M), Clancy and Whelan [5] found that glyoxylic acid (IV) was not oxidised. Similarly, glycollic acid was not attacked [2d] after 8 days by 0·015M-periodate over the range pH 1—7·5, but with 0·047M-periodate slow oxidation did occur at pH 1 and pH 3·6 (0·14—0·15 mol. of carbon dioxide was liberated after 140 hr.) though not at pH 7·5. With higher concentrations of periodate, glycollic acid is oxidised more rapidly, but the reaction is complex, as in the case of lactic acid, since formaldehyde, carbon dioxide, and formic acid are released, the proportions varying with the temperature of the reaction.[6]

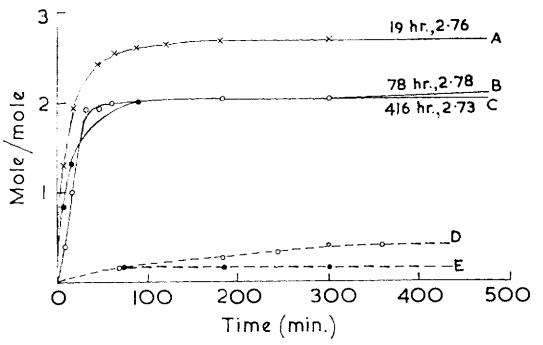


FIG. 1.

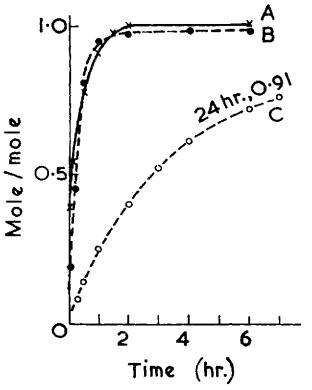


FIG. 2.

FIG. 1. Oxidation of 0·0038M-benzyloxymalonaldehyde with 0·015M-periodate at 24—25°; A, uptake of IO_4^- at pH 7·6; B, uptake of IO_4^- at pH 1·0; C, uptake of IO_4^- at pH 3·6; E, release of CO_2 at pH 3·6.

The oxidation of 0·006M-benzyloxymalonaldehyde with 0·047M-periodate at 24—25°; D, release of CO_2 at pH 1·0.

Note: In all the Figures, full lines denote uptake of IO_4, broken lines production of CO_2, and dotted lines production of CH_2O; also pH is denoted as ×, ○, and ● for 7·6, 3·6, and 1·0, respectively.

FIG. 2. The oxidation of 0·0038M-glyoxylic acid with 0·015M-periodate at 24—25°; A, uptake of IO_4^- at pH 7·6; B, release of CO_2 at pH 3·6; C, release of CO_2 at pH 1·0.

When benzyloxymalonaldehyde (I; R = Ph·CH_2) was oxidised at pH 7·6, 2·75 mol. of oxidant were quickly consumed owing to the rapid hydroysis of the intermediary ester (III; R = Ph·CH_2) (Fig. 1, curve A), whereas at pH 1 only 2 mol. of oxidant were readily used, with later slow reduction of 0·78 mol. in 70 hr. with concomitant liberation of 1 mol. of carbon dioxide (Fig. 1, curve B). The latter effect is explained by the slow oxidation of glyoxylic acid (IV) at pH 1 (Fig. 2).

Since malonic acid (VI) is oxidised [7] as a result of activation of the methylene group by the flanking carboxyl groups, it was of interest to examine the pH-dependence of this reaction. The first mol. of oxidant reacted most rapidly at pH 3·6. Over the range of pH 2·5—5·0, complete oxidation occurred within 30 hr., 3 mol. of oxidant (0·015M)

[4] Head, *Chem. and Ind.*, 1958, **36**, 38.
[5] Clancy and Whelan, *Chem. and Ind.*, 1959, **37**, 673.
[6] Fleury, Courtois, Perles, and Le Dizet, *Bull. Soc. chim. France*, 1954, **21**, 347.
[7] Huebner, Ames, and Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621; Fleury and Courtois, *Compt. rend.*, 1946, **223**, 633.

reacting to give 2 mol. of carbon dioxide, but in strongly acidic and weakly alkaline

$$\underset{\text{(VI)}}{CH_2(CO_2H)_2} \longrightarrow \underset{\text{(VII)}}{HO{\cdot}CH(CO_2H)_2} \longrightarrow CO_2 + \underset{\text{(IV)}}{OHC{\cdot}CO_2H} \longrightarrow CO_2 + H{\cdot}CO_2H$$

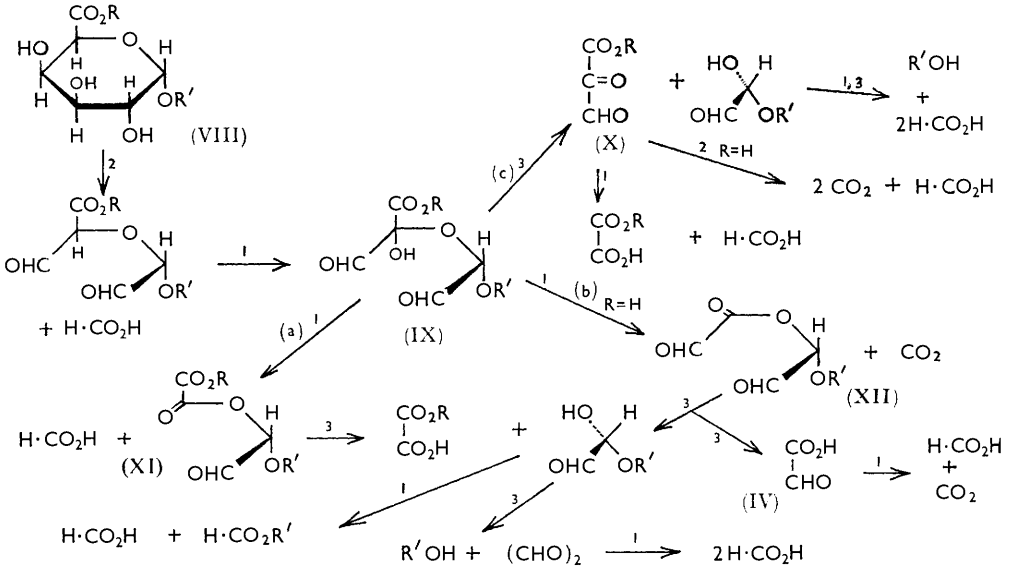
solution, the rate of reaction was considerably retarded (Table). These results are in agreement with the participation of hydroxymalonic acid (tartronic acid) (VII) and glyoxylic acid (IV) as intermediates. Phosphate promoted a faster reaction of the methylene group with periodate, as it does with some dialdehydes.[2c,8] Wolfrom and

The effect of pH and phosphate on the oxidation of malonic acid (30 mg.) with buffered 0·015M-sodium metaperiodate (100 ml.) at 24—25°.

		Time (hr.) for the reduction of IO_4^- per mole of malonic acid					Time (hr.) for the reduction of IO_4^- per mole of malonic acid		
pH	Buffer	1 mole IO_4^-	2 mole IO_4^-	3 mole IO_4^-	pH	Buffer	1 mole IO_4^-	2 mole IO_4^-	3 mole IO_4^-
1·2	H_2SO_4	51	136	340 (2·9) *	5·0	Phosphate	4·0	11	72 (2·9) *
2·5	Phosphate	2·4	6·4	24	5·0	Acetate	3·3	9·2	22 (2·6) *
2·5	Acetate	2·8	6·2	25	~7·5	$NaHCO_3$	115	130 (1·1) *	—
3·65	Acetate	2·0	5·8	29	7·9	Phosphate	41	282	511 (2·6) *
3·65	Phosphate	1·7	5·3	21					

* Oxidation incomplete; the figures in parentheses are the final values for the periodate consumption.

Bobbitt [9] found satisfactory oxidation of 1,3-diketones between pH 3 and pH 8 with a rate maximum at pH 5. As in the case of glycollic acid, increased concentrations of periodate hastened the oxidation of malonic acid. In 50% aqueous *NN*-dimethylformamide, no oxidation occurred after 4 days, suggesting that the participating periodate species in oxidation of the active methylene group is the same as that in cleavage of an α-glycol.[10]



1, 1 mol. of IO_4^-. 2, 2 mol. of IO_4^-. 3, Hydrolysis.

The oxidation of methyl α-D-galactopyranosiduronic acid (VIII; R = H, R' = Me) was then studied, since on the previous evidence various routes are feasible. In sodium

[8] Lindstedt, *Nature*, 1945, **156**, 448.
[9] Wolfrom and Bobbitt, *J. Amer. Chem. Soc.*, 1956, **78**, 2489.
[10] Guthrie, *Chem. and Ind.*, 1960, **38**, 691.

hydrogen carbonate solution, rapid hydrolysis of intermediary esters was expected and the uronic acid (VIII; R = H, R′ = Me) consumed 5·05 mol. of periodate in 20 hr. with the liberation of 0·92 mol. of oxalic acid (Fig. 3). Huebner, Ames, and Bubl [7] similarly found that bornyl α-D-glucopyranosiduronic acid reacted with 5 mol. of periodate, giving 1 mol. of bornyl formate, 1 mol. of oxalic acid, and 3 mol. of formic acid. Whilst this evidence rules out reaction (b) as a major route above pH 7, the small amounts of carbon dioxide detected when the oxidation was carried out at pH 3·6 (0·28 mol. of CO_2) and pH 1 (0·12 mol. of CO_2) suggested that some 15—30% of the hemiacetal (IX; R = H, R′ = Me) breaks down according to reaction (b) or (c) through glyoxylic acid (IV) and mesoxalic semialdehyde (X; R = H), respectively. Increase in the concentration of periodate from 0·015M to 0·05M caused a slight increase in the yield of carbon dioxide (0·42 mol. at pH 3·6; 0·33 mol. at pH 1), suggesting an analogy between the oxidations of the hydroxy-acid (IX; R = H, R′ = Me) and glycollic acid. At pH 1 and pH 3·6, the

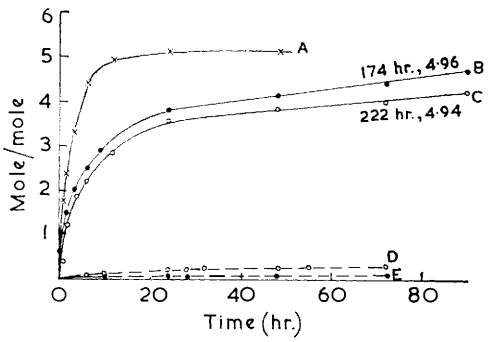


FIG. 3.

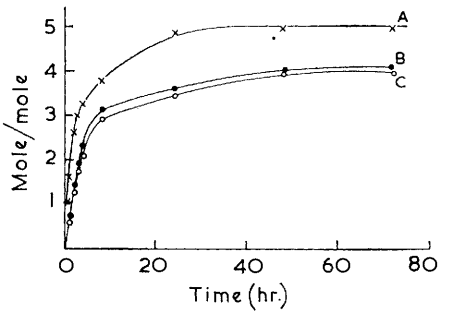
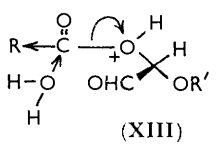


FIG. 4.

FIG. 3. Oxidation of 0·0015M-methyl α-D-galactopyranosiduronic acid with 0·015M-periodate at 24—25°; A, uptake of IO_4^- at pH 7·6; B, uptake of IO_4^- at pH 3·6; C, uptake of IO_4^- at pH 1·0; D, release of CO_2 at pH 1·0; E, release of CO_2 at pH 3·6.

FIG. 4. Oxidation of 0·0015M-methyl (methyl α-D-galactopyranosid)uronate by 0·015M-periodate at 24—25°; A, uptake of IO_4^- at pH 7·6; B, uptake of IO_4^- at pH 3·6; C, uptake of IO_4^- at pH 1·0.

fifth mol. of periodate reacted very slowly (Fig. 3), suggesting that the oxalyl (XI; R = H, R′ = Me) and the glyoxylyl (XII; R′ = Me) ester are relatively stable in acid media and thereby determine the rate of reaction of the last mol. of periodate. The electron-withdrawing inductive effect of the electrophilic carboxyl and aldehyde substituents in the acid components of these esters would account for the relative stability to hydrolysis by reducing the rate of cleavage of the C–O ester bond (XIII). In agreement with this suggestion, determination of the free oxalic acid liberated after 48 hr. at pH 1 and pH 3·6 gave values of 0·11 and 0·24 mol., respectively.[11]



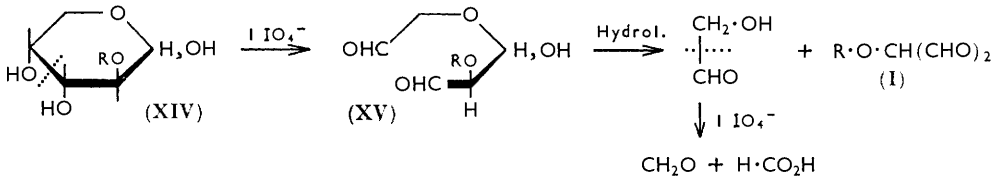
(XIII)

Methyl (methyl α-D-galactopyranosid)uronate (VIII; R = R′ = Me) also reduced 5 mol. of periodate in sodium hydrogen carbonate, and only 4 mol. at pH 1 and pH 3·6 (Fig. 4), supporting the previously suggested pathway [7] through the methyl oxalate (XI; R = Me) which will be labile above pH 7 but relatively stable in acidic solution because of the electron-withdrawing inductive effect of carbomethoxycarbonyl group in the ester (XIII; R = CO_2Me). Neither carbon dioxide nor oxalic acid was liberated at pH 3·6 or pH 1·0, but, as was expected, in sodium hydrogen carbonate 0·96 mol. of oxalic acid was found at the end of the reaction.[11]

The oxidation of 2-*O*-benzyl-D-arabinose (XIV; R = Ph·CH_2) was of interest because

[11] Cantley, Hough, and Peters, unpublished results.

initial reaction of 1 mol. of periodate would give a dialdehyde hemiacetal (XV; R = Ph·CH_2), the rate of hydrolysis of which determines the rate of further oxidation. As judged by the rate of formaldehyde formation, hydrolysis was significantly slower at



pH 3·6 than at other pH values (Fig. 5). Apart from this effect, the results for 2-*O*-benzyl-D-arabinose resembled those obtained for benzyloxymalonaldehyde (I; R = Ph·CH_2).

3-*O*-Methyl-D-glucose (XVI; R = Me) was oxidised and the rate of formation of carbon dioxide from the intermediary methoxymalonaldehyde (I; R = Me) followed; at pH 1, 1 mol. was released in 30 hr., whereas at pH 3·6 it appeared very slowly, reaching only 0·2 mol. in 60 hr. (Fig. 6). This result confirms the previous observations [1] on the stability of the intermediate ester, 4-*O*-formyl-2-*O*-methyl-D-arabinose (XVII; R = Me).

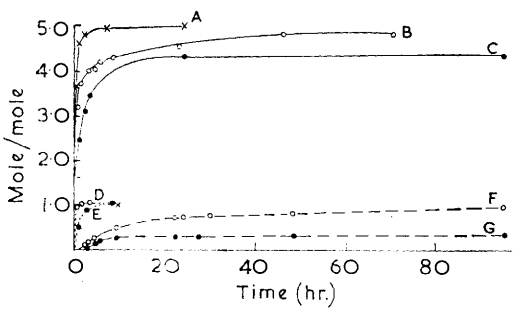


FIG. 5.

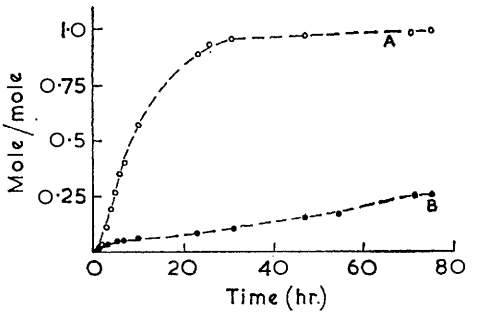


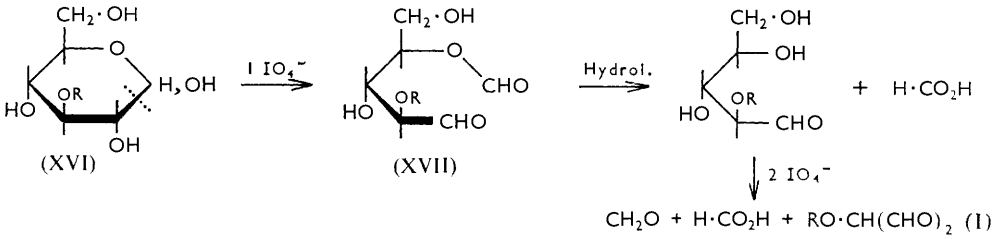
FIG. 6.

FIG. 5. Oxidation of 0·0014M-2-*O*-benzyl-D-arabinose by 0·015M-periodate at 24—25°; A, uptake of IO_4^- at pH 7·6; B, uptake of IO_4^- at pH 1·0; C, uptake of IO_4^- at pH 3·6; D, release of CH_2O at pH 7·6 and pH 1·0; E, release of CH_2O at pH 3·6; F, release of CO_2 at pH 1·0; G, release of CO_2 at pH 3·6.

FIG. 6. Oxidation of 0·0011M-3-*O*-methyl-D-glucose by 0·047M-periodate at 24—25°; A, release of CO_2 at pH 1·0; B, release of CO_2 at pH 3·6.

Oxidation of 3-*O*-methyl-D-glucitol (XVIII) was expected to give 2 mol. of formaldehyde and methoxymalonaldehyde (I; R = Me), the latter breaking down to methyl glyoxylate (III; R = Me) and mesoxalaldehyde (V), eventually giving 1 mol. of carbon dioxide. Results obtained at pH 1 and in sodium hydrogen carbonate (Fig. 7) were in general agreement with this reaction sequence. At pH 3·6, however, oxidation was incomplete since 3·4 mol. of periodate were consumed with liberation of 1·5 mol. of formaldehyde and 0·1 (3 hr.) to 0·27 mol. (80 hr.) of carbon dioxide. The possibility of more than one reaction pathway must therefore be considered. The results may be accounted for as follows, by assuming that 50% of the hexitol ether (XVIII; R = Me) is oxidised to the methoxymalonaldehyde (I; R = Me), a little of which undergoes hydrolysis, as outlined, to mesoxalaldehyde (V) and thence carbon dioxide (0·2 mol.); the majority of aldehyde (I; R = Me) would be directly oxidised to methyl glyoxylate (III; R = Me) which is relatively stable at pH 3·6. The other 50% of the hexitol ether reacts with 1 mol. of periodate with selective cleavage of the 5,6-bond, as can be predicted on steric grounds, to give 1 mol. of formaldehyde and 3-*O*-methyl-L-xylose which by analogy with 3-*O*-methyl-D-glucose (XVI; R = Me) would be oxidised in the pyranose

form (XIX; R = Me) to the stable ester, 4-*O*-formyl-2-*O*-methyl-L-threose (XX; R = Me), and consequently no further carbon dioxide or formaldehyde is released. Combining these two pathways, in theory 3-*O*-methyl-D-glucitol (XVIII; R = Me) would react at



pH 3·6 with 3·6 mol. of periodate, giving 1·5 mol. of formaldehyde and 0·1 mol. of carbon dioxide in good agreement with the results (Fig. 7). It appears, therefore, that 50% of the

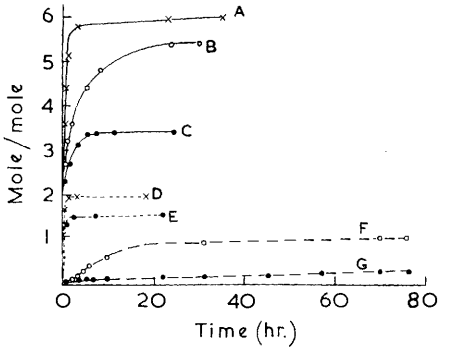


FIG. 7.

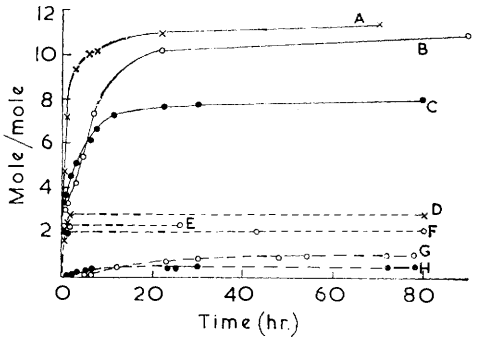
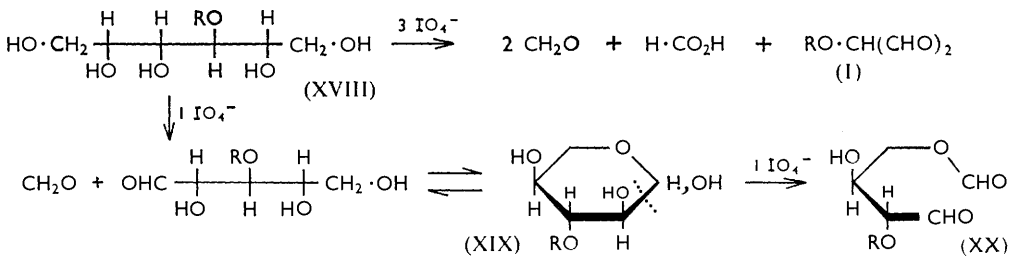


FIG. 8.

FIG. 7. Oxidation of 0·0013M-3-*O*-methyl-D-glucitol by 0·047M-periodate at 24—25°; A, uptake of IO_4^- at pH 7·6; B, uptake of IO_4^- at pH 1·0; C, uptake of IO_4^- at pH 3·6; D, release of CH_2O at pH 7·6; E, release of CH_2O at pH 3·6; F, release of CO_2 at pH 1·0; G, release of CO_2 at pH 3·6.

FIG. 8. The oxidation of 0·0009M-cellobi-itol by 0·015M-periodate at 24—25°; A, uptake of IO_4^- at pH 7·6; B, uptake of IO_4^- at pH 1·0; C, uptake of IO_4^- at pH 3·6; D, release of CH_2O at pH 7·6; E, release of CH_2O at pH 1·0; F, release of CH_2O at pH 3·6; G, release of CO_2 at pH 1·0; H, release of CO_2 at pH 3·6.

pentose formed by the initial selective cleavage of the polyol undergoes cyclisation and that the rate of cyclisation is at least as great as the rate of oxidation of the open-chain form. A similar cyclisation was observed with 2,3-di-*O*-methyl-D-arabinose.[12]

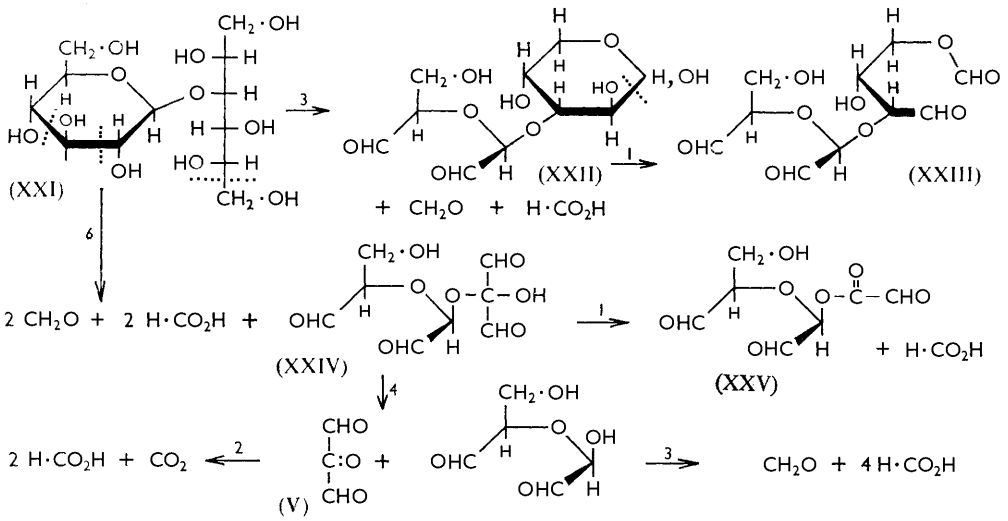


A study of the oxidation of cellobi-itol (XXI) was undertaken in order to investigate the rate of oxidation through the malonaldehyde (XXIV) without the intervention of formyl esters that arise in the case of the reducing disaccharides. Cellobi-itol (XXI)

[12] Heinz, Sorger-Domenigg, Goldstein, and Smith, *J. Amer. Chem. Soc.*, 1959, **81**, 444.

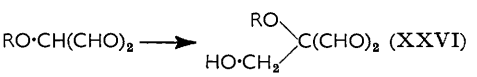
consumed 11 mol. of periodate at pH 1 and in sodium hydrogen carbonate, to give, at pH 1, 1 mol. of carbon dioxide and, above pH 7, 3 mol. of formaldehyde (Fig. 8). As in the case of 3-*O*-methyl-D-glucitol (XVIII; R = Me), the reaction was complex at pH 3·6, since only 8 mol. of periodate were consumed. The release of only 0·5 mol. of carbon dioxide at pH 3·6 suggested that 50% of the cellobi-itol (XXI) was completely oxidised by 11 mol. of periodate through a hydroxymalonaldehyde (XXIV) which gave, by hydrolysis, mesoxalaldehyde (V) and thence carbon dioxide. As in the case of 3-*O*-methyl-D-glucitol, the intervention of esters was indicated. The oxidation of the remaining 50% of cellobi-itol (XXI) was probably arrested at either the glyoxylyl ester (XXV) or the formyl ester (XXIII) stage when only 7 mol. and 4 mol., respectively, of periodate would have been consumed. If 25% of the cellobi-itol were oxidised to the formyl ester (XXIII) through the D-arabinopyranose (XXII), if 25% of the cellobi-itol gave the glyoxylyl ester (XXV), and if the remaining 50% were completely eroded by way of mesoxalaldehyde (V), an uptake of 8·25 mol. of oxidant with the formation of 0·5 mol. of carbon dioxide and 2·25 mol. of formaldehyde should be observed. The results (Fig. 8) obtained at pH 3·6 are in close agreement with these figures.

O'Dea [13] suggested that the low yields of formaldehyde obtained from the periodate oxidation of lactose and maltose are due to combination of formaldehyde with a malon-



1, 1 mol. of IO_4^-. 2, 2 mol. of IO_4^-. 3, 3 mol. of IO_4^-. 4, Hydrolysis. 6, 6 mol. of IO_4^-.

aldehyde intermediate, but that this reaction was prevented, giving theoretical quantities of formaldehyde, by the inclusion of *p*-hydroxybenzaldehyde, possibly as a result of its preferential combination at the activated centre:



This explanation cannot be correct, however, since oxidations of 2-*O*-benzyl-D-arabinose (XIV; R = Ph·CH_2) and 3-*O*-methyl-D-glucitol (XVI; R = Me) gave theoretical yields of formaldehyde in both the presence and the absence of *p*-hydroxybenzaldehyde. Theoretical yields of formaldehyde were reported for the periodate oxidation of 3-*O*-methyl-D-galactitol [14] and 3-*O*-methyl-D-glucose [15] in the absence of the aromatic aldehyde.

[13] O'Dea, *Chem. and Ind.*, 1953, **31**, 1338; O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.
[14] McKeon and Haywood, *Canad. J. Chem.*, 1955, **33**, 1392.
[15] Bell, *J.*, 1948, 992.

Further, the oxidation of malonaldehydes is much more rapid than their rate of combination with formaldehyde.[3] The product (XXVI) from the reaction with formaldehyde would not be expected to be significantly oxidised, whereas the oxidation of malonaldehydes usually proceeds to completion.

As in the cases of cellobiose, maltose, and lactose, low yields of formaldehyde were obtained on oxidation of cellobi-itol (XXI) at various pH, unless *p*-hydroxybenzaldehyde was included in the reaction mixture. These results suggest that it is the dialdehyde residue arising from the non-reducing pyranosyl unit that is responsible for the loss of formaldehyde.

Oxidations by periodate have been carried out at low temperatures (<5°) as a means of minimising the non-Malapradian reactions.[16] It appears that the rate of oxidation of malonaldehydes has a greater temperature coefficient than glycol cleavage, perhaps because the formation of a six-membered cyclic complex in the oxidation of malonaldehydes, proposed by Bose, Foster, and Stephens,[17] is a strongly endothermic process. In sodium hydrogen carbonate, benzyloxymalonaldehyde consumed 1·7 mol. of periodate in 23 hr. at 0°, whereas at 27° the same consumption was recorded after 10 min., reaching a final value of 2·75 mol. after 3 hr.

Experimental

2-*O*-Benzyl-D-arabinose and benzyloxymalonaldehyde were prepared by the method of Schwarz and MacDougall.[3] Methyl α-D-galactopyranosiduronic acid dihydrate was isolated by alkaline hydrolysis of its methyl ester.[18] Cellobi-itol was obtained by reduction of cellobiose with sodium borohydride followed by purification through its acetate, as described for 2-acetamido-2-deoxy-D-glucitol;[19] crystallisation from methanol gave a monohydrate,[20] m. p. 106—107°, $[\alpha]_D$ −6·8° (*c* 1·45 in water) (Found: C, 39·9; H, 7·5. Calc. for $C_{12}H_{26}O_{12}$: C, 39·8; H, 7·2%). Prolonged reduction of 3-*O*-methyl-D-glucose with several portions of sodium borohydride[19] gave 3-*O*-methyl-D-glucitol as a syrup, $[\alpha]_D$ +16·1° (*c* 6·5 in water) (Found: C, 42·7; H, 8·0; OMe, 16·1. $C_7H_{16}O_6$ requires C, 42·8; H, 8·2; OMe, 15·9%). Another specimen was prepared by heating a mixture of 3-*O*-methyl-D-glucose (1·1 g.) and Raney nickel (settled vol., 41 ml.) in 70% ethanol (500 ml.) under reflux for 50 min.[21] and purifying the product by chromatography on sheets of Whatman No. 1 filter paper with butan-1-ol–ethanol–water (40 : 11 : 19 v/v) as mobile phase. After de-ionisation with Amberlite IR-120 (H) and IRA-400 (OH) resins, the polyol was obtained as a syrup (402 mg.) with $[\alpha]_D$ +7·0° (*c* 3·74 in water) (Found: C, 42·8; H, 8·2; OMe, 16·0%). Treatment with acetic anhydride–sulphuric acid failed to give a crystalline acetate.

Compounds to be oxidised were dried at 60° under reduced pressure. The compound (accurately weighed) was washed into a mixture of the appropriate sodium acetate buffer, 0·1N-sulphuric acid (25 ml.), or saturated aqueous sodium hydrogen carbonate (10 ml.), and sodium metaperiodate solution (5 ml.) and made up to 100 ml. with water. The reaction mixtures for the determination of carbon dioxide and formaldehyde were made up in smaller volumes of solution but contained each component in the same proportions. The oxidations were carried out in the dark at 24—25°, unless otherwise stated.

By using serial samples, periodate was determined by the neutral thiosulphate method,[1,22] formaldehyde by the chromotropic acid reagent,[1,13] oxalic acid as its calcium salt,[11,23] and carbon dioxide in the Warburg apparatus.[1,2c]

[16] Jackson and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 959; Jeanloz and Forchielli, *J. Biol. Chem.*, 1951, **188**, 361; Fleury, Courtois, and Beider, *Bull. Soc. chim. France*, 1952, 118; 1953, 543; Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488.
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[18] Morell and Link, *J. Biol. Chem.*, 1933, **100**, 385.
[19] Bragg and Hough, *J.*, 1957, 4347.
[20] Wolfrom and Fields, *Tappi*, 1958, **41**, 204.
[21] Karabinos and Ballun, *J. Amer. Chem. Soc.*, 1953, **75**, 4501; Wolfrom and Schumacher, *ibid.*, 1955, **77**, 3318.
[22] Neumüller and Vasseur, *Arkiv Kemi*, 1953, **5**, 235.
[23] Powers and Levatin, *J. Biol. Chem.*, 1944, **154**, 207.

The behaviour of malonic acid (15 mg.) in a mixture of 0·03M-sodium metaperiodate solution (25 ml.) and *NN*-dimethylformamide (25 ml.) was examined by a spectrophotometric method.[24]

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[24] Aspinall and Ferrier, *Chem. and Ind.*, 1957, **35**, 1216.