Radical Reactions of Carbohydrates. Part 2.[1] An Electron Spin Resonance Study of the Oxidation of D-Glucose and Related Compounds with the Hydroxyl Radical

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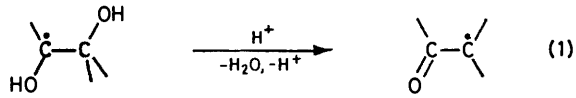
E.s.r. spectra are described for a variety of radicals obtained from the reactions of α-D-glucose, β-D-glucose, and some related substrates with the hydroxyl radical (generated from the Ti^{III}–H_2O_2 reaction in a flow system). Evidence is presented that attack of ·OH is largely unselective; for both glucose anomers, all six radicals which can be formed *via* C–H abstraction are identified. A study has also been made of the relative ease with which these αβ-dioxygen-substituted radicals undergo acid-catalysed fragmentation; this appears to be governed not only by the nature of the leaving group (OH^-, OR^-) but also by the geometry of the radical (in particular, an axial β-OH group is much more readily lost than an equatorial group). Potential routes for carbohydrate degradation *via* glycosidic cleavage of first-formed radicals have been identified.

CONSIDERABLE recent attention has been paid to the study of radical reactions of carbohydrates; this reflects, at least in part, interest in the important physiological roles of these compounds, in their wide occurrence in foods, and in the possibility that radicals are involved in some industrially important degradations of plant polysaccharides.[2] Much of the work has been concerned with the radiation chemistry of carbohydrates [3] (for example, on the determination [4] of the products resulting from the irradiation of aqueous sugar solutions) but photolytic studies [5] and reactions carried out in the presence of hydrogen peroxide, especially Fenton's reagent [6] (Fe^{II}–H_2O_2), have also been reported. The related biomimetic [7] Ti^{III}–H_2O_2 system, which also produces the hydroxyl radical, has been used in conjunction with e.s.r. spectroscopy and a simple flow technique to detect short-lived radicals formed in the reactions between ·OH and some simple sugars.[8,9] Although some conformational information was obtained in this way (see *e.g.* ref. 8) and instances of radical rearrangements were revealed, the site selectivity reported for ·OH in its reaction with, *e.g.* glucose,[8] appears to be in contrast with the conclusion from product studies [4] that attack of ·OH is unselective.

We have previously studied [1] the reaction of the model compound myoinositol with ·OH (generated from Ti^{III} and H_2O_2 in a three-way mixing system in an e.s.r. spectrometer) and concluded that attack on this substrate is indeed unselective: all possible radicals formed by C–H abstraction were detected and their conformations deduced. However, a stereoelectronic requirement for acid- and base-catalysed rearrangement of first-formed radicals was established. As an extension to this study we set out to investigate in detail the reaction between ·OH and glucose and some of its derivatives; the latter were chosen in an attempt to ' block,' activate, or deactivate various positions within the basic monosaccharide skeleton in order that a complete analysis of the spectra, which were expected to comprise mixtures of complex, overlapping signals, could be obtained. We hoped to obtain evidence for differences in behaviour of anomeric and epimeric structures, and detailed information about the conformations of the radicals produced; we also aimed to discover more about the selectivity of ·OH in such systems and the factors affecting the rates of subsequent transformations (*e.g.* acid-catalysed loss of water, possible ring-opening) of first-formed radicals.

RESULTS AND DISCUSSION

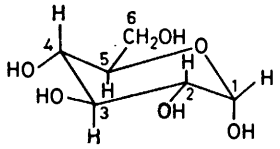
E.s.r. spectra were mostly obtained from the reaction of ·OH (from Ti^{III}–H_2O_2) with individual monosaccharides added to the third stream of a three-way continuous flow system whose details have been described previously.[1] Studies were generally carried out at pH *ca.* 4 (in the presence of EDTA to sequester titanium), this pH being chosen so as to minimize the effects of acid- and base-catalysed rearrangements of first-formed hydroxy-conjugated radicals; studies were also carried out at lower pH (down to pH *ca.* 1) where the operation of acid-catalysed loss of hydroxide ion from αβ-dihydroxy-substituted radicals [reaction (1)] was expected to be important.[1,8] Some experiments were also carried out in which the hydroxyl radical was generated by the photolysis of solutions of hydrogen peroxide and the appropriate sugars as these were flowed slowly through the cavity of the spectrometer.



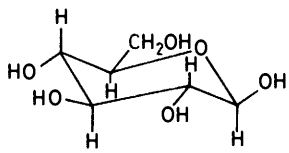
(1)

(a) D-*Glucose*.—When ·OH was reacted with a commercial sample of D-glucose in the flow system at pH 4, a complex spectrum was obtained, with many overlapping signals centred on *g ca.* 2.0031. This spectrum proved to be the superposition of somewhat less complicated spectra obtained from the anomers α-D-glucose [shown in the predominant pyranose form (1)] and β-D-glucose (2) when these were studied separately; provided that ice-cooled solutions of these sugars were kept free from acid or base, the spectrum from each anomer was not contaminated by a significant contribution from the other

until up to 2 h after dissolution. Under our conditions, then, mutarotation is evidently slow.



(1)



(2)

(i) α-D-*glucose. Reaction at* pH 4. The spectrum obtained from α-D-glucose at pH 4 is shown in Figure 1; the following analysis, in terms of *all* the possible radicals (3)—(8) which can be formed by C–H hydrogen abstraction, is based in part on conformational considerations (*e.g.* the $B\cos^2\theta$ dependence for β-proton splittings [10]) the orbital of the unpaired electron at the radical centre. Such splittings are observed in other radicals with eclipsing OH groups including myoinositol- and glycol-derived radicals;[1] as anticipated on this basis it is absent in the spectra of the analogous radicals from β-D-glucose and 1-*O*-methyl-α-D-glucopyranoside. The two lines in the centre of the spectrum are attributed to the superposition of signals from the C(3)- and C(4)-derived radicals (5) and (6), in both of which there are two axial β-protons subtending small dihedral angles with the orbital of the unpaired electron and hence giving rise to substantial (though not quite equivalent) splittings in the range *ca.* 2.5—3 mT; one of this pair of radicals, which appears to be dominant, probably on account of its smaller line-width, also possesses a small doublet splitting. The individual assignments to (5) and (6) are

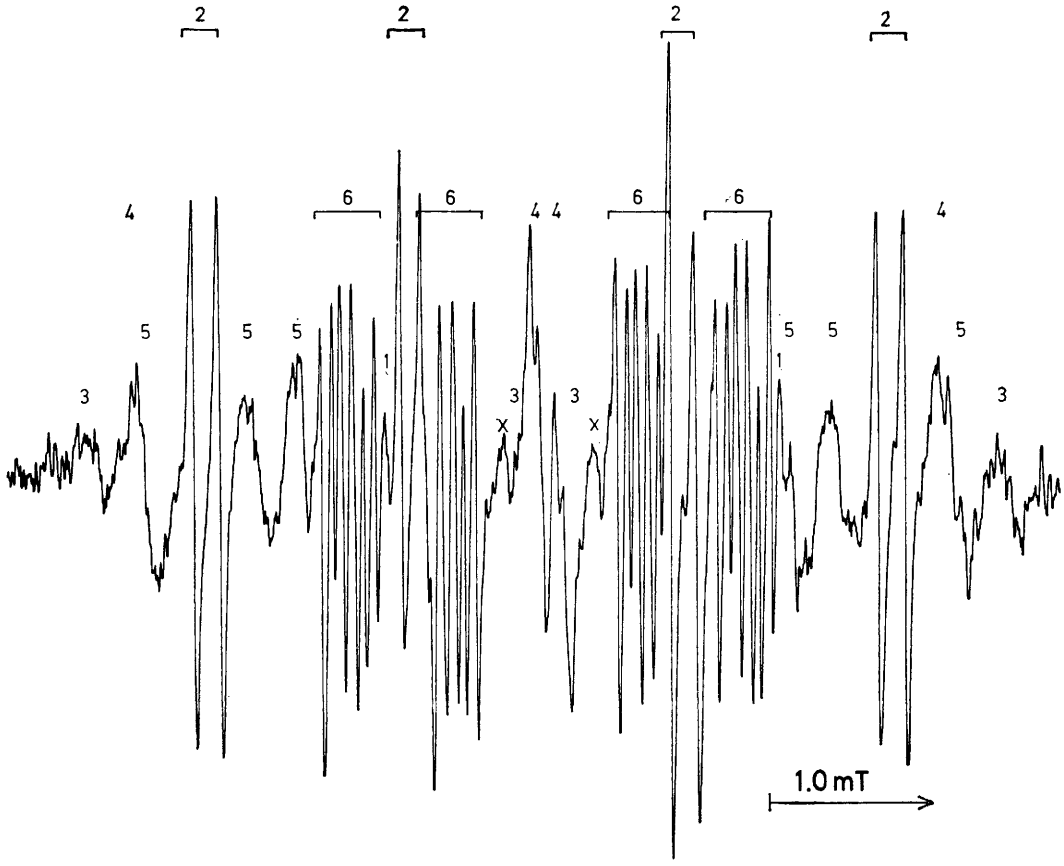


FIGURE 1 E.s.r. spectra of radicals obtained from the oxidation of α-D-glucose with ·OH at pH 4. The radicals are identified by numbers which refer to the carbon atom from which the hydrogen atom was abstracted. Peaks marked × are from the C(2)-radical derived from β-D-glucose, which forms by mutarotation

as well as our findings for β-D-glucose, substituted and related substrates (see later), and on the changes observed as the pH was lowered.

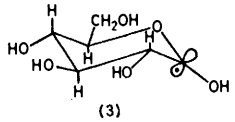
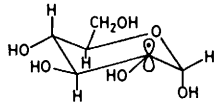
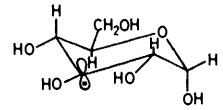
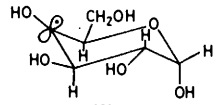
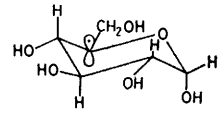
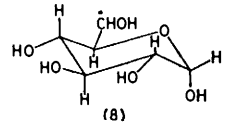
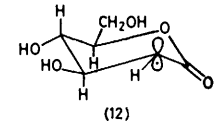
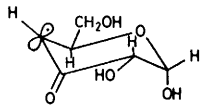
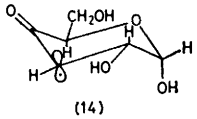
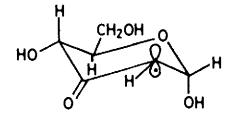
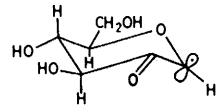
Abstraction of hydrogen from C(2) gives radical (4) (see Table 1) which is recognized in the spectrum by the characteristic splittings from axial and equatorial β-protons (2.975 and 1.295 mT, respectively); the additional doublet of 0.160 mT evidently derives from the axial hydroxy-group at C(1), which presumably eclipses

made on the basis of the spectra observed for the 2-amino-substituted analogue; for this substrate the single spectrum observed with two axial β-proton splittings (and which is virtually identical to one of those obtained for α-D-glucose itself) is attributed to the C(4)-derived radical [*cf.* (6)], as attack at C(3) by the electrophilic hydroxy-radical will be inhibited by the presence of the NH_3^+ substituent.[11]

The radical formed by attack at C(6), (8), is clearly

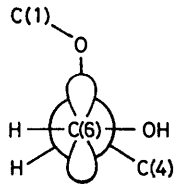
TABLE 1

E.s.r. spectra of radicals detected in the reaction of α-D-glucose with ·OH

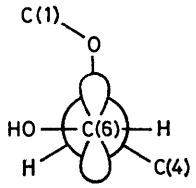
	Hyperfine splittings (mT) [a]			
	a (α-H)	*a* (β-H)	*a* (other)	*g* [b]
(i) at pH 4				
 (3)		2.470 (1 H)	0.260 (5-H) [c] 0.165 (1-OH) [c]	2.0031
 (4)		2.975 (1 H) 1.295 (1 H)	0.160 (1-OH) [c]	2.0031
 (5)		2.950 (1 H) 2.745 (1 H)		2.0031
 (6)		2.605 (1 H) 2.455 (1 H)	0.040 (γ-H) [c]	2.0031
 (7)		3.330 (1 H) 0.990 (1 H) 0.710 (1 H)		2.0031
 (8)	1.845 (1 H)	0.625 (1 H)	0.140 (1 H) [d] 0.125 (1 H) [d] 0.075 (1 H) [d]	2.0032
(ii) at pH *ca.* 1				
 (12)	2.020 (1 H)	3.840 (1 H)	0.075 (1 H)	2.0035
 (13)	1.80 (1 H) [e]	3.70 (1 H) [e]	0.05 (1 H)	2.0045
 (14)				
 (15)	1.84 (1 H) [f]	1.10 (1 H) [f]		2.0045
 (16)	1.395 (1 H)		0.540 (1 H) [g] 0.500 (1 H) [g] 0.020 (2 H) [g]	2.0049

[a] ±0.005 mT except where indicated otherwise. [b] ±0.0001. [c] Suggested assignments: see text. [d] Not individually assigned: see text. [e] Approximate splittings (±0.01 mT): only the outer lines of the spectrum could be clearly measured. [f] Spectrum indistinct; suggested assignment (further long-range couplings were incompletely resolved): ±0.01 mT. [g] Probably the γ-protons: see text.

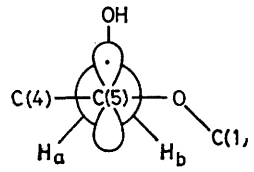
recognized by the occurrence of the splitting of 1.845 mT, typical of an α-proton in radicals of this type [*cf.*[12] ·$CH(OH)CH_2OH$]; the splitting of 0.625 mT is evidently from the single β-proton, and its low magnitude is associated with the preference of a conformation or conformations [locked, and probably distorted, *cf.* ·$CH(OH)CH_2OH$] in which the β-C–O bond eclipses the orbital of the unpaired electron, so that the β-C–H bond subtends a large dihedral angle (60°), *cf.* structures (9) and (10). The extra hyperfine pattern associated with this radical, which appears to be six lines in Figure 1 is revealed as eight lines under conditions of higher resolution, and spectrum simulation confirmed our analysis in terms of three proton splittings; one of these is expected to be the α-OH proton, and the other two are probably from 1-H and 1-OH (a somewhat different pattern was obtained from the corresponding radical from β-glucose), but no unambiguous analysis was possible. It is not clear whether the observed spectrum corresponds to an individual conformation [(9) or (10)] or to an average of the spectra from these individual conformers.



(9)



(10)



(11)

We believe that the radical formed by attack at C(5), (7), is characterized by the rather broad-lined spectrum (as indicated in Figure 1) with a large β-proton coupling, typical of interaction with an axial proton, and two non-equivalent β-protons [*i.e.* the hydrogens on C(6)]; the relatively small magnitude of the latter splittings is again consistent with the conformational locking found for radicals with both α- and β-oxygen substituents. The marked preference for conformation (11) probably contributes to the non-equivalence of the splittings from the C(6)-β-protons, which are in any case diastereotopic.* The spectrum from the analogous radical appears rather more clearly in the spectrum from the β-anomer (see later).

The remaining radical to be identified is that obtained by abstraction from C(1) [radical (3)]. Some rather weak lines from this species can just be discerned in Figure 1 (the weakness of these signals may reflect, at least in part, the ease with which an αα-dioxygen-substituted radical would be expected to be oxidized by hydrogen peroxide [13]). Proof of our assignment, and analysis in terms of three doublets, with splittings from the axial β-proton, the axial γ-proton across oxygen (*cf.* ref. 14) and the 1-OH proton, is provided by the spectrum from β-D-glucose in which the same lines and their associated resonances occur rather more clearly (see later). Oxidation of both anomers at C(1) should, of course, produce the same radical as long as the stereochemical integrity of the C(1)–oxygen substituent is lost when the hydrogen is removed; although the radical is not expected to be strictly planar, the bending induced by two α-oxygen-substituents is not thought [15] to be sufficient to prevent a common equilibrium geometry being established.

Reaction at lower pH. As the pH was lowered in the reaction of ·OH with α-D-glucose, signals from (3)—(8) were removed. Radical (4) was the first to be reduced in concentration (its signal had virtually disappeared by pH *ca.* 2.2), to be followed by (5) and (6), and then by radical (7) (by pH *ca.* 1.5); at pH 1 the C(6)-derived radical (8) persisted, with somewhat reduced concentration, but on lowering the pH further this signal also disappeared.†

Several new radicals were detected (see Figure 2 and Table 1). The spectrum detected in the wings which has *g* 2.0035, typical of a radical with conjugation of the unpaired electron to a carboxy-group and which also possesses the appropriate α-H (2.020 mT) and axial β-H (3.840 mT) splittings is attributed to radical (12). This, as noted previously,[8] is presumably formed *via* acid-catalysed loss of water from (3) [reaction (2)]; its detection provides further evidence that the precursor radical (3) is indeed present in the mixture of first-formed radicals.

Other carbonyl-conjugated radicals (with *g* 2.0044) could be recognized, including either or both (13) or (14); these, which could not be distinguished, would be expected to result from the acid-catalysed rearrangement of (5) and (6), respectively. Reaction of (5) should also produce (15), a spectrum from which [with typical *g*-value, α-proton splitting and low (equatorial) β-splitting] is tentatively assigned. A further dominant spectrum, which under conditions of higher resolution was shown to comprise eight lines, has *g* 2.0049, and a proton splitting (1.395 mT) typical [16] of that expected for an α-proton adjacent to both carbonyl and oxygen functions (–CO–ĊH–O–); it is assigned to (16), formed *via* rearrangement of (4).‡ The two substantial long-range couplings of 0.54 and 0.50 mT are attributed to the two axial γ-protons across oxygen and the carbonyl

* See *e.g.* K. Mislow and M. Raban, *Top. Stereochem.*, 1967, **1**, 1; we thank a referee for drawing this point to our attention.

† It was not possible to establish the appropriate pH for the weak signal attributed to (3).

‡ This e.s.r. signal also dominated the spectrum obtained from flow photolysis experiments with mixtures of α-D-glucose and hydrogen peroxide at pH *ca.* 1.5. Both at this pH and at pH 4, other much weaker signals were recognized as those detected in the appropriate experiments with Ti^{III}–H_2O_2.

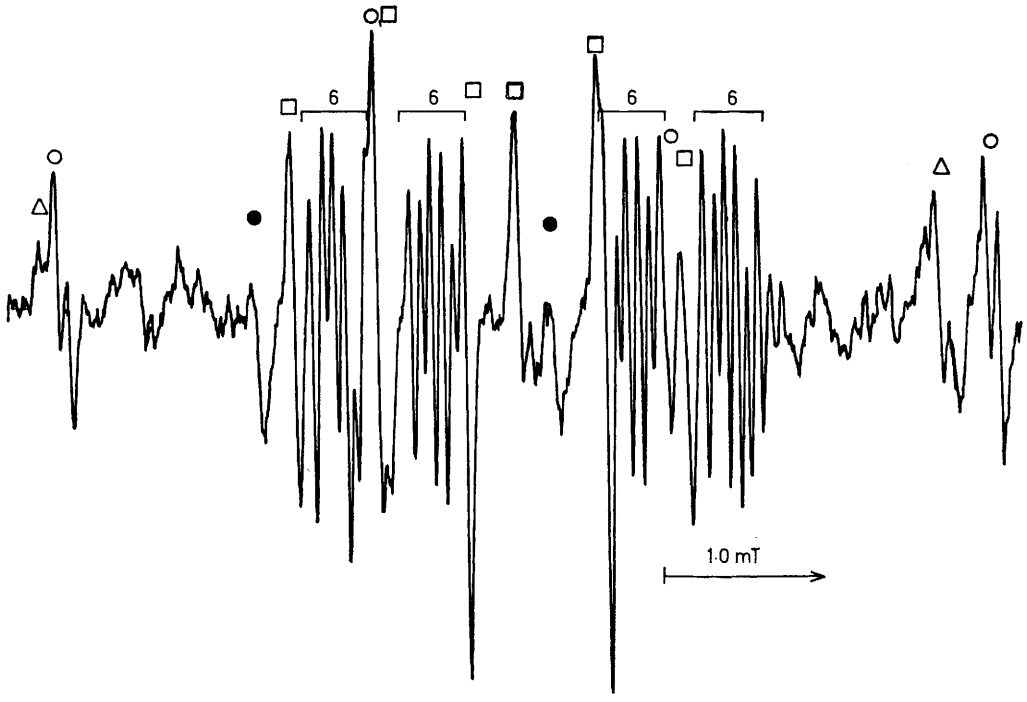
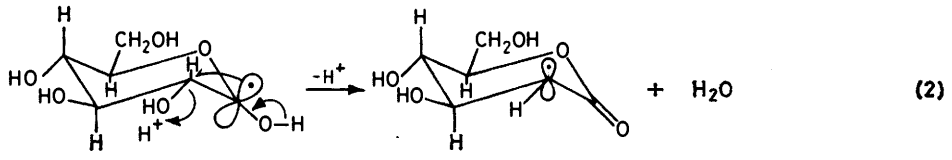


FIGURE 2 E.s.r. spectra of radicals derived from the oxidation of α-D-glucose with ·OH at pH 1: 6, C(6)-derived primary radical; ○, (12); △, (13), (14); ●, (15); □, (16)

group. Precedents for significant long-range couplings to such axially sited protons exist (*cf. e.g.* refs. 13 and 17); the enhanced values observed here may be either an electronic or a steric consequence (*e.g. via* the adoption of planarity by the C–CO–Ċ–O–C fragment) of the juxtaposition of both $+M$ and $-M$ substituents at the radical centre.

The fact that the radicals (12) and (16) are present in the orbital of the unpaired electron (*cf.* the similar stereoelectronic requirement demonstrated [1] for the appropriate radical from myoinositol). We have employed the kinetic analysis described in ref. 1 to calculate the rate of this rearrangement. From an estimate of the radical–radical dimerization rate ($2k_t$) for (4) (*i.e. ca.* 3×10^8 dm^3 mol^{-1} s^{-1}; this would be expected to be similar to that for the structurally related radicals



(3) (12) (2)

greater concentrations than (13)—(15) is ascribed to the fairly ready reduction of the latter carbonyl-conjugated radicals by Ti^{III} (*cf.* ref. 18); such a reaction would be expected to be retarded by the ($+M$) oxygen substituents in (12) and (16).

The following points of mechanistic significance may be noted at this stage. First, ·OH evidently attacks α-D-glucose rather unselectively: all possible radicals formed by C–H abstraction can be detected (in relative concentrations which are probably not far removed from those expected on a statistical basis). Secondly, all these radicals appear to undergo acid-catalysed transformations. The observation that radical (4) is the first to rearrange, to (16), is attributed, at least in part, to the ease of loss of the axial β-hydroxy-group which eclipses derived from myoinositol) and the pH at which the radical's concentration is 50% depleted *via* rearrangement (*i.e.* 2.85), we calculate that k_r is *ca.* 5×10^6 dm^3 mol^{-1} s^{-1}.

Thirdly, it is significant that when the C(5)-radical (7) is removed at low pH no recognizable radical is detected in its place. For this species, acid-catalysed loss of OH^- would be expected [19] to proceed readily (it possesses the necessary α-oxygen and β-OH groups) to give a radical-cation [reaction (3)]; whereas for α-hydroxy-substituted analogues ready loss of the α-OH proton [reaction (4)] leads directly to carbonyl-conjugated radicals, no such pathway is possible here. Instead we believe that reduction of the intermediate radical-cation by Ti^{III} occurs, so that neither the radical-cation nor any radical

derived from it is detected. Lastly, the observation that the C(6)-radical virtually disappears, but not until pH *ca.* 1, suggests that fragmentation of the β-C–O bond in this radical (which leads to ring-opening) occurs [reaction (5)]. We estimate that k_r is *ca.* 10^5 dm^3 mol^{-1} s^{-1} and note that this reaction is considerably slower than the loss of a β-hydroxy-group. Our failure to detect the appropriate ring-opened radical is not unexpected in view of the ready reduction by titanium(III) of radicals of this type.

(ii) β-D-*Glucose.* The structural assignments and mechanistic analyses based on the observations of e.s.r. spectra from α-D-glucose are supported by our findings for the anomer β-D-glucose. The differences between the results for the two are small, but of some significance.

Reaction of ·OH with β-D-glucose at pH 4 in the presence of EDTA led to a complex e.s.r. spectrum which was closely similar to the somewhat better spectrum obtained at pH *ca.* 2.5 in the absence of EDTA (see Figure 3). We conclude that none of the first-formed radicals has undergone significant rearrangement by pH 2.5, in contrast to our finding for radical (4) from α-D-glucose. The spectrum can again be analysed in terms of signals from all six possible radicals formed by C–H abstraction [(17)—(22)] (see Table 2). Three of these radicals, (18)—(20), possess two axial β-protons, and the expected spectra (with two large β-splittings) are detected. Unambiguous assignment of the C(4) radical (20) is possible on the basis of detecting precisely the same spectrum during the oxidation of the epimeric

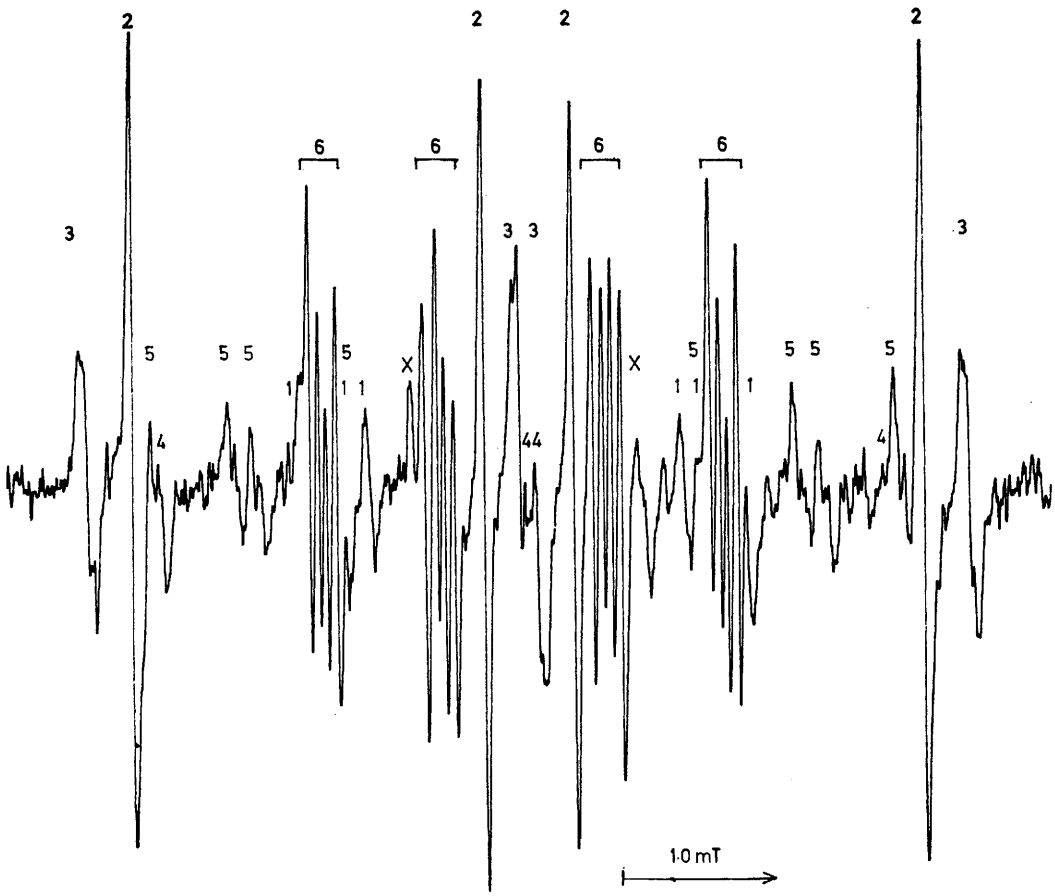
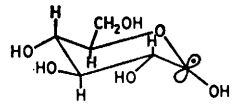
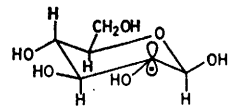
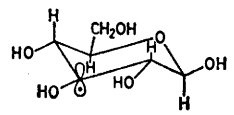
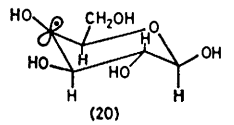
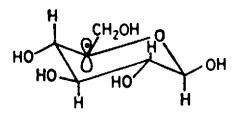
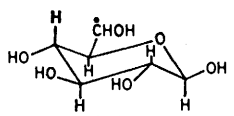
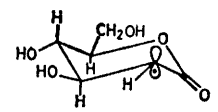
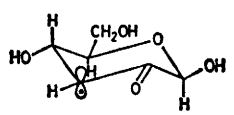
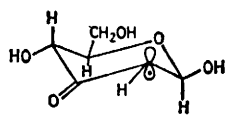
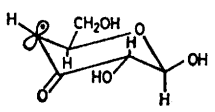
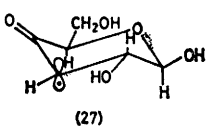


FIGURE 3 E.s.r. spectra of radicals obtained from the oxidation of β-D-glucose with ·OH at pH 2.5

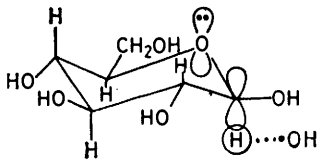
TABLE 2

E.s.r. spectra of radicals detected from the reaction of β-D-glucose with ·OH

	Hyperfine splittings (mT) [a]			
	a (α-H)	*a* (β-H)	*a* (other)	*g* [b]
(i) at pH 2.5—4				
 (17) [≡(3)]		2.470 (1 H)	0.260 (5-H) [c] 0.165 (1-OH) [c]	2.0031
 (18)		2.860 (1 H) 2.280 (1 H)		2.0031
 (19)		2.925 (1 H) 2.810 (1 H)	0.030 (2γ-H) [c]	2.0031
 (20)		2.390 (1 H) 2.340 (1 H)		2.0031
 (21)		3.680 (1 H) 0.660 (1 H) 0.510 (1 H)	0.040 (1 H)	2.0031
 (22)	1.840 (1 H)	0.745 (1 H)	0.110 (1 H) [d] 0.065 (1 H) [d]	2.0032
(ii) at pH *ca.* 1				
 (12)	2.020	3.840	0.075	2.0035
 (24)				
 (25)				
 (26)	1.80 (1 H) [e]	3.70 (1 H) [e]		2.0045
 (27)				

[a] ±0.005 mT. [b] ±0.0001. [c] Suggested assignments; see text. [d] Not assigned; see text. [e] Approximate values (±0.01 mT): individual spectra indistinguishable.

compound D-galactose [the latter was largely present in the β-form: oxidation of C(4) thus produces (20)]. Of the two remaining spectra of this type, the sharp-lined spectrum is assigned to the C(2)-derived radical (18) [*cf.* its anomeric counterpart (4), but with no hydroxy-splitting]; radical (19) shows interaction with two more protons (probably the axial γ-H). Radical (21), from C(5), appears more clearly than does its counterpart (7); the non-equivalence of the C(6) β-proton splittings, as well as their small magnitude, is readily revealed and again provides evidence for restricted rotation around the C(5)–C(6) bond [*cf.* (11)]. The C(6) radical (22) is also clearly detected *via* its characteristic α- and β-proton splittings (with a further splitting pattern somewhat different from that of the anomeric radical). Lastly, the radical formed in common from both α- and β-anomers, (17), can be more easily identified in the spectra from the β-anomer; the crucial evidence for its assignment derives not only from the recognition of appropriate lines in common from both α- and β-glucose but also from the



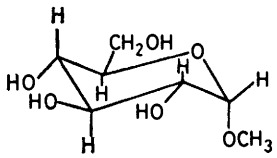
(23)

magnitude of the observed parameters (*i.e.* the large axial-type β-proton splitting and the long-range splitting from the γ-proton adjacent to the ring-oxygen). The possible significance of the enhancement of this signal obtained from β-glucose compared to that obtained from α-glucose may lie in the fact that in the former it is the axial C(1)–H bond which is broken; this may be somewhat assisted by overlap in the transition state of the incipient orbital of the unpaired electron and the alicyclic oxygen atom [*cf.* (23)]. A similar effect has been proposed [20] to account, at least in part, for the more rapid hydrogen-abstraction from the C(2) position in the *cis*- than from the corresponding *trans*-2-methoxy-4-methyltetrahydropyran; the C–H bond at the incipient radical centre is *axial* in the former. With the possible exception of this finding, attack of ·OH on α- and β-D-glucose appears to be relatively unselective.

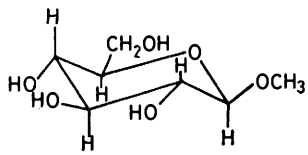
Our analysis accounts for all the lines detected from β-D-glucose, with the exception of those marked × in Figure 3. These, and, presumably, some matching resonances hidden under other peaks, could have splittings typical of a radical of partial structure ·CH(OH)–CH(OR)–; amongst the possibilities which might account for this observation is the occurrence of a second conformer of the C(6) radical (22) [*cf.* comments earlier on (9) and (10) and the evidence for restricted rotation in this type of radical] or alternatively the acyclic radical $HOCH_2C(O)CHOHCHOHCHOH–\dot{C}HOH$ formed by ring-opening of the C(5) radical (21) [*cf.*[21] fragmentation of radicals of type $\cdot CR^1R^2OR^3$ where $\cdot R^3$ is stabilized]. Evidence against the latter assignment is the observation that at higher temperatures the signal from this species was not enhanced, nor was [(21)] significantly reduced. We believe that further speculation is unjustified.

As the pH was lowered, all the spectra of first-formed radicals were removed [including, finally, that from radical (22), by pH *ca.* 0.8], as described earlier for the α-anomer. Again, the rearranged carboxy-conjugated radical (12) appeared as the C(1) radical (17) disappeared [*cf.* reaction (2)]; other weaker carbonyl-conjugated radicals were detected, but no distinction among (24)—(27), all of which should have similar a(α-H) and a(β-H) values, could be made. One very clear difference, however, between the behaviour of α- and β-D-glucose at low pH is that in the latter there is no trace of radical (16) which would result by loss of the 1-OH group from radical (18) [since signals from (18) disappear as the pH is lowered we believe that its rearrangement evidently involves loss of 3-OH rather than 1-OH]. This observation supports our assertion, made earlier, that radical (16) is formed particularly readily during the oxidation of α-glucose by rearrangement of radical (4) because of the favourable overlap between the orbital of the unpaired electron and the axial C–O bond in this anomer, a process which is presumably unfavourable for the equatorial 1-OH in the corresponding radical from β-glucose.

(b) *Methyl Glycosides of* D-*Glucose.*—Reactions of both 1-*O*-methyl-α-D-glucose (28) and 1-*O*-methyl-β-D-glucose (29) with ·OH at both *ca.* pH 4 and 1 were investigated; the results, spectral analyses, and conclusions were essentially as anticipated on the basis of the findings outlined for α- and β-D-glucose.



(28)

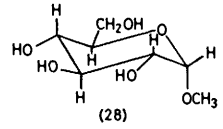
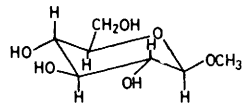
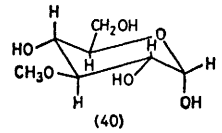


(29)

The spectrum from (28) at pH 4 was dominated by a 1 : 2 : 1 triplet of 1.800 mT with an extra (γ-proton) doublet (0.140 mT) which is assigned to the radical formed by hydrogen-abstraction from the methoxy-group [(30); see Table 3]. Also fairly prominent was the spectrum of the radical formed from attack at the oxygen-substituted exocyclic carbon, namely the C(6) radical (34). The radical from attack at C(2), (31), was again clearly seen; it lacks the characteristic doublet splitting detected from the axial 1-OH proton in the analogue, (4), from α-D-glucose itself. Radicals formed by abstraction at C(3) and C(4), (32) and (33), respectively, could be identified; peaks from the C(5)-derived radical were clearly present, though the spectrum could not be measured accurately, but no peaks could be unambiguously attributed to the C(1)-derived radical. This

TABLE 3

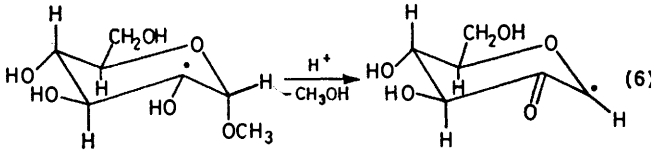
E.s.r. spectra of radicals formed from reactions of methyl glycosides of D-glucose with ·OH at pH 4

Substrate	Position of abstraction	Radical	Hyperfine splittings (mT) [a, b] a (α-H)	a (β-H)	a (other)
 (28)	OMe	(30) [c]	1.800 (2 H)		0.140 (1γ-H)
	C(2)	(31)		3.020 (1 H) 1.220 (1 H)	
	C(3)	(32)		3.00 (1 H) [d] 2.81 (1 H) [d]	
	C(4)	(33)		2.73 (1 H) [d] 2.45 (1 H) [d]	
	C(6)	(34) [c]	1.835 (1 H)	0.590 (1 H)	0.140 (1 H) [e] 0.115 (1 H) [e] 0.075 (1 H) [e]
 (29)	OMe	(35) [c]	1.805 (2 H)		0.075 (1γ-H)
	C(2)	(36)		2.765 (1 H) 2.200 (1 H)	
	C(3)	(37)		2.77 (1 H) [d] 2.85 (1 H) [d]	
	C(4)	(38)		2.42 (1 H) [d] 2.53 (1 H) [d]	
	C(6)	(39) [c]	1.825 (1 H)	0.765 (1 H)	0.120 (1 H) [e] 0.065 (1 H) [e]
 (40)	OMe	(41) [c]	1.690 (2 H)		0.040 (1γ-H)
	C(2)	(42)		3.05 (1 H) 0.94 (1 H)	0.130 (1-OH)
	C(4)	(43)		2.60 (1 H) [d] 2.23 (1 H) [d]	
	C(6)	(44) [c]	1.830 (1 H)	0.600 (1 H)	0.140 (1 H) 0.125 (1 H) 0.065 (1 H)

[a] Typically ±0.005 mT except where indicated otherwise. [b] *g* 2.0031 ± 0.0001 unless otherwise stated. [c] *g* 2.0032. [d] ± *ca.* 0.01 mT; peaks partly obscured; more accurate measurements impossible. [e] For possible assignments, see comments in text concerning C(6) radicals from α- and β-glucose.

may reflect several factors related to, for example, radical production [C(1)–H may now be sterically hindered], radical removal (as noted above, this radical is expected to be readily removed by oxidation with hydrogen peroxide on account of its two oxygen substituents) and the effect of multiple splittings in lowering the individual peak heights.

When the pH was lowered, few discernible changes to the spectrum occurred. The exocyclic radicals (30) and (34) evidently do not undergo significant rearrangement (at least down to pH *ca.* 1), and for this substrate, unlike α-D-glucose, no carboxy-conjugated radical akin to (12) can be formed. Though radicals (32) and (33) can presumably rearrange to carbonyl-conjugated radicals, the intensity of signals from the latter are expected to be low on account of the ready reduction of such radicals by Ti^{III}.[18] The only rearranged radical to be clearly discerned was (16) (see Table 1) formed by rearrangement of (31) [reaction (6)]; the fact that the pH at which this becomes detectable (1.7) is significantly lower than the appropriate pH for the α-D-glucose-derived analogue (*ca.* 3.3) reflects the poorer leaving-group ability of methanol compared with water. We estimate that k_6 is *ca.* 3.5×10^5 dm^3 mol^{-1} s^{-1}.



(31) (16) (6)

Table 3 also contains details of the spectra of the radicals obtained from 1-*O*-methyl-β-D-glucose at pH 4; the overall spectrum is shown in Figure 4. It is again dominated by the signals from the radicals formed by attack at the methoxy-group (35) and the exocyclic hydroxymethyl group (39); signals from the C(2)-, C(3)-, and C(4)-derived radicals (36)—(38) could also be distinguished, but those from C(1)- and C(5)-derived radicals could not be reliably assigned. At low pH no traces of signals from (16) were obtained [contrast reaction (6)]; this presumably again reflects the necessity for orbital overlap between the unpaired electron on C(2)

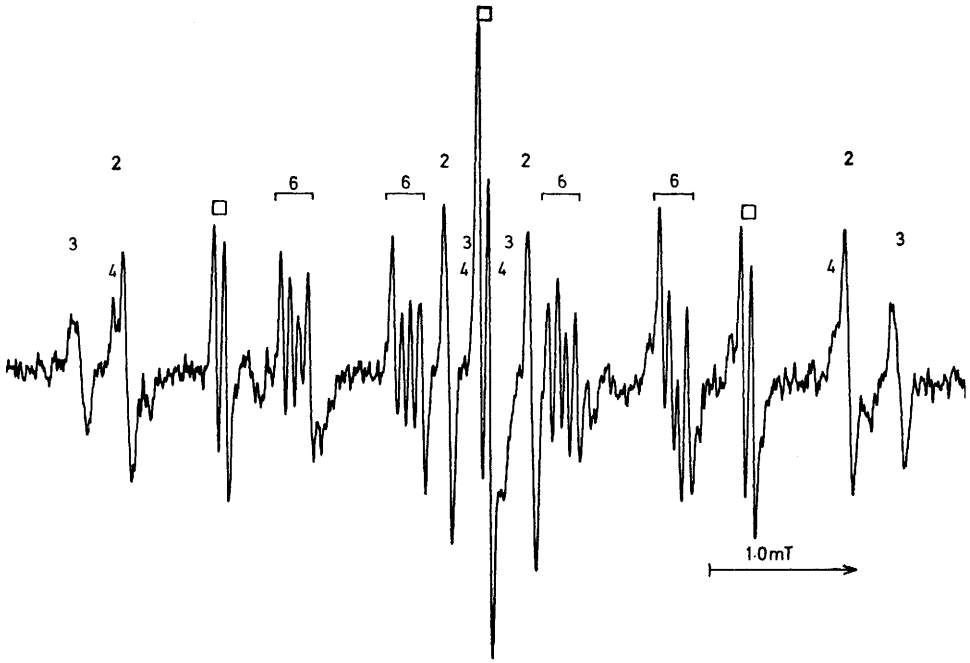


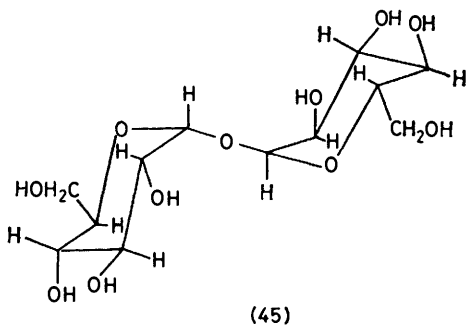
FIGURE 4 E.s.r. spectra of radicals obtained from the oxidation of 1-*O*-methyl-β-D-glucose at pH 3.5. The signal marked □ is attributed to the radical derived by attack at the methoxy-group

and the C–O bond and the consequent difficulty attaining the transition state for an equatorial methoxy-group. Nor, as expected, was the ester-conjugated radical (12) detected. Weak signals were detected from two carbonyl-conjugated radicals [with *a*(1H) 1.78, *a*(1H) 3.73, and *a*(1H) 0.075 mT, *g* 2.0044 and *a*(1H) 1.78, *a*(1H) 3.47, and *a*(1H) 0.055 mT, *g* 2.0044], these evidently being derived by rearrangement of (36), (37), or (38); no further distinction can be made.

Reaction of 3-*O*-methyl-α-D-glucose (40) at pH 4 led to the detection of the radicals derived by attack at C(2), C(4), and C(6), (42)—(44), as well as at OCH_3, (41); data are given in Table 3. Other weaker lines could not be unambiguously analysed. In the complex spectra obtained at low pH, conjugated radicals of both ĊH–CO–O– and –CO–ĊH–O– type could be detected, suggesting that acid-catalysed rearrangement of radicals obtained by C–H abstraction at C(1) and C(2) (*cf.* α-D-glucose) had occurred.

Here, as with the other methoxy-substituted compounds studied, attack of hydroxyl is directed chiefly to the exocyclic OCH_3 and CH_2OH groups; this presumably reflects, at least in part, the statistical factor involved, though steric hindrance of the tertiary C–H bonds on the rings close to the methoxy and hydroxymethyl substituents, and, possibly, faster oxidative pathways for the radicals so formed, may play a part.

(c) D-*Trehalose* (1-O-α-D-*Glucopyranosyl*-α-D-*glucopyranoside*).—Particularly clear spectra were obtained at pH *ca.* 4 for the disaccharide αα-trehalose (45), chosen for study since it comprises two 1,1-linked α-glucose moieties, thus providing only six different sites for C–H abstraction and no possibility of mutarotation. Evidence for the formation of at least five of these radicals, (46)—(50), formed by C–H abstraction from positions C(2)—C(6) (see Table 4) was obtained; no signals attributed to the radical formed *via* 1-H abstraction could be clearly detected (these may be present, obscured by other lines, though it is also possible that steric hindrance seriously inhibits its formation). The e.s.r. parameters of radicals (46)—(50) closely resemble those for the related α-D-glucose radicals (see Table 1) and lend



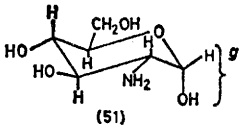
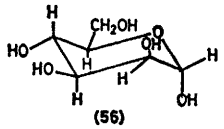
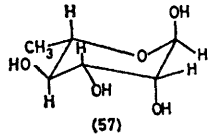
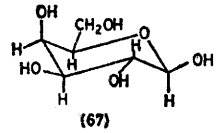
(45)

support to our analysis for the latter. As with the α-glucose-derived analogues, radicals from C(5) and C(6), (49) and (50) respectively, demonstrate the conformational locking associated with radicals with both an α- and a β-oxygen substituent.

As the pH was lowered the C(5)-derived radical (49) was the first to be removed, followed by those from C(3), (47), and C(4), (48), and then the C(2)-derived species (46). The ready removal of (49) probably reflects the ease of loss of the β-OH group [locked into an eclipsing position, and therefore favourable for elimination: the resulting radical-cation is presumably removed by reaction with titanium(III)]. In (47) and (48) the loss of the

TABLE 4

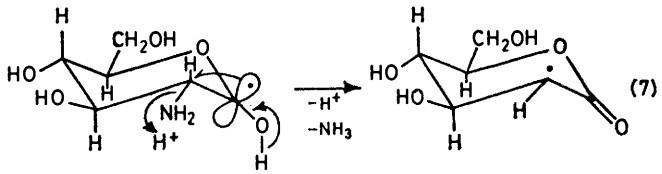
E.s.r. parameters of radicals formed from the reaction of a variety of carbohydrates with ·OH [a]

Substrate	Position of abstraction	Radical	Hyperfine splittings (mT) [b, c] a (α-H)	a (β-H)	a (other) [d]
ααα-Trehalose (45)	C(2)	(46)		3.175 (1 H); 1.075 (1 H)	0.060 (1 H)
	C(3)	(47)		3.100 (1 H); 2.800 (1 H)	0.065 (2 H)
	C(4)	(48)		2.550 (1 H); 2.600 (1 H)	0.05 (1 H)
	C(5)	(49)		3.35 (1 H); 1.04 (1 H) [e]; 0.76 (1 H) [e]	*ca.* 0.05 [f]
	C(6)	(50)	1.85 (1 H)	0.588 (1 H)	0.145 (1 H); 0.125 (1 H); 0.070 (1 H)
 (51) [g]	C(4)	(52)		2.515 (1 H); 2.650 (1 H)	
	C(5)	(53)		3.375 (1 H); 1.015 (1 H) [e]; 0.665 (1 H) [e]	
	C(6)	(54)	1.84 (1 H)	0.600 (1 H)	0.130 (2 H); 0.070 (1 H)
 (56)	C(2)	(58)		2.975 (1 H); 1.295 (1 H)	0.160 (1 H)
	C(3)	(59)		3.310 (1 H); 0.690 (1 H)	
	C(4)	(60)		2.850 (1 H); 2.265 (1 H)	
	C(6)	(61)	1.875 (1 H)	0.625 (1 H)	0.130 (1 H); 0.125 (1 H); 0.075 (1 H)
 (57)	C(2)	(62)		2.960 (1 H); 1.300 (1 H)	0.15 (1 H)
	C(3)	(63)		3.310 (1 H); 0.690 (1 H)	
	C(4)	(64)		2.850 (1 H); 2.290 (1 H)	
 (67)	C(1)	(68)		2.85 (1 H)	0.28 (γ-H)
	C(2)	(69)		3.430 (1 H); 2.070 (1 H)	
	C(3)	(70)		3.290 (1 H); 0.775 (1 H)	0.040 (3 H)
	C(4)	(71)		2.390 (1 H); 2.340 (1 H)	
	C(5)	(72)		0.900 (1 H); 0.630 (1 H); 0.515 (1 H)	
	C(6)	(73)	1.765 (1 H)	1.345 (1 H)	0.110 (1 H); 0.050 (1 H)

[a] pH *ca.* 4.0. [b] ±0.005 mT. [c] *g* 2.0031 ± 0.0001 [except for C(6) radicals (*g* 2.0032)]. [d] Not assigned unambiguously; splittings expected (in some cases, see text) from α-OH, β-OH, γ-H. [e] Conformational locking, see text. [f] Extra small splittings; not analysed in detail. [g] Probably mainly α-form, see text.

β-equatorial hydroxy-groups is expected to be much more difficult. As (46) was removed, the signal characteristic of (16), as detected from α-D-glucose at low pH, was observed. This implies that (46) rearranges *via* loss of the β-axial alkoxy-group [*cf.* reaction (6) for the corresponding methyl glucoside]; this observation has particular significance in that it characterizes a mechanism for glycosidic cleavage, and hence for the radical degradation of polysaccharides under acidic conditions.

(d) *2-Amino-2-deoxy-D-glucose Hydrochloride* (*2-Aminoglucose Hydrochloride*).—As expected for this substrate (51), attack of the electrophilic hydroxyl radical is directed away from the amino-group, which is protonated at the pH values employed; at pH *ca.* 4 (the highest pH studied) only the radicals formed by C–H abstraction at C(4)—C(6) could be unambiguously detected [(52)—(54), respectively]. These radicals have parameters which are nearly identical with those from the corresponding radicals from α-D-glucose, which is as expected since this substrate is reported [22] to exist predominantly in its α-form. We can also infer that the C(1)-radical (55)



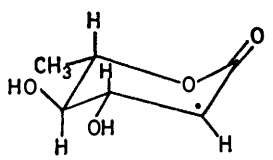
(55) (12)

is formed, even though its resonances could not be clearly discerned; thus, as the pH was lowered signals from the ester-conjugated radical (12) (see Table 1) appeared, to join these from the C(6) radical (54) which does not rearrange significantly under these conditions. Evidently (55), presumably formed, despite the deactivating effect of NH_3^+, on account of the activating effect of two α-oxygen substituents, rearranges *via* loss of ammonia [reaction (7)], a process analogous to the loss of hydroxide ion from the corresponding radical from α-D-glucose [reaction (2)].

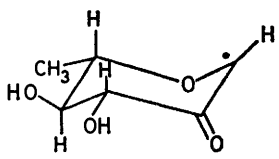
(e) *Other Hexoses and Related Compounds.*—(i) *D-Mannose and α-L-rhamnose.* We have also studied the reactions of ·OH with D-mannose, which differs from D-glucose only in its configuration at C(2) [it exists predominantly in its α-form (56)] and α-L-rhamnose (57) (6-deoxymannose).

In the spectrum from D-mannose, the radical (58), formed by abstraction of 2-H, is recognized since the e.s.r. parameters are identical within experimental error to those of the same species (4) derived from α-D-glucose (see Table 4). Radicals formed by abstraction from C(3), C(4), and C(6) could also be readily identified. Signals from the C(5)- and C(1)-derived radicals could not be clearly discerned, though evidence for the formation of the latter is provided by the detection of the radical (12), formed from it *via* rearrangement, as the pH was lowered. Radical (16), from rearrangement, was also detected as (58) disappeared; signals from (59) and (60) were also readily removed, but the resultant carbonyl-conjugated radicals were not detected (see earlier). The order of disappearance of (58)—(61), *i.e.* first (58), then (59), and then (60) [and, ultimately, at very low pH (61)] probably reflects, at least in part, the ease of loss of the axial β-OH group in the first two of these. As judged by the appearance of (12), the C(1)-derived radical rearranges faster than (59) but slower than (58).

Our findings for L-rhamnose (see Table 4) confirm these assignments and those presented earlier; the spectra obtained were less complicated than those of most of the other sugars studied, notably because no C(6)-derived radical was detected (presumably because the activating effect of the 6-hydroxy-group has been lost). Nor could signals due to the C(5)-derived radical be found, despite the fact that an expected spectrum width of *ca.* 7.5 mT (*ca.* 3.0 mT from an axial β-H splitting and *ca.* 1.5 mT from the three protons of the CH_3 group attached to the radical centre) should have made recognition straightforward; this may be because the position is relatively deactivated, or because this α-alkyl-substituted radical is readily oxidized, or, possibly, because the effect of the methyl-group splitting is to render the outer lines too weak for detection. Clear analyses for radicals (62)—(64) were obtained. When the pH was lowered signals from these were removed, that from (62) first, then that from (63) and then (64) (exactly as with mannose). The two new radicals detected [(65) from the (undetected) C(1)-derived radical, and (66), from (62)] have parameters similar to the analogues from D-glucose and D-mannose [*i.e.* (12) and (16) in both cases].



(65)



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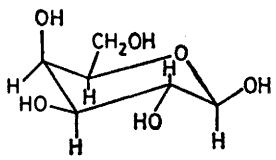
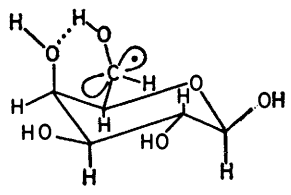


(67)

(ii) D-*Galactose.* This, the last compound to be studied, was chosen since it is epimeric with D-glucose [the configuration is reversed at C(4)]. The spectra from this substrate proved more readily analysable when the substrate-containing solution was allowed to stand for at least 1 h at room temperature before the flow experiments were conducted; we associate this with the occurrence of mutarotation to give, ultimately, a mixture which is predominantly (*ca.* 75%) the β-form indicated [23] [structure (67)].

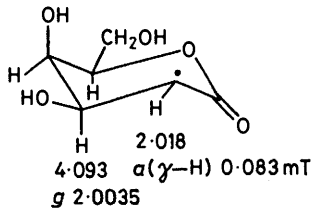
Spectra were detected from all six possible radicals obtained from (67) (see Table 4). The spectrum from the C(2) (β)-derived species (69) contains splittings from two axial β-protons, the difference between these (3.430 and 2.070 mT) being somewhat greater than that in the analogous radical from β-D-glucose (18) (2.860 and 2.280 mT). The individual assignments are made to 1- and 3-H, respectively; the particularly low value of the latter in (69) [even in comparison with that from (18)] presumably reflects the effect of the *axial* 4-hydroxy-group in distorting the ring geometry such as to increase the dihedral angle between the orbital of the unpaired electron and 3-H. A spectrum from the corresponding C(2)-α radical [with g 2.0031 and a(1H) 2.72, a(1H) 1.65 mT, together with incompletely resolved fine structure] could be detected from samples studied immediately after dissolution (*i.e.* before mutarotation had occurred).

The spectrum from (70) showed the anticipated axial and equatorial β-H splittings, in addition to some unassigned small splittings, and (71), as expected, has parameters identical with those of the same radical generated by attack at C(4) in β-glucose (20). The C(5)-derived radical (72) possesses a typical equatorial β-proton splitting, together with two non-equivalent β-proton splittings from the conformationally locked protons of the hydroxymethyl group. The comparatively intense spectrum from the C(6)-radical (73) is recognized by its characteristic α-H proton splitting of 1.765 mT; somewhat surprising here, in view of the low β-H splittings (*ca.* 0.7 mT) in analogous radicals from other substrates, is the enhanced value of 1.345 mT for a(β-H). This evidently derives from the influence of the axial 4-OH group: we suggest that internal hydrogen-bonding involving the 4- and 6-hydroxy-groups favours a conformation (74) in which the ring-oxygen no longer eclipses the orbital of the unpaired electron so the β-C–H bond makes a smaller dihedral angle with the orbital of the unpaired electron than the 60° associated with, *e.g.*, (9) and (10). A weak signal attributed to the C(1)-abstraction radical (68) was also detected, though since the resonances were largely obscured by those from (70) and (73) only approximate measurements were possible.



(74)

As the pH was lowered, signals of radical (68)—(72) from β-D-galactose were removed; by comparison, the C(6)-derived radical (73) appeared to rearrange particularly slowly and its signal was hardly reduced in intensity by pH *ca.* 1.0 [this may reflect the finding that in this radical the β-C–O bond does not eclipse the orbital of the unpaired electron, see (74)]. Three new signals were analysed, one the carboxy-conjugated radical (75) evidently derived by rearrangement of the C(1)-radical (68) and two carbonyl-conjugated radicals which possess axial β-proton splittings; these have g 2.0045, a(α-H) 1.805, a(β-H) 3.685, a(1H) 0.070 mT and g 2.0045, a(α-H) 1.805 and a(β-H) 3.80 mT with further long-range coupling. Of these two, the former, unusually intense spectrum is assigned to (76) since elimination is expected to occur particularly rapidly for the axial 4-OH in the precursor C(3)-derived radical (70). The latter may be the signal from either the radical (77) [derived from (70) *via* loss of the equatorial 2-OH] or (78) [derived from (71) *via* loss of the equatorial 3-OH].



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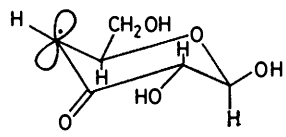

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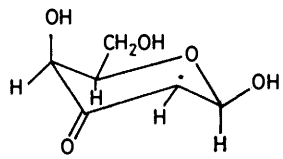

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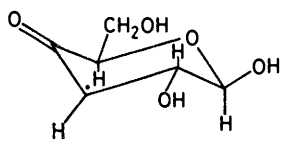

(78)

Conclusions.—We have established that when the hydroxyl radical reacts with many relatively simple carbohydrates at pH *ca.* 4 in aqueous solution attack is essentially unselective. Even in examples where the C(1)-derived radical is itself difficult to characterize, the detection at low pH of a radical evidently derived *via* its rearrangement, suggests that the precursor is indeed generated in (approximately) the statistically expected concentration (this αα-dioxygen-substituted radical may suffer preferential oxidation by H_2O_2). Some degree of selectivity is observed for an amino-substituent (where the NH_3^+ group apparently deactivates adjacent C–H bonds to attack) and in some methyl glucosides, as judged by the apparent ease of attack at the exocyclic OCH_3 substituents.

The spectra of the first-formed radicals and the radicals derived from them by acid-catalysed rearrangement provide good examples of the angular dependence of β-proton splittings and, in some cases, of longer range interactions with axial γ-protons (across oxygen and carbonyl) or β-axial OH groups.

We have studied in detail the rearrangement of a variety of αβ-dioxygen-substituted radicals as the pH is lowered and have identified a route for glycosidic cleav-

age under acid conditions. The following order of reactivity for acid-catalysed loss of different β-substituents has also been found:

axial $OH^- >$ equatorial $OH^- \sim$ axial $OR^- >$ equatorial OR^-

Elimination of an axial β-group is evidently favoured by the overlap between the orbital of the unpaired electron and the bond joining the β-carbon to the leaving group. For radicals of different structure which possess in common a β-axial hydroxy-group, rearrangement proceeds somewhat faster for the C(2)-derived radical (with loss of 1-OH) than for the C(1)-derived radical (with loss of the 2-OH) which in turn is faster than the loss of hydroxy from C(3)- and C(4)-derived species; examples are provided by the C(1)-radical, (58), and (59) from mannose. The enhancement of the former two compared with the last-named may reflect the production of radicals with more extensive conjugation [·C–C(O)O and C(O)–ĊH–O] than the simple carbonyl-conjugated radicals [·C–C(O)]; the particular readiness with which the C(1) radicals in this category react is likely to be associated with the presence at the α-carbon of *two* mesomerically electron-donating oxygen substituents.

EXPERIMENTAL

E.s.r. spectra were recorded on Varian E-104 and E4 spectrometers equipped with X-band klystrons and 100 kHz modulation. Hyperfine splittings were measured to within 0.005 mT (except in cases where peaks were partially obscured, for which the accuracy is estimated as ±0.01 mT), by comparison with Fremy's salt [a(N) 1.309 mT [24]; g values (±0.0001) were also measured by comparison with the same standard (g 2.0055 [25]). Most experiments employed a flattened aqueous sample cell and a mixing chamber which allowed three reagent streams to be mixed simultaneously; the solutions were pumped through the cell, using a Watson–Marlowe H.R. flow inducer, such that the dead time (between mixing and observation) was *ca.* 60 ms. Measurements (to within ±0.05 units) were made continuously with a Pye PW9410 digital pH meter coupled to a Russell pH Ltd. electrode inserted into the effluent stream.

For reactions at pH < 2, stream (i) contained 0.008 mol dm^{-3} titanium(III) [added as 12.5% (w/v) titanium(III) chloride solution (Fisons technical grade)], stream (ii) contained *ca.* 0.05 mol dm^{-3} hydrogen peroxide [added as 100 volume hydrogen peroxide (Fisons)], and stream (iii) contained the substrate (*ca.* 0.035 mol dm^{-3}). Concentrated sulphuric acid was added to the first stream to bring the final pH to the required value. For experiments at higher pH, EDTA (4 g dm^{-3}) was added to stream (i) and the pH was adjusted as required with either concentrated sulphuric acid or ammonia (d 0.880). All solutions were made up in water which had been deoxygenated with a nitrogen purge, and nitrogen was bubbled through the solutions prior to mixing. Where it was necessary to avoid mutarotation, the solutions containing the sugars were made up with ice-cold water.

Flow-photolysis experiments were conducted by the continuous irradiation (with an Hanovia 977B-1 1 kW mercury–xenon compact arc) of a deoxygenated aqueous solution containing the sugar (*ca.* 1 mol dm^{-3}) and hydrogen peroxide (*ca.* 0.5 mol dm^{-3}) as it flowed slowly through a flattened aqueous sample cell in the cavity (flow rate *ca.* 1 ml min^{-1}).

The following reagent-grade chemicals were employed: α-D-glucose, β-D-glucose, 1-*O*-methyl-α-D-glucose, 1-*O*-methyl-β-D-glucose, 3-*O*-methyl-α-D-glucose, D-trehalose dihydrate, D-mannose (at least 80% α-form), 2-amino-D-glucose (believed [22] to be mainly in the α-form) (all Sigma), and D-galactose (B.D.H.), which was largely present as the β-anomer.[23]

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