Fragmentation Reactions of Radicals formed from Sugar Phosphates and the Hydroxyl Radical: an Investigation by Electron Spin Resonance Spectroscopy and Pulse Radiolysis

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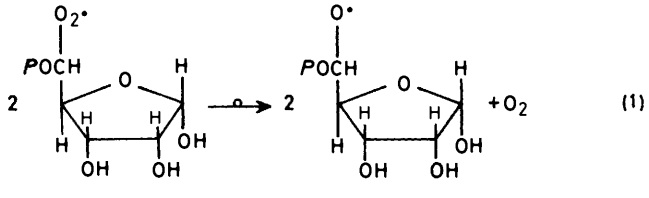
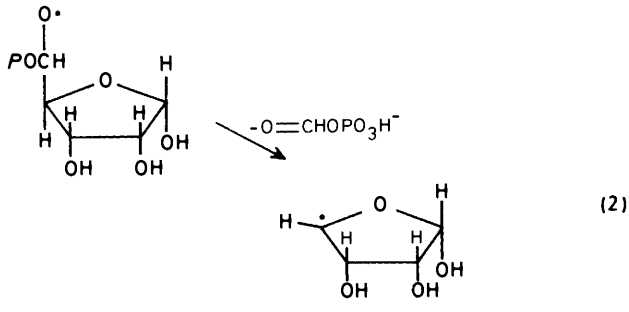
E.s.r. spectroscopy has been employed with the radiomimetic Ti^{III}–H_2O_2 couple and an aqueous flow system to study the reactions between the hydroxyl radical and some sugar phosphates. For the furanose compounds hydrogen-atom abstraction from the carbon adjacent to the alicyclic oxygen is preferred: subsequent phosphate loss occurs when the radical centre is adjacent to the carbon bound to phosphate, and, as judged by steady-state e.s.r. and time-resolved pulse-radiolysis experiments, is faster for radicals from ribose 5-phosphate, α-D-glucose 1-phosphate, and glycerol phosphates (*k ca.* 10^4 s^{-1}) than for fructose phosphates (*k ca.* 10^2—10^3 s^{-1}).

Phosphate-ester cleavage *via* radiolysis has excited considerable interest,[1–4] in part because the process is believed to play a key role in radiation-induced damage to DNA.[5] The phosphate group itself is relatively unreactive towards attack by 'OH and 'H,[6] and scission of the phosphoric ester bond by the action of the solvated electron is believed to be negligible.[7] The primary steps in radiolytic damage to sugar phosphates should thus be analogous to those of the parent sugars, namely reaction of 'OH radicals with the carbohydrate moiety by hydrogen abstraction from C–H bonds [the hydroxyl radical reacts with fructose 1,6-diphosphate and fructose 1- and fructose 6-phosphate[2] at rates similar to that for D-glucose (*ca.* 2×10^9 dm^3 mol^{-1} s^{-1})[8]].

Product studies have largely been based on the measurement of inorganic phosphate and dephosphorylation products, though modified sugars (which retain the phosphate group) have been isolated following irradiation of, for example, fructose phosphates[4] and D-ribose 5-phosphate.[9] It has been suggested that dephosphorylation largely occurs from the first-formed radical which has the unpaired electron on the carbon atom β to the phosphate group. Other reactions evidently also contribute, *e.g.* radical formation at C(5), α to the phosphate group: thus phosphate loss in oxygenated solution is believed[10] to occur along with the loss of C(5) following peroxyl radical formation at C(5) [reactions (1) and (2)].



(1)



(2)

Results from e.s.r. studies, both for solids[2] and in solution,[11,12] and pulse-radiolytic investigations of alkyl phosphates[13] appear to be in accord with conclusions based on product studies, although the compounds studied are only relatively simple models for the reactions of ribofuranose-phosphate-derived radicals.

In order to obtain a fuller understanding of the free-radical chemistry of the ribose phosphate backbone of DNA, we have employed complementary techniques of e.s.r. spectroscopy and pulse radiolysis to investigate the reactions of 'OH with a variety of phosphate-substituted sugars. As with the corresponding study of sugars themselves,[14] structural information on first-formed radicals was obtained *via* e.s.r. spectra obtained under steady-state conditions [using the radiomimetic Ti^{III}–H_2O_2 reaction as a source of 'OH, reaction (3)]. Kinetic information on rearrangements and fragmentation processes was obtained *via* steady-state analysis of e.s.r. experiments and also *via* time-resolved pulse radiolysis. A preliminary account of some of this work has been published.[15]

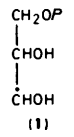
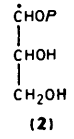
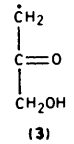
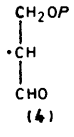
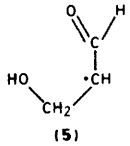
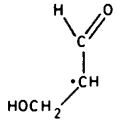
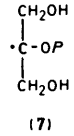
Results and Discussion

E.s.r. Results.—A three-way continuous-flow system allowed the mixing of aqueous solutions of Ti^{III} (typically 0.008 mol dm^{-3}), H_2O_2 (0.05 mol dm^{-3}), and the substrate (0.02 to 0.1 mol dm^{-3}) *ca.* 50 ms before the combined solution entered the spectrometer. In experiments at pH > 2.5, the disodium salt of ethylenediaminetetra-acetic acid (EDTA) was included to sequester Ti^{III}. The pH of the solution was adjusted by the addition of sulphuric acid or aqueous ammonia to the Ti^{III} stream; to obtain information on first-formed radicals the majority of experiments were carried out initially at pH *ca.* 4, to avoid (where possible) the occurrence of subsequent acid- and base-catalysed transformations.

$$Ti^{III} + H_2O_2 \longrightarrow Ti^{IV} + OH^- + {}^{\bullet}OH \qquad (3)$$

(a) *Glycerol Phosphates.*—Although e.s.r. has been utilised in several studies of the reactions of 'OH (generated radiolytically) with both glycerol 1- and 2-phosphates,[11,12] the characterisation of all the primary radicals has not previously proved possible. It was expected that use of the Ti^{III}–H_2O_2 couple might allow detection of all such radicals produced, as a result of the higher radical concentrations which can normally be generated in this way.

Table 1. E.s.r. parameters of radicals formed from reaction of 'OH with glycerol phosphates

Substrate	Radical	Hyperfine splitting (mT)[a] a(α-H)	a(β-H)	a(other)	g Value[b]
Glycerol 1-phosphate $POCH_2CH(OH)CH_2OH$ pH 2.5—6	 (1)	1.782	1.155		2.0031
pH 1.5—6	 (2)	1.883	1.205	0.678 (^{31}P)	2.002 95
pH 1—6	 (3)	1.970(2)		0.030 (2γ-H)	2.0041
pH 1—2.0	 (4)	1.807	2.673(2) 0.120 (*CHO*)		2.0042
Glycerol 2-phosphate $HOCH_2CH(OP)CH_2OH$ pH 1—6	 (5)	1.710	2.780(2) 0.120 (*CHO*)		2.0045
pH 1—6	 (6)	1.840	2.545(2) 0.140 (*CHO*)		2.0043
pH 1.5—6	 (7)	1.250(4)	2.520 (^{31}P)		2.0028

[a] ±0.005 mT. [b] ±0.000 1.

Reaction of 'OH and *glycerol 1-phosphate* at pH 4 (at which the phosphate group is singly charged) led to the detection of radicals (Table 1)* assigned as either α-oxyalkyl radicals (*g* 2.003), formed by the initial reaction of 'OH, or carbonyl-conjugated radicals (*g ca.* 2.004) resulting from rearrangement of α-oxyalkyl radicals (*via* a mechanism related to the acid-catalysed rearrangement of α,β-dihydroxyalkyl radicals[16] [*e.g.* $^{\cdot}CH(OH)CH_2OH$ into $^{\cdot}CH_2CHO$]. The spectra also indicate that reaction of 'OH by hydrogen abstraction is essentially unselective.

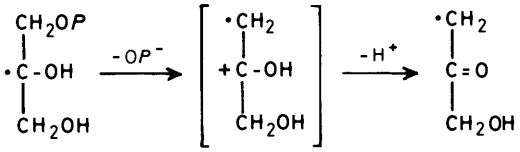
The main feature of the spectrum is a signal with α-proton (1.782 mT) and β-proton (1.155 mT) splittings assigned to (**1**), formed by attack at C(3). The radical (**2**), which also shows α- and β-proton couplings, is further characterised by a doublet (*ca.* 0.7 mT) from the interaction of the unpaired electron with the α-O-^{31}P nucleus. This was reduced to 0.55 mT at pH 8.5 (see ref. 12), presumably a result of ionisation of the phosphate group to the dianion [the pK_α for the radical would not be expected to differ significantly from that for the parent, *i.e. ca.* 6 (ref. 17)]. The radical formed by oxidation at C(2) was not directly detected but the signal with *g* 2.0041 is assigned to the radical (**3**) formed, as noted previously, *via* rapid loss of the β-phosphate group [reaction (4)]. A simple steady-state kinetic analysis indicates that for such a process (when the transformed radical, rather than the first-formed species, is detected), the rate constant must be greater than *ca.* 10^3 s^{-1}.†

* Here and throughout the paper –O*P* = –OP(O)(OH)O$^-$.

† Consider the situation in which the radical A$^{\cdot}$ rearranges to B$^{\cdot}$ which is then destroyed by dimerisation or disproportionation:

$$A^{\cdot} \xrightarrow{k_1} B^{\cdot} \xrightarrow{2k_t} \text{products}$$

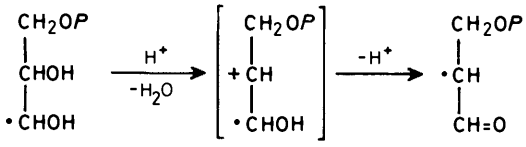
Under steady-state conditions, it can be shown that $k_1 = 2k_t[B^{\cdot}]\{([B^{\cdot}]/[A^{\cdot}]) + 1\}$; for $2k_t$ *ca.* 10^9 dm^3 mol^{-1} s^{-1} (typical of small alkyl radicals), the conditions for $[A^{\cdot}] = [B^{\cdot}] = 10^{-6}$ mol dm^{-3} (the concentration required for detection) is $k_1 = 10^3$ dm^3 mol^{-1} s^{-1}.



(4)

(3)

As the pH was lowered, signals from the other first-formed radicals disappeared, the radical (**1**) being the first to be affected (its spectrum was removed by pH *ca.* 2.1). Signals from (**2**) persisted at pH 1, but at even lower pH these too disappeared. Only one new signal was detected, assigned to (**4**) (Table 1), formed through acid-catalysed loss of water from (**1**) [reaction (5)]. [By overlapping the spectra from glycerol 1-phosphate



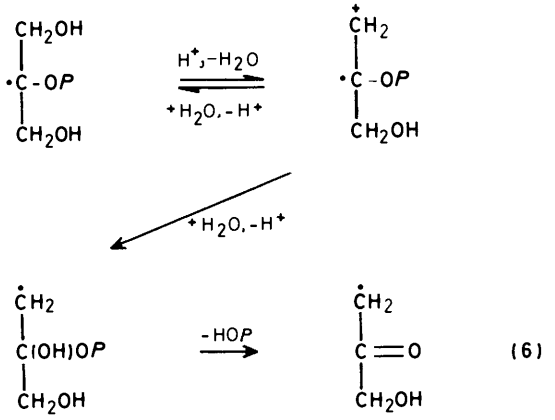
(5)

(1) (4)

with those obtained for glycerol, it was clear that the parameters of this radical differ from those of $HOCH_2\dot{C}HCHO$ [*a*(α-H) 1.845, α(β-H) 2.600, *a*(CHO) 0.145 mT, *g* 2.0044], indicating that the phosphate group is retained]. Furthermore the rearrangement of the radical $^{\bullet}CH(OH)CH(OH)CH_2OH$ [*a*(α-H) 1.76, *a*(β-H) 1.085, *a*(OH) 0.080 mT, *g* 2.0031] occurs at a similar pH to that of (**1**); it can be concluded therefore that the rate constants for these acid-catalysed rearrangements are similar[18] (*ca.* 5×10^6 dm^3 mol^{-1} s^{-1}).*

Two of the radicals from $^{\bullet}OH$ and *glycerol 2-phosphate* at pH 4 (see Table 1) have *g* values consistent with carbonyl-conjugated radicals formed following H-atom abstraction at the terminal carbon atom(s): rapid loss of the β-phosphate ($k > 10^3$ s^{-1}) [*cf.* reaction (4)] leads to the formation of a secondary radical which can apparently exist as two isomeric forms (**5**) and (**6**). Individual assignment has previously been made[11] on the basis of expected hydrogen bonding and restricted rotation of the CH_2OH group about the C(2)–C(3) bond in the *cis*-isomer (**5**), resulting in an increased β-splitting. A third radical, assigned structure (**7**), was not previously observed.[11,12] An unusual feature of this spectrum is a very large ^{31}P splitting of 2.5 mT: α-O-^{31}P splittings in phosphate radicals are typically in the range 0.5—1.0 mT, though values up to 4.5 mT have been observed in analogous radicals in the solid state.[19] For radicals of this type a conformation with a planar arrangement of the $C_\beta C_\alpha$–O–P atoms (normally favoured) would result in low ^{31}P couplings (*ca.* 0.4 mT) (*via* π-delocalisation of spin-density onto the α-oxygen); on the other hand, a conformation in which the phosphate group is lifted out of the plane could give ^{31}P splittings of up to 10 mT as a result of hyperconjugative interaction involving the O–P bond. The splitting of *ca.* 2.5 mT for (**7**) suggests that such a conformation is preferred in this radical.

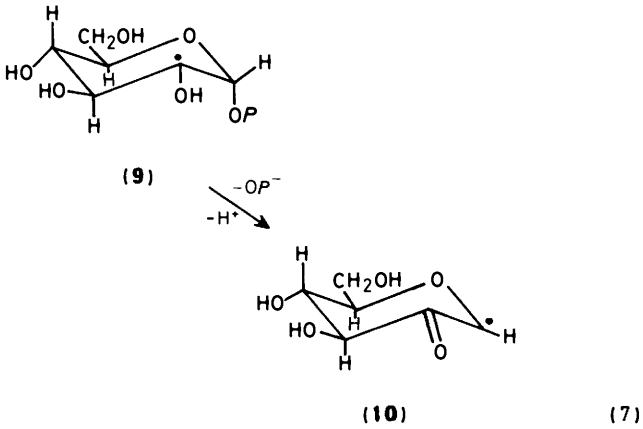
Signals from (**7**) dominate the spectra for pH 3—4. This is not thought to be a result of regioselectivity in the reaction of $^{\bullet}OH$ but a result of removal of the carbonyl-conjugated radicals (**5**) and (**6**) by the effective reductant Ti^{III}–EDTA; below pH 3 and in the absence of EDTA signals from (**5**) and (**6**) dominate, presumably as such reduction becomes less significant.[20] As the pH was lowered to *ca.* 1 signals from (**7**) disappeared. No product resulting from its rearrangement was detected, but it is possible that the acid-catalysed decay of this radical involves loss of the β-hydroxy group to give a radical-cation intermediate [reaction (6)], which may undergo hydration or reduction by Ti^{III}.



(6)

(b) *Pyranose Sugar Phosphates.*—Analysis of the complex spectra obtained following reaction of $^{\bullet}OH$ with α-D-*glucose 1-phosphate* (**8**) indicates that the radicals formed (see Table 2) closely resemble species previously detected from α-D-glucopyranose (and that oxidation occurs at all carbon atoms):[21] evidently phosphorylation of the 1-OH group has little effect on the conformation or reactivity of the sugar. Signals attributable to radicals formed by reaction of $^{\bullet}OH$ at C(3) and C(5) are more distinct for the phosphate, but this is associated with a decrease in linewidth, rather than a change in the selectivity of attack.

The radical (**9**) was not observed, and its initial presence can only be inferred from the detection of a carbonyl-conjugated radical (**10**), evidently formed by elimination of phosphate [reaction (7)]. The presence of this species over the whole pH



(9) (10) (7)

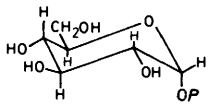
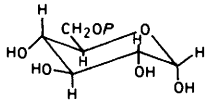
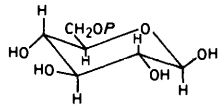
range studied is an indication of the ease with which phosphate is lost (steady-state analysis sets a lower limit of *ca.* 10^3 s^{-1} for the rearrangement) and it forms the major distinction between the free-radical chemistry of simple sugars and their phosphate esters.

Only approximate measurements were possible for the weak and partially obscured signal attributable to the C(1)-abstraction radical. The splittings are consistent with a $^4C_1$ chair conformation. The small splitting from ^{31}P in the α-phosphate group (0.3 mT) is in keeping with a planar conformation of the C(1)–O–P system, similar to that observed in glycerol 1-phosphate.

As the pH was lowered to *ca.* 1.5 signals from the carbonyl-

* Rate constants for acid-catalysed rearrangements were obtained *via* steady-state calculations: the pH of half-removal is essentially governed by the competition between dimerisation of the initial radical ($2k_t$ *ca.* 10^9 dm^3 mol^{-1} s^{-1}) and acid-catalysed destruction (see ref. 18).

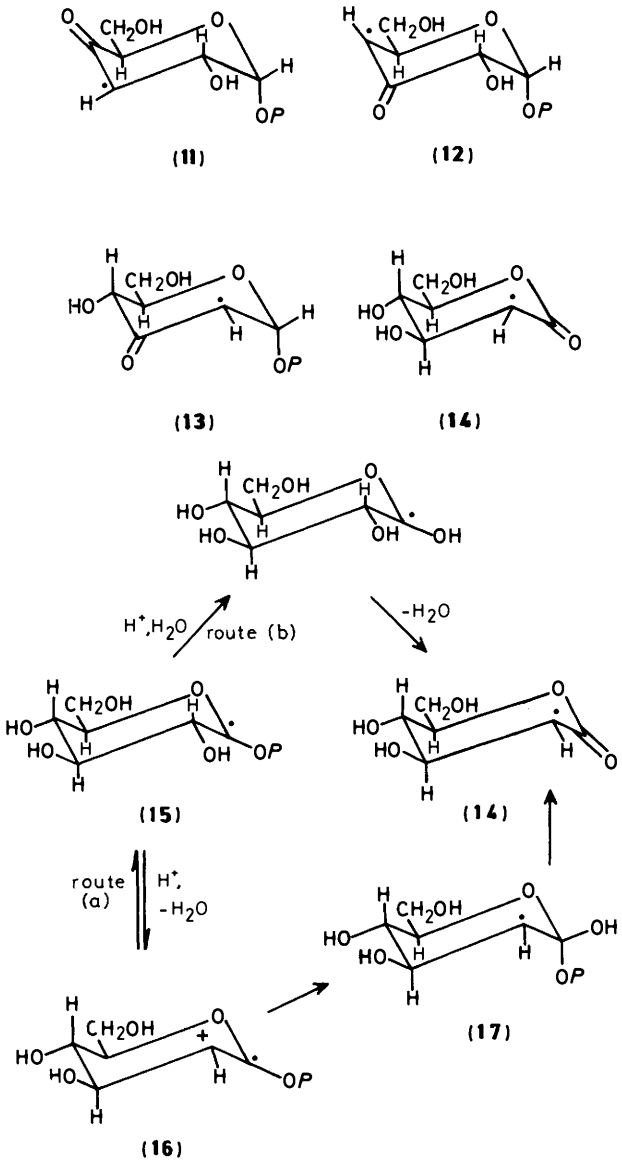
Table 2. E.s.r. parameters of radicals formed following reaction of 'OH with glucose phosphates

Substrate	Position of abstraction	Radical[a]	Hyperfine splittings[b] (mT) a(α-H)	a(β-H)	a(other)
 (8) α-D-Glucose 1-phosphate pH *ca.* 4	C(1)			3.470(1)	0.250 (γ-H) 0.300 (^{31}P)
	C(2)[c]	(10)[d]	1.395(1)		0.540(1) 0.500(1) 0.020(1)
	C(3)			3.010(1) 2.830(1)	
	C(4)			2.635(1) 2.445(1)	
	C(5)			3.405 1.015 0.700	
	C(6)		1.835	0.650(1)	0.130(1) 0.140(1) 0.075
pH *ca.* 1	C(1)[c]	(14)[e]	2.020(1)	3.840(1)	0.075(1)
	C(4)[c]	(11)[f]	1.800(1)	3.700(1)	0.05(1)
	C(3)[c]	(12)[f]			
	C(3)[c]	(13)[f]	1.840(1)	1.100(1)	
 (18) α-D-Glucose 6-phosphate pH *ca.* 4	C(1)	(20)		3.470(1)	0.180(1) 0.145(1)
	C(2)	(21)		2.975(1) 1.295(1)	0.160(1)
	C(3)[g,h]			2.86(1) 2.77(1)	
	C(4)[g,h]			2.51(1) 2.38(1)	
	C(6)[g]		1.877(1)[g]	0.625(1)	0.725 (^{31}P)[i] 0.078(1)
pH *ca.* 1	C(1)[c]	(25)[e]	2.020(1)	3.840(1)	0.075(1)
	C(2)[c]	(26)[d]	1.395(1)		0.540(1) 0.500(1) 0.020(1)
	C(5)[c]	(27)[f]	1.985(2)		0.115(1)
 (19) β-D-Glucose 6-phosphate pH *ca.* 4	C(1)	(20)			
	C(2)	(22)		2.860(1) 2.280(1)	
	C(3)[g,h]			2.86(1) 2.77(1)	
	C(4)[g,h]			2.51(1) 2.38(1)	
	C(6)[g]		1.877(1)	0.625(1)	0.725 (^{31}P) 0.078(1)
pH *ca.* 1	C(1)[c]	(25)[e]		See above	
	C(2)[c]	(26)[d]		See above	
	C(5)[c]	(27)[f]		See above	

[a] Radicals formed by C–H abstraction at the carbon atom stated, except where rearrangement is indicated. [b] ±0.005 mT; g = 2.0031 except where otherwise stated. [c] Secondary radical indicates initial position of abstraction. [d] g = 2.0049. [e] g = 2.0035. [f] g = 2.0045. [g] No distinction between α- and β-anomers possible. [h] ±0.01 mT. [i] 0.530 mT at pH 7.

conjugated radicals (**11**)—(**13**) replaced those of their precursors (see Table 2). These signals, associated with rearrangements distant from the phosphate group, are identical with those *via* acid-catalysed rearrangements of radicals formed from α-D-glucose,[21] and indicate that the phosphate group does not affect radicals formed at remote sites. At even lower pH the appearance of a signal with three doublet splittings (2.02, 3.84, and 0.075 mT, g 2.0035) characterises the carboxy-conjugated radical (**14**), evidently formed *via* acid-catalysed hydrolysis of the C(1)-centred radical. [It is unlikely that (**14**) arises *via* hydrolysis of the parent ester: although glucosyl phosphates are hydrolysed more rapidly than other phosphate esters, this reaction is still quite slow in strong acid (with a half-life of *ca.* 1 h at pH 1; see ref. 22). However, the formation of a radical centre α to a glycosidic bond has been observed to increase the rate of hydrolysis of such glycosides,[23] supporting the suggestion that (**14**) is formed from the C(1) radical (**15**)]. Two possible mechanisms are presented in Scheme 1.

Route (a) involves a radical-cation (**16**), which can subsequently undergo hydration in either of two possible ways. Addition of water to C(2) followed by proton loss would lead to the regeneration of (**15**), whereas hydration at C(1) leads to the formation of an intermediate (**17**) with three oxygen substituents on C(1): rapid elimination of phosphate from such a species would give (**14**). Such a mechanism is consistent with the results of e.s.r. studies on radical-cations from 2,3-dihydrofuran and 1-methoxyprop-1-ene, which have been shown to undergo hydration at both possible positions.[1] Alternatively the reaction may proceed *via* nucleophilic attack by water on (**15**) itself [route (b)]. Any such reaction is clearly not fast enough when the leaving group is OPO_3H^- (since no hydrolysis product is detected at higher pH), but an increase in

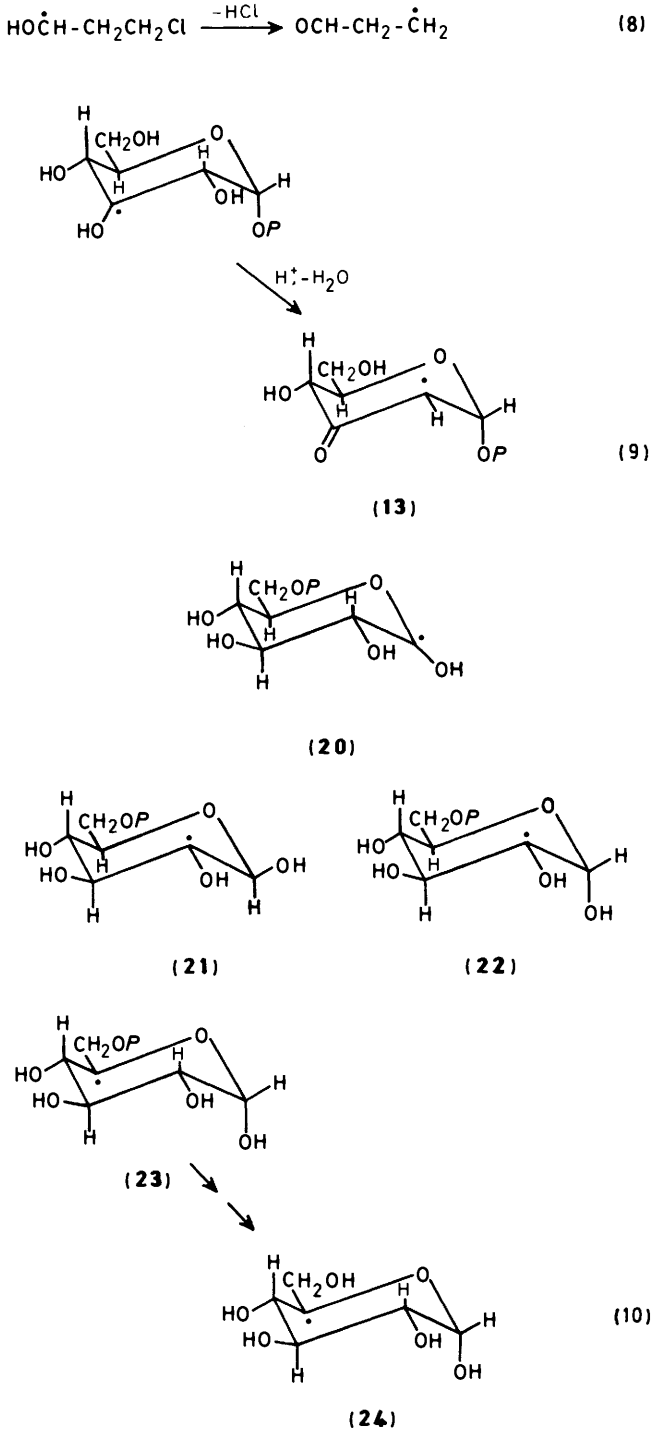


Scheme 1.

the rate of reaction may occur on protonation. Once formed, the product of this reaction would undergo an acid-catalysed dehydration to give the detected species.

No examples of 1,3-eliminations were observed: such reactions have been shown to occur in certain radicals with good leaving groups γ to the radical centre[24] [see *e.g.* reaction (8)]. Rearrangement of the C(3)-derived radical at low pH to give (**12**) and (**13**) [reaction (9)] clearly indicates that, in this instance at least, loss of β-OH is preferred.

The spectrum obtained when $^{\bullet}OH$ reacts with D-*glucose* 6-*phosphate* at pH 4 (Figure 1) is analysed in terms of radicals from both α- and β-anomers of the sugar [(**18**) and (**19**), respectively]. The parameters of most of the radicals correspond to analogous radicals detected from α- and β-D-glucose,[21] their structures not being significantly affected by the phosphate group. In contrast to α- and β-D-glucose, however, signals from (**20**) are clearly observed [a result of changes in the spectrum of the C(6)-centred species which removes most of the overlapping signals which hamper observation of the non-phosphorylated analogue]: the long-range γ-proton splittings of 0.180 and 0.145 mT characterise interaction with *axial*-protons on C(5) and C(3), respectively.



The radicals (**21**) and (**22**), formed by abstraction from C(2) (from α- and β-anomers, respectively), are separately detected, though signals from the C(3)- and C(4)-centred radicals could not be assigned to individual anomers. No signals could be detected from the radical formed by abstraction at C(5) [(**23**)]; nor was there evidence for the formation of (**24**) [which would result from the hydrolysis of the former; reaction (10)]. It is possible that a phosphate-containing precursor (**23**) is present but that its resonances are obscured by other signals. Elimination of phosphate from this radical is probably not as rapid as observed in the glycerol phosphates or α-D-glucose 1-phosphate, where rearrangement leads directly to a carbonyl-conjugated radical.

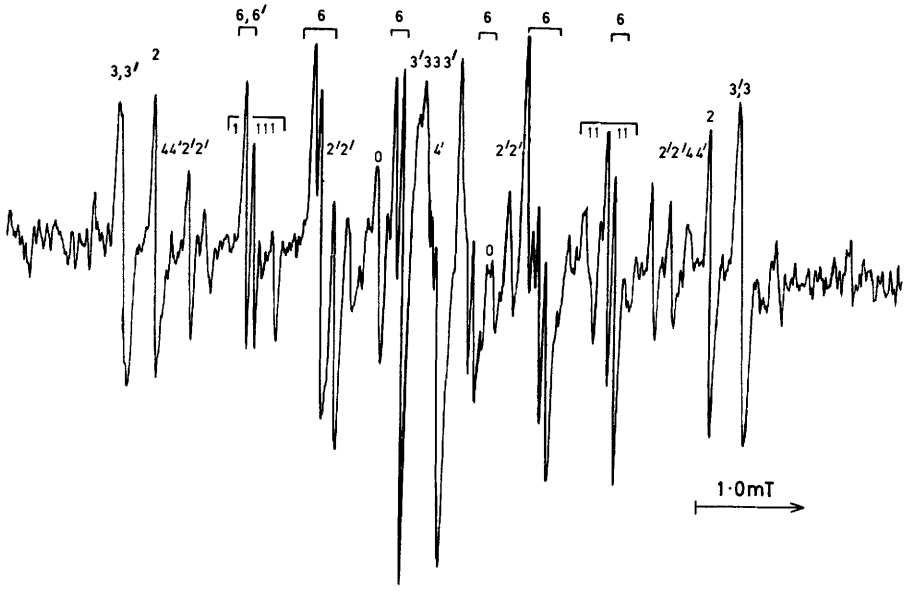
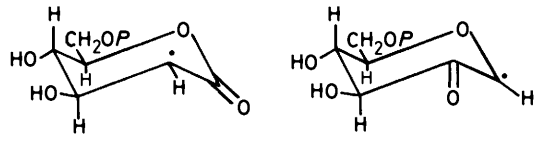


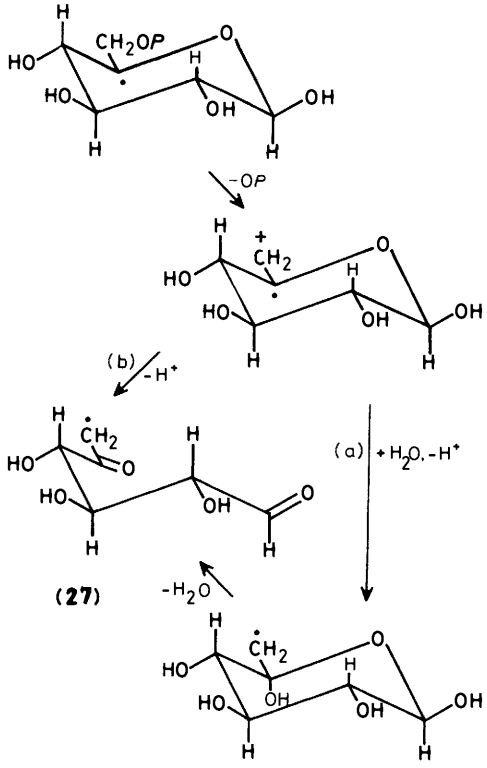
Figure 1. E.s.r. spectrum obtained at pH *ca.* 4 from the reaction of 'OH with a mixture of α- and β-D-glucose 6-phosphate; numbers refer to the carbon atoms from which hydrogen is abstracted. Radicals derived from the α-anomer are indicated by a prime. Peaks marked ○ are unassigned

Finally, the product of hydrogen-atom abstraction at C(6) is characterised by an α-proton coupling and β-coupling to the proton on C(5) (distinguished from the ^{31}P coupling of similar magnitude by the change in the latter splitting at pH *ca.* 6 following ionisation).

As the pH was lowered, rearrangement (*cf.* α-D-glucose 1-phosphate) led to the detection of signals attributed to (**25**), (**26**) (formed by acid-catalysed dehydration), and the ring-opened species (**27**), the detection of which confirms that the C(5)-centred radical is indeed formed in the initial reaction. It is suggested that phosphate loss gives a radical-cation, which then reacts in one of two ways to give (**27**) (Scheme 2): either hydration at C(5) leads to a hemiacetal which subsequently undergoes acid-catalysed ring-opening [route (a)], or the radical-cation fragments directly [possibly concerted with proton loss from C(1)OH; route (b)]. These two processes will be discussed later: however, we note that since no ring-opened radical is detectable from D-glucose[21] or α-D-glucose 1-phosphate, radical-cations are evidently not easily formed by the acid-catalysed loss of C(6)–OH in these compounds.



(**25**) (**26**)



(**27**)

Scheme 2.

(c) *Furanose Sugars and Sugar Phosphates.*—Fructose 1,6-diphosphate (**28**), fructose 6-phosphate (**29**), and ribose 5-phosphate (**30**), in which the sugar is present in the furanose configuration, were chosen as models since they possess structural features similar to those of the ribose phosphate backbone of DNA and RNA. Experiments were first conducted with D-fructose, of which solutions which have achieved equilibrium at room temperature contain both the β-pyranose form (**31**) and the β-furanose anomer (**32**) (though freshly dissolved solutions contain solely the β-pyranose form).[25]

(i) *Reaction of* 'OH *with* D-*fructose.* Reaction of 'OH with a freshly prepared ice-cooled solution of β-D-fructopyranose (**31**) at pH *ca.* 4 gave a product showing a complex spectrum comprising many overlapping lines [Figure 2(a)]. This is analysed in terms of radicals formed by hydrogen abstraction from all positions in the pyranose ring (Table 3).

The C(1) radical is characterised by an α-hydrogen coupling of 1.815 mT and fine structure (splittings of 0.1 and 0.05 mT) typical of the interaction of an exocyclic radical centre with ring protons [the couplings are attributed to the interaction of the radical centre with the hydroxylic proton on C(1) and the axial γ-hydrogen on C(3) of the ring]. The *g*-value (2.00315) is slightly higher than that of corresponding alicyclic radicals (*g* 2.00310), as previously observed in α-D-glucose and other saccharides.[21] The radical formed by hydrogen abstraction from C(3) has an

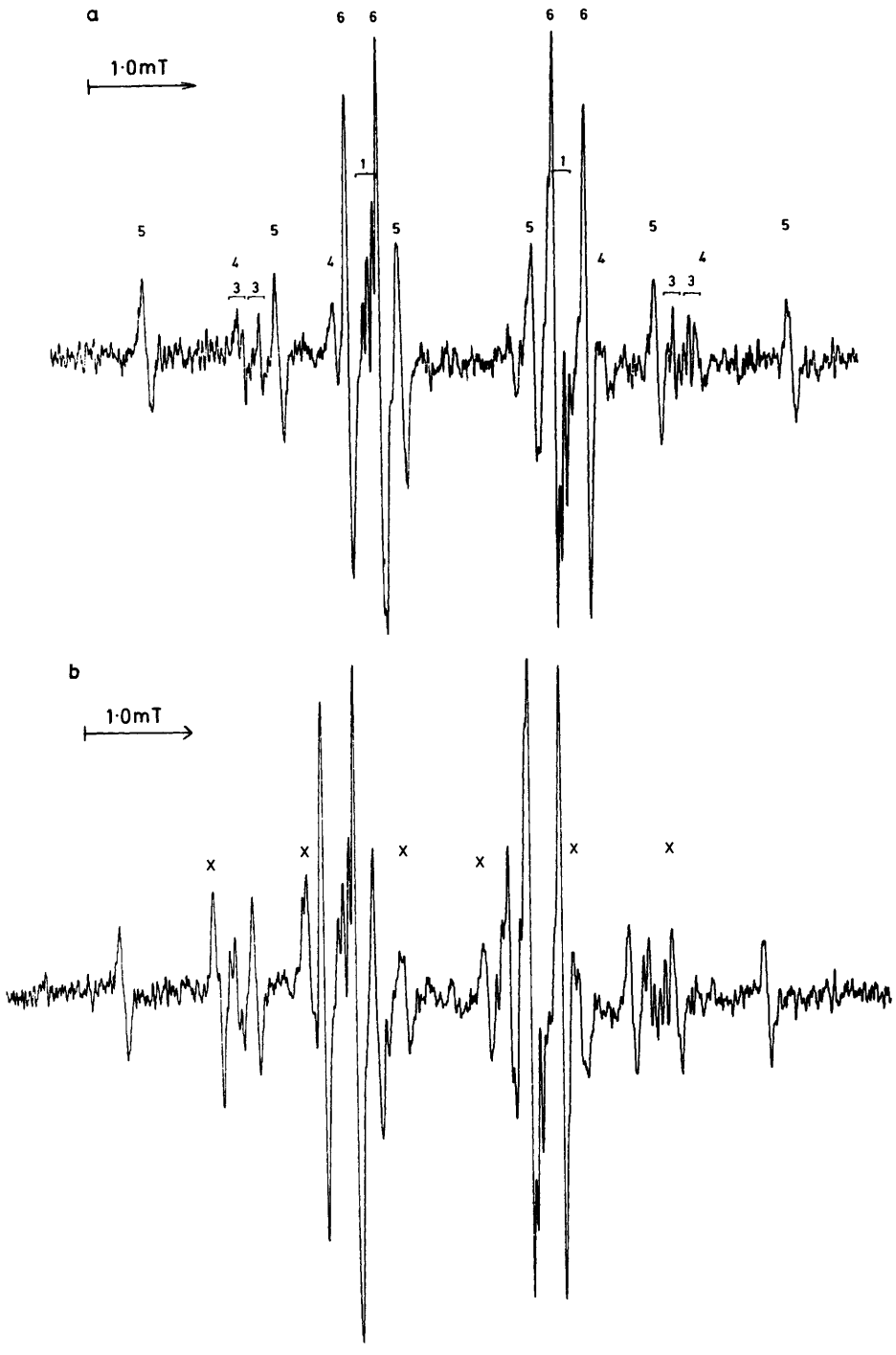
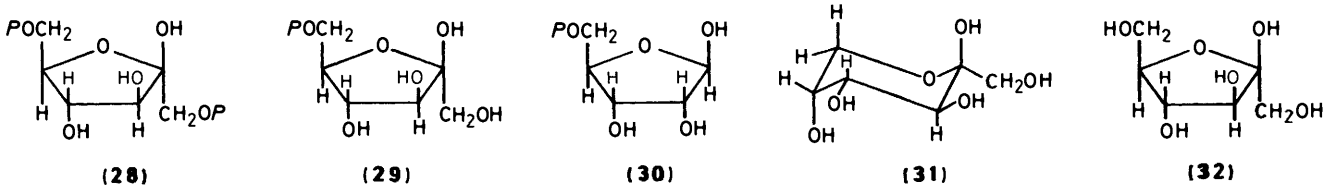


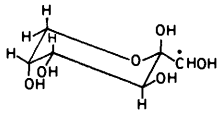
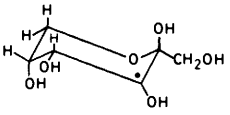
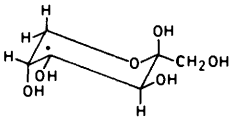
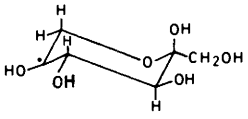
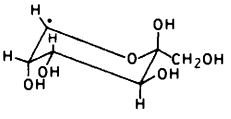
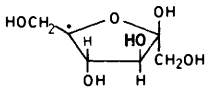
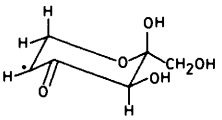
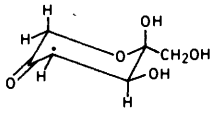
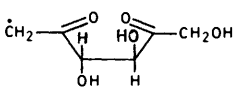
Figure 2. E.s.r. spectrum obtained from (a) the reaction of $^{\bullet}OH$ with β-D-fructopyranose at pH 4 (numbers refer to the carbon atom from which the hydrogen atom is abstracted) and (b) the reaction of $^{\bullet}OH$ with β-D-fructose at pH 4 following mutarotation of the sugar. Peaks marked × are from the radical (**33**)



(**28**) (**29**) (**30**) (**31**) (**32**)

axial β-proton splitting, and further fine structure; the 0.155 mT coupling is assigned to the α-OH proton, whilst the coupling of 0.055 mT is attributed to interaction with the two methylene protons on C(1). The spectrum attributed to the C(5)-derived radical was also clearly identified in the spectrum obtained from the epimer L-sorbose [which differs from (**31**) in the

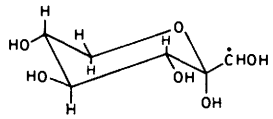
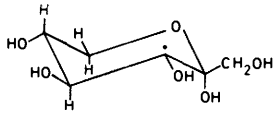
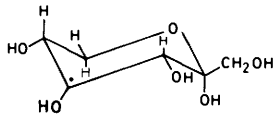
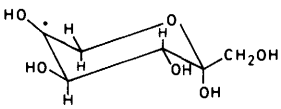
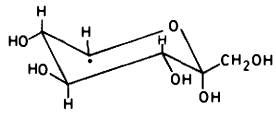
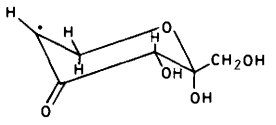
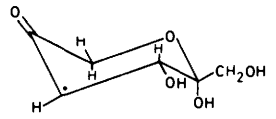
Table 3. E.s.r. parameters of radicals detected from the reaction of β-D-fructose with 'OH

	Hyperfine splittings (mT) [a]			
	a(α-H)	*a*(β-H)	*a*(other)	*g* Value [b]
(i) at pH 4				
	1.815(1)		0.100(1) 0.050(1)	2.003 15
		3.750(1)	0.155(1) 0.050(2)	2.0031
		3.360(1) 0.785(1)		2.0031
		2.380(2) 1.240(1)		2.003 05
	1.930(1)	0.290(1)		2.0031
 (33)		2.520(1) 0.885(2)		2.0031
(ii) at pH *ca.* 1				
 (34)	1.765(1)	4.010(1) 3.520(1)	0.370(1)	2.0042
 (35)	1.820(1)	3.602(1)	0.095(2)	2.0042
 (36)	1.925(2)		0.115(1)	2.0041

[a] ±0.005 mT. [b] ±0.0001.

configuration at C(5)]. The radical formed by hydrogen abstraction from C(4) is expected to have a spectrum characterised by splittings corresponding to one axial and one equatorial β-proton and is tentatively assigned the spectrum with *a*(β-H) 3.360 and 0.785 mT. Abstraction at C(6) also gives a spectrum comprising two doublets: an unusually high α-proton coupling is observed, evidently a consequence of the effect of the axial β-OH group modifying the geometry of the radical [*cf.* the locked conformation of $^{\cdot}CH(OH)CH_2OH$].[26] Such radicals which have a conformation in which the radical centre eclipses a β-oxygen function adopt a flatter configuration than their counterparts which cannot;[27] an unusual geometry is also suggested by the small coupling to the equatorial proton on C(5). These parameters clearly show that no ring-flipping (*e.g.*

Table 4. E.s.r. parameters of radicals detected from the reaction of α-L-sorbose with 'OH

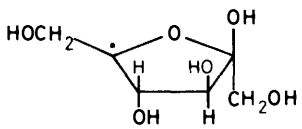
	Hyperfine splittings (mT) [a]			
	a(α-H)	*a*(β-H)	*a*(other)	*g* Value [b]
pH *ca.* 4				
	1.705(1)		0.100(1) 0.055(1)	2.0032
		2.805(1)	0.090(1)	2.0031
		3.04(1) 2.99(1)		2.0031
		2.380(2) 1.240(1)		2.003 05
	1.705(1)	3.540(1)	0.075(1)	2.0031
pH *ca.* 1				
 (39)	1.765(1)	{4.010(1) 3.520(1)	0.370(1)	2.0042
 (40)	1.820(1)	3.602(1)	0.095(2)	2.0042

[a] ±0.005 mT. [b] ±0.0001.

to either a $^5C_2$ chair or a $B_{2,5}$ boat conformation) has occurred, consistent with previous results which suggest that carbohydrate-derived radicals mostly adopt the structural conformation of the parent sugar (contrast the finding that certain tetramethylated and -acetylated glucosyl radicals are transformed into a boat conformation [28]).

The intensities of the individual spectra from the five possible pyranose radicals are consistent with essentially unselective attack.

Solutions of D-fructose which had been set aside for several minutes gave somewhat different spectra [Figure 2(b)]. Since solutions which have achieved equilibrium at room temperature comprise mainly β-D-fructopyranose (70%) and β-D-fructofuranose (23%),[25] the new lines evidently originate from 'OH attack on β-D-fructofuranose obtained by mutarotation. The spectra in particular show a signal from a radical with parameters characterising it as resulting from 'OH radical attack at C(5) in the furanose ring (**33**) [*i.e.* with a β-proton with pseudo-axial orientation (*a* 2.520 mT) and two equivalent β-protons in an exocyclic CH_2OH group]. No other furanose-derived radicals could be unambiguously characterised; this is probably attributable in part to the overlapping of lines from radicals of similar type [*e.g.* the furanose-derived C(1) radical is expected to have an e.s.r. spectrum very similar to that of its pyranose counterpart].



(**33**)

The intensity of the signals from (**33**) suggests that 'OH is *selective* in its reaction with the furanose ring, preferentially abstracting the hydrogen atom adjacent to the alicyclic oxygen: if the solution is assumed to contain 70% β-pyranose and 23% β-furanose sugar, unselective attack by 'OH would lead to the

Table 5. E.s.r. parameters of radicals formed following reaction of 'OH with furanose phosphates

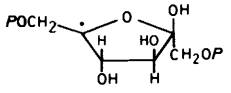
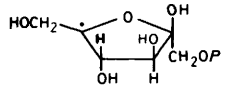
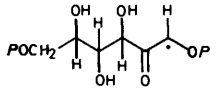
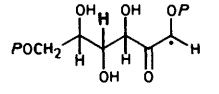
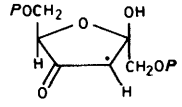
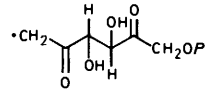
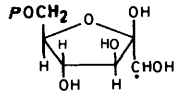
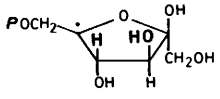
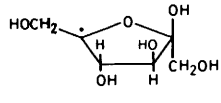
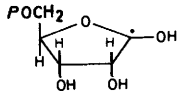
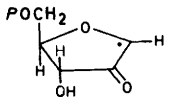
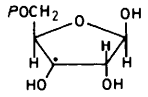
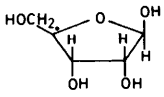
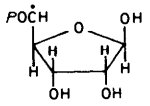
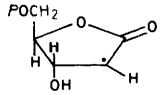
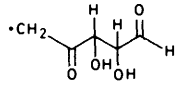
	Hyperfine splittings (mT) [a]			
	a(α-H)	*a*(β-H)	*a*(other)	*g* Value [b]
Fructose 1,6-diphosphate				
 (41)		1.978(1) 0.595(2)	0.07 (^{31}P) 0.025(1)	2.0031
 (42)		2.520(1) 0.885(2)		2.0031
 (43)	1.525(1)		0.350 (^{31}P)	2.0047
 (44)	1.513(1)		0.253 (^{31}P)	2.0047
 (45)	1.800(1)		0.140(1)	2.0045
 (46)	1.925(2)		0.113(1)	2.004 05
Fructose 6-phosphate				
 (47)	1.963			2.0031
 (48)		1.978(1) 0.595(2)	0.07 (^{31}P) 0.025(1)	2.0031
 (33)		2.520(1) 0.885(2)		2.0031
Ribose 5-phosphate				
 (49)		2.050(1)	0.175(1)	2.0031

Table 5 (*continued*)

	Hyperfine splittings (mT) [a]			
	a(α-H)	*a*(β-H)	*a*(other)	*g* Value [b]
Ribose 5-phosphate				
 (50)	1.343(1)		0.530(1) 0.253(1)	2.0049
 (51)		4.860 (1 + 1)		2.0031
 (52)		1.350(1) 0.780(2)	0.171(1)	2.0030
 (53)	1.80(1)	1.40(1)		2.0032
 (54)	2.050(1)	2.880(1)		2.0032
 (55)	1.944(2)			2.0043

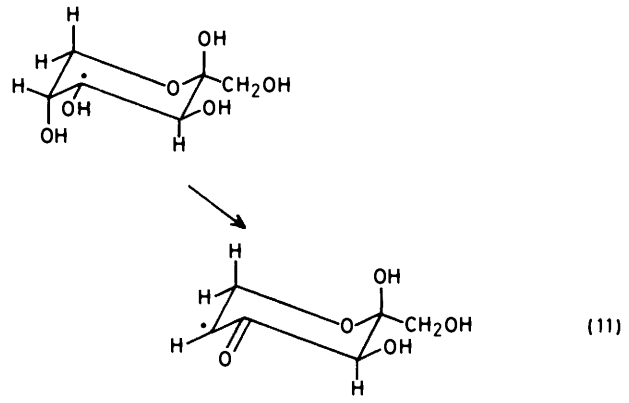
[a] ±0.005 mT. [b] ±0.0001.

formation of the C(5) (furanose) radical as *ca.* 3% of the total radical yield [*cf. ca.* 10% for the C(5) (pyranose) radical]. The decrease in the intensity of the C(5) (pyranose) signals following mutarotation to 64% of their original value is consistent with a change in solution composition from 100% β-pyranose to 70% β-pyranose, and suggests that the overall reactivities of furanose and pyranose sugars are approximately the same.* The intensity of the signal found for attack at C(5) in the furanose ring represents *ca.* 50% of the total 'OH attack in this ring (*cf.* also the reaction of sucrose[30]). This indicates a degree of selectivity in the reaction of 'OH not encountered in the free-radical chemistry of pyranose sugars (this may hold particular importance for the free-radical chemistry of polynucleotides, where radical formation at a site adjacent to an alicyclic oxygen in a furanose sugar has been implicated in radiation-induced damage). Malatesta and Ingold have observed a similar preference for the reaction of Bu^{t}O$^\cdot$ with hydrogen atoms adjacent to the alicyclic oxygen of furanose ethers as compared with pyranose and acyclic analogues,[31] a result interpreted in terms of the interaction between the lone pair of electrons on the alicyclic oxygen[32] and the developing radical centre, coupled with the relief of ring-strain which occurs in furanose rings on formation of the radical. The reaction of 'OH and fructofuranose is interpreted on a similar basis.

When freshly dissolved solutions of β-D-*fructopyranose* were treated with 'OH at low pH, signals from the first-formed radicals were replaced by lines attributable to species formed by an acid-catalysed dehydration (Table 3). The first new signals arise from rearrangement of the C(4)-(pyranose)-derived radical to give (**34**) [reaction (11)]: loss of an axially orientated β-OH group thus occurs more readily than comparable rearrangements where the leaving group is equatorial (in accord with previous findings for radicals formed from 'OH and both glucose and myoinositol). Signals from the precursor had disappeared by pH *ca.* 2.2, consistent with a rate constant for reaction (11) of *ca.* 10^6 dm^3 mol^{-1} s^{-1}.

At lower pH a second carbonyl-conjugated species (**35**) (*g* 2.0042) results from rearrangement of the C(5)-derived radical [an identical radical is formed through acid-catalysed transformation of a precursor radical from L-sorbose (see later), showing that the stereochemistry at C(5) is lost]. The pH for half-removal of signals from the precursor (1.2) is typical of the loss of an equatorial OH group, with k_r *ca.* 10^5 dm^3 mol^{-1} s^{-1}. The splittings of (**34**) and (**35**) show that the integrity of the chair conformations of the first-formed radicals has to some extent

* The rate constant for the reaction of hydroxyl radical with sucrose is the same as that for the reaction of 'OH with the exclusively pyranose disaccharide maltose,[29] suggesting that the *overall* reactivities of carbohydrates are independent of configuration.

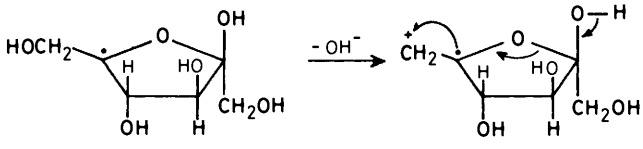


(34)

been lost: the carbonyl group and radical centre on C(4) and C(5) presumably force a more planar arrangement of the atoms C(3) to C(6) in the pyranose ring (a similar flattening occurs in cyclohexenyl[33]).

No products derived from the rearrangement of pyranose radicals formed by reaction at C(2) and C(3) could be detected.

Lowering the pH in experiments with D-fructose after mutarotation gave rise to extra signals attributable to the radical (**36**) derived from an acid-catalysed ring-opening of (**33**) [reaction (12)]. At least two possible mechanisms may be envisaged: (i) an acid-catalysed loss of β-OH would lead to the formation of a radical-cation which can undergo rehydration to give the hemiacetal which in turn can ring-open to give the detected product, as previously postulated for the ring-opening of the analogous radical derived from tetrahydrofurfuryl alcohol.[1] Alternatively (ii) the ring-opening reaction could occur directly from the radical-cation [as depicted in reaction (12)]. The failure of the related radical (**37**) from sucrose to



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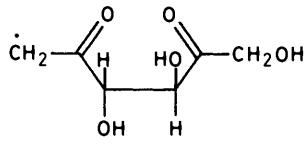

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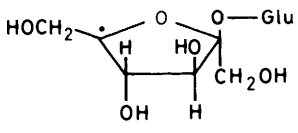
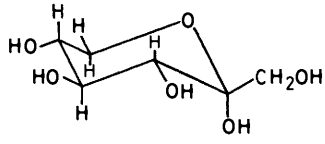


(37)

undergo ring-opening at low pH[30] suggests that deprotonation of the C(2)OH group plays an important part in the reaction, *i.e.* that ring-opening and deprotonation are concerted.

Solutions of L-*sorbose* [epimeric with L-fructose, the configuration at C(5) being reversed] which have achieved equilibrium consist almost entirely of the α-pyranose anomer[25] (**38**), and the spectra obtained from reaction of $^{\bullet}OH$ are consistent with radicals derived from this anomer. All five radicals which can be obtained by direct hydrogen-abstraction were detected at pH 4 (Table 4). The C(1)-derived species has an α-hydrogen coupling of 1.705 mT, notably lower than the analogous radical from β-D-fructopyranose [such as decrease is difficult to rationalise simply in terms of epimerisation at C(5), although a similar effect has been noted for the radicals formed by hydrogen-atom abstraction at the exocyclic C(6) in the epimers D-glucose and D-galactose[21]]. Other hyperfine couplings are essentially the same as those from β-D-fructose and are attributed to interactions with the hydroxy proton on C(1) and the axial γ-H on C(3). The spectrum of the C(3)-derived radical has the pattern expected for a radical with an axial β-H; small couplings to the α-OH and a γ-proton are also observed. The latter interaction is assigned to the axial proton on C(5); the absence of a similar coupling in the analogous radical from β-D-fructose (where this proton is equatorial) supports such an assignment. No coupling to the two methylene protons on C(1) is observed, but the large linewidth suggests the presence of some unresolved interactions. Attack of $^{\bullet}OH$ at C(4) gives rise to a radical with coupling to two almost equivalent axial protons, though the assignment is tentative since the outer lines of the spectrum are partially hidden. The C(5)-derived radical is identical with its counterpart detected following reaction of $^{\bullet}OH$ with D-fructose. Finally, the product of oxidation at C(6) shows couplings to an α-H and axial β-H.



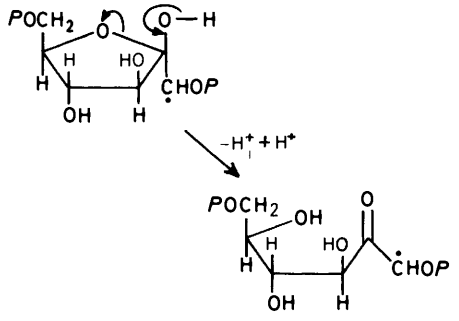
(38)

As the pH was lowered, signals from the first-formed α-hydroxyalkyl radicals gradually disappeared until at pH 0.5 signals from the C(5)-derived radical were finally removed. The two new radicals, (**39**) and (**40**), have parameters identical with those observed at low pH following reaction of $^{\bullet}OH$ with D-fructose. However, it is notable that signals from (**39**) in the reaction of sorbose appear at a lower pH than for (**34**) from fructose, indicating that the loss of the equatorial C(5)–OH group from the C(4)-derived radical is slower than the loss of an axial OH group from the corresponding fructose-derived radical.

(ii) *Fructose* 1,6-*diphosphate*. The dominant first-formed radical (**41**), the formation of which appears to be favoured for stereoelectronic reasons [*cf.* the analogous radical (**33**) from fructose] shows the large axial-proton splitting and small β-methylene proton splitting of the latter (together with a long-range phosphorus splitting). The lower value of a(β-H) for (**41**) is indicative of its adoption of a more-nearly locked conformation in which the β-C–O bond more effectively eclipses the orbital containing the unpaired electron (such conformational locking is interpreted in terms of a favourable interaction between the SOMO and the σ* orbital of the β-C–O bond, encouraged by the $+M$ effect of the α-alkoxy substituent and the increased $-I$ effect of the β-phosphate.[26,27]) It is also clear that the radical detected following reaction at C(5) retains the β-phosphate group. However, weaker lines with parameters almost identical to those from (**33**) suggest the presence of a minor radical (**42**) which has lost the phosphate group. We therefore suggest that elimination of the phosphate group from (**41**) occurs at a rate which leads to the detection of both initial (major) and rearranged radicals, *i.e.* that the rate constant is in the region 10^2—10^3 s^{-1}. This value is in agreement with the rate

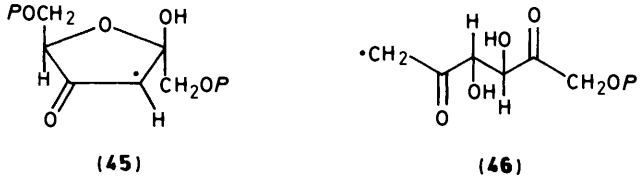
of elimination of phosphate groups from several 2-methoxyethyl phosphate 2-radicals[34] (but lower than those observed for glycerol phosphates and α-D-glucose 1-phosphate).

The isomeric radicals (**43**) and (**44**) (*g* 2.0047) were formed at all pH values studied, although the signals were more intense above pH 6 [presumably a consequence of further ionisation of the phosphate groups to the dianions, slowing radical dimerisation reactions through electrostatic repulsion: a decrease in $a(^{31}P)$ was observed, as with other radicals with α-phosphate groups]. It is suggested that these radicals are formed *via* initial hydrogen-abstraction from C(1) followed by rapid ring-opening ($k > 10^3$ s^{-1}) [reaction (13)]. The C(1)-phosphate group is a critical influence in this ring-opening reaction, since similar radicals formed from furanose sugars without this substituent do not show such reactions (see later).* No signals originating from attack at C(3) or C(4) were directly characterised, although spectra recorded at low pH indicate the initial formation of the C(4)-centred radical. Two new signals are observed at pH *ca.* 1.5, both with *g ca.* 2.004, consistent with carbonyl-conjugation, and are assigned to structures (**45**) and (**46**). The former is evidently derived from attack at C(4) and rearrangement as described for pyranose radicals: the apparent absence of the precursor at higher pH (though its spectrum may have been obscured) may indicate that this process is faster for the furanose ring. The radical (**46**) is believed to arise *via* acid-catalysed ring-opening of the initially formed radical (**41**), either directly from the radical-cation formed both by rapid elimination of the β-phosphate group (faster at lower pH) and the β-hydroxy group which replaces it [in (**42**)] [*cf.* reaction (12)] or *via* hydration to give a hemiacetal and subsequent fragmentation.



(13)

(**43**), (**44**)

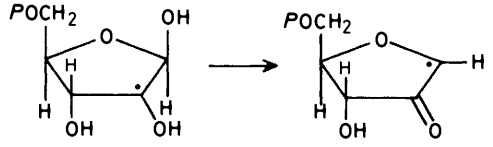


(**45**) (**46**)

(iii) *Fructose 6-phosphate.* Attack takes place chiefly at C(1), to give (**47**) [though subsequent ring-opening is not observed in the absence of the C(1)-phosphate substituent] and C(5) [to give (**48**) and (**33**) and, at low pH, a signal identical with that from (**46**)]. Poor resolution hampered further analysis and identification of any radicals resulting from attack at C(3), C(4), or C(6).

(iv) *Ribose 5-phosphate.* Signals from radicals formed by oxidation at all positions in the carbon skeleton of ribose 5-phosphate (a model for the ribofuranose moiety in nucleotides) have been assigned; the spectra indicate that although 'OH reacts selectively, favouring abstraction of the C(4)-bond, the preference is not as great as that observed in β-D-fructofuranose or fructose 1,6-diphosphate.

A radical with *a*(H) 2.050 and 0.175 mT is assigned to structure (**49**), formed by hydrogen-abstraction at C(1), with splittings from a β-proton and the C(4) γ-H, respectively. The most striking feature of the overall spectrum is an eight-line signal with *g* 2.0049, indicative of conjugation to both α-oxygen and carbonyl substituents, and consistent with structure (**50**), formed by 'OH attack at C(2) and subsequent elimination of water [equation (14)]. This process occurs throughout the pH range studied (2—6), and does not require acid catalysis. Such a spontaneous elimination of water has not previously been encountered in the free-radical chemistry of sugars (although several examples from substituted α,β-dihydroxyalkyl radicals have been described [*e.g.*[18] 'CH(OH)CMe$_2$OH], and is evidently a result of favourable overlap between the lone pair of electrons on the furanose alicyclic oxygen and the incipient radical centre (note that the pyranose equivalent requires acid-catalysis[21]).



(14)

(**50**)

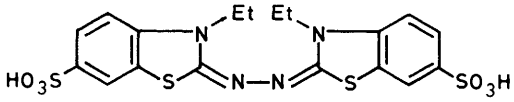
Two broad lines at the extremities of the spectrum (total width 4.8 mT) are assigned to the C(3)-centred radical (**51**), with couplings to one pseudoaxial and one pseudoequatorial proton (individual splittings could not be assigned). The radical (**52**) has a pseudoequatorial proton and exocyclic methylene-proton splitting, as well as a splitting of *ca.* 0.2 mT from H(1), which evidently arises through delocalisation of spin density *via* the ether oxygen (a comparable splitting does not occur in the fructofuranose-derived radicals). The radical (**52**) is formed by phosphate loss *via* a radical-cation in a manner similar to the formation of (**42**) from (**41**). Since the initial radical is not detected, phosphate elimination in ribose 5-phosphate is faster than from the corresponding radical (**41**) from fructose 1,6-diphosphate ($> 10^3$ s^{-1}). The C(5)-derived radical (**53**) is characterised by small long-range couplings [*cf.* the C(6)-centred radical from D-glucose] and a typical phosphorus coupling.

At low pH the C(1)-centred radical rearranged to give a carboxy-conjugated radical (**54**), through an acid-catalysed dehydration analogous to those observed with the pyranose saccharides. (It was impossible to record spectra over a range of pH values; consequently the rate of elimination in pyranose and furanose saccharides cannot be compared, although available evidence suggests that these rates are similar.) Lines from a spectrum consistent with the radical (**55**) appear in conjunction with the disappearance of the signal from (**52**). Clearly the acid-catalysed ring-opening observed in the fructose phosphates also occurs with this substrate [reaction (12)].

Determination of the Rate of Rearrangement of Sugar-derived Radicals by Pulse Radiolysis.—The technique of pulse radiolysis has previously been employed to monitor rearrangements from relatively simple α,β-dihydroxyalkyl radicals (ref. 18 and references therein), utilising the absorption of the carbonyl-conjugated radicals produced ($\lambda_{max.}$ *ca.* 210 nm; ε *ca.* 5×10^3 dm^3 mol^{-1} cm^{-1}). The monitoring of reactions at such low

* Rate constants for ring-opening of aldose and ketose phosphates are higher than those of their non-phosphorylated analogues.[35] Thus 6-*O*-methylfructose 1-phosphate anomerises with $k = 130$ s^{-1} at pH 7.5 and 40 °C, whilst 6-*O*-methylfructofuranose has $k = 1.3$ s^{-1} under similar conditions; there is a marked decrease (by a factor of *ca.* 60) in the rate of ring-opening on lowering the pH to 4.2.

wavelengths however is particularly difficult for radicals from sugars, and it was therefore considered preferable to design indirect methods to study rearrangements described here. For example, it should be possible to differentiate between first-formed (α,β-dihydroxyalkyl) and secondary (carbonyl-conjugated) radicals on the basis of their redox properties. Thus α,β-dihydroxyalkyl radicals are (relatively) easily oxidised, while the secondary carbonyl-conjugated radicals are more easily reduced (see *e.g.* ref. 20). The reagent 2,2′-azinobis-(3-ethyl-2,2′,3,3′-tetrahydrobenzothiazole-6-sulphonate) [ABTS (**56**)] is known[36] to yield a particularly stable radical-cation ($ABTS^{+\bullet}$), with a high extinction coefficient at 414 nm, following reaction with oxidising radicals (*e.g.* with $^{\bullet}OH$; k 1.2×10^{10} dm^3 mol^{-1} s^{-1}). It was expected that α,β-dihydroxy radicals would react slowly, if at all, with ABTS, while carbonyl-conjugated radicals (formed through subsequent rearrangements) would react rapidly, to give $ABTS^{+\bullet}$. Monitoring the appearance and yield of $ABTS^{+\bullet}$ should thus allow determination of the occurrence (and possibly the rate) of dehydration reactions.



(**56**)

Conversely, it has been previously shown that α,β-dihydroxyalkyl radicals are readily *oxidised* by the one-electron oxidant 1,1-dimethyl-4,4-bipyridinium dichloride (methylviologen, MV^{2+}).[18,37] Thus, in principle, the kinetics of dehydration could also be monitored using competition between rearrangement and reduction with MV^{2+}. These complementary approaches have been employed here to study the rates of rearrangements of sugar-derived radicals.

(a) *Reactions of* α-D-*glucose in the presence of* ABTS. Solutions typically contained *ca.* 0.1 mol dm^{-3} of the sugar (which ensures complete scavenging of both the hydroxyl radicals and hydrogen atoms), and ABTS (*ca.* 1×10^{-4} mol dm^{-3}). Solutions were deoxygenated and saturated with N_2O prior to pulse radiolysis; the formation of $ABTS^{+\bullet}$ was monitored at 414 nm (ε_{414} 3.6×10^4 dm^3 mol^{-1} cm^{-1}).[36] The rate of build-up of optical absorption* was analysed to give kinetic information on the reaction under study, while the asymptotic value of the optical absorption is directly related to the concentration (or yield) of transients produced. The calculation of yields is based on 100% conversion of $^{\bullet}OH$ to give $ABTS^{+\bullet}$.

The yield of $ABTS^{+\bullet}$, determined over a range of pH, was found to be a minimum between pH *ca.* 3 and 5 [Figure 3(a)]. The small yield in this pH range is attributed to the direct reaction of $^{\bullet}OH$ with ABTS, and the rate constant for the build-up of this absorption was in keeping with this conclusion; carbonyl-conjugated radicals are apparently not formed at this pH (see earlier). The marked increase in the yield below pH *ca.* 3 is interpreted as resulting from acid-catalysed rearrangement of first-formed radicals, producing carbonyl-conjugated species which subsequently react with ABTS. In some cases two components of the formation of $ABTS^{+\bullet}$ could be distinguished [see Figure 3(b)], both of which showed first-order kinetic behaviour with a rate constant linearly dependent on [ABTS]; the fast (and dominant) process produces $ABTS^{+\bullet}$ in high yield with a second-order rate constant determined as $8.8 \pm 0.2 \times 10^8$ dm^3 mol^{-1} s^{-1} (at pH 1.25 and 20 °C), while the second component is characterised by k $1.1 \pm 0.2 \times 10^6$ dm^3 mol^{-1} s^{-1}.

* This was usually found to be pseudounimolecular (first-order): second-order kinetics are not expected since [ABTS] ≫ [oxidising radicals].

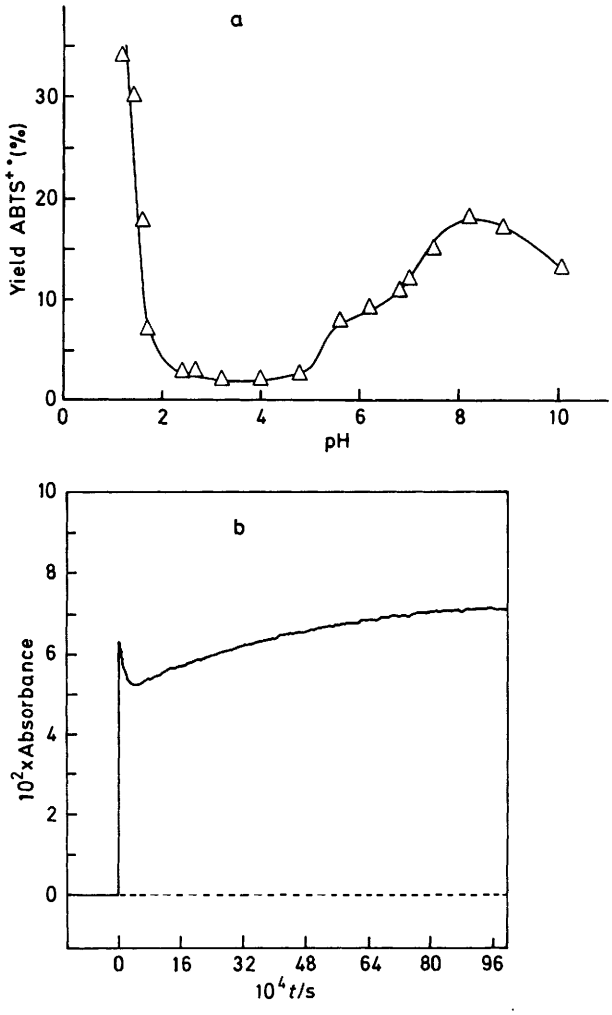


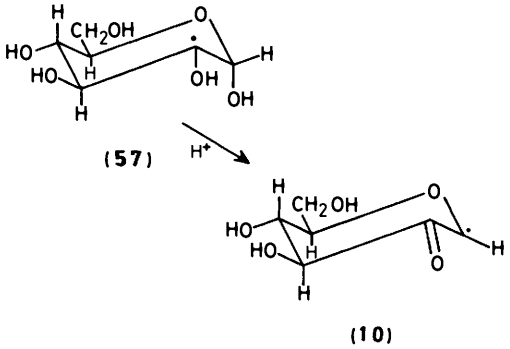
Figure 3. (a) Variation in the yield of $ABTS^{+\bullet}$ (based on 100% conversion of $^{\bullet}OH$ into $ABTS^{+\bullet}$) with pH in the reaction of $^{\bullet}OH$ (generated *via* pulse radiolysis) with α-D-glucose. (b) Variation with time of the absorption from $ABTS^{+\bullet}$ following pulse radiolysis of a solution containing α-D-glucose and ABTS at pH 1.7

The separate components may arise *via* reactions in which the rate-determining step in $ABTS^{+\bullet}$ production is the rearrangement leading to the oxidising (carbonyl-conjugated) radical, *i.e.* carbonyl-conjugated radicals (presumably of different structure) are formed *via* two distinct rearrangements, one of which is significantly faster than the other (*e.g.*, as suggested by e.s.r. results, that loss of axial β-OH is faster than loss of equatorial β-OH). A second possibility is that rearrangements lead to two discrete types of oxidising radical, and that the two processes observed result from different rates of reaction with ABTS.

However if the rate-determining step in $ABTS^{+\bullet}$ production is the *formation* (*via* acid-catalysed dehydration) of oxidising radical, then the rate of formation of $ABTS^{+\bullet}$ should be independent of [ABTS], whereas if oxidation of ABTS is the rate-determining step, the rate of formation of $ABTS^{+\bullet}$ will have a linear dependence upon [ABTS] (as observed). We conclude that the different rates of formation of $ABTS^{+\bullet}$ reflect the rates of subsequent reactions of carbonyl-conjugated radicals of different type. The rate constants are consistent with this: thus the rate of generation of carbonyl-conjugated radicals is given by $k_r[H^+]$, and with $[H^+]$ *ca.* 0.08 mol dm^{-3}, and k_r (estimated from e.s.r. observations) *ca.* 10^6 dm^3 mol^{-1} s^{-1}, the rate of formation of carbonyl-conjugated species is *ca.* 8×10^4 s^{-1}, significantly greater than the observed rate of appearance of

$ABTS^{+\bullet}$ at the concentrations of ABTS used (*ca.* 0.2 to 1.5 × 10^{-4} mol dm^{-3}). [In addition, the ratio of axial to equatorial OH groups in α-D-glucose implies that the slower component in the formation of $ABTS^{+\bullet}$ should give significantly higher yields of $ABTS^{+\bullet}$ if this component arises *via* a slower (rate-determining) dehydration, contrary to observation.]

The C(2)-derived radical (**57**) undergoes the most rapid rearrangement of any of the first-formed radicals from α-D-glucose, *via* loss of the axial C(1)OH group, to give (**10**) [reaction (15)]. This radical is unique amongst the (detected) rearrangement products in having conjugation to both carbonyl and α-oxygen substituents. It is therefore expected to be less susceptible to reduction than other carbonyl-conjugated radicals produced. We suggest that the slower build-up of $ABTS^{+\bullet}$ represents its reaction with ABTS (k 1.1 × 10^6 dm^3 mol^{-1} s^{-1}), in contrast with the higher value of 1.8 × 10^8 dm^3 mol^{-1} s^{-1} for other carbonyl-conjugated radicals. Supporting evidence is described later.



(15)

The yield of $ABTS^{+\bullet}$ also increased at pH > 5. A rearrangement which involves ionisation of the α-hydroxy group[16] again gives carbonyl-conjugated radicals [not detectable in the e.s.r. system owing to rapid reduction by Ti^{III}–EDTA at this pH (ref. 20)]. The somewhat lower yield of $ABTS^{+\bullet}$ (in comparison with low pH) is thought to arise from competing base-catalysed rearrangements which give semidione radicals.[38]

Although the experiments with ABTS indicate the presence of rapid acid-catalysed rearrangements of the first-formed α,β-dihydroxyalkyl radicals, the rates of rearrangement could not be determined by this technique. However, the acid-catalysed rearrangement of α,β-dihydroxyalkyl radicals [reaction (16)] should, in the presence of methylviologen (MV^{2+}), be in competition with reaction (17).[18] Kinetic analysis then leads to equation (18): in this expression $[MV^{+\bullet}]$ and $[MV^{+\bullet}]_0$ represent the concentrations (directly proportional to the observed optical densities) of the methylviologen radical-cation in experiments at pH values where there is competition and no significant competition respectively. The value k_{17} was measured by kinetic analysis of the formation of the absorption due to $MV^{+\bullet}$ at 602 nm (ε_{602} $MV^{+\bullet}$ 1.37 × 10^4 dm^3 mol^{-1} cm^{-1})[39] at pH 5 (where studies using ABTS indicate there to be no significant rearrangement).

$$R^\bullet + H^+ \xrightarrow{k_{16}} S^\bullet \quad (16)$$

$$R^\bullet + MV^{2+} \xrightarrow{k_{17}} MV^{+\bullet} + R^+ \quad (17)$$

$$\frac{[MV^{+\bullet}]_0}{[MV^{+\bullet}]} - 1 = \frac{k_{16}[H^+]}{k_{17}[MV^{2+}]} \quad (18)$$

The concentration of $MV^{+\bullet}$ was determined at a variety of pH values following pulse radiolysis of a solution of *ca.* 0.1 mol dm^{-3} α-D-glucose and *ca.* 1 × 10^{-4} mol dm^{-3} MV^{2+} (and sufficient acid to achieve the required pH). A plot of

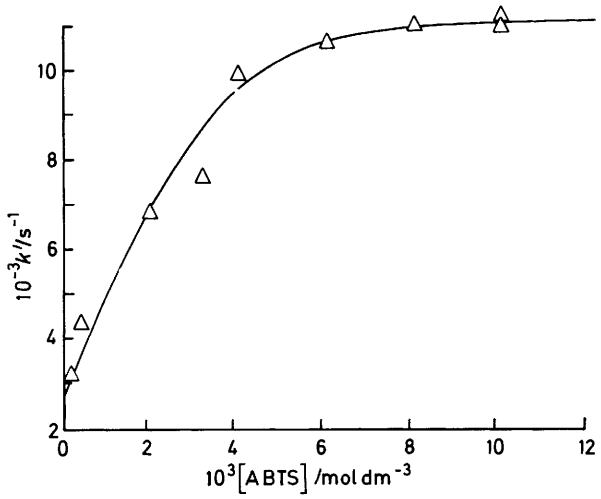


Figure 4. Variation with [ABTS] in the pseudo-first-order rate constant (k') for the build-up of the absorption from ($ABTS^{+\bullet}$) following reaction of $^\bullet OH$ with α-D-glucose 1-phosphate at pH 4

$[MV^{+\bullet}]_0/[MV^{+\bullet}] - 1$ *versus* $[H^+]/[MV^{2+}]$ gave a straight line (provided that only low values of $[H^+]$ were used*).

The bimolecular rate-constant k_{17} was determined from a plot of the first-order rate constant k'_{17} *versus* $[MV^{2+}]$, and was found to be 1.20 ± 0.1 × 10^8 dm^3 mol^{-1} s^{-1}, in good agreement with the rate of oxidation of other similar α,β-dihydroxyalkyl radicals (*e.g.* myoinositol, k = 1.0 × 10^8 dm^3 mol^{-1} s^{-1}). Substituting the value of k_{17} into equation (18) gives k_{16} 1.10 ± 0.06 × 10^6 dm^3 mol^{-1} s^{-1}. This rate evidently represents an average value for the dehydration reactions of structurally different first-formed radicals (*e.g.* with axial *versus* equatorial leaving group): the number of reactions which contribute to this value precludes its resolution into its individual components. The value is within the range of rate constants measured by e.s.r. for different radicals from α-D-glucose and fructose (see earlier), and in keeping with those for structurally similar radicals (*e.g.* values of 7 × 10^5 and 6 × 10^4 dm^3 mol^{-1} s^{-1} for the radicals for myoinositol[18]). That the value is somewhat less than those for alicyclic analogues such as glycerol (*ca.* 10^7 dm^3 mol^{-1} s^{-1}) may reflect the increased number of (−*I*) hydroxy groups, the ring strain incurred in forming the transition state from the cyclic species, or the relative difficulty in protonating β-equatorial groups.[18]

(b) *Determination of rate constants for the elimination of phosphate.* The e.s.r. results presented earlier suggest that fragmentation of β-phosphate-substituted radicals occurs with k *ca.* 10^3—10^4 s^{-1} (in the pH range 2.5—6: more rapid fragmentation is expected at lower pH). Although the competition between fragmentation and reduction of MV^{2+} is inappropriate for studying this reaction (since it is expected that elimination is too rapid), it should be possible to employ ABTS to scavenge carbonyl-conjugated radicals (if these are formed) at a rate determined by the rate of the rearrangement.

On pulse radiolysis of solutions containing glycerol 1-phosphate (*ca.* 0.1 mol dm^{-3}) and ABTS (typically 2 × 10^{-5} to 1.2 × 10^{-2} mol dm^{-3}), deoxygenated and saturated with N_2O, the rate of formation of $ABTS^{+\bullet}$ was found to depend upon [ABTS], with a linear relationship at low [ABTS]. Kinetic analysis leads to a pseudounimolecular (first-order) rate constant for the appearance of $ABTS^{+\bullet}$ and hence to a rate-constant of 2.13 ± 0.14 × 10^7 dm^3 mol^{-1} s^{-1} for the oxidation

* This is interpreted in terms of the reversibility of the protonation of $R^\bullet$ (see, *e.g.* ref. 18).

of ABTS by $^{\bullet}CH_2COCH_2OH$. This is notably lower than the rate of reaction of ABTS with the majority of carbonyl-conjugated radicals from α-D-glucose (see earlier), and may reflect the absence of β-hydroxy substituents in the former radical. At higher [ABTS], the linear relationship between [ABTS] and the pseudounimolecular (first-order) rate constant was found to break down, suggesting that the formation of the carbonyl-conjugated species is now rate-determining. From the limiting value of the first-order rate constant a value for the loss of phosphate [reaction (4)] of k_r 1.4×10^4 s^{-1} (20 °C; pH 3.5) was obtained, in agreement with the e.s.r. results, which set a lower limit of k_r of 10^3 s^{-1}, and similar to the rates observed in some related radicals [*e.g.* $^{\bullet}CH(OMe)CH_2OPO_3H^-$, *k ca.* 10^3 s^{-1} [34]].

Pulse radiolysis was also employed to determine the rate of phosphate elimination from radicals derived from α-D-glucose 1-phosphate at pH *ca.* 4, *via* formation of $ABTS^{+\bullet}$. At low [ABTS] the rate of production of $ABTS^{+\bullet}$ was found to be directly proportional to the concentration of ABTS (Figure 4), and a value of $1.8 \pm 0.4 \times 10^6$ dm^3 mol^{-1} s^{-1} was obtained for the rate constant for reduction of (**10**). This is almost identical with the rate of the slower process observed in the oxidation of ABTS by radicals produced *via* acid-catalysed rearrangement of radicals from α-D-glucose, and supports the assertion that (**10**) is responsible for the second component of the formation of $ABTS^{+\bullet}$ observed. At high concentrations of ABTS the linear relationship breaks down, and from the maximum rate of production of $ABTS^{+\bullet}$ a rate of 1.2×10^4 s^{-1} for reaction (7) is obtained. This is similar to the rate observed for the elimination of phosphate from glycerol 1-phosphate: although it might be expected that cyclic species would undergo elimination more slowly than their acyclic analogues (as a result of ring strain incurred in forming the transition state), it is believed that the axial orientation of the β-phosphate group in (**9**) compensates for this.

Conclusions

In the reaction of the hydroxyl radical with some sugar phosphates, elimination of phosphate follows attack to produce a radical centre adjacent to the carbon bound to the phosphate. Such a mode of initial attack is preferred for fructose 1,6-diphosphate, fructose 6-phosphate, and β-D-fructofuranose itself, less marked for ribose 5-phosphate, and absent in the corresponding pyranose sugars. Selectivity is believed to result from overlap between the incipient radical centre and the lone pair of electrons on the alicyclic oxygen, an interaction which may also account for the rapid elimination of water from the C(2)-centred radical from ribose 5-phosphate.

Fructose 1,6-diphosphate and fructose 6-phosphate give rise to β-phosphate-substituted radicals which rearrange by loss of phosphate (*k ca.* 10^2—10^3 s^{-1}) somewhat slower than analogous radicals from glycerol phosphates, ribose 5-phosphate, and α-D-glucose phosphate (*k* 10^4 s^{-1}, as suggested by pulse radiolysis and steady-state e.s.r. experiments). A set of analogous reactions at low pH lead to ring-opening, probably *via* reaction to give a radical-cation [see reaction (12)].

These observations lend considerable further support to the suggestion[40] that radical-induced strand-breakage in irradiated DNA involves analogous hydrogen-abstraction at the carbon atom attached to oxygen in the furanose ring (either directly, by $^{\bullet}OH$, or *via* base-derived radicals) followed by rapid cleavage of the β(C)-phosphate bond.

Experimental

E.s.r. spectra were recorded with a Varian E104 spectrometer employing 100 kHz modulation and equipped with an *X*-band klystron. Hyperfine splittings were measured from the spectrometer field scan, which was calibrated using an aqueous solution of Fremy's salt [a(N) = 1.3091 mT (ref. 41)]. *g*-Factors were measured (to ±0.0001) by comparison with Fremy's salt [*g* 2.005 50 (ref. 42)], or with $^{\bullet}CHMeOH$ [*g* 2.003 21 ± 0.000 05 (ref. 43)], itself checked with Fremy's salt. In some cases absolute radical concentrations were obtained by comparison of the doubly integrated e.s.r. signal with that of $^{\bullet}CH_2OH$ from methanol (under identical spectrometer settings and flow conditions), this in turn being checked by comparison with the signal from vanadyl sulphate (*ca.* 10^{-3} mol dm^{-3}). Double integrations were performed using a Datalab DL4000 microcomputer. Computer simulation was carried out with a DEC system KL-10 computer using a program kindly supplied by Dr. M. F. Chiu.

Flow experiments employed a mixing chamber which allowed the simultaneous mixing of three reagent streams *ca.* 50 ms before passing through a flattened cell in the cavity of the e.s.r. spectrometer. The flow was maintained by use of a Watson-Marlow 5025 flow-inducer on the inlet tubing and the pH was monitored with a Pye Unicam PW9410 pH meter (with the electrode positioned in the effluent). pH Values were measured to within ±0.05 except in the pH range 4—8 where poorer buffering of the solution limited the accuracy to ±0.1. Solutions were made up in distilled or deionised water and were deoxygenated prior to and during use with oxygen-free nitrogen. For the generation of hydroxyl radical, solutions were made up containing in the first stream titanium(III) chloride (0.008 mol dm^{-3}), in the second stream hydrogen peroxide (0.05 mol dm^3), and the substrate (up to 0.1 mol dm^{-3} depending on solubility) in the third stream. For reactions at pH < 2.5 the first stream contained enough concentrated sulphuric acid to give the desired pH. Reactions above pH 2.5 were performed with the addition of the disodium salt of ethylenediaminetetra-acetic acid (EDTA) (3—6 g cm^{-3}) to the first stream [in order to sequester Ti^{III}] along with sufficient ammonia (*d* 0.880).

The pulse-radiolysis results were obtained using the Brunel University linear accelerator, details of which have been described.[44] Optical detection techniques were employed to monitor the transient absorption changes; the dosimeter voltage was calibrated by KSCN dosimetry. Solutions for study were made up in doubly distilled or millipore grade water, and the substrates were commercial samples of the highest purity readily available and used without further purification. Solutions were deoxygenated using dinitrogen oxide [nitrous oxide, N_2O (BOC)], which also serves to facilitate the production of $^{\bullet}OH$.

All chemicals employed were commercial samples of the highest available purity and were used without further purification.

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