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THE FORMATION OF MALONALDEHYDE IN IRRADIATED CARBOHYDRATES

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ABSTRACT

Pulse radiolysis and ^{60}Co γ-radiolysis have been used to elucidate the mechanism of formation of malonaldehyde in irradiated carbohydrates. Reaction of HO· at C-5 and C-6 of D-glucose may produce 3 mol of malonaldehyde. Substitution of C-2, C-3, and C-6 decreases the yield of malonaldehyde, but substitution of C-1 by phosphate (as in α-D-glucopyranosyl phosphate) has no effect. Mechanisms are proposed for the formation of malonaldehyde in irradiated pentoses, disaccharides, and polyhydroxy alcohols.

INTRODUCTION

The cytotoxicity of irradiated carbohydrates and polyhydroxy alcohols has been attributed to the formation of deoxy compounds and α,β-unsaturated ketones[1–5]. Malonaldehyde is the principal α,β-unsaturated ketone formed[6–10], particularly at alkaline pH, and it is mainly responsible for the characteristic u.v. absorption generated during irradiation[10].

We now report elucidation of the mechanism of formation of malonaldehyde on pulse radiolysis and ^{60}Co γ-radiolysis of carbohydrates and their derivatives.

MATERIALS AND METHODS

α-D-Glucopyranosyl dipotassium phosphate dihydrate (Glc-1-P) and D-glucose 6-phosphate barium salt heptahydrate (Glc-6-P) were obtained from Koch–Light Laboratories. Potassium D-glucose 6-sulphate (Glc-6-S), potassium D-glucose 3-sulphate (Glc-3-S), potassium D-galactose 6-sulphate (Gal-6-S), and potassium 2-deoxy-2-sulphoamino-D-glucose (GlcN-S) were gifts from Dr. D. Bain, Department of Biochemistry, University College, Cardiff. All the other chemicals were of the highest purity commercially available.

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Pulse-radiolysis experiments were performed with a Febetron 705B. Full details are given elsewhere[11]. All solutions were prepared from water distilled from alkaline permanganate, and sodium hydroxide was used to adjust the pH of the solutions where desired. Prior to irradiation, the solutions were deaerated with nitrogen or saturated with nitrous oxide. Second-order rate constants for the reaction of e_{aq}^- were determined from the disappearance of its absorption at 650 nm; for HO·, the thiocyanate competition method was employed[12], based[13] on k_2 (HO· + CNS^-) = 1.08×10^{10} l.mol^{-1}.sec^{-1}.

Steady-state radiolysis was performed with a 10,000-Ci ^{60}Co γ-source at a dose rate of 5.3×10^{17} eV.ml^{-1}.min^{-1}. Malonaldehyde was determined colorimetrically by the method of Waravdekar and Saslow[14].

RESULTS

The second-order rate constants for the reaction of e_{aq}^- and HO· with the carbohydrates are given in Table I. As is typical for such compounds, the values for e_{aq}^- are extremely low (10^6–10^7 l.mol^{-1}.sec^{-1})[15], but introduction of a sulphate group tends to increase the values. The rate constants for reaction of HO· are greater by a factor of at least 10^2, typical of the value of H· abstraction, and indicate that it is the latter species which is primarily responsible for chemical change, as confirmed by the transient spectra formed immediately after the pulse. Typical data obtained for Glc-1-P are given in Fig. 1. A transient was always observed with absorption maximum at ~250 nm in N_2-saturated solution and with low extinction coefficient. The absorption was approximately doubled in N_2O-saturated solutions, where all e_{aq}^- are effectively converted into HO·. The species decay by second-order kinetics (Table I), except for Glc-6-P where no appreciable decay was observed during 1 msec. As the pH was increased, the shape of the decay of the oscilloscope trace changed

TABLE I

KINETIC AND SPECTRAL DATA OBTAINED FOLLOWING PULSE RADIOLYSIS OF CARBOHYDRATES AND RELATED COMPOUNDS (pH 6.5)

	$k_2\, e_{aq}^-$ ($\times 10^{-6}$) (*l mol^{-1} sec^{-1}*)	k_2(HO·) $\times 10^9$ (*l mol^{-1}.sec^{-1}*)	ε *at* λ_{max} ($\times 10^{-3}$) (*l.mol^{-1}.cm^{-1}*)[a]	*Second-order decay of transient* ($\times 10^{-6}$) (*l.mol^{-1}.sec^{-1}*)
D-Glucose	< 4	2 3	1.6 (250 nm)	2 3
D-Galactose	< 6	2.0	1.5 (250 nm)	1.5
Lactose	< 4	3 0	1.4 (250 nm)	1.6
Maltose	<10	2 3	1 8 (250 nm)	1.3
Glc-1-P	< 4	1.4	1.7 (250 nm)	2.7
Glc-6-P	40.0	1.4	1.4 (250 nm)	No decay
Glc-3-S	18.0	1.8	—	—
Glc-6-S	66 0	1.4	—	—
GlcN-S	18 0	2.1	—	—

[a]Based on G(OH) = 5.6 in N_2O-saturated solution.

markedly with formation of a permanent product, λ_{max} ~270 nm; the yield (Fig. 2) and first-order rate constant (Fig. 3) for its formation increased with pH. We consider that this product is mainly malonaldehyde (MA), $\varepsilon_{265} = 3 \times 10^4$ l.mol^{-1}.cm^{-1}; G(MA) is plotted as a function of pH in Fig. 2. The values of G(MA) were also calculated, in certain instances, from linear yield–dose curves, using the method of Waravdekar[14], following steady-state radiolysis of N_2O-saturated, aqueous solutions of the sugars. G(MA) for glucose and galactose were approximately the same over the pH range studied. The yields from the pentoses were similar, but lower than those from hexoses. The yields from the disaccharides investigated were considerably lower than those from the pentoses. The yields from the alditols erythritol and arabinitol were similar and, at alkaline pH, comparable to the values obtained from the pentoses.

The yield of malonaldehyde from D-glucose is considerably modified on substitution at C-6, C-3, or C-2 (Fig. 4). For Glc-6-P, Glc-6-S, Gal-6-S, and GlcN-S, the yields appear to be the same, but considerably lower than that from D-glucose, particularly above pH 9. Substitution at C-3 (as in Glc-3-S) has less effect than substitution at C-6 and C-2.

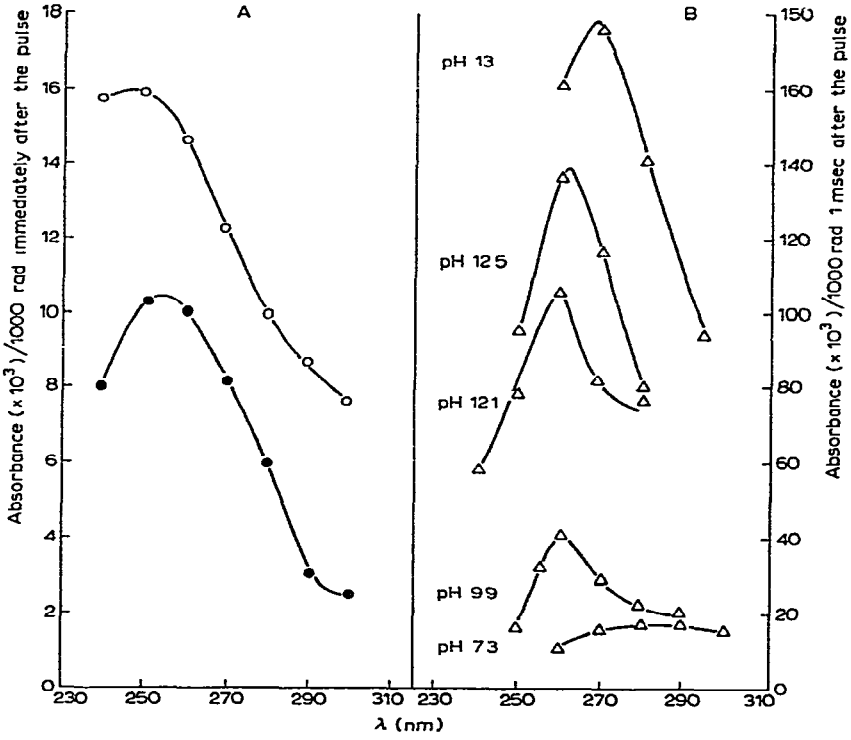


Fig. 1. Spectra produced following pulse radiolysis of aqueous solutions of Glc-1-P (10^{-3}M): *A* immediately after the pulse, —○— saturated with N_2O, —●— deaerated; *B* 1 msec after the pulse, saturated with N_2O.

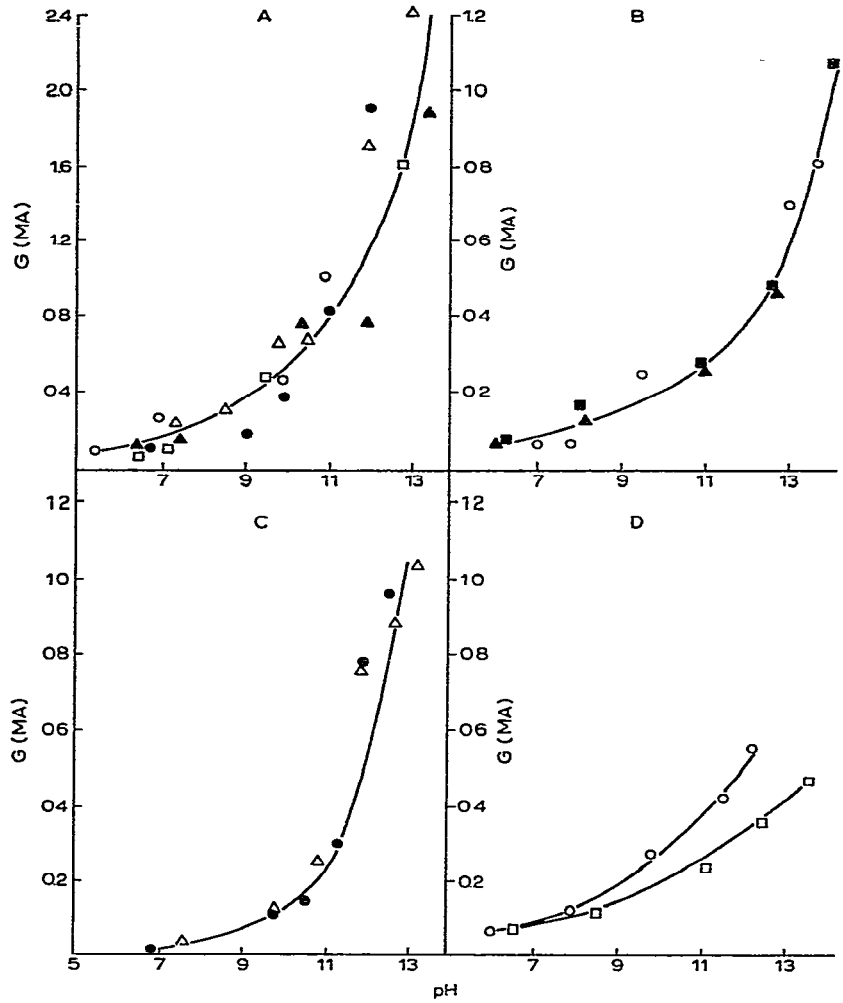


Fig. 2. Effect of pH on the yield of malonaldehyde following pulse radiolysis of aqueous solutions of *A* Glc-1-P (△), glucose (▲), galactose (○), and fructose (□); glucose (●), after ^{60}Co γ-radiolysis, and assayed using thiobarbituric acid; *B* xylose (○), ribose (■), and arabinose (▲); *C* arabinitol (△) and erythritol (●); *D* maltose (○) and lactose (□).

Substitution at C-1 by phosphate (as in Glc-1-P) does not significantly affect the yield of malonaldehyde, but the first-order rate of formation is very low (k_1 $\sim 3.4 \times 10^3$ sec^{-1}) and independent of dose over the range 140–1600 rad/pulse at pH 12.1 (Fig. 5).

DISCUSSION

The nature of the characteristic u.v. absorption produced during the radiolysis of carbohydrates is now generally accepted to be due to α,β-unsaturated carbonyl

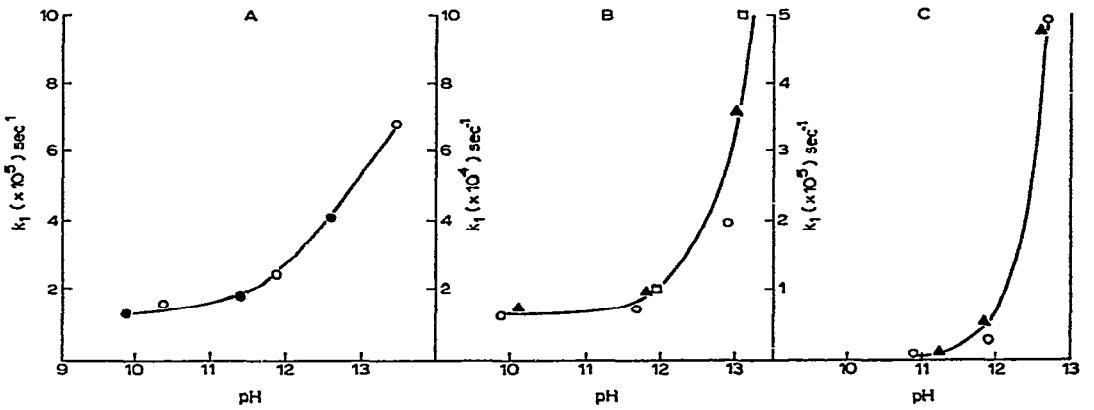


Fig. 3. Effect of pH on the first-order rate constant for formation of malonaldehyde following pulse radiolysis of *A* glucose (○) and galactose (●); *B* ribose (○), arabinose (▲), and xylose (□); and *C* arabinitol (○) and erythritol (▲).

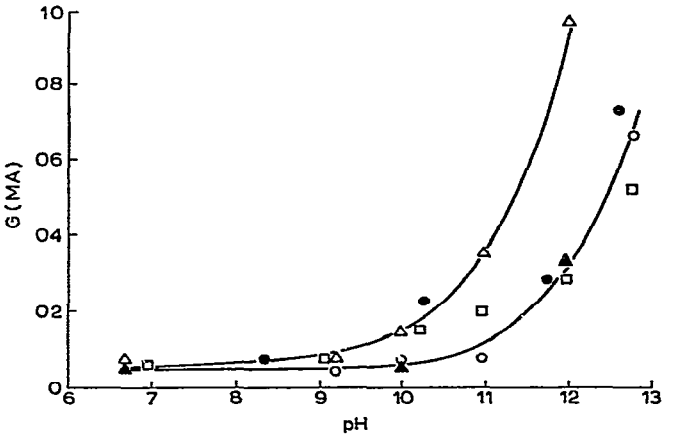


Fig. 4. Effect of pH on the yield of malonaldehyde following radiolysis of Glc-3-S* (△); Glc-6-P* (●); Glc-6-S* (▲); Gal-6-S* (○); and GlcN-S** (□). *Assayed using thiobarbituric acid following ^{60}Co γ-radiolysis; **data obtained from pulse radiolysis.

products. At neutral pH, G(MA) is low[9,10], but considerably greater in alkaline solution. This observation is consistent with the low yield of malonaldehyde found during the radiolysis of solutions of 2-deoxy-D-*erythro*-pentose[16] (2-deoxyribose) ($G \leq 0.01$). Detection is facilitated by the high extinction coefficient at λ_{max} 265 nm (ε 3 × 10^4 $M^{-1}.cm^{-1}$, pH 12)[17]. The permanent product observed following pulse radiolysis always had λ_{max} ~270 nm. Furthermore, those carbohydrates assayed with thiobarbituric acid following γ-radiation produced the red chromophore (λ_{max} 532 nm), identical to that obtained using authentic malonaldehyde. Thus, malonaldehyde is considered to be the major product responsible for the permanent product observed.

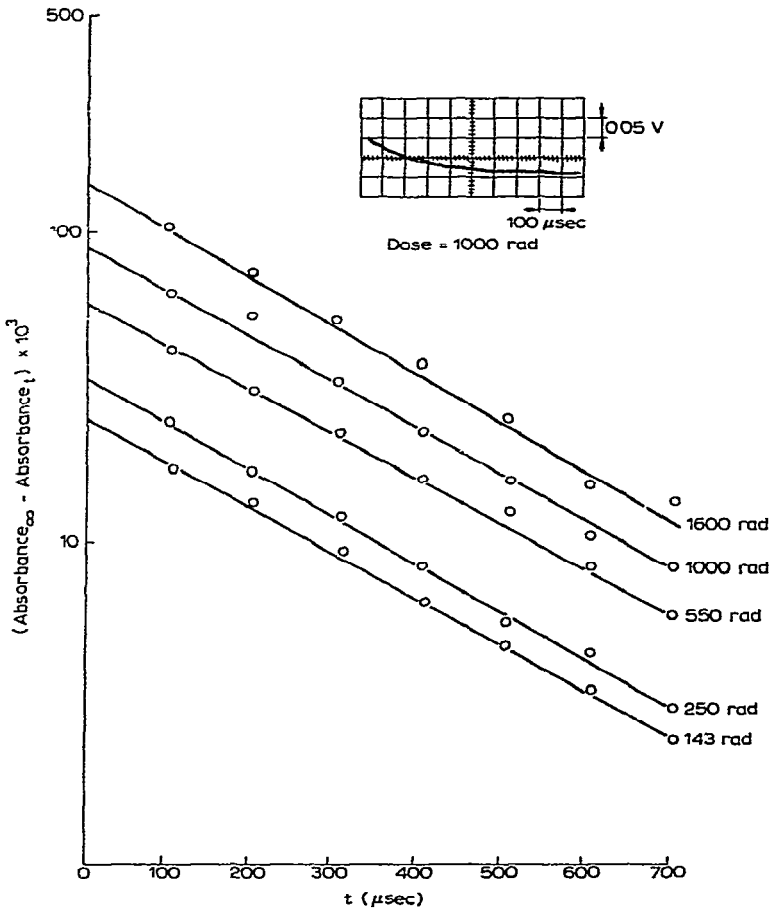
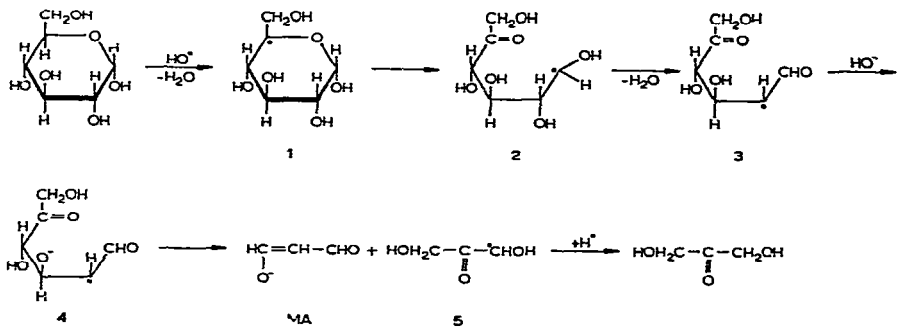


Fig. 5. Effect of increasing dose on the first-order rate of formation of malonaldehyde following pulse radiolysis of N_2O-saturated solutions of Glc-1-P (pH 12.1), λ 260 nm.

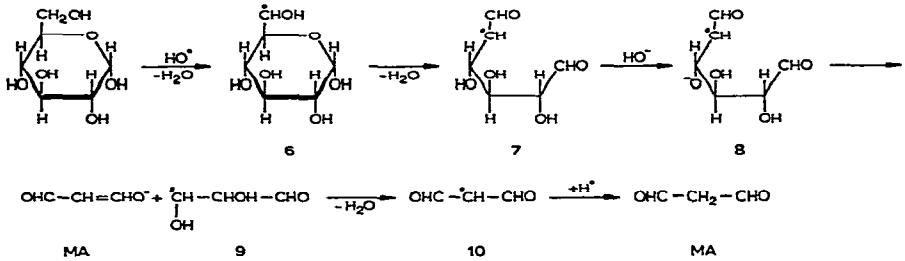
The high rate constant for reaction of HO· (and presumably $O^{-\cdot}$ at pH > 12; pK HO· $\rightleftharpoons$ $O^{-\cdot}$ = 11.8[18]) and the nature of the transient spectra confirm that it is



Scheme 1. Reaction of HO· at C-5 in D-glucose

this species which is ultimately responsible for the formation of malonaldehyde in solutions of D-glucose, *via* reaction of HO· and C-6 and C-5, as shown in Scheme 1.

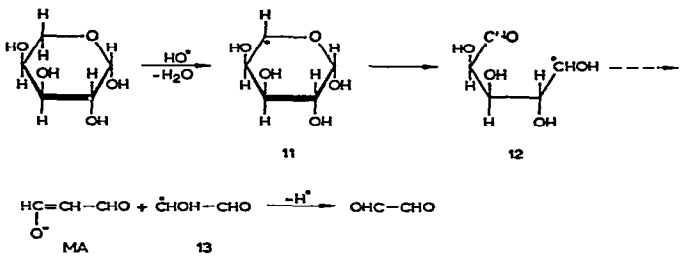
Radical **1** undergoes ring-opening and rearranges into **2**, which undergoes an alkali-catalyzed water-elimination process[19,20]. The resulting radical **3** has a planar configuration, making H-3 slightly acid[9], and thus produces **4** in the presence of HO^-. Malonaldehyde is then split off (as β-hydroxyacrolein anion), leaving **5**, which can disproportionate, *e.g.*, to dihydroxyacetone (an established radiolytic product of glucose[21]).



Scheme 2. Reaction of HO· at C-6 in D-glucose

Evidence is available that radical **6** undergoes rearrangement followed by elimination of water[22,25] to produce radical **7**, which gives malonaldehyde and radical **9**, *via* radical **8** (Scheme 2), in a manner similar to that described above. Radical **9** can also lose water to form radical **10**, which then disproportionates to produce a further molecule of malonaldehyde. The yield of malonaldehyde from glucose and galactose is the same (Fig. 2), indicating that variation in the configuration at C-4 does not influence the mechanism.

The yield of malonaldehyde from aldopentoses is lower than from the aldohexoses, as only 1 mol. is produced following reaction of HO· at C-5 in L-arabinose as shown in Scheme 3.

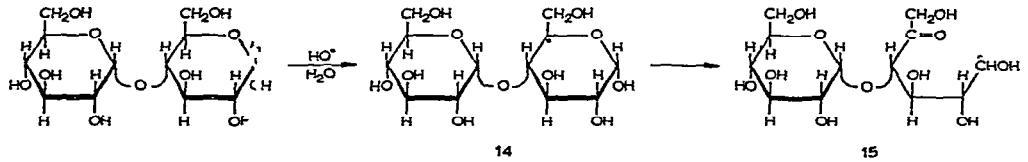


Scheme 3. Reaction of HO· at C-5 in L-arabinose

Rearrangement of radical **11** to give radical **12** may be followed by a series of reactions, as described for D-glucose, to produce malonaldehyde and radical **13**, which disproportionates to produce glyoxal, another well-established product of radiolysis of carbohydrates[21].

The yield of malonaldehyde from erythritol and arabinitol is similar to that from the aldopentoses, as only reaction of HO· and the CH_2OH groups leads to its formation *via* a series of reactions similar to those described previously.

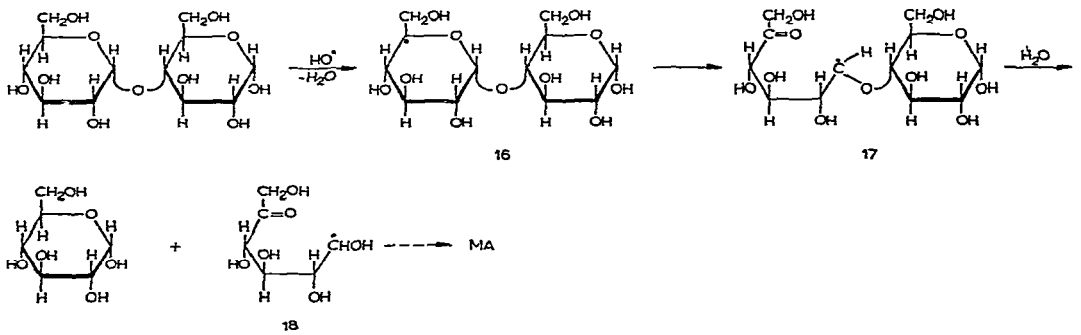
Malonaldehyde may be produced from maltose and lactose by reaction of HO· at C-5′ and C-6′ of the non-reducing sugar and at C-5 of the reducing sugar.



14 15

Scheme 4 Reaction of HO· at C-5 in maltose

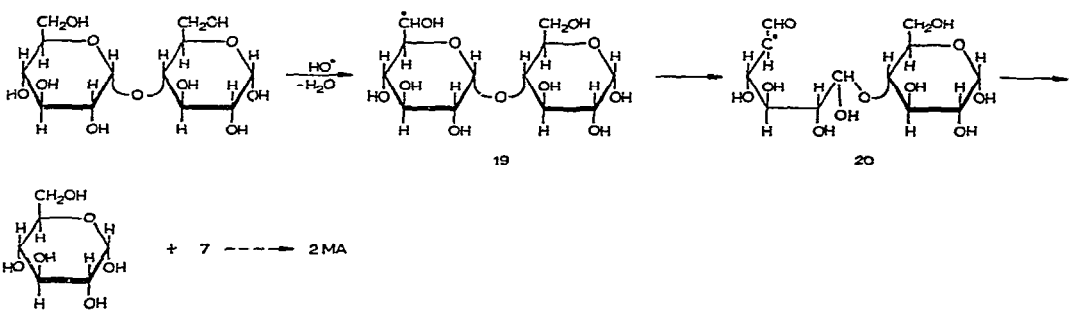
Oxidation at C-5 produces radical **14** (Scheme 4), which rearranges into radical **15** and then produces malonaldehyde as previously described.



16 17 18

Scheme 5 Reaction of HO· at C-5′ in maltose

Following H· abstraction from C-5′ in maltose (Scheme 5) to produce radical **16** and rearrangement into radical **17**, rapid hydrolysis occurs, probably *via* a carbonium ion[26–28], to give glucose and radical **18**, which then yields malonaldehyde as previously described.

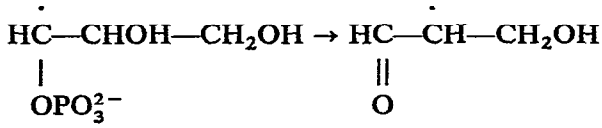


19 20

Scheme 6 Reaction of HO· at C-6′ of maltose

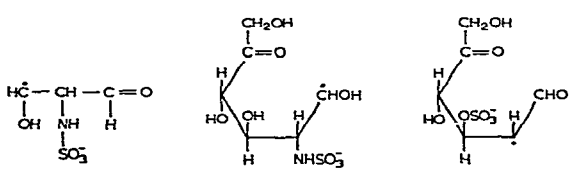
Reaction of HO· at C-6′ in maltose gives radical **19**, which could be the precursor of radical **20** formed in a manner similar to that described by Dizdaroglu *et al.*[24]. Radical **20** contains a labile hemiacetal function and will readily produce D-glucose and radical **7**, which is the species produced following initial reaction of HO· at C-6 of D-glucose (Scheme 2), and thus yields two mol of malonaldehyde.

The yields of malonaldehyde from irradiated Glc-6-S and Glc-6-P are very similar, and considerably lower than that obtained from D-glucose. Following initial reaction of HO· at C-6 in Glc-6-P and Glc-6-S, reactions analogous to the formation of radical **7** from radical **6** appear to be inhibited. It is well known that elimination of phosphate



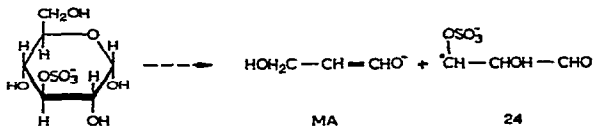
is a very slow process[29], and such radicals may undergo dimerization and disproportionation. For Glc-6-S and Glc-6-P, only one mol of malonaldehyde may be produced, *i.e.*, following initial reaction at C-5 *via* a series of reactions similar to those previously described.

The yield of malonaldehyde from GlcN-S is similar to that obtained from Glc-6-S and Glc-6-P. In this instance, initial reaction of HO· at C-6 would yield 1 mol of malonaldehyde in a manner similar to that for glucose (Scheme 2) and produce radical **21**. Formation of malonaldehyde from radical **21** would require elimination of $^{N}H_2SO_3^-$. No evidence for such a reaction is presently available. The absence of acetamide following radiolysis of 2-acetamido-2-deoxy-D-glucose[30], which would require an elimination process similar to that required above, indicates that radical **21** probably disproportionates. Reaction of HO· at C-5 of GlcN-S, *via* a series of reactions similar to those proposed for D-glucose, would produce the radical **22** which, for reasons already given, would prevent the formation of malonaldehyde.



21 **22** **23**

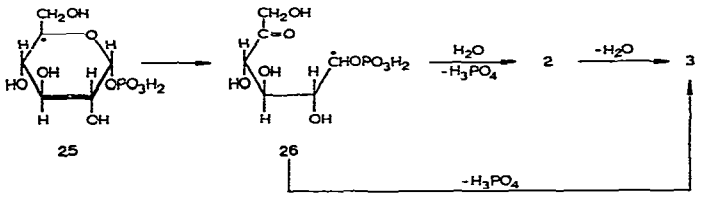
Substitution of C-3 (as in Glc-3-S) also decreases the yield of malonaldehyde, although the yield is higher than for Glc-6-S, Glc-6-P, and GlcN-S. In this instance, reaction of HO· at C-5 would ultimately produce radical **23**, which would effectively prevent the formation of malonaldehyde. However, malonaldehyde may be produced following reaction of HO· at C-6.



Scheme 7 Formation of 24 from Glc-3-S

The higher yield of malonaldehyde from Glc-3-S can only be rationalized on the basis of elimination of sulphate from **24** (Scheme 7). Such a process may well occur, as *G*(sulphate) for Glc-3-S is[31] 1.1.

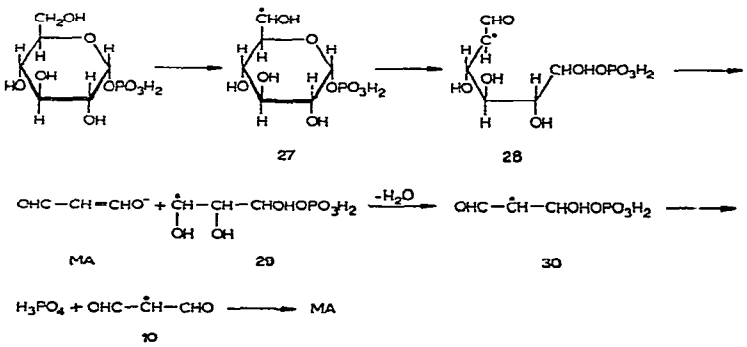
Substitution of phosphate on C-1, as in Glc-1-P, apparently does not affect the yield of malonaldehyde (Fig. 2). However, at alkaline pH, malonaldehyde is formed extremely slowly (Fig. 4), unlike in glucose. Following abstraction of H· from C-5 by HO·, malonaldehyde may be formed as shown in Scheme 8.



Scheme 8 Formation of 3 from radical 25

Radical **25** rearranges into radical **26**, and phosphate is eliminated to produce **3** [the radical derived from D-glucose (Scheme 1)] which then yields malonaldehyde. Such a process is slow[29] and may be the process observed here. However, an alternative may be hydrolytic elimination of phosphate to produce **2**, which then undergoes an alkali-catalyzed elimination of water.

Two further moles of malonaldehyde may be formed from Glc-1-P following reaction of HO· at C-6 (Scheme 9).



Scheme 9 Formation of malonaldehyde from Glc-1-P

The precursor radicals (**27** and **28**) of malonaldehyde also yield radical **29**, from which water may be eliminated to yield the alkali-labile radical **30**. Slow loss of phosphate from **30** produces **10**, which may then disproportionate to yield malonaldehyde (Scheme 2).

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