Radiation Chemistry of Carbohydrates. Part 14.† Hydroxyl Radical Induced Oxidation of D-Glucose in Oxygenated Aqueous Solution

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D-Glucose (5×10^{-3}M) has been γ-irradiated in N_2O–O_2 (80 : 20 v/v) saturated aqueous solutions (dose rate 1.1×10^{15} eV g^{-1} s^{-1}), and the *G* values of 22 products determined. Major products (*G* values in parentheses) are: D-gluconic acid (1) (0.9), D-*arabino*-hexosulose (2) (0.9), D-*ribo*-hexos-3-ulose (3) (0.57), D-*xylo*-hexos-4-ulose (4) (0.50), D-*xylo*-hexos-5-ulose (5) (0.60), D-*gluco*-hexodialdose (6) (1.55), and L-*threo*-tetrodialdose (12) (0.20). Products (1)—(6) are thought to be formed by OH attack at C-1—C-6, followed by oxygen addition and and HO_2 elimination. In competition, reactions between the glucose peroxyl radicals and HO_2· (O_2^{-}·) give rise to fragmentation of C–C bonds. The major product from these fragmentation reactions is (12) (precursor, radical at C-5). From a dose rate study using electron pulses from a van de Graaff generator it has been shown that the rates of HO_2· elimination are in the order: C-1 ≫ C-2, C-3, C-4 > C-6 ≫ C-5. Furthermore, it has been calculated that OH radicals abstract a hydrogen atom from C-1 and C-2 with a probability of *ca.* 20%, from C-3 and C-4 of *ca.* 10% from C-5 of *ca.* 15%, and from C-6 *ca.* 30%.

γ-IRRADIATION is a powerful tool for food sterilization. Although only very small quantities of irradiation products are formed by this procedure,[1] it is of interest to know the nature of the products and their approximate yields in order to evaluate their toxicity. Food consists of a high proportion of carbohydrates. Glucose has always been considered as the best model. Hence, the γ-radiolysis of D-glucose is of considerable interest, and a

† Part 13, G. A. W. Bradbury and C. von Sonntag, *Carbohydrate Res.*, in the press.

[1] J. F. Diehl and H. Scherz, *Internat. J. Appl. Radiation Isotopes*, 1975, **26**, 499.

series of papers appeared on the radiolysis of solid D-glucose [2-5] and its aqueous solutions both in the absence [6-12] and in the presence of oxygen.[8,13-15]

In the γ-radiolysis of D-glucose in dilute aqueous solution the radiation energy is absorbed by the solvent water. The primary species are OH radicals, solvated electrons (e_{aq}^-), and H atoms. Solvated electrons can be converted by N_2O into OH radicals [reaction (1)], or scavenged by O_2 [reaction (2)]. In N_2O saturated

$$e_{aq}^- + N_2O \longrightarrow {}^{\cdot}OH + N_2 + OH^- \quad (1)$$

$$e_{aq}^- + O_2 \longrightarrow O_2^{-\cdot} \quad (2)$$

$$H^{\cdot} + O_2 \longrightarrow HO_2^{\cdot} \quad (3)$$

solutions the system then consists of *ca.* 90% OH radicals and 10% H atoms. In O_2 saturated (or air saturated) solutions there is about an equal yield of OH and $O_2^{-\cdot}$ radicals. OH radicals and H atoms attack the glucose by abstracting carbon bound hydrogen atoms,[16] generating six different primary glucosyl radicals. In the absence of oxygen, products then arise from the subsequent reactions of these radicals as has been discussed in detail.[12] If present in concentrations $>10^{-5}$M oxygen adds to the primary glucosyl radicals. In this case peroxyl radicals derived from the primary glucosyl radicals are the precursors of the products.

In the present study the solutions were either saturated with N_2O–O_2 in the ratio of 4 : 1, or with oxygen only. Because of the high solubility of N_2O and the high rate constant for reaction (1) this reaction still prevails in the N_2O–O_2 saturated system, oxygen merely scavenging the H atoms [reaction (3)] and converting the glucosyl radicals into the corresponding peroxyl radicals (*G ca.* 5.3 *). In solutions saturated with oxygen alone, the yield of glucosyl radicals is about halved. $O_2^{-\cdot}$ radicals are the other primary radicals under these conditions. The identification of a number of products in the radiolysis of glucose in oxygenated aqueous solutions has been reported by Kawakishi *et al.*[14] while our study was going on, but they did not carry out quantitative measurements and could not identify some products not accessible by their method. Kinetic data also became available.[17] We therefore present a more detailed study.

RESULTS

Aqueous solutions of D-glucose (5×10^{-3}M) were irradiated with ^{60}Co-γ rays. Prior to as well as during irradiation the solutions were either saturated with 4 : 1 nitrous oxide–oxygen or with oxygen alone. After irradiation the samples were dried by rotary evaporation and either reduced with $NaBD_4$, followed by trimethylsilylation (or acetylation) or methoximated and trimethylsilylated, and subsequently analysed by g.l.c.–m.s. On reduction hexosuloses yield two stereoisomers, *e.g.* D-*arabino*-hexosulose yields D-glucitol and D-mannitol. One of the stereoisomers is always D-glucitol which is also formed from the starting material, D-glucose. However, quantitative measurements are possible if the ratio of formation of the two isomers after reduction is known. Reference material for all hexosuloses has been synthesized and this ratio determined (Table 1). With the reduction technique a number of products cannot be determined. D-Gluconic acid (1), D-glucuronic acid (7) and D-*gluco*-hexodialdose (6) are all reduced to D-glucitol. The methoximation technique allows the identification of these products. However, methoximation yields a rather complex mixture (Figure 1). The methoximino-group can be either *syn* or *anti* giving rise to up to four isomers which in most cases have been

TABLE 1

Reduction of hexosuloses by $NaBH_4$ to hexitols. Ratio of the stereoisomers formed

Hexosulose	Hexitols	Ratio
D-*arabino*-Hexosulose	D-Mannitol–D-Glucitol	44 : 56
D-*ribo*-Hexos-3-ulose	Allitol–D-Glucitol	64 : 36
D-*xylo*-Hexos-4-ulose	Galactitol–D-Glucitol	40 : 60
D-*xylo*-Hexos-5-ulose	L-Iditol–D-Glucitol	56 : 44

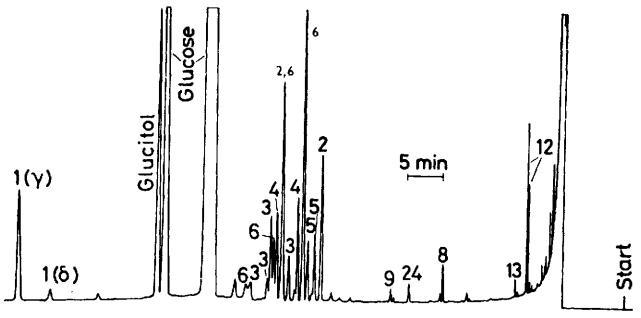


FIGURE 1 Gas chromatogram of trimethylsilylated or methoximated and trimethylsilylated products in γ-irradiated N_2O–O_2 (80 : 20 v/v) saturated aqueous solution of D-glucose: (1) D-gluconic acid lactones; (2) D-*arabino*-hexosulose; (3) D-*ribo*-hexos-3-ulose; (4) D-*xylo*-hexos-4-ulose; (5) D-*xylo*-hexos-5-ulose; (6) *gluco*-hexodialdose; (8) D-arabinose; (9) D-arabinonic acid γ-lactone; (12) L-*threo*-tetrodialdose; (13) D-erythrose; (24) D-*arabino*-hexulosonic acid (Dexsil 300; 123 m; glass capillary column; 0.25 mm i.d.; 170° isothermal)

* The *G* value is defined as the number of particles formed per 100 eV absorbed energy.

[2] G. O. Phillips and P. J. Baugh, *Nature*, 1963, **198**, 282.
[3] G. O. Phillips, P. J. Baugh, and G. Löfroth, *J. Chem. Soc.* (*A*), 1966, 377.
[4] G. Löfroth, *Acta Chem. Scand.*, 1967, **21**, 1997.
[5] M. Dizdaroglu, D. Henneberg, K. Neuwald, G. Schomburg, and C. von Sonntag, *Z. Naturforsch.*, 1977, **32b**, 213.
[6] P. J. Baugh, K. Kershaw, and G. O. Phillips, *J. Chem. Soc.* (*B*), 1970, 1482.
[7] P. M. Grant and R. B. Ward, *J. Chem. Soc.*, 1959, 287.
[8] G. O. Phillips, *Radiation Res.*, 1963, **18**, 446.
[9] S. Kawakishi and M. Namiki, *Carbohydrate Res.*, 1973, **26**, 252.
[10] S. Kawakishi, Y. Kito, and M. Namiki, *Carbohydrate Res.*, 1973, **30**, 220.
[11] H. Scherz and G. Stehlik, *Monatsh.*, 1968, **99**, 1143.
[12] M. Dizdaroglu, D. Henneberg, G. Schomburg, and C. von Sonntag, *Z. Naturforsch.*, 1975, **30b**, 416.
[13] G. O. Phillips, G. J. Moody, and G. L. Mattok, *J. Chem. Soc.*, 1958, 3522.
[14] S. Kawakishi, Y. Kito, and M. Namiki, *Carbohydrate Res.*, 1975, **39**, 263.
[15] I. Gotlieb and P. Markakis, *Radiation Res.*, 1968, **36**, 55.
[16] K.-D. Asmus, H. Möckel, and A. Henglein, *J. Phys. Chem.*, 1973, **77**, 1218.
[17] E. Bothe, D. Schulte-Frohlinde, and C. von Sonntag, *J.C.S. Perkin II*, in the press.

separated under the conditions applied. With such a considerable number of g.l.c. peaks some overlap of the various peaks could not be avoided. The structure assignment of these products has been confirmed by g.l.c.–m.s. analysis.[18,19]

D-Gluconic acid (1) was determined as the trimethylsilyl ethers of its γ- and δ-lactones There it was preferable to work under methoximation conditions rather than direct silylation. On direct silylation the α-D-glucose peak (starting material, large excess) overlaps with the γ-lactone peak. There might be a systematic error in $G(1)$ through incomplete lactonisation of the acid.

There is a large number of products with a shorter carbon chain than glucose itself. Most of these fragment products were identified by the above techniques. Reductions were carried out with $NaBD_4$ in order to label the carbonyl or carboxy-functions.[20] Glyceric acid (16) was converted into the trimethylsilyl ether of its methyl ester, and also

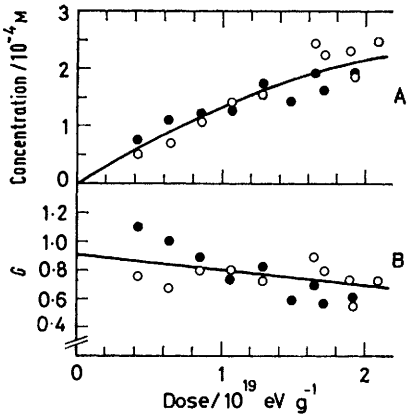


FIGURE 2 A, Dose dependence of the D-*arabino*-hexosulose yield and B, of the corresponding G value from 5×10^{-3}M-D-glucose γ-irradiated in N_2O–O_2 (80 : 20 v/v) saturated aqueous solution at room temperature; dose rate 1.1×10^{15} eV g^{-1} s^{-1} (○, product as acetylated D-mannitol after reduction with $NaBH_4$; ●, product as the methoximated and trimethylsilylated derivative)

determined by g.l.c. For other products (with one or two carbon atoms) colorimetric methods were used. The method to determine glycolic acid gives the same reaction also for glyoxylic acid. Hence, the yield of these two compounds (18) has been given as their sum.

A typical yield–dose plot is given for D-*arabino*-hexosulose (2) in Figure 2A. It can be seen that the reduction technique (closed circles) and the methoximation technique (open circles) give the same results within experimental error. A different representation is given in Figure 2B, where the G value calculated for every sample measured is plotted against the dose. This plot gives a better idea of the errors involved. The scatter of the data is considerably less for the other major products. The values in Table 2 are taken from the extrapolation to zero dose of the best fit in the G value–dose plots. At the highest dose employed the conversion of the starting material was *ca.* 35%. Therefore, attack of the OH radicals on the products is not negligible, causing the curvature in Figure 2A and the negative slope in Figure 2B.

[18] M. N. Schuchmann, Doctorial Thesis, Bochum, 1976.

[19] M. Dizdaroglu, D. Henneberg, C. von Sonntag, and (in part) M. M. Schuchmann, *Org. Mass Spectrometry*, in the press.

In most cases the secondary products do not appear to a significant extent over this dose range because of the great variety of possible secondary products. An exception appears to be the formation of D-*arabino*-hexulosonic acid

TABLE 2

γ-Radiolysis of 5×10^{-3}M aqueous solutions of glucose in the presence of oxygen. Dose rate 1.1×10^{15} eV g^{-1} s^{-1}. Products and their G values

No.	Product	N_2O–O_2 [g]	O_2 [h]	O_2 or air
(1)	D-Gluconic acid	0.90 [a]	0.37 [a]	0.4[13]
(2)	D-*arabino*-Hexosulose	0.90 [b,c]	0.48 [b,c]	
(3)	D-*ribo*-Hexos-3-ulose	0.57 [b,c]	0.33 [b,c]	
(4)	D-*xylo*-Hexos-4-ulose	0.50 [b,c]	0.31 [b,c]	
(5)	D-*xylo*-Hexos-5-ulose	0.60 [b,c]	0.23 [b,c]	
(6)	D-*gluco*-Hexodialdose	1.55 [c]	0.79 [c]	
(7)	D-Glucuronic acid	0.05 [c]	n.d.	0.9[13]
(8)	D-Arabinose	0.07 [b]	0.015 [b]	
(9)	D-Arabinonic acid	0.03 [b]		
(10)	*xylo*-Pentodialdose	0.07 [b]	0.02 [b]	
(11)	D-Xylose	0.01 [b]		
(12)	L-*threo*-Tetrodialdose	0.20 [b]	0.12 [b]	0.25 [i,8]
(13)	D-Erythrose	0.01 [b]	0.01 [b]	
(14)	D-Erythronic acid	0.01 [b]		
(15)	D-Glyceraldehyde	0.06 [b]	n.d.	
(16)	D-Glyceric acid	0.07 [d]	n.d.	
(17)	Glyoxal	0.11 [e]	n.d.	1.8[13] 0.35[15] (0.8 [j,8])
(18)	Glyoxylic–glycolic acid	0.4 [e]	n.d.	
(19)	Formaldehyde	0.12	0.07 [e]	
(20)	Formic acid	0.6 [e]	0.2 [e]	
(21)	Hydrogen peroxide	3.0 [e]	3.0 [e]	3.0[13]
(22)	Hydrogen	0.37 [f]	n.d.	
(23)	Carbon dioxide	n.d.*	sec. prod. [f]	
(24)	D-*arabino*-Hexulosonic acid	sec. proc. [c],†	n.d.	

[a] Determined as trimethylsilyl ether of the lactones. [b] Determined as trimethylsilylated or acetylated polyhydric alcohols after reduction with $NaBH_4$. [c] Determined as trimethylsilyl ether after methoxyimation. [d] Determined as trimethylsilyl ether of the methyl ester. [e] Determined photometrically. [f] G.l.c. [g] Saturated 4 : 1 v/v, irradiated at room temperature. [h] Irradiation at 0°. [i] Four-carbon fragments. [j] Two-carbon fragments.

* n.d. not determined. † sec. prod. = Secondary product.

(24) and carbon dioxide (23). The latter is easy to determine (in the absence of N_2O) because of its volatility; the former has two abundant precursors, D-gluconic acid (1) and D-*arabino*-hexosulose (2). Figure 3 shows the yield–dose dependence of D-*arabino*-hexulosonic acid (24). In the inset $G(24)$ is plotted against the dose. In this plot a straight line through the origin is obtained indicating that D-*arabino*-hexulosonic acid (24) is a secondary product An analogous result has been obtained for carbon dioxide.

The total yield of acidic compounds was obtained by titration with NaOH. In order to open the lactones an excess of base was added and the yield determined by back titration. This procedure yielded G(acidic products) = 4.0 in N_2O–O_2 saturated solutions. The value is higher than that of the sum of the carbonic acids listed in Table 2. However, it was found that under these conditions D-*ribo*-hexos-3-ulose (3) could also be titrated, and possibly other compounds [*e.g.* D-*arabino*-hexosulose (2)] might be degraded under these conditions giving rise to acidic compounds. Therefore, this value is at best an upper limit and we consider it to be not sufficiently indicative to draw further conclusions.

[20] M. Dizdaroglu, D. Henneberg, and C. von Sonntag, *Org. Mass Spectrometry*, 1974, **8**, 335.

Some experiments were also done with electron pulses from a van de Graaff accelerator which gives a higher dose

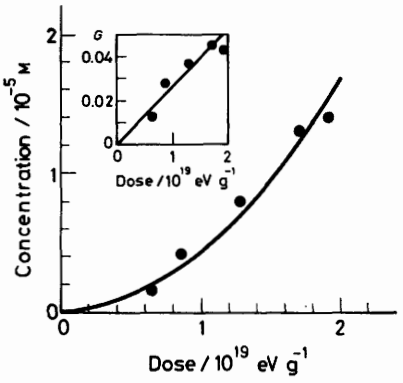


FIGURE 3 Dose dependence of D-*arabino*-hexulosonic acid yields and of the corresponding G values (inset) in the γ-radiolysis of a N_2O–O_2 (80 : 20 v/v) saturated aqueous solution of D-glucose (for conditions see caption to Figure 2)

rate than the ^{60}Co-γ source. The results are compiled in Table 3. The errors in the absolute G values of the experiments with electron pulses are thought to be larger than with ^{60}Co-γ, but the errors in the G values relative to one another within one set of experiments are the same in either case.

TABLE 3

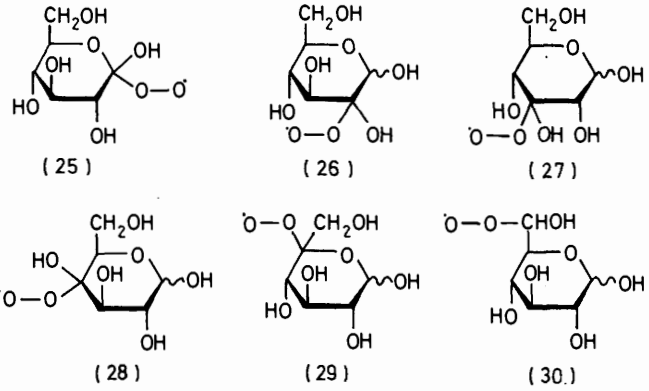
Dose rate effect on the product formation of N_2O–O_2 (4 : 1) saturated aqueous solutions of D-glucose (5×10^{-3}M) at room temperature. (a) ^{60}Co-γ, 1.1×10^{15} eV g^{-1} s^{-1}; (b) 1 μs electron pulses from a van de Graaff generator, 250 rad pulse^{-1}, frequency 4 Hz

No.	Product	G Values ^{60}Co-γ (a)	G Values Electron pulses (b)
(1)	D-Gluconic acid	0.90	0.80
(2)	D-*arabino*-Hexosulose	0.90	0.61
(3)	D-*ribo*-Hexos-3-ulose	0.57	0.40
(4)	D-*xylo*-Hexos-4-ulose	0.50	0.41
(5)	D-*xylo*-Hexos-5-ulose	0.60	0.20
(6)	D-*gluco*-Hexodialdose	1.55	0.90
(8), (9)	D-Arabinose–D-arabinonic acid	0.10	0.16
(10), (11)	*xylo*-Pentodialdose–D-xylose	0.08	0.08
(12)	L-*threo*-Tetrodialdose	0.20	0.28
(13), (14)	D-Erythrose–D-erythronic acid	0.02	0.08
(21)	Hydrogen peroxide	3.0	1.6

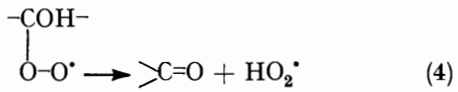
DISCUSSION

Monomolecular Decay of the Glucose Peroxyl Radicals.—The products listed in Table 2 arise from the subsequent reactions of the glucose peroxyl radicals (25)—(30). Radicals (26)—(28) and (30) are α-hydroxy-peroxyl radicals. Radical (25) is also an α-hydroxy-peroxyl radical but with a neighbouring ether function. Radical (29) is a peroxyl radical, which might be considered as a crypto-α-hydroxy-peroxyl radical with some features of the ether type peroxyl radicals.

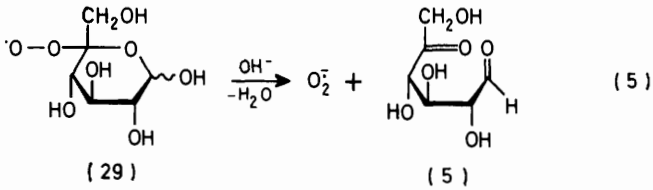
A considerable effort has been made to study the kinetics of α-hydroxy-peroxyl and ether peroxyl radicals using the pulse radiolysis technique. The high dose rates used in most of these experiments favour second-order processes. However, more recently data at relatively low dose rates also became available [17] which, we



believe, allow conclusions to be made on the reaction mechanisms prevailing also at dose rates as low as those in ^{60}Co-γ experiments. It has been found that the peroxyl radicals of simple alcohols [18,21,22] decompose into the carbonyl compounds and $HO_2^{\bullet}$ [reaction (4)]. Superimposed on this spontaneous decomposition is a base



catalysed reaction which is virtually diffusion controlled in the case of OH^-. For those peroxyl radicals which eliminate $HO_2^{\bullet}$ slowly the base catalysed reaction appears to determine the rate at pH 7 and even below. It is suggested that the major products (1)—(4) and (6) arise from the glucose peroxyl radicals (25)—(28) and (30) respectively, *via* $HO_2^{\bullet}$ elimination as described by reaction (4). Product (5) is expected to be formed according to reaction (5). The contribution of the open chain form of the peroxyl radical at C-5 to give (5)



according to reaction (4) is thought to be negligible because of the low steady-state concentration of the open chain form and the comparatively slow mutarotation of D-glucose.

Fragmentation of the Carbon Skeleton.—In contrast to the behaviour of deoxygenated solutions, substantial fragmentation of the carbon skeleton is observed. These reactions lead to products (8)—(20). Taking $k_4 > 200$ s^{-1}, for at least (26)—(28) and (30), the steady-state

[21] Y. Ilan, J. Rabani, and A. Henglein, *J. Phys. Chem.*, 1976, **80**, 1558 and references cited therein.

[22] E. Bothe, G. Behrens, and D. Schulte-Frohlinde, *Z. Naturforsch.*, 1977, **32b**, 886.

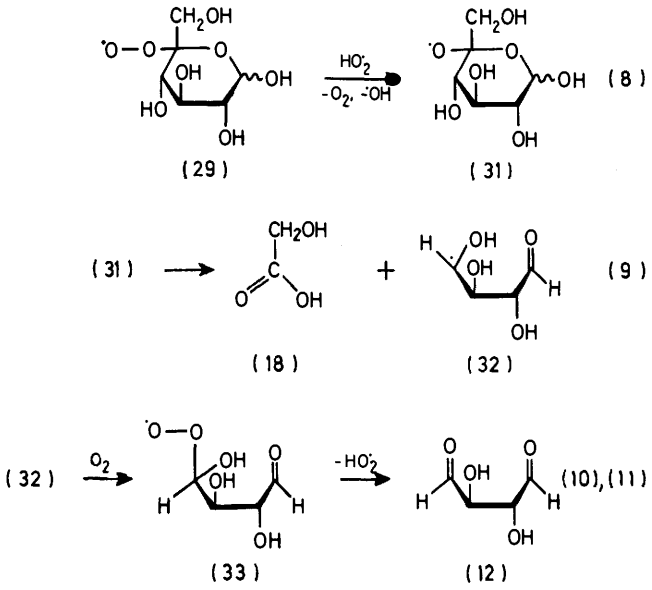
concentrations of the primary peroxyl radicals ($RO_2^{\bullet}$) is too low under our ^{60}Co-γ-conditions to give the bimolecular reaction (6). However, $O_2^{-\cdot}$ and $HO_2^{\bullet}$ radicals

$$RO_2^{\bullet} + RO_2^{\bullet} \longrightarrow \text{products} \qquad (6)$$

decay much more slowly and there is the possibility that they build up a sufficient steady-state concentration to interact with the primary peroxyl radicals according to reaction (7). Fragmentation reactions on the basis of

$$RO_2^{\bullet} + O_2^{-\cdot}\ (HO_2^{\bullet}) \longrightarrow \text{products} \qquad (7)$$

bimolecular reactions of peroxyl radicals have been postulated for a number of compounds with similar structural elements.[23-27] It is observed that the most



prominent fragment product is L-*threo*-tetrodialdose (12) (*cf.* ref. 27). From stereochemical considerations its precursor must be radical (29). A possible reaction could be reaction (8) which gives rise to the oxyl radical (31). Oxyl radicals are well known to undergo fragmentation reactions [24] as depicted in reaction (9) for radical (31) which yields glycolic acid (18), and radical (32). (32) adds oxygen [reaction (10)]. The resulting peroxyl radical (33) is expected to give (12) *via* reaction (11) which is analogous to reaction (4).

Similar reactions can be written to account for D-arabinonic acid (9) and formic acid (20) from radical (26), *xylo*-pentodialdose (10) and formic acid from radical (30), D-erythrose (13) and glyoxylic acid (18) from radical (26), D-erythronic acid (14) and D-glyceraldehyde (15) from radical (27), and glyoxal (17) and D-glyceric acid (16) from radical (28).

D-Arabinose (8) is thought not to arise from such a fragmentation process [from radical (25)] because the other expected product, carbon dioxide (23), has been shown not to be a primary product. Its route of formation remains an open question at this stage.

The higher the steady-state concentration of a given peroxyl radical (25)—(30), the greater the probability to undergo the bimolecular fragmentation. Assuming equal rate constants of reaction (7) for all peroxyl radicals (25)—(30), we can give relative rates of $HO_2^{\bullet}$ eliminations on the basis of the dose rate effects (Table 3). It has been observed that the dose rate effect is smallest in D-gluconic acid (1) [precursor (25)] followed by the products (2)—(4) [precursors (26)—(28)]. This effect is more pronounced in (6) [precursor (30)] and especially in (5) [precursor (29)]. This suggests that k_4 (25) $\gg$ k_4 (26)—(28) $>$ k_4 (30) $\gg$ k_4 (29). These conclusions have been substantiated recently by the direct measurement of the rates of $HO_2\cdot$ elimination.[17]

$G(H_2O_2)$ decreases markedly with increasing dose rate (Table 3). This is thought to be due to two competing processes. At low dose rates the $HO_2^{\bullet}$ radicals largely react with one another (or with $O_2^{-\cdot}$) according to reaction (12). Their steady-state concentration increases with increasing dose rate. Reactions with other

$$HO_2^{\bullet} + HO_2^{\bullet} \longrightarrow H_2O_2 + O_2 \qquad (12)$$

peroxyl radicals become increasingly likely, first with (29), then with (30) and (33), and at even higher steady-state concentrations with (26)—(28). The reaction sequence (8)—(11) does not consume $HO_2^{\bullet}$ radicals. However, it has been observed [18,28] that the 1-hydroxyethylperoxyl radical (from ethanol) gives rise to considerable acetic acid formation, concomitant with a low yield of H_2O_2, possibly according to reaction (13).

$$RCH(OH)O_2^{\bullet} + HO_2^{\bullet}\ (O_2^{-\cdot}) \longrightarrow RCO_2H\ (RCO_2^{-}) + H_2O + O_2 \qquad (13)$$

A similar sequence is expected to occur with the primary peroxyl radical (30) and the fragment radicals such as (33) in competition to fragmentation [*e.g.* reaction (8)]. Some D-glucuronic acid (7) [from (30)] has already been found under ^{60}Co-γ conditions.

Distribution of OH *Attack.*—There is a fair agreement between G(OH) and G(products). On the basis of the proposed mechanstic concept the relative abundance of the radicals (25)—(30) can be calculated, and hence also the ratio of OH attack at the various carbon atoms: $G(25) = G(1) = 0.90$; $G(26) = G[(2) + (9) + (13)] = 0.94$; $G(27) = G[(3) + (14) + (15)] = 0.64$; $G(28) = G[(4) + (16)] = 0.57$; $G(29) = G[(5) + (12)] = 0.80$ and $G(30) = G[(6) + (7) + (10)] = 1.67$. These values suggest that primary OH attack at C-1 and C-2 is *ca.* 20%,

[23] D. Lindsay, J. A. Howard, E. C. Horswill, L. Iton, K. U. Ingold, T. Cobbley, and A. Ll, *Canad. J. Chem.*, 1973, **51**, 870.
[24] C. von Sonntag, K. Neuwald, H.-P. Schuchmann, F. Weeke, and E. Janssen, *J.C.S. Perkin II*, 1975, 171.
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at C-3 and C-4 *ca.* 10%, at C-5 *ca.* 15%, and at C-6 *ca.* 30%.

EXPERIMENTAL

Materials.—Nitrous oxide–oxygen (80 : 20 v/v) (Messer-Griesheim), D-glucose, D-xylose, D-glucurono-1,4-lactone, D-glucitol (all biochemically pure; Merck), D-erythrose (purum), D-arabinose (puriss), D-glucono-1,5-lactone (purum), D-glucuronic acid (purum), sodium borohydride (>98%), sodium borodeuteride (97 atom % D), Dowex 50 W × 8 (200—400 mesh; H^+ form) (Roth), methoxyamine hydrochloride (>97%; Serva), hexamethyldisilazane (purum), and trimethylchlorosilane (puriss) (Fluka) were available.

Synthetized reference materials were D-*arabino*-hexosulose,[29] D-*ribo*-hexos-3-ulose,[30] D-*xylo*-hexos-4-ulose,[31] D-*xylo*-hexos-5-ulose,[32] and D-*gluco*-hexodialdose.[33] The mass spectra of the $NaBD_4$-reduced and trimethylsilylated derivatives, and the methoximated and trimethylsilylated derivatives of these compounds are given elsehwere.[18,19]

Analyses.—Separation of the products by liquid chromatography, reduction with $NaBH_4$ and $NaBD_4$, trimethylsilylation, methoximation, and acetylation were carried out as described previously.[12,18]

G.l.c. analyses for the trimethylsilylated samples were carried out in a Varian 1 400 equipped with a flame ionization detector on a thin film glass capillary column [Dexsil 300 (Analabs); 123 m, 0.25 mm i.d.] operating isothermally at 170 °C, with hydrogen as carrier gas. Acetylated polyhydric alcohols were separated on a OV-101 glass capillary column (68 m, 0.25 mm i.d.) operating isothermally at 180 °C. The details of g.l.c.–m.s. instrumentation including the computer link-up are available elsewhere.[34]

The quantitative determinations of the products as trimethylsilylated or acetylated derivatives were carried out by g.l.c. using a suitable reference material as internal standard. The relative response factors (or relative sensitivities) of the products with respect to the internal standard were determined experimentally whenever pure crystalline materials were available. In the case of the synthesized hexosuloses as well as D-*gluco*-hexodialdose, where these compounds were obtained as syrups, the relative response factors of their methoximated derivatives with respect to D-glucitol as internal standard were calculated on an incremental basis.[35] Hydrogen and carbon dioxide were determined by g.l.c. using stainless steel columns packed with activated carbon, operated at room temperature.

Formic acid was analysed colorimetrically [36] as well as alkalimetrically after being separated from other acids in the samples by distillation. Formaldehyde was determined by means of the Hantzsch reaction,[37] $\lambda_{max.}$ (20 °C) 412 nm (ε 7 530 l mol^{-1} cm^{-1}) (lit.,[37] 8 000 l mol^{-1} cm^{-1}). Glycolic acid and glyoxylic acid were determined together colorimetrically by the method of Sarfati and Szabó,[38] $\lambda_{max.}$ (20 °C) 520 nm (ε 6 550 l mol^{-1} cm^{-1}). Glyoxal was determined colorimetrically,[15,39] $\lambda_{max.}$ (20 °C) 600 nm (ε 1.92 × 10^4 l mol^{-1} cm^{-1}) (lit.,[39] 2.3 × 10^4 l mol^{-1} cm^{-1}). Total peroxides were determined iodometrically,[40] $\lambda_{max.}$ (20 °C) 350 nm (ε 2.63 × 10^4 l mol^{-1} cm^{-1}) (lit.,[40] 2.5 × 10^4 l mol^{-1} cm^{-1}). Hydrogen peroxide was determined by the method of Eisenberg,[41] $\lambda_{max.}$ (20 °C) 408 nm (ε 635 l mol^{-1} cm^{-1}) (lit.,[41] 689 l mol^{-1} cm^{-1}). Both methods gave similar results.

Irradiations.—Irradiations were carried out in a Nuclear Engineering Ltd. 7000 Curie ^{60}Co-γ-source at dose rates 1.6 × 10^{14}—4.7 × 10^{15} eV g^{-1} s^{-1}. For higher dose rates irradiations were carried out with a van de Graaff electron-accelerator (High Voltage Engineering Corp.; type K).

We are grateful to Univ.-Doc. Dr. M. Dizdaroglu for generous assistance, and to Miss U. Rauhnt for g.l.c. analyses.

[7/576 *Received*, 1st *April*, 1977]

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