504. *Malonaldehyde Derivatives as Intermediates in the Periodate Oxidations of Amino- and Acetamido-sugars.*

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Between pH 1 and pH 7·5, the major pathway for the oxidation of 2-acetamido-2-deoxy-D-glucose proceeds exclusively through acetamidomalonaldehyde. Oxidation of 2-deoxy-2-(2,4-dinitroanilino)-D-glucose has been investigated and, in accordance with this pathway, 2,4-dinitroaniline was recognised as one of the products.

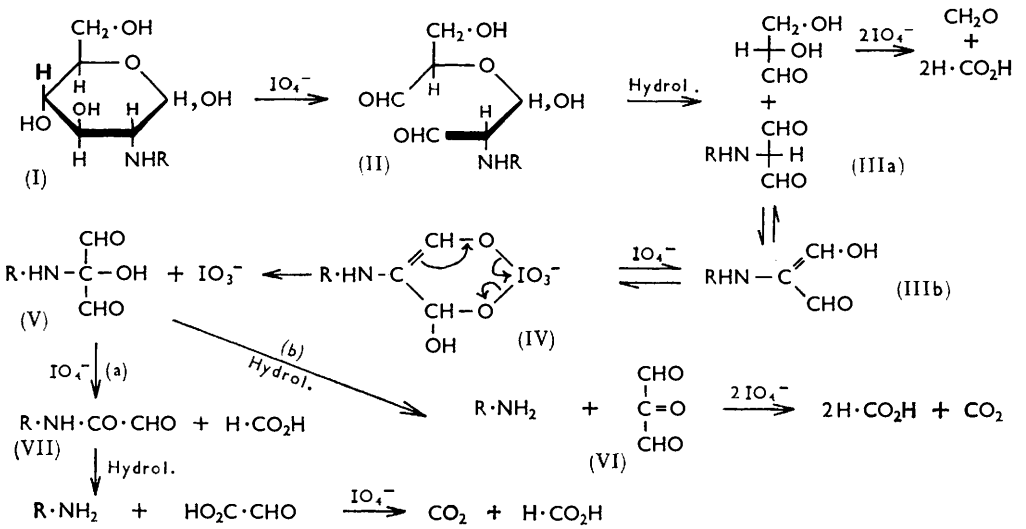
Oxidation of 2-amino-2-deoxy-D-glucose and -D-galactose by sodium metaperiodate is pH-dependent and involves more than one route. In alkaline solution oxidation is rapid and proceeds almost exclusively in the normal Malapradian manner, but as the pH is lowered (thus increasing the proportion of the ammonium form), oxidation *via* ammoniomalonaldehyde, $^{+}NH_3 \cdot CH(CHO)_2$, becomes significant. The influence of pH on the rate of oxidation of 2-aminoethanol has also been studied.

PERIODATE oxidations of 2-amino-2-deoxy- and 2-acetamido-2-deoxy-derivatives of reducing monosaccharides at various temperatures, concentrations, and pH values have shown that the reactions are complex and usually proceed further than would be predicted for normal scission of αβ-glycol and α-amino-β-hydroxy-structures.[1-4] At room temperature and in the dark, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside rapidly consumes one mol. of oxidant, very slow over-oxidation following; thus the α-acetamidoaldehyde structure is relatively stable to periodate.[2,5] Under the same conditions, 2-acetamido-2-deoxy-D-glucose (I; R = Ac) rapidly consumes *ca.* 5·5 mol. of periodate.[5] On lowering the temperature of the reaction mixture to 5°, Jeanloz and Forchielli [1] found that the over-oxidation was considerably reduced, 2-acetamido-2-deoxy-D-glucose consuming 5 mol. of oxidant. This behaviour suggested the participation in the reaction of malonaldehyde intermediates,[6,7] oxidation of which is relatively rapid at room temperature but slow below 5°.[8]

On this basis, the oxidation of 2-acetamido-2-deoxy-D-glucose (I; R = Ac) would

[1] Jeanloz and Forchielli, *J. Biol. Chem.*, 1951, **188**, 361.
[2] Neuberger, *J.*, 1941, 47.
[3] Aminoff and Morgan, *Biochem. J.*, 1949, **44**, xxi.
[4] Cantley and Hough, *Biochem. J.*, 1960, **77**, 6P.
[5] Hough and Taha, *J.*, 1956, 2042.
[6] Zilliken, Smith, Tomarelli, and György, *Arch. Biochem. Biophys.*, 1955, **54**, 398.
[7] Hough and Perry, *Chem. and Ind.*, 1956, 768; Hough and Woods, *ibid.*, 1957, 1421; Cantley, Hough, and Pittet, *ibid.*, 1959, 1126.
[8] Cantley, Hough, and Pittet, *J.*, 1963, 2527.

proceed by oxidation to the hemiacetal (II) with subsequent hydrolysis to glyceraldehyde and acetamidomalonaldehyde (IIIa; R = Ac) which would be further oxidised to acetamidohydroxymalonaldehyde (V) and thence by either route (a) or (b). If both reactions (a) and



(b) go to completion, 1 mol. of carbon dioxide would be released with consumption of 6 mol. of periodate per mol. of hexosamine. At pH 7·5, these results were obtained (Fig. 1), thus establishing acetamidomalonaldehyde (IIIa; R = Ac) as an intermediate in the process.

However, at pH 5 and pH 1, 2-acetamido-2-deoxy-D-glucose (I; R = Ac) gave only 0·85 and 0·72 mol. of carbon dioxide, respectively (Fig. 1), with the utilisation of 5·7—5·8 mol. of periodate. This behaviour suggested that the amide (VII; R = Ac) is stable to hydrolysis within the range of pH 1—5, thus preventing the formation of carbon dioxide via route (a). Since the hydrolysis of the hydroxymalonaldehyde (V; R = Ac) will, by analogy with glycosylamines and ketone-ammonias, proceed in acidic solution [subsequent oxidation of the resulting mesoxaldialdehyde (VI) giving carbon dioxide], the results can be interpreted by assuming that the relative rates of the oxidation reaction (a) and the hydrolysis (b) vary with pH. On this basis, at pH 5 about 85% of the hydroxymalonaldehyde (V; R = Ac) breaks down via route (b), giving 0·85 mol. of carbon dioxide and 15% is oxidised to the amide (VII) by route (a), whereas at pH 1 about 72% proceeds via route (b) with the formation of 0·72 mol. of carbon dioxide and 28% by route (a).

The oxidation of 2-acetamido-2-deoxy-D-glucose was slower at pH 3·5 than at other pH values (Fig. 1). This effect is attributed to the rate of hydrolysis of the dialdehyde (II) which appears to be at a minimum at this pH. It is noteworthy that at about this pH, the mutarotation of D-glucose and D-galactose, which is a measure of the hydrolysis of the hemiacetal, is at a minimum.

Application of the mechanism proposed by Bose, Foster, and Stephens [9] to the hydroxylation of the acetamidomalonaldehyde would give a cyclic complex (IV; R = Ac) of the enolic form (IIIb; R = Ac) with periodate. The results obtained are consistent with this mechanism since the oxidation will be facilitated by the electron-withdrawing inductive effect of the acetamido-group, which favours the ionisation of the hydrogen on C-2 and hence the formation of the enol. As in the case of other malonaldehydes,[8] the rate of oxidation of the acetamido-derivative (IIIa; R = Ac) was considerably reduced at 0°, as judged by the carbon dioxide liberated at pH 5 (Fig. 1).

[9] Bose, Foster, and Stephens, *J.*, 1959, 3314.

Oxidation of 2-acetamido-2-deoxy-D-ribose with unbuffered sodium metaperiodate gave rise after *ca.* 10 hr. to 0·8 mol. of carbon dioxide at 25° and 0·7 mol. at 19°, thus showing that it is oxidised in a similar manner to 2-acetamido-2-deoxy-D-glucose.

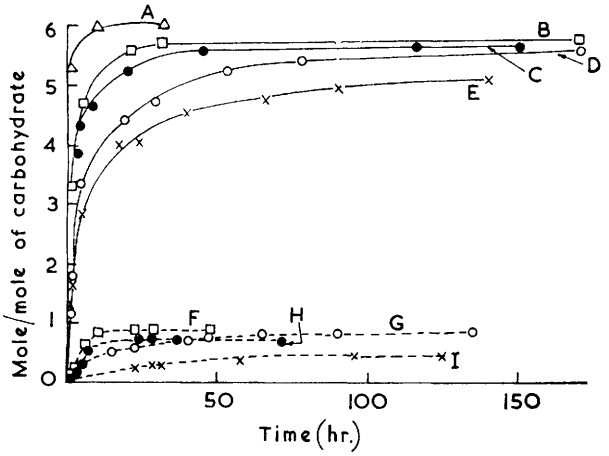


FIG. 1 Oxidation of 0·0015M-2-acetamido-2-deoxy-D-glucose with 0·015M-periodate in the dark and, unless stated otherwise, at 24—25°; A, uptake of IO_4^- at pH 7·6 (when 6 mol. of IO_4^- had been consumed, acidification of the oxidation mixture gave 1 mol. of CO_2 per mol. of sugar); B, uptake of IO_4^- at pH 5·0; C, uptake of IO_4^- at pH 1; D, uptake of IO_4^- at pH 3·6; E, uptake of IO_4^- at pH 5·0 and at 0°; F, release of CO_2 at pH 5·0; G, release of CO_2 at pH 3·6; H, release of CO_2 at pH 1·0; I, release of CO_2 at pH 5·0 and at 0°.

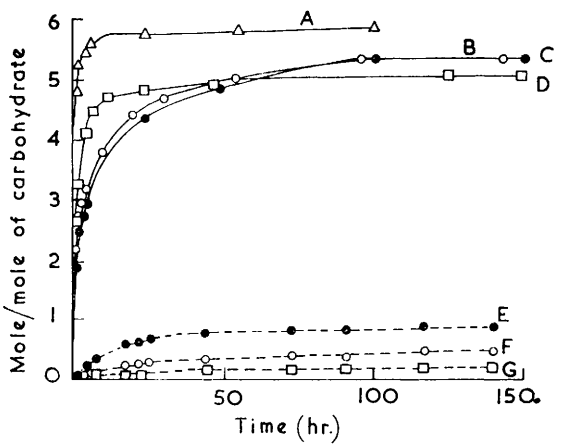


FIG. 2 Oxidation of 0·0015M-2-deoxy-2-(2,4-dinitrophenylanilino)-D-glucose with 0·015M-periodate; A, uptake of IO_4^- at pH 7·5; B, uptake of IO_4^- at pH 3·6; C, uptake of IO_4^- at pH 1·0; D, uptake of IO_4^- at pH 5·0; E, release of CO_2 at pH 1·0; F, release of CO_2 at pH 3·6; G, release of CO_2 at pH 5·0.

2-Deoxy-2-(2,4-dinitroanilino)-D-glucose [I; R = $C_6H_4(NO_2)_2$] would also be expected to be oxidised through a malonaldehyde intermediate on account of the strongly electron-withdrawing character of the 2,4-dinitrophenyl group. It was first established that 2-(2,4-dinitroanilino)ethanol did not react with periodate. At pH 7·5, the periodate uptake of the dinitrophenyl derivative (I) approached 6 mol. and 2,4-dinitroaniline separated, as required for complete oxidation (Fig. 2), but lower values (*ca.* 5 mol.) were

obtained at pH 1—5. Increasing quantities of carbon dioxide were liberated as the pH was lowered from pH 5 to pH 3·6 and then to pH 1. As in the case of 2-acetamido-2-deoxy-D-glucose (I; R = Ac) it appears that the rates of reactions (a) and (b) are pH-dependent, a higher proportion proceeding by path (b) at pH 1 than at higher pH values, and that the amide [VII; R = $C_6H_4(NO_2)_2$] is stable to hydrolysis from pH 1 to pH 5. Thus, at pH 1, 0·8 mol. of carbon dioxide was liberated and 2,4-dinitroaniline separated, whereas at pH 5 and pH 3·6 only 0·2—0·4 mol. of carbon dioxide was released (Fig. 2) and 2,4-dinitroaniline did not separate until the solution of the amide (VII) was acidified with 2N-sulphuric acid. This suggested the accumulation at pH 5·0 of a high proportion of *N*-2,4-dinitrophenylglyoxylamide [VII; R = $C_6H_4(NO_2)_2$] and a compound with the properties of such an intermediate was detected.

It was of interest to examine corresponding periodate oxidations of 2-amino-2-deoxy-D-glucose (VIII) since if experimental conditions under which it yielded no carbon dioxide could be established, then 2-acetamido-2-deoxy-D-glucose could be quantitatively estimated in the presence of the free amino-sugar. In aqueous solution, 2-amino-2-deoxy-D-glucose (VIII) can exist in equilibrium with the protonated form (IX). Oxidation of the latter would be expected to give ammoniomalonaldehyde (X) and hence carbon dioxide, through the intermediates (IV; R = H) and (V; R = H), as a result of the inability of periodate to cleave the 1,2- and 2,3-bonds of the protonated amino-sugar. As in the case of 2-acetamidomalonaldehyde (III; R = Ac) the hydroxylation step will be favoured by the

TABLE 1.

Oxidation of 0·0019M-2-aminoethanol with 0·015M-sodium metaperiodate at 25° in the dark.

pH	Free base at equilibrium * (%)	Second order rate constant (l. mole^{-1} min.$^{-1}$)
1·2	0·049	0·0166
3·6	11·5	0·461
5·0	76·2	4·79
7·5	99·9	261

* The relative proportions of the protonated and non-protonated forms in the equilibrium mixtures were calculated from the pK_a value of the NH_3^+ group.

electrophilic inductive effect of the ammonium group. McCasland and Smith [10] have shown that the rate of oxidation of 2-aminocyclanols increases with increasing pH. Confirmation has now been obtained by relating the rate of oxidation of 2-aminoethanol to the proportion of free base at various pH values (Table 1).

The amount of carbon dioxide produced in the periodate oxidation of 2-amino-2-deoxy-D-glucose was found to decrease as the pH was raised from 1 to 5, in agreement with the intermediate formation of ammoniomalonaldehyde (X) in decreasing quantities (Table 2).

TABLE 2.

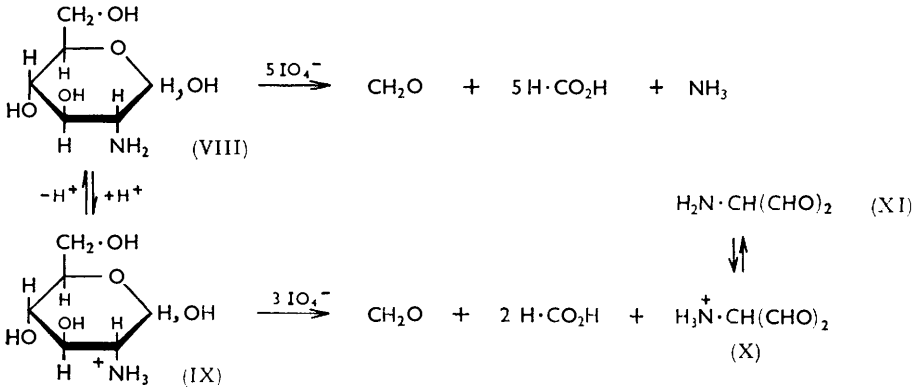
Oxidation of 0·0016M-2-amino-2-deoxy-D-glucose with 0·015M-sodium metaperiodate at 25° in the dark.

		Carbon dioxide (mol.)				Carbon dioxide (mol.)	
Buffer	pH	Calc.*	Found †	Buffer	pH	Calc.*	Found †
H_2SO_4	1·2	1·0	0·71	Phosphate	7·8	0·50	0·07
Acetate	3·6	1·0	0·29	Borate	8·5	0·17	0·09
Acetate	5·0	0·99	0·07	Borate	9·3	0·03	0·08

* The protonated (IX) and the unprotonated (VIII) forms are assumed to be oxidised at the same rate, and the ammoniomalonaldehyde (X) is assumed not to equilibrate with its unprotonated species (XI). † Final value.

At pH 1, some 70% of the monosaccharide was oxidised through this intermediate to give carbon dioxide, whereas in the range pH 5—9 less than 0·1 mol. of carbon dioxide was

[10] McCasland and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5164.



produced and about 90% of the amino-sugar was oxidised in the Malapradian manner (Fig. 3).

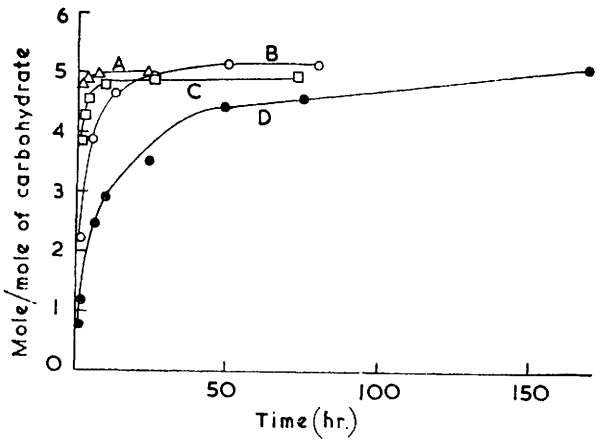


FIG. 3. Oxidation of 0·0016M-2-amino-2-deoxy-D-glucose with 0·015M-periodate; A, uptake of IO_4^- at pH 7·5; B, uptake of IO_4^- at pH 3·6; C, uptake of IO_4^- at pH 5·0; D, uptake of IO_4^- at pH 1·0.

There is apparently a considerable difference in the rates of oxidation of the unprotonated (VIII; XI) and the protonated (IX; X) species and if, as would be expected, the former (VIII) is oxidised faster than the latter (IX), this would account for the discrepancy between the observed and the calculated yields of carbon dioxide (Table 2), since the equilibrium will be shifted in favour of the uncharged species, thus decreasing the yield of carbon dioxide.

2-Amino-2-deoxy-D-galactose reacted with sodium metaperiodate similarly to 2-amino-2-deoxy-D-glucose (VIII). The only significant difference was observed at pH 1, where the initial rate of oxidation for the former was greater than for the latter, probably because the *galacto*-isomer possesses *cis*-hydroxyl-groups at positions 3 and 4 in contrast to the *trans*-system in the *gluco*-isomer.

EXPERIMENTAL

M. p.s were determined on a Kofler micro-heating stage.

Periodate oxidations and associated methods of analysis were carried out as described previously.[8]

Potentiometric titration of 0·1N-2-aminoethanol (25 ml.) with 0·1N-sulphuric acid gave the half-neutralisation point at pH 9·5, from which pK_a 4·5 was calculated.

2-(2,4-*Dinitroanilino*)*ethanol*.—2-Aminoethanol (1·0 g.) in ethanol (12 ml.) was shaken with 1-fluoro-2,4-dinitrobenzene (3·1 g.) in the presence of anhydrous potassium carbonate (1·15 g.) for 6 hr., diluted with water (100 ml.), and extracted with chloroform (3 × 100 ml.). After

being washed with water (100 ml.), the combined chloroform extracts were evaporated to a solid which crystallised from ethanol as orange needles of the *derivative*, m. p. 90—92° (Found: C, 42·6; H, 3·7; N, 18·2. $C_8H_9N_3O_5$ requires C, 42·3; H, 3·9; N, 18·5%).

2-*Deoxy*-2-(2,4-*dinitroanilino*)-D-*glucose*.[11]—Preparation of this derivative as above and recrystallisation from ethyl acetate–ethanol, gave bright yellow crystals, m. p. 193—196°, $[\alpha]_D^{25}$ +50° (equil. value; *c* 0·96 in 80% aqueous ethanol) (Found: C, 41·7; H, 4·3; N, 12·2. Calc. for $C_{12}H_{15}N_3O_9$: C, 41·6; H, 4·5; N, 12·3%).

During oxidation of this derivative (150 mg.) at pH 1 or pH 7·5, 2,4-dinitroaniline (40 mg.) separated as the free amine on account of its very low basicity.[12] Recrystallisation from aqueous ethanol gave yellow crystals, m. p. 174—175° (Found: C, 39·9; H, 2·9; N, 22·6. Calc. for $C_6H_5N_3O_4$: C, 39·4; H, 2·7; N, 22·9%), with the correct infrared spectrum.

After oxidation of 2-deoxy-2-(2,4-dinitrophenylanilino)-D-glucose (0·57 g.) at pH 5 for 37 hr., the mixture was extracted with ether (3 × 250 ml.), giving, on evaporation of the extract, pale yellow crystals (0·16 g.) with m. p. 82—84°, unchanged on recrystallisation from ethanol and from water. Apart from $\nu_{max.}$ (C=O stretching) 1730 cm.$^{-1}$., the infrared spectrum was similar to that of 2,4-dinitroaniline. The *N*-(2,4-dinitrophenyl)glyoxylamide could not be freed from traces of 2,4-dinitroaniline, as indicated by paper chromatography, and a satisfactory analysis for elements was not obtained. Both the amide and 2,4-dinitroaniline gave negative results in the phenylhydrazine–hydrogen peroxide test [13] for glyoxylic acid, but after hydrolysis with 2N-hydrochloric acid at room temperature the amide gave the transient red colour characteristic of glyoxylic acid. Further, a high yield of 2,4-dinitroaniline was obtained when the amide was hydrolysed.

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[11] Kent, *Research*, 1950, **3**, 427; Wang-Yu and Tai Hsing-I, *Acta Chim. Sinica*, 1958, **24**, 368; Meyer and Schwartz, *Helv. Chim. Acta*, 1950, **33**, 1651; Annison, James, and Morgan, *Biochem. J.*, 1951, **48**, 477.

[12] Hine, " Physical Organic Chemistry," McGraw-Hill, New York, 1956, p. 59.

[13] Paget and Berger, *Bull. Biol. Pharm.*, 1938, 70; *Chem. Abs.*, 1938, **32**, 4908.