Radical Reactions of Carbohydrates. Part 6.[1] An Electron Spin Resonance. Study of the Reaction of the Hydroxyl Radical with Some Lactones derived from Sugars and with Ascorbic Acid

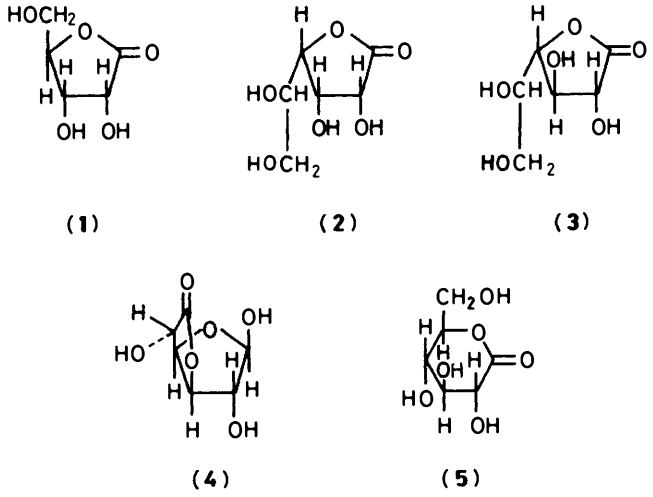
Mark Fitchett and Bruce C. Gilbert*
Department of Chemistry, University of York, Heslington, York YO1 5DD

For a series of lactones derived from sugars it is shown that reaction of 'OH (from the Ti^{III}–H_2O_2 couple) takes place preferentially at C(2) to give the appropriate carboxy-conjugated radical. Radicals formed by abstraction of C(3)–H in the γ-lactones are shown to react readily both by ring-opening [*via* fission of the C(β)–O(acyloxy) bond] and by rapid oxidation with H_2O_2, evidently to give enediols related to ascorbic acid. Oxidation of the latter with 'OH is suggested to proceed both *via* direct electron transfer (at pH > 5) and, at lower pH, addition to the enolic double bond followed by either acid- or base-catalysed elimination of water.

The hydroxyl radical ('OH) has been implicated in many processes believed to be involved in the *in vivo* degeneration of mono- and poly-saccharides, including the loss of structural integrity in *e.g.* hyaluronic acid, the sugar–phosphate backbone of DNA, and some plant polysaccharides.[1-3] Our interest in the primary processes occurring during radiation damage (*via* the generation of 'OH), and in the possibility that similar reactions occur *in vivo* through the reaction between metal ions (*e.g.* Fe^{II}) and either oxygen or H_2O_2, has led us to employ the Ti^{III}–H_2O_2 couple [reaction (1)] to investigate the oxidation of carbo-

$$Ti^{III} + H_2O_2 \longrightarrow Ti^{IV} + {}^{\cdot}OH + OH^- \qquad (1)$$

hydrates with 'OH. In previous studies we have shown, for example, that 'OH attacks C–H bonds in pyranose sugars in an essentially indiscriminate manner[4] (in contrast to the selectivity apparently exhibited in attack on the furanose ring in sucrose at the C–H bond adjacent to the oxygen atom in the ring[5]) and that both base-catalysed[6] and acid-catalysed rearrangements occur (including those leading directly to depolymerisation *via* fragmentation of the C_2-derived radicals in *e.g.* dextran[1]). We report here the results of an investigation of the corresponding reactions of a series of sugar-derived lactones (**1**)—(**5**), chosen so as to provide a further number of furanose model compounds and to reveal any effects of the carboxy groups on both the selectivity of attack of 'OH and the fate of first formed radicals. Our findings prompted a reinvestigation of the oxidation of ascorbic acid under similar conditions.



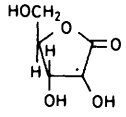
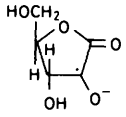
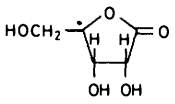
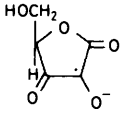
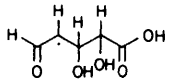
Results and Discussion

E.s.r. spectra were obtained using a three-way rapid-mixing flow system in which solutions of Ti^{III} (8 mmol dm^{-3}), H_2O_2 (30 mmol dm^{-3}), and the substrate (typically 0.05—0.1 mol dm^{-3}) were mixed shortly before the combined solution flowed through the cavity of the spectrometer. Experiments were normally carried out in the pH range 2—4 and at pH *ca.* 7 [in the presence of ethylenediaminetetra-acetic acid (EDTA) as sequestering agent for titanium] as well as at pH *ca.* 1 (*via* the addition of sulphuric acid). In all the experiments, the lactone was contained separately in a stream with pH maintained at *ca.* 7; since the time between mixing and observation was typically *ca.* 50 ms, it is clear that no significant hydrolysis of the lactones occurs prior to reaction with 'OH (see *e.g.* ref. 7: equilibration between lactone and ring-opened acid is achieved in *ca.* 24 h for D-glucono-5-lactone and even less rapidly with γ-lactones).

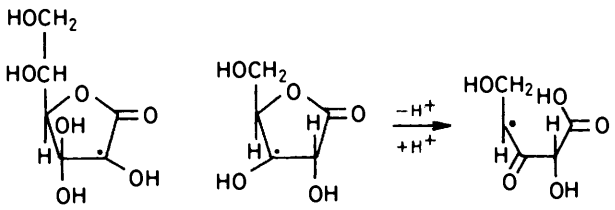
(a) *Reaction of Ribonolactone* (D-*Ribono*-γ-*lactone*) (**1**).— (i) *Primary radicals.* E.s.r. spectra obtained following oxidation of (**1**) by 'OH are interpreted in terms of the selective reaction of 'OH by hydrogen-atom abstraction from the carbon adjacent to the carbonyl group [*cf.* the behaviour of galacturonic acid[1]], though radicals resulting from abstraction of each carbon-bound hydrogen are also detected (see Table 1).

Thus the dominant feature of the spectra recorded at low pH is the signal attributed to (**6**). This is most clearly identified at pH values below 2.5 and has a spectrum which is characterised by couplings to two protons, centred at *g* 2.003 95 [typical[8] of a radical with the structure $R\dot{C}(OH)CO_2R'$], and a linewidth of 0.1 mT. The larger of the two couplings is clearly attributable to an interaction with the β-proton on C(3), whilst the small doublet is assigned to the γ-proton on C(4). The significant linewidth is believed to result from an unresolved coupling to the hydroxylic proton on the α-carbon atom. Raising the pH above 2.5 led to the loss of this signal and the detection of new lines attributed to the appropriate radical anion (**7**): deprotonation is accompanied by a change in *g* value to 2.0045 and a lowering of the β-coupling constant, both of which are consistent with increased delocalisation of spin density from C(2). The small (γ-H) coupling remains, confirming that this splitting does indeed arise from the interaction with the C(5)–H, rather than α-OH; the loss of this latter proton considerably reduces the linewidth, presumably because of the removal of an unresolved coupling. The pK_a value for (**6**) is estimated as *ca.* 2.5, in

Table 1. E.s.r. parameters of radicals detected from reaction of ribonolactone with •OH

	Hyperfine splittings[a] (mT)[b]			
	a(α-H)	*a*(β-H)	*a*(other)	*g*[c]
 (6)		1.885	0.065	2.003 95
 (7)		1.375	0.050	2.0045
 (11)		2.235 1.670(2)		2.0032
 (12)			0.215	2.0053
 (19)	2.025	1.425 0.125		2.0044

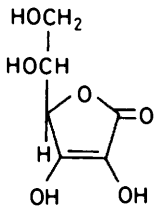
[a] Numbers given in parentheses indicate numbers of nuclei (one unless otherwise stated). [b] ±0.005. [c] ±0.000 05.

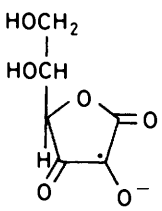

(8) (9) (10)

good agreement with that reported for the related radical (**8**) from ascorbic acid (2.0).[9]

The radical (**9**) which would be formed by hydrogen abstraction at C(3) was not directly detected, though experiments to be described suggest that its participation may nevertheless be inferred. We note that cleavage of such radicals *via* β-fission [(**9**) ⟶ (**10**)] might be expected to be rapid by analogy with the ready loss of *e.g.* Cl^-, PO_4^{3-}, or acetate as β-substituents in substituted acyclic α-alkoxyl radicals[10] [though the radical (**10**) might itself prove difficult to detect on account of its expected[11] ease of reduction by Ti^{III}].

Oxidation at C(4) leads to the detection at all pH values studied of a signal assigned to (**11**), with a large coupling of


(13)


(14)

2.235 mT, attributed to the β-proton on C(3), and two equivalent splittings of 1.670 mT from the methylene protons on C(5). This splitting is significantly higher than those (*ca.* 0.8 mT) in corresponding radicals derived from oxidation of sucrose at the position adjacent to the oxygen in the furanose ring.[5] Low β-couplings in this type of radical are generally indicative of a preference for a conformation in which the β-C–O bond eclipses the orbital of the unpaired electron[12] (as a result of an interaction involving the $-I$ effect of the β-hydroxy group and the $+M$ effect of the α-alkoxy substituent). A decrease in the magnitude of this interaction (caused by the lower $+M$ effect of the α-O–C=O substituent in the lactone) presumably reduces the tendency to adopt such a conformation and hence leads to an increase in the β-coupling constant.

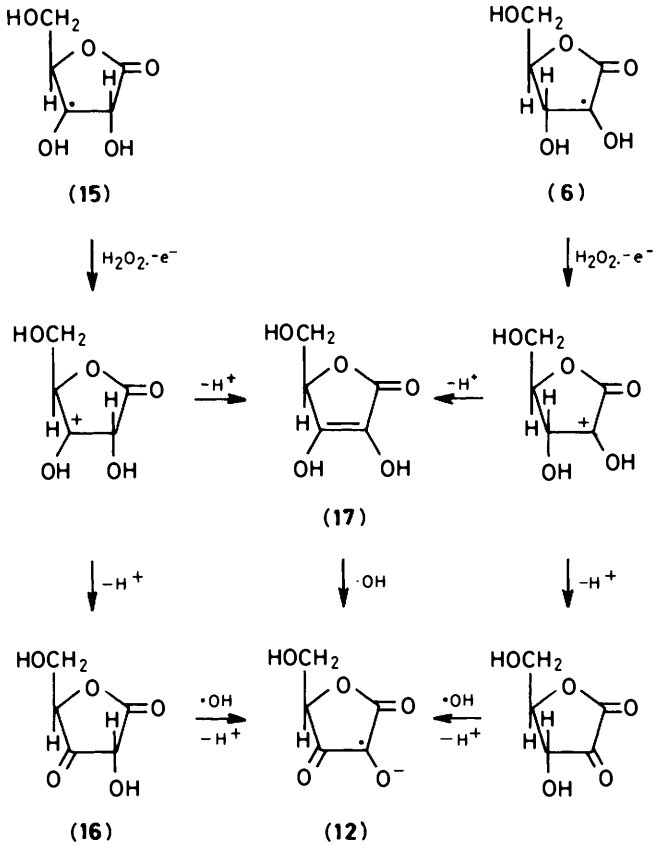
No radical product resulting from oxidation at C(5) could be detected at pH >2.5, although the product of a rearrangement of this species was observed at lower pH in the absence of EDTA (see later).

(ii) *Secondary radicals.* A surprising observation in experiments over a wide range of pH was a radical with *g* 2.0053 and a coupling to one proton (0.215 mT). The *g* value is consistent[8] with a radical of the type RC(O)–$\dot{C}$(O$^-$)–C(O)R′, and on this basis the radical is assigned structure (**12**). Evidence to support this assignment follows from studies[9,13,14] of the reaction of •OH with ascorbic acid (**13**), from which the detection of a radical with *g* 2.0052 and *a*(H) = 0.176 mT is attributed to the formation of the radical anion (**14**) (for which the conjugate acid has pK_a −0.45).[9] Ascorbic acid and ascorbate absorb strongly in the u.v. region[15] ($\lambda_{max.}$ 245 and 265 nm, respectively); solutions of ribonolactone failed to show any absorption in this region and the possibility that signals from (**12**) arise as a result of an adventitious ascorbate-type impurity are dismissed. Furthermore experiments in which the lactone was set aside in the hydrogen peroxide solution prior to flowing resulted in no alteration in the intensity of e.s.r. spectrum, indicating that the signal from (**12**) does not arise as a result of oxidation of the parent compound by hydrogen peroxide itself.

It is suggested that (**12**) arises *via* initial reaction of the lactone with •OH, and possible mechanisms are outlined in Scheme 1. Hydrogen-atom abstraction from C(2) or C(3) would give α-hydroxyalkyl radicals which may be expected to react further with a one-electron oxidant (*e.g.*[16] H_2O_2) to give the appropriate cations, which can be rapidly deprotonated before a second hydrogen atom is abstracted by •OH.* Although oxidation of both (**6**) and (**15**) can in principle lead ultimately to the same precursor (Scheme 1), it is expected that oxidation of (**15**) will be faster than that of (**6**), since in the latter carbocation character is developed adjacent to the $-M$ carbonyl substituent.

The proposed mechanism was tested in experiments where [H_2O_2] was increased (up to 0.1 mol dm^{-3}). This led to a decrease in the intensity of signals from all first formed radicals, presumably as a result of oxidation by hydrogen

* The rate of oxidation of a number of α-hydroxyalkyl radicals by H_2O_2 has been measured[16] as *ca.* 10^5 dm^3 mol^{-1} s^{-1}, and the rapid formation of (**12**) suggests a similar, if not greater, rate in this instance.



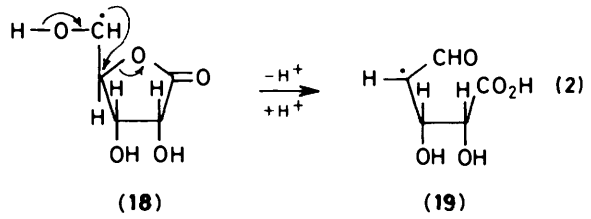
Scheme 1.

peroxide, but signals from (**6**) were more persistent, in keeping with the assertion that this is oxidised more slowly than other α-hydroxyalkyl radicals. The decrease in concentration of the first formed radicals was also accompanied by a notable increase in the intensity of signals from (**12**), supporting the suggestion that this is formed *via* oxidation of a first formed radical believed to be the (undetected) radical (**15**).

It is not clear whether (**12**) is formed by further reaction with $^{\bullet}OH$ as the keto (**16**), or enol (**17**) tautomer: (**16**) would be expected to react by abstraction of a carbon-bound hydrogen atom, which will lead to the protonated form of the detected species, whereas it is likely that the enol would react *via* addition of $^{\bullet}OH$ (followed by elimination of OH^-) or direct electron transfer. A parallel study of the oxidation of ascorbic acid by $^{\bullet}OH$, designed to distinguish these possibilities, is described later.

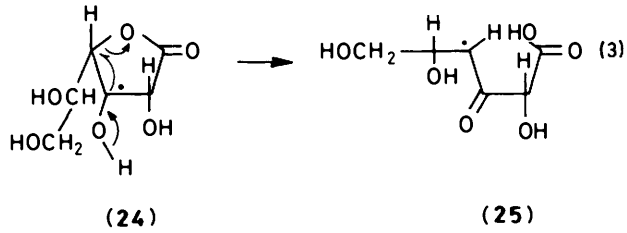
Although no radical resulting from oxidation at C(5) in ribonolactone could be characterised at pH > 2.5, signals were detected, below this pH, of a radical with *g* 2.0045 (clearly attributable to a carbonyl-conjugated species) and assigned structure (**19**); this is evidently formed *via* the rapid ring-opening (which effectively involves loss of an acyloxy group; see above) of the C(5)-centred radical (**18**) [reaction (2)]. It is not clear whether this rearrangement requires acid-catalysis, since the failure to detect the rearrangement product at higher pH (> 2.5) could be a result of rapid reduction of (**19**) by the Ti^{III}–EDTA complex (the sequestering agent is necessary above this pH): however, the failure to detect the precursor (**18**) at any pH suggests that the reaction is indeed rapid ($k \geqslant 10^3\ s^{-1}$) under all conditions studied.

No products of acid-catalysed rearrangement could be unambiguously assigned, although reduction in the intensity of



signals from (**6**) and (**11**) at low pH (< 1.5) suggests that acid-catalysed loss of β-OH groups may be occurring.

(b) *Reaction of Guluronolactone* (D-*Gulurono-γ-lactone*), (**2**).—Signals from two radicals dominate the e.s.r. spectra which result from $^{\bullet}OH$ attack at pH *ca.* 3; these are assigned structures (**20**) and (**21**), the products of hydrogen-atom abstraction from C(6) and C(2) respectively (Table 2). Those of the latter are the more intense, consistent with preferential hydrogen-atom abstraction from the carbon adjacent to the carbonyl group. The radical (**21**) has parameters similar to those of the analogous radical (**6**) from ribonolactone and is similarly deprotonated to give the related radical anion (**22**) (above pH *ca.* 4). A weaker signal with *g* 2.0031 and *a* 1.304 (1H) and 0.702 mT (1H) is assigned to (**23**). The failure to detect *directly* the radicals which would be formed by hydrogen-atom abstraction at either C(5) or C(3) (the latter of which would have been revealed by the detection of large β-proton splittings typical of a radical centre in a five-membered ring) is attributed to the rapidity of ring opening *via* heterolytic fission of the bond to the (β)-acyloxy substituent [see *e.g.* reaction (3)]: the clear identification of (**25**) [from (**24**)] and (**26**) [from attack at C(5)] at low pH (in the



absence of EDTA: signals were much weaker at higher pH in its presence) is believed to reflect the less efficient removal of carbonyl-conjugated radicals by Ti^{III} as compared with Ti^{III}–EDTA.

Finally a signal from a radical with *g* 2.0053 and *a*(H) 0.175 mT was detected at all pH values (its concentration reaching a minimum at pH *ca.* 2.5). This is assigned structure (**27**), and we suggest that it is formed in an analogous manner to (**12**) from ribonolactone, *i.e.* that oxidation at C(3) is followed by a further one-electron oxidation and subsequent reaction with a second hydroxyl radical (*cf.* Scheme 1).

(d) *Reaction of Galactonolactone* (D-*Galactono-γ-lactone*) (**3**).—Following reaction of $^{\bullet}OH$ at pH *ca.* 4 with D-galactonolactone (**3**), which is epimeric with D-guluronolactone [the configuration about C(3) being reversed], signals from three radicals were clearly distinguished (see Table 2). The spectrum assigned to the C(2)-derived species (**28**) has a splitting from one β-proton, and a further coupling assigned to the γ-proton on C(4) [the *g* value (2.003 85) and magnitude of the β-proton coupling clearly distinguish this from the related radical anion (**29**), formed at pH > 4]. A second radical, with *g* 2.0031, is assigned structure (**30**) and it is interesting to compare its proton splittings with those in the epimeric radical (**23**) from

Table 2. E.s.r. parameters of radicals detected from reaction of some sugar lactones with 'OH

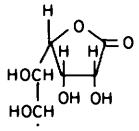
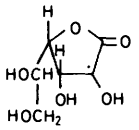
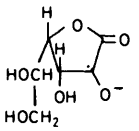
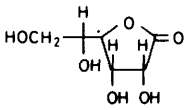
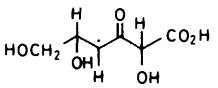
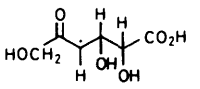
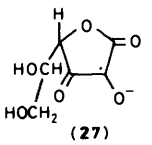
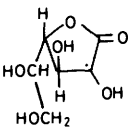
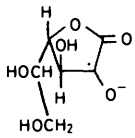
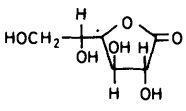
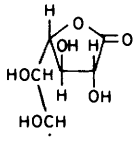
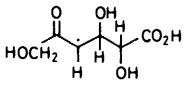
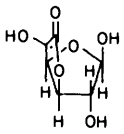
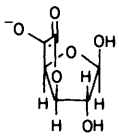
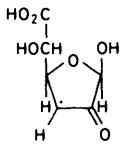
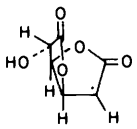
Substrate	Radicals detected	Hyperfine splittings[a] (mT)[b]			g Value[c]
		a(α-H)	a(β-H)	a(other)	
(2)	 (20)	1.765	0.760	0.090	2.003 15
(2)	 (21)		1.742	0.050	2.003 85
(2)	 (22)		1.035	0.090	2.0046
(2)	 (23)		1.304 0.702		2.0031
(2)	 (25)	1.935	1.240	0.095	2.0044
(2)	 (26)	1.905	1.815	0.100(2)	2.0044
(2)	 (27)			0.175	2.0053
(3)	 (28)		1.870	0.050	2.003 85
(3)	 (29)		1.405	0.050	2.0046
(3)	 (30)		3.010 0.750	0.063	2.0031

Table 2 (*continued*)

Substrate	Radicals detected	Hyperfine splittings[a] (mT)[b] a(α-H)	a(β-H)	a(other)	g Value[c]
(3)	 (31)	1.935	0.955		2.0032
(3)	 (32)	1.905	1.70[d]	0.100(2)	2.0044
(3)	(25), (27)				
(4)	 (33)		1.475	0.195 0.075	2.0041
(4)	 (34)		1.065	0.125 0.075	2.004 55
(4)	 (36)	1.780	3.345	0.180 0.085	2.0045
(4)	 (38)	2.140	1.185	0.050	2.0031

[a] Numbers given in parentheses indicate numbers of nuclei (one unless otherwise stated). [b] ±0.005. [c] ±0.000 05. [d] ±0.05.

guluronolactone: the observation of a coupling of *ca.* 3 mT (*cf.* 1.3 mT) is consistent with a change in the orientation of the C(3) proton from pseudoequatorial in guluronolactone to pseudo-axial in galactonolactone. The coupling assigned to the proton on C(5) is virtually unaffected by this change.

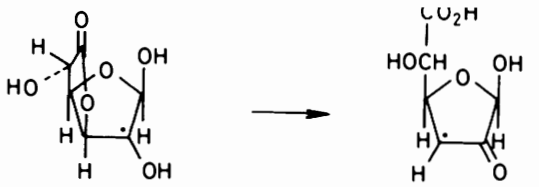
The C(6)-derived radical (**31**), readily detected at pH *ca.* 4, is typified by its g value of 2.0032 and an α-proton coupling of 1.935 mT [somewhat different from the corresponding radical from guluronolactone – evidently the change in stereochemistry at C(3) effects the parameters of this radical, although it is formed at a site somewhat removed from C(3)].

No radical products resulting from hydrogen-atom abstraction at C(3) or C(5) could be clearly detected at this pH, consistent with the results obtained with other lactones. However, lowering the pH to 2.5 and omission of EDTA from the Ti^{III} stream allowed signals from two radicals with g *ca.* 2.0045 to be observed. These are assigned structures (**25**) and (**32**), formed *via* ring-opening of the C(3)- and C(5)-centred radicals respectively. One [*i.e.* (**25**)] is identical with that detected under similar conditions with glucuronolactone and is evidently derived from hydrogen-atom abstraction at C(3). The second, with parameters similar to those of (**26**), formed from the former lactone, is assigned the diastereoisomeric structure (**32**); it is evidently formed by ring-opening following hydrogen-atom abstraction at C(5). Experiments in which the pH was raised to *ca.* 3 in the absence of EDTA as a sequestering agent gave poorly resolved spectra, but the presence of both (**25**) and (**32**) could be inferred by comparison with spectra recorded at low pH. This supports the conclusion that (**25**) and (**32**) are formed via a *spontaneous* (*i.e.* uncatalysed) fragmentation of the C(β)–O bond and that failure to detect stronger signals from these radicals is a result of their reduction by Ti^{III}–EDTA.

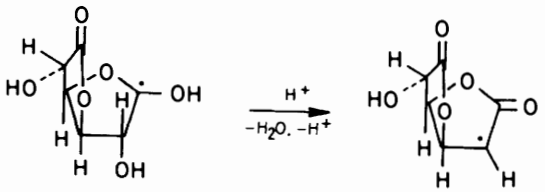
A radical with g 2.0053 and a(H) 0.175 mT (indistinguishable from one of those observed for guluronolactone) is assigned the structure (**27**); its formation *via* attack at C(3) is interpreted as already outlined.

(e) *Reaction of Glucuronolactone* (α-D-*Glucurono-γ-lactone*) (**4**).—Only two radicals could be clearly detected at pH >2 following reaction of ·OH (see Table 2). These are assigned the conjugate acid and base structures (**33**) and (**34**), formed by oxidation at the carbon atom adjacent to the carbonyl group; pK_a is estimated as *ca.* 4.0. Closer examination of the e.s.r. spectra shows that oxidation of the lactone at positions other than C(5) also occurs, but signals from these radicals were too weak to allow unambiguous assignment.

Evidence that attack by ·OH also occurs at C(1) and C(2) is provided by observations made at lower pH, when signals from two extra radicals accompany those from (**33**). The first is assigned structure (**36**) on the basis of the α-, β-, and γ-proton splittings and the *g* value; we believe that this is formed *via* oxidation at C(2) to give (**35**), which rapidly undergoes β-fission [reaction (4)] [(**36**) is evidently rapidly reduced by Ti^{III}–EDTA at higher pH]. It is interesting that under the conditions employed ring-fission is faster than the alternative acid-catalysed loss of β-OH [from C(1)], a reaction described for a variety of sugar-derived radicals and for which a rate constant of *ca.* 10^6 dm^3 mol^{-1} s^{-1} would be expected.[4] The second radical is assigned structure (**38**), formed *via* acid-catalysed loss of the β-OH group from the C(1)-derived radical (**37**) [reaction (5)]: the absence of (**37**) at higher pH is attributed to the expected rapid oxidation of this α-dioxygen-substituted radical



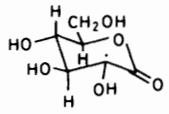
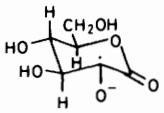
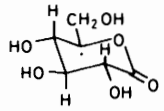
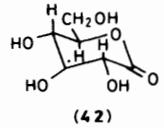
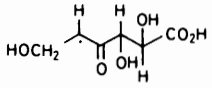
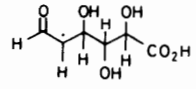
(35) (36) (4)



(37) (38) (5)

by H_2O_2. Two other features are notable: the first is the observation that the rate of the elimination reaction (5) is similar to that of the related rearrangement in glucopyranose-derived radicals, indicating that furanose rings behave similarly to their pyranose counterparts, and the second is the magnitude

Table 3. E.s.r. parameters of radicals detected from reaction of D-glucono-lactone with ·OH

	Hyperfine splittings[a] (mT)[b]			
	a(α-H)	*a*(β-H)	*a*(other)	*g* Value[c]
 (39)		2.410		2.0042
 (40)		1.556		2.004 65
 (41)		1.030 1.180 2.440		2.0027
 (42)		2.055 1.460		2.0031
 (44)	1.855	2.630(2)	0.176	2.0045
 (46)	2.105	1.760	0.075	2.0042

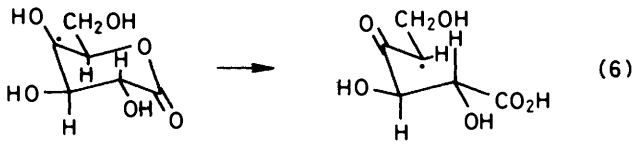
[a] Numbers in parentheses indicate numbers of nuclei (one unless otherwise indicated). [b] ±0.005. [c] ±0.000 05.

of the β-proton splitting in (**38**), this being typical of a pseudoequatorial orientation.

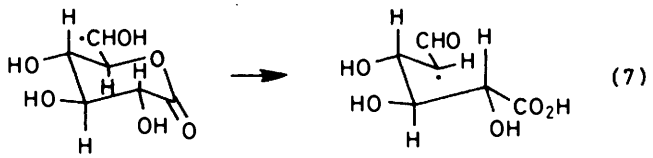
The failure to detect radicals resulting from oxidation at C(3) or C(4) is consistent with the suggestion that radical formation at bridgehead carbon atoms is unfavourable, as a consequence of the strain which results from their production.[5] In marked contrast to the other γ-lactones studied, no signal from an analogue of (**12**) and (**27**) was obtained under any conditions. This again is interpreted in terms of the unreactive nature of the C(4) hydrogen atom towards abstraction by ·OH (which is the first step in the formation of such a radical if Scheme 1 is correct); further, initial radicals once formed would be expected to be relatively slowly oxidised, as a result of the strain which occurs in adopting a conformation whereby cationic character can be stabilised by delocalisation onto the α-oxygen.

(f) *Reaction of Gluconolactone* (D-*Glucono-δ-lactone*) (**5**).—Radicals which result from attack at all five possible sites for C–H abstraction were characterised (see Table 3), though only three of the first formed radicals were *directly* observed. The preference for attack of ·OH at the hydrogen adjacent to the carbonyl groups is confirmed by the dominance of the spectrum from radical (**39**) which is characterised by *g* 2.0042 and *a*(β–H) 2.41 mT (both somewhat higher than in corresponding radicals from γ-lactones). The magnitude of the β-coupling constant indicates that the proton on C(3) has an axial orientation, which is expected if this radical has a conformation which is close to the $^4C_1$ chair conformation of D-glucose (with all OH and CH_2OH groups equatorially orientated). Deprotonation of (**39**) gives (**40**) (with pK_a *ca.* 4). A signal with *g* 2.0027 is assigned to (**41**), formed *via* oxidation at C(5): the large β-(CH) coupling of 2.44 mT is assigned to the interaction of the unpaired electron with the pseudoaxial proton on C(4), and two couplings of *ca.* 1.1 mT are attributed to the β-methylene protons [comparison of this splitting with the significantly higher value for the furanose analogue (**11**) suggests that conjugation between oxygen and the carbonyl group is less effective in the puckered pyranose ring]. This radical shows a marked resistance to acid-catalysed rearrangement; its resonances remain unchanged below pH *ca.* 1.

A broad set of resonances is assigned to (**42**), formed by oxidation at C(3): the magnitude of the β-C(H) couplings (2.055 and 1.46 mT) indicates a significant flattening of the pyranose ring as compared with related radicals from α- and β-D-glucose.[4] An alternative assignment to the radical formed by oxidation at C(4) [(**43**)] is dismissed following the detection of radical (**44**) formed by rearrangement of (**43**) [reaction (6)]. Although signals from this radical were more easily characterised below pH *ca.* 2, it is clear from e.s.r. spectra recorded at higher pH that



(**43**) (**44**) (6)



(**45**) (**46**) (7)

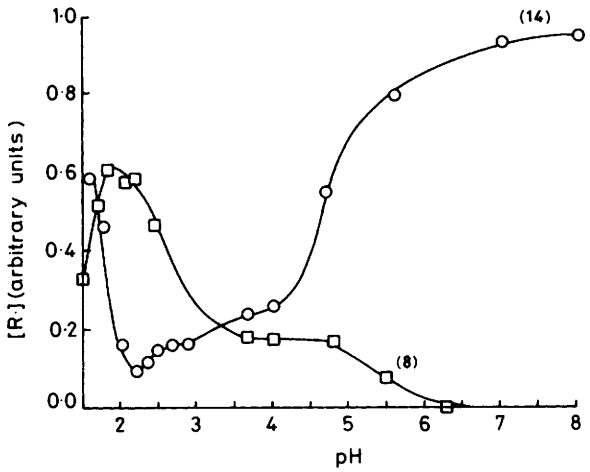


Figure 1. Variation with pH in the steady-state concentrations of (**14**) and (**8**) in the oxidation of ascorbic acid with ·OH

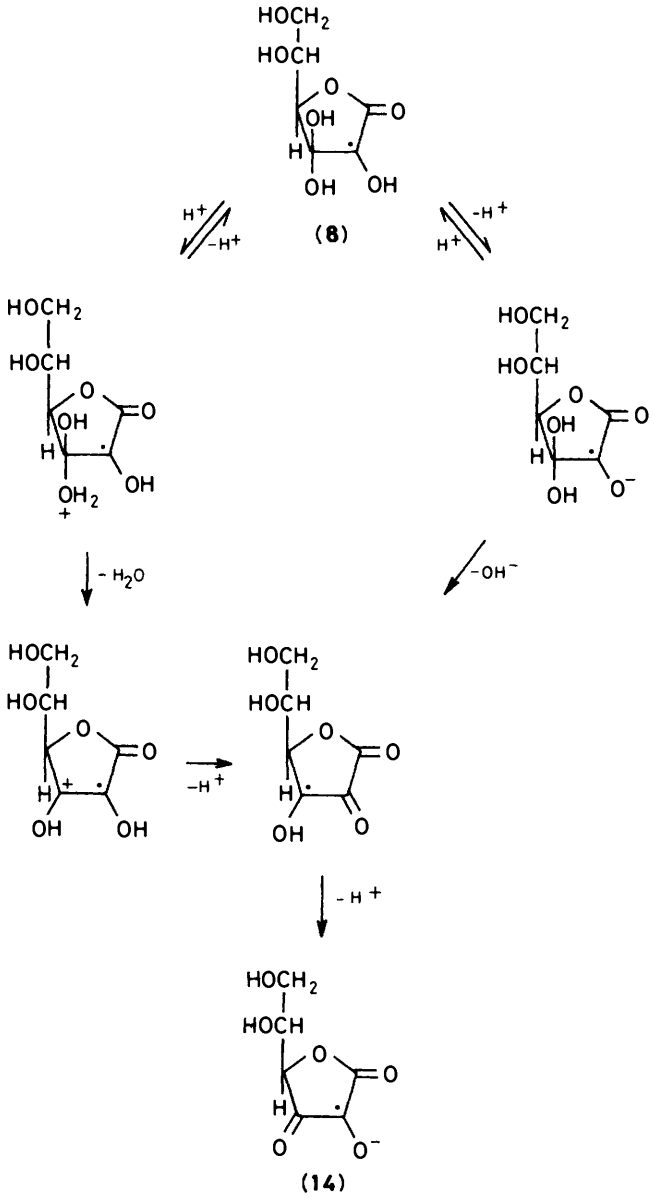
(**44**) is formed at all pH values, and that rearrangement does not require acid catalysis.

No signal corresponding to (**45**) could be characterised following reaction of ·OH; however, below pH 2, a signal with *g* 2.0042, *a* 2.105 (1 H), 1.760 (1 H), and 0.075 mT (1 H) was detected. This is assigned structure (**46**), formed from (**45**) *via* reaction (7). Unfortunately resonances from this radical were obscured at higher pH, and it was impossible to ascertain whether (**46**) results from an acid-catalysed or spontaneous rearrangement of (**45**). The failure to observe signals from the first formed radicals, (**43**) and (**45**), is consistent with the observations on similar radicals formed from γ-lactones in which the radical centre is formed on a carbon atom adjacent to the acyloxy group, and suggests that these radicals undergo a rearrangement (involving ring fission) which does not require acid catalysis. In contrast to the behaviour of the majority of γ-lactones, the signal from (**46**) was readily detectable at pH *ca.* 4: this may reflect a higher rate of generation of this species *via* ring-opening than for the corresponding γ-lactones. No evidence for a radical analogous to (**12**) could be obtained following oxidation of gluconolactone.

(g) *Oxidation of Ascorbic Acid by* ·OH.—When ascorbic acid (**13**) reacted with ·OH in the cavity of the e.s.r. spectrometer, a spectrum consistent with the presence of two radicals, (**14**) and the adduct (**8**), was obtained (see also ref. 9). The former has *a*(1H) 0.175, *a*(2H) 0.02 mT, *g* 2.0052; the latter has *a*(1H) 0.07 mT, with *g* 2.0038 at pH <2 and 2.0044 at pH >3 (for the conjugate base).

The signal from (**14**) reached its minimum value at pH *ca.* 2.2 (see Figure 1), at which resonances from (**8**) dominate the spectrum (this radical has been observed in an *in-situ* radiolysis–e.s.r. experiment[9] as a minor feature of the spectrum). Higher and lower pH values resulted in a marked increase in the concentration of (**14**), at the expense of (**8**).

The increase in [(**14**)] below pH 2.2 is interpreted in terms of an acid-catalysed rearrangement of the precursor (**8**), presumably *via* protonation of a β-hydroxy group (*cf.* refs. 1, 4, and 10). The increase in [(**14**)] at pH values just above 2 is believed to result from deprotonation of (**8**) and analogous rapid loss of OH^- as shown in Scheme 2 [(**8**) has pK_a *ca.* 2.0]. The more marked increase in the concentration of (**14**) at higher pH suggests that there is another mechanism for generating this radical which becomes dominant above pH *ca.* 5. This pH is similar to the pK_a of ascorbic acid itself and we therefore suggest



Scheme 2.

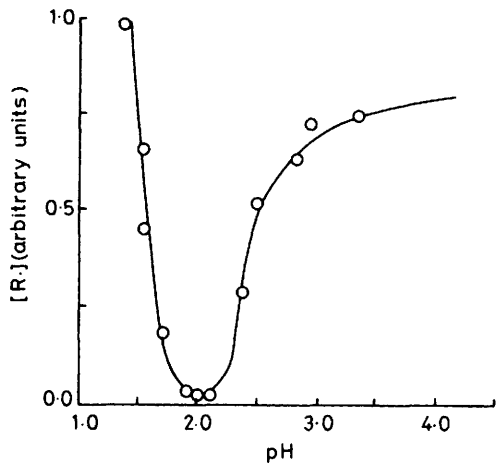
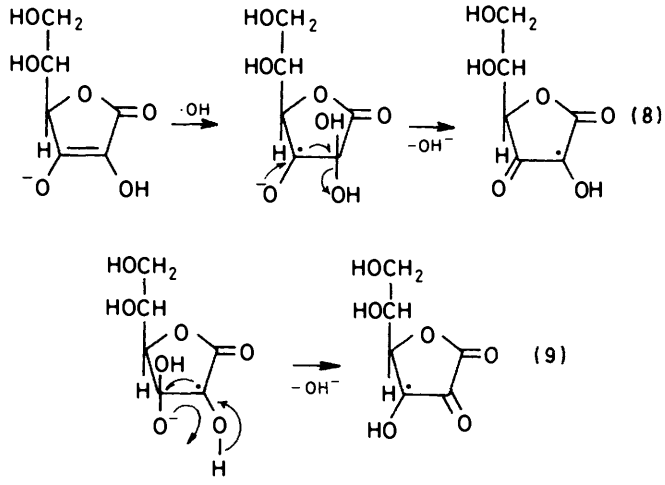


Figure 2. Variation with pH in the steady-state concentration of (12) in the oxidation of ribonolactone with 'OH



that the increase results from ionisation to the ascorbate anion and subsequent faster reaction with the electrophilic hydroxyl radical. Although we cannot rule out a change in the regioselectivity of attack by 'OH [reaction (8)] or of the occurrence of an alternative base-catalysed reaction of (**8**) [reaction (9)] our results are also consistent with occurrence of *direct* electron transfer from the ascorbate anion to 'OH at high pH (a suggestion previously made on the basis of a radiolytic investigation[9]).

Finally, in the light of the results for ascorbic acid, particular attention was paid to the formation of the secondary radical (**12**) from ribonolactone. The variation in its concentration over the pH range 1—4 (Figure 2) suggests that this radical is formed in a manner analogous to that for (**14**) from ascorbic acid [although no (precursor) radical similar to (**8**) was detected from ribonolactone]: we propose that oxidation of the first formed radical (**15**) is followed by addition of 'OH to (**17**) (see Scheme 1), and that (**12**) is formed through subsequent rearrangement of the appropriate adduct.

We also note that if (**12**) and its analogues indeed arise *via* oxidation of the C(3)-centred radicals, then such reaction may also be in competition with a radical rearrangement involving heterolytic fission of the β-acyloxy group (a reaction identified for several lactones).

Summary

For all the lactones investigated, hydrogen-atom abstraction occurs preferentially from the carbon atom adjacent to the carbonyl substituent. The radicals which result are stabilised by both the $+M$ effect of the α-hydroxy group and the $-M$ effect of the carboxy group: a combination of these mesomeric effects, giving a so-called 'capto-dative' radical, is generally believed to give rise to enhanced stability (which may also be reflected in the lifetimes of the radicals);[17] it seems likely that the activation energy for hydrogen-abstraction is lowered accordingly. These radicals are acidic species (with pK_a *ca.* 4).

Other first formed radicals (except where the radical centre would be formed at a bridgehead carbon) are either directly detected (at relatively low concentrations) or transformed into detectable secondary radicals in several different ways. For example, α-hydroxyalkyl radicals, with the radical centre β with respect to the lactone oxygen, apparently undergo rapid ring-opening, which reflects the excellent leaving-group ability of the acyloxy group: the fission is faster for the radical (**43**) from

glucono-δ-lactone than from the analogous radicals from the γ-lactones, which may reflect the relative ease for the former in achieving a conformation with the β-C–O bond eclipsing the orbital of the unpaired electron (see *e.g.* refs. 1, 4, and 18). Exocyclic analogues also appear to react somewhat less rapidly than (**43**).

In contrast to the apparent selectivity in the reactions of sucrose and some related compounds,[5] abstraction from the C–H bond adjacent to the ring oxygen does not appear to be favoured: this presumably reflects the reduced availability of the lone pair on oxygen in the lactone for stabilisation *via* overlap with the incipient radical centre.

For the γ-lactones investigated, radicals formed *via* attack at C(3) are readily oxidised by hydrogen peroxide, evidently with subsequent deprotonation to generate stabilised enolic intermediates which are structurally related to ascorbic acid (and which readily undergo further reaction with $^{\bullet}OH$).

For ascorbic acid itself our investigation suggests that the radical formed by overall one-electron oxidation (**14**) probably arises by *direct* electron transfer from the anion (above pH *ca.* 5) but that at lower pH reactions proceed *via* a first formed *adduct* which can undergo both acid- and base-catalysed fragmentation.

Experimental

E.s.r. spectra were recorded with a Varian E-104 spectrometer equipped with an *X*-band klystron and 100 kHz modulation. Hyperfine splittings were measured to within 0.005 mT from the spectrometer field scan, which itself was calibrated using the spectrum of Fremy's salt [a(N) 1.309 mT];[19] g values were measured by comparison with the same standard[20] (g 2.0055) or $^{\bullet}CHMeOH$ (g 2.0032 ± 0.000 05), itself standardised with Fremy's salt.

All the experiments utilised a flattened, aqueous-sample cell and a mixing chamber which allowed three reagent streams to be mixed simultaneously. The flow was maintained by a Watson-Marlowe HR Flow-Inducer positioned upstream of the sample cell; the flow rate was adjusted so that the mixing time was *ca.* 50 ms. The pH of the reaction was measured to within 0.05 unit by means of a Pye-Unicam PW9410 digital pH meter coupled to a Russell pH Ltd. glass electrode, inserted into the effluent stream immediately above the cavity of the spectrometer. Three streams of the flow system contained, typically, (i) 8 mmol dm^{-3} titanium(III) [added as aqueous 12.5% (w/v) titanium(III) chloride], (ii) 30 mmol dm^{-3} hydrogen peroxide (added as 100-volume hydrogen peroxide), and (iii) the carbohydrate at the required concentration, typically 0.05—0.1 mol dm^{-3}. Stream (i) also included conc. sulphuric acid or ammonia solution (d 0.880) to give the required pH; the disodium salt of EDTA (3—4 g dm^{-3}) was added to sequester the titanium(III) when a pH of > 2.5 was required. The solutions were made up in water deoxygenated with a nitrogen purge and were held under nitrogen atmosphere during use.

All the substrates were commercial materials of the highest purity readily available and used without further purification.

Acknowledgements

We thank the Association for International Cancer Research for financial support.

References

1 Part 5, B. C. Gilbert, D. M. King, and C. B. Thomas, *Carbohydr. Res.*, 1984, **125**, 217.
2 W. Pigman, S. Rizvi, and H. L. Holley, *Arthritis Rheum.*, 1961, **4**, 240.
3 See, *e.g.*, F. Hutchinson, *Cancer Res.*, 1965, **26**, 2045; R. B. Painter, in 'Radiation Biology in Cancer Research,' eds. R. E. Meyn and H. R. Pointer, Raven Press, New York, 1980; C. L. Greenstock, *Radiat. Res.*, 1981, **86**, 196; G. O. Phillips, *Adv. Carbohydr. Chem.*, 1961, **16**, 13; G. O. Phillips, *Radiat. Res. Rev.*, 1972, **3**, 335.
4 B. C. Gilbert, D. M. King, and C. B. Thomas, *J. Chem. Soc., Perkin Trans. 2*, 1981, 1186.
5 B. C. Gilbert, D. M. King, and C. B. Thomas, *J. Chem. Soc., Perkin Trans. 2*, 1983, 675.
6 B. C. Gilbert, D. M. King, and C. B. Thomas, *J. Chem. Soc., Perkin Trans. 2*, 1982, 169.
7 J. W. Green, in 'The Carbohydrates,' ed. W. Pigman, Academic Press, New York, 1977.
8 A. J. Dobbs in 'Electron Spin Resonance,' Specialist Periodical Report, The Royal Society of Chemistry, London, 1974, vol. 2, p. 281.
9 G. P. Laroff, R. W. Fessenden, and R. H. Schuler, *J. Am. Chem. Soc.*, 1972, **94**, 9062.
10 G. Behrens, G. Koltzenburg, and D. Schulte-Frohlinde, *Z. Naturforsch., Teil C*, 1982, **37**, 1205.
11 B. C. Gilbert, R. O. C. Norman, and R. C. Sealy, *J. Chem. Soc., Perkin Trans. 2*, 1973, 2174.
12 A. J. Dobbs, B. C. Gilbert, and R. O. C. Norman, *J. Chem. Soc., Perkin Trans. 2*, 1972, 786.
13 I. Yamazaki, H. S. Mason, and L. H. Piette, *J. Biol. Chem.*, 1960, **235**, 2444.
14 Y. Kirino and T. Kwan, *Chem. Pharm. Bull.*, 1971, **19**, 718.
15 B. H. J. Bielski, D. A. Comstock, and R. A. Bowen, *J. Am. Chem. Soc.*, 1971, **93**, 5624.
16 B. C. Gilbert, R. O. C. Norman, and R. C. Sealy, *J. Chem. Soc., Perkin Trans. 2*, 1974, 824.
17 See, *e.g.*, H. G. Viehe, Z. Janousek, R. Merenyi, and L. Stella, *Acc. Chem. Res.*, 1985, **18**, 148.
18 A. L. J. Beckwith and G. Phillipou, *Aust. J. Chem.*, 1976, **29**, 123; A. L. J. Beckwith and W. B. Gara, *J. Chem. Soc., Perkin Trans. 2*, 1975, 795.
19 R. J. Faber and G. K. Fraenkel, *J. Chem. Phys.*, 1967, **47**, 2462.
20 J. Q. Adams, S. W. Nicksic, and J. R. Thomas, *J. Chem. Phys.*, 1966, **45**, 654.

Received 23*rd December* 1985; *Paper* 5/2258