Radical Reactions of Carbohydrates. Part 4.[1] Electron Spin Resonance Studies of Radical-induced Oxidation of Some Aldopentoses, Sucrose, and Compounds containing Furanose Rings

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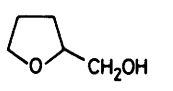
E.s.r. spectra detected when ·OH reacts with the aldopentoses xylose and ribose are attributed to pyranose ring radicals, formed in essentially unselective attack; in contrast, in the corresponding reactions of the model compound tetrahydrofurfuryl alcohol, as well as some isopropylidene derivatives of glucofuranose, the hydrogen atoms on the carbon atoms adjacent to oxygen in a furanose ring are activated towards abstraction. A similar activation, evidently stereoelectronic in origin, is also proposed to account for the enhanced reactivity of C(5')–H in the *furanose* ring of sucrose, a finding which may have particular relevance to the mechanism of radiation damage in DNA.

In aqueous solution the majority of monosaccharides adopt preferentially pyranose ring configurations;[2] consequently most studies of the free-radical chemistry of simple sugars have been chiefly concerned with species containing six-membered rings.[3] However, many biologically important compounds which are built up from a sugar unit combined with other groups have the carbohydrate locked into its furanose form, as for example in DNA. The free-radical chemistry of DNA assumes particular significance in the light of the recognition[4] that this is the cellular component most susceptible to radiation damage; further it has been suggested[5] that radical reactions of the deoxyribose phosphate moiety lead to strand-breakage and subsequent damage to the cell.

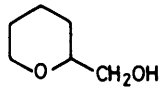
We have previously shown that e.s.r. spectroscopy may be used in conjunction with a continuous-flow system and the radiomimetic Ti^{III}–H_2O_2 couple to study the reactions between ·OH and a series of carbohydrates containing six-membered rings.[1,6,7] It was possible to demonstrate directly in this way that ·OH reacts relatively unselectively with such sugars: for example, with D-glucose all six radicals resulting from abstraction of hydrogen at each carbon atom in the α- and β-anomers could be characterized and distinguished under pseudo steady-state conditions. In contrast the subsequent acid- and base-catalysed transformations of the first-formed radicals were found to be particularly sensitive to the steric and electronic effects of substituents.[7]

We have now extended this investigation to the reactions of the hydroxyl radical with some furanose compounds: the substrates chosen include the model compound tetrahydrofurfuryl alcohol, some aldopentoses (*e.g.* ribose) in which pyranose and furanose forms exist in equilibrium in solution, some isopropylidene derivatives of glucofuranose, and the disaccharide sucrose (1-*O*-α-D-glucopyranosyl-β-D-fructofuranoside). Our concern has mainly been with the selectivity, or otherwise, of ·OH, generated *via* reaction (1) in an aqueous flow system, though we have also studied where possible the corresponding reactions of Bu^tO·, generated photolytically from di-t-butyl peroxide in non-aqueous solvents.[8]

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



(1)



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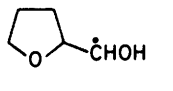

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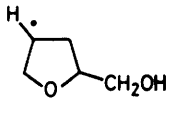


(6)

Results and Discussion

E.s.r. spectra were generally obtained by mixing in a three-way flow system (with a dead-time of *ca.* 50 ms) solutions containing the substrate (at concentrations typically up to *ca.* 0.05 mol dm^{-3}, where solubility and availability allowed), hydrogen peroxide, and titanium(III) ions. Reactions were generally carried out in the pH range *ca.* 2—4 (in the presence of edta to sequester titanium above pH 2.5), so as to minimize the effects of acid- and base-catalysed rearrangements of αβ-dioxygen-substituted radicals. Photolytic experiments were carried out by irradiating *in situ* mixtures of the substrate (dissolved in dichloromethane where necessary) and a few drops of di-t-butyl peroxide, with the unfiltered output from an Hanovia 9770-1B 1 kW mercury–xenon lamp in the temperature range −30 to 0 °C.

(a) *Tetrahydrofurfuryl Alcohol* (1) *and 2-Hydroxymethyltetrahydropyran* (2).—We first studied the reaction with ·OH in a flow system of the model compounds (1) and (2), chosen so that we could compare the reactivity of hydrogens at different positions in five- and six-membered rings containing a single oxygen atom and an adjacent hydroxymethyl group. It was of particular interest to determine whether attack by ·OH and the reactivity of (1) and (2) towards radicals shows evidence of ring-strain and stereoelectronic effects as previously noted[9] for the relative rates of hydrogen-atom abstraction by Bu^tO· from a variety of cyclic and acyclic ethers, acetals, and orthoformates at −60°.

Reaction of an aqueous solution of (1) (at a concentration of *ca.* 0.1 mol dm^{-3}) with ·OH led to the detection of an e.s.r. spectrum comprising signals from a mixture of radicals. Better signals were obtained at pH *ca.* 2 in the absence of edta than at pH *ca.* 4 in its presence (which can be understood in terms of the ability of Ti^{IV}–edta to act as an oxidant for oxygen-conjugated radicals under the latter conditions[10]). The spectrum was dominated by the signal from (3) (see Table 1), as noted previously.[11] This is characterized by a rather low α-proton splitting (1.250 mT), indicative of there being some

Table 1. E.s.r. parameters of a variety of radicals containing five-membered rings and some related species

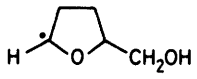
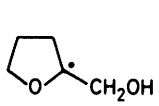
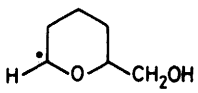
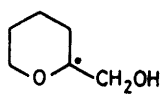
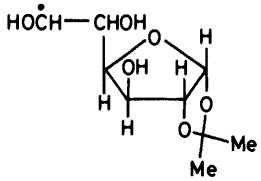
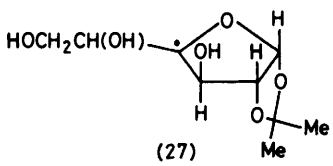
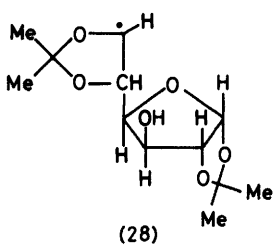
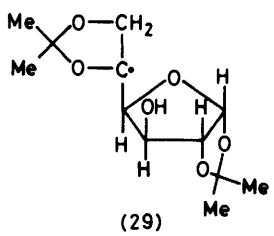
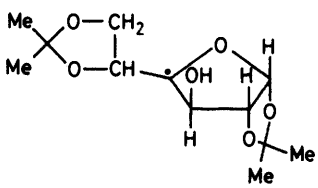
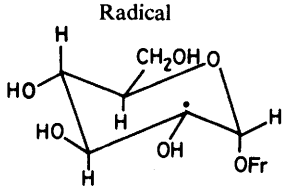
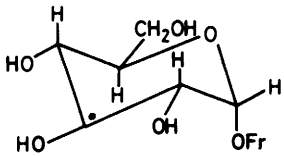
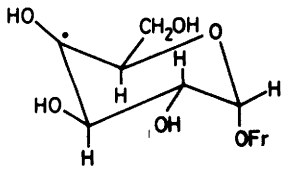
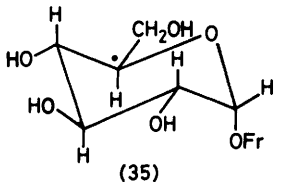
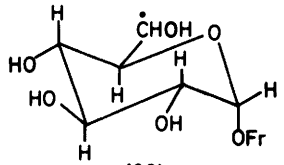
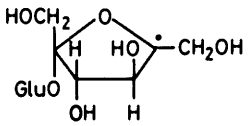
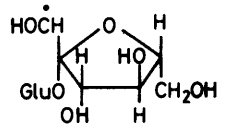
Substrate	Method [a]	Radical	T/°C	Hyperfine splittings (mT) [b] a(α-H)	a(β-H)	a(other)	g [c]
(1)	·OH	 (3)	18	1.250(1)	3.170(1) 2.560(1)	0.075(s, γ) 0.210(1, OC*H*)	2.0031
	Bu^{t}O·	 (4)	−13	1.320(1)	3.090(1) 2.565(1)	0.075(2, γ) 0.235(1, OC*H*)	2.0031
					2.595(2) 0.750(2)	0.040(1, O*H*) 0.065(2, γ) 0.180(2, OC*H*)	2.0031
(2)	Bu^{t}O·	 (7)	−10	1.630(1)	2.440(2) [d]	0.070(2, γ) 0.185(1, OC*H*)	2.0031
		 (8)			2.280(2) [d] 0.965(2)	0.075(2, OC*H*)	2.0031
(24)	·OH	 (26)	18	1.780(1)	0.825(1 H)	0.045(2) [e] 0.090(2) [e]	2.0032
		 (27)			1.075(1) 0.940(1)	0.095(1) [f]	2.0031
(25) [g]	·Bu^{t}O·	 (28)	−20	1.235(1)	2.670(1)	0.075(1) [h]	2.0032
		 (29)			2.645(1) 2.540(1) 0.430(1)	0.150(1) [i] 0.075(1) [i]	2.0032
	·OH	 (30)	18		1.075(1) 0.440(1)		2.0031

Table 1. (*continued*)

Substrate	Method [a]	Radical	*T*/°C	Hyperfine splittings (mT) [b] *a*(α-H)	*a*(β-H)	*a*(other)	g [c]
Sucrose (31)	·OH	 (32)	18		{3.100(1), 1.270(1)}	0.050(2) [f]	2.0031
		 (33)			{3.100(1), 2.820(1)}	0.045(2) [f]	2.0031
		 (34)			{2.590(1), 2.515(1)}		2.0031
		 (35)		*j*	*j*		
		 (36)		1.850(1)	0.585(1)	{0.140(1) [e], 0.125(1) [e], 0.075(1) [e]}	2.0032
		 (37)			{2.950(1), 0.965(1), 0.940(1)}	0.050(2) [k]	2.0031
		 (38)		1.850	*i*		

[a] ·OH, aqueous solutions, flow system; Bu^{t}O·, photolytic decomposition of di-t-butyl peroxide. For experimental details see text. [b] ±0.005 mT. [c] ±0.0001. [d] Only the average splittings of the pairs of β-protons could be recorded on account of line broadening of the central lines in the splitting patterns. [e] Long-range and/or hydroxy-splittings typical of this type of radical (see ref. 7): individual assignments not possible. [f] Assignment ambiguous: probably γ-OCH. [g] Radicals also detected with ·OH: see text. [h] Assignment ambiguous: probably γ-H. [i] Long-range splittings unassigned. [j] Weak and broad resonances: no analysis possible. [k] γ-H and β-hydroxy-proton splittings (the latter is removed at pH <2).

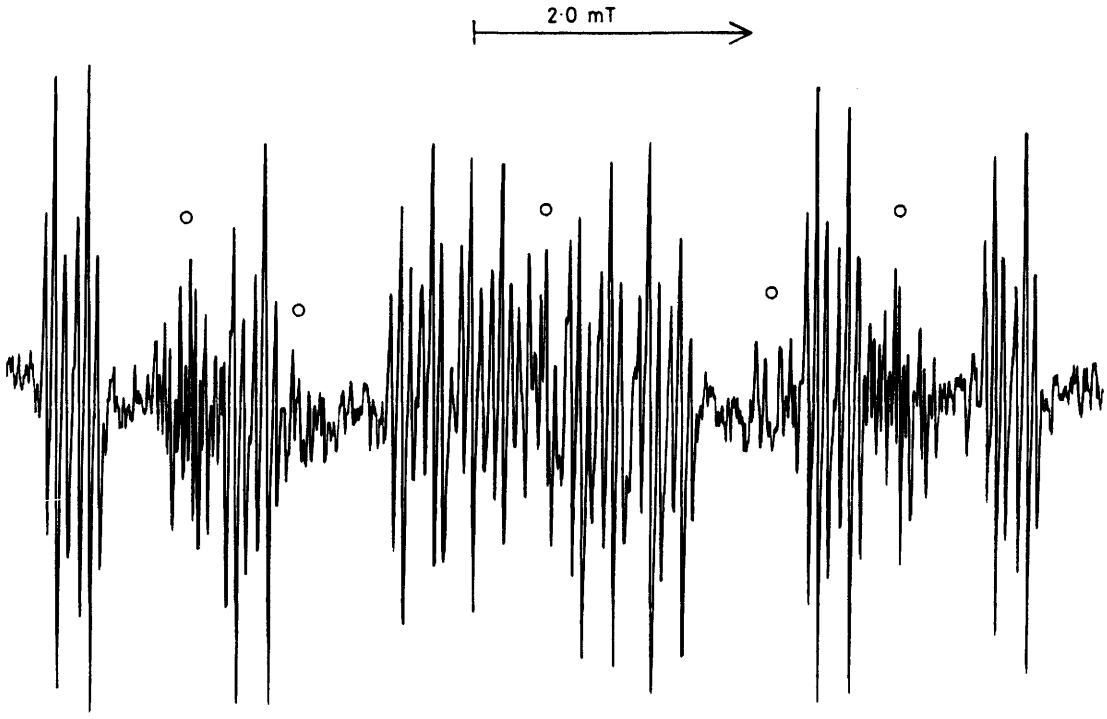
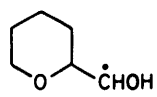


Figure 1. E.s.r. spectra of radicals (3) and (4) derived from the reaction of Bu^{t}O· with tetrahydrofurfuryl alcohol at −13 °C. Peaks from (4) are indicated by O

degree of bending at the radical centre, and two non-equivalent β-protons (3.170, 2.560 mT) whose difference reflects the role of the exocyclic substituent in establishing a conformational preference in a non-planar ring (probably a half-chair, with the substituent pseudo-equatorial [11]). Comparison of the spectra with those obtained under photolytic conditions (see below) enabled the presence of a relatively weak spectrum from (4) to be identified, though the parameters could not be measured with certainty. The radical (5) obtained by hydrogen abstraction from the hydroxymethyl group was also characterized as present in low concentrations though its resonances were largely obscured by those from (3). No signal could be identified from radicals formed by attack at β-C–H bonds [*e.g.* (6)].

Under photolytic conditions signals from (3) and (4) were observed (see Figure 1 and Table 1). The dominant spectrum from the former radical is similar to that obtained in aqueous solution; as the temperature was lowered, no significant changes in splittings occurred but the inner lines of the 1 : 1 : 1 : 1 pattern from the β-protons began to broaden (at −27°) and had virtually disappeared by −60° (below this the mixture froze). The much weaker spectrum from (4) is characterized by a triplet from the exocyclic methylene β-protons [whose low magnitude is indicative of there being a 'locked' conformation with the β-C–O(H) bond eclipsing the orbital of the unpaired electron] and two large and equivalent β-proton splittings from the cyclic methylene group (the ring is presumably undergoing rapid flipping). No significant changes with temperature were discerned. Nor was there any trace of (5) or of radicals from β-abstraction [*e.g.* (6)].

2-Hydroxymethyltetrahydropyran (2) was studied under similar circumstances, though with less clear-cut results. For example, the flow-system oxidation of a solution of this substrate (*ca.* 0.1 mol dm^{-3}) with ·OH led to a more complex and weaker spectrum than was obtained from (1). Signals from the oxygen-conjugated radicals (7)—(9) were present, but an



(9)

unambiguous analysis proved impossible. The weakness of the spectrum is believed to reflect not only a relative lack of selectivity compared with (1) but also, at least in part, the expected occurrence of selective line-broadening processes which result from 'flipping' of the six-membered ring at intermediate exchange rates (see *e.g.* ref. 12). In photolysis experiments, signals from both (7) and (8) could be clearly detected (see Table 1) though the occurrence of broad lines in the centre of the spectrum allowed only the *sum* of the splittings of the β-protons to be established for each radical.

In a series of experiments designed to study the relative reactivity of (1) and (2) towards ·OH (and their reactivity compared with an open-chain oxygen-substituted analogue) the flow system was employed with equimolar mixtures of (1) and (2) as well as with equimolar mixtures of each of (1) and (2) with methanol. Analysis of the results,* *via* measurement of the intensities of overmodulated spectra, indicates that (1) and (2) show a comparable reactivity towards ·OH but that the *overall* reactivity in each case is *ca.* 2.5 times that of methanol (for which a rate constant of 1×10^9 dm^3 mol^{-1} s^{-1} has been reported [13]).

Our results for the reactions of (1) and (2) with ·OH suggest that, as found for Bu^{t}O· in its reactions with a series of cyclic ethers,[9] the α-(C–H) bonds in the ring systems are activated to attack; this is particularly noticeable for reaction

* Our analysis (*cf.* also ref. 9) is based on the assumption that the radicals involved, being of the same charge and type, possess similar termination rate constants.

of (1) with ·OH, where radical (3) predominates (in contrast, for glucose and many of its derivatives, attack at the exocyclic methylene group and at the ring C–H atoms appears to proceed at comparable rates in more or less statistical fashion). This selectivity presumably reflects in part the loss of a certain amount of ring strain in abstraction from the tetrahydrofuran but also the fact that abstraction of C–H is evidently favoured by a small dihedral angle between the C–H bond and the *p*-type lone-pair orbital on oxygen (a five-membered ring has each hydrogen in the adjacent methylene group subtending a formal angle of 30°; * for the tetrahydropyran the angles are 30 and 90°). The fact that [(3)] is considerably greater than [(4)] (*e.g.* a ratio of *ca.* 4 : 1 for ·OH) reflects, in part, the statistical factor of 2; other factors may include a reduction in the reactivity of the single α-hydrogen in (4) as a result of either (or both) steric hindrance by the CH_2OH group or deactivation to attack by the electrophilic hydroxyl radical at positions β to an oxygen substituent (see *e.g.* ref. 14).

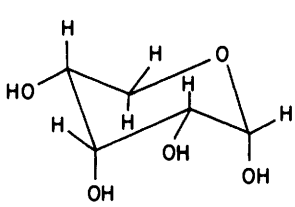
(b) D-*Ribose and Related Compounds.*—Experiments were also conducted to study the reaction between ·OH and a variety of pentoses (D-ribose, L-arabinose, D-xylose, and deoxy-D-ribose), some of which can exist to a significant extent in solution in both pyranose and furanose forms.

The spectra from freshly prepared solutions of D-ribose at pH *ca.* 3 (in the presence of edta) comprise a complex overlapping set of weak signals with one fairly prominent signal [with *a*(3H) 0.075 mT, *g* 2.0031] which was somewhat less intense when solutions which had been standing for some time were oxidized. In the background signal, lines from radicals with *a*(1H) 1.37, *a*(1H) 2.71 mT, *g* 2.0031 and *a*(1H) 1.95, *a*(1H) 2.25 mT, with further splittings and *g* 2.0031, could be distinguished.

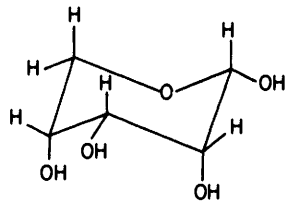
We attribute the complexity and time-dependent behaviour to the facts that a variety of different forms are present under equilibrium conditions (after mutarotation) and that the composition changes between dissolution of the solid and achievement of equilibrium. After mutarotation ribose comprises [15] (at 31°) a complex mixture of 21.5% α-pyranose form [with two possible conformations, $^4C_1$ (10a) and $^1C_4$ (10b), the latter apparently predominating] and 58.5% β-pyranose form [with $^4C_1$ conformation (11a) preferred over $^1C_4$ (11b), with a calculated ratio of *ca.* 3 : 1], together with 6.5% of the α-furanose form (12) and 13.5% of the β-furanose form (13). Though the overall complexity is therefore hardly surprising, we identify the radicals detected as being derived from the major conformer present, namely the β-pyranose $^4C_1$ isomer (11a). The two weaker sets of signals can be seen to have parameters as expected for radicals formed by abstraction from C(2) and C(5) respectively (the former with equatorial and axial β-proton splittings, the latter with an α-proton and an axial β-proton splitting). The origin of the signal comprising the very small quartet, which does not appear attributable to any corresponding radical from (11a), is discussed later.

As the pH was lowered the weak resonances from ribose were reduced in intensity even further and were replaced by signals characterized by their *g*-values as being from carbonyl-conjugated radicals [·CH–C(O)–; *g* 2.0045] and, as previously noted by Norman and Pritchett,[16] a carboxy-conjugated radical [·CH–C(O)O–; *g* 2.0035]. These radicals are formed by acid-catalysed rearrangement of first-formed αβ-dihydroxy-substituted radicals [reaction (2)]; the carboxy-conjugated species evidently derives from the C(1)-abstraction radical *via* loss of the β-OH group on C(2).

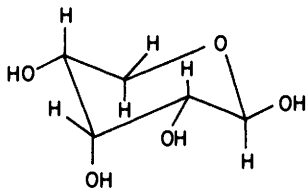
2-Deoxyribose and arabinose gave weak complex spectra



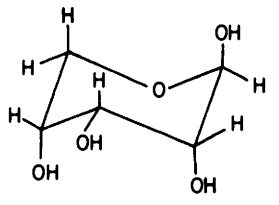
(10a)



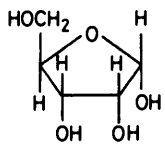
(10b)



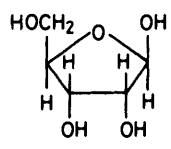
(11a)



(11b)

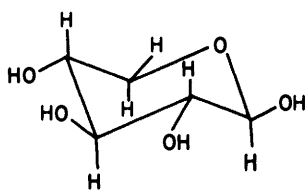


(12)

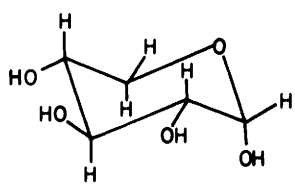


(13)

$$R^1-CH(OH)-\dot{C}(OH)-R^2 \xrightarrow[-H^+,-OH^-]{H^+} R^1-\dot{C}H-C(O)-R^2 \quad (2)$$



(14)



(15)

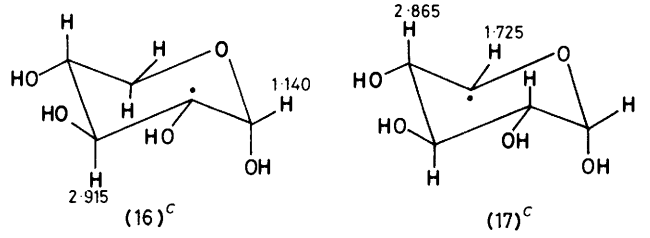
at pH *ca.* 3, and evidence for rearranged radicals at pH *ca.* 1, more or less as noted for ribose. On the other hand, reaction of xylose with ·OH at pH *ca.* 4 led to the detection of several radicals whose spectra could be clearly analysed and assigned (*cf.* the behaviour of D-glucose). Again, the exact nature of the signals depended upon the time elapsed between dissolution and the recording of the spectra. The significance of the relative simplicity of the spectra compared with those from ribose is, we believe, that this substrate exists in aqueous solution at equilibrium (*i.e.* after mutarotation has occurred) almost exclusively in pyranose forms [15] [at 31 °C there is 63% β (14) and 36.5% α (15), both shown in the predominant [2] $^4C_1$ form].

Prominent signals from freshly prepared solutions (which contain a greater preponderance of the α-form than at equilibrium, see Experimental section) are assigned to the C(2)(α)- and C(5)(α)-derived species, namely (16) and (17) respectively (see Table 2). Other lines in the overall spectrum are presum-

* For a half-chair geometry the angles are 10 and 50°.

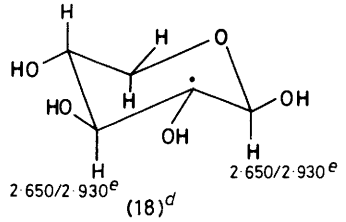
Table 2. E.s.r. spectra of radicals obtained by reaction of D-xylose with ·OH [a,b]

(a) pH *ca.* 4

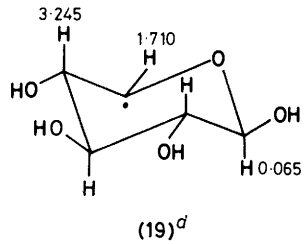


(16)[c]

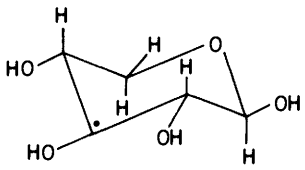
(17)[c]



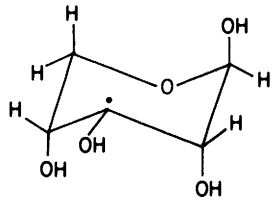
(18)[d]



(19)[d]

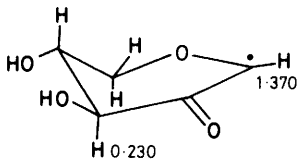


(20)[d,f]

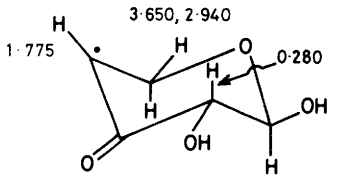


(21)[d,f]

(b) pH *ca.* 1.5



(22)[c]



(23)[d]

[a] Hyperfine splittings in mT; ±0.005. [b] *g* for (16)—(21) 2.0031; *g* for (22), 2.0049; *g* for (23) 2.0044 (all ±0.0001). [c] Maximum intensity for freshly prepared solutions. [d] Maximum intensity for solutions for which mutarotation had occurred. [e] No distinction possible. [f] Spectrum has *a*(3H) 0.075 mT: see text.

ably from C(1)-, C(3)-, and C(4)-derived radicals (though detailed analysis was impossible) together with radicals from the β-form (14). Analysis of the spectrum recorded after the substrate solution had been allowed to stand for some time (*ca.* 4 h) prior to flowing led to *different* parameters and assignments attributed to radicals from the now dominant β-form: of these the spectra from the C(2)- and C(5)-derived species (18) and (19) were prominent though the C(4)-derived radical could also be recognized. Lastly, a spectrum *identical* with the signal with the small quartet splitting detected for D-ribose also became more prominent. This observation is particularly significant since it indicates that the spectrum is associated with a radical which can be derived from D-ribose *and* β-D-xylose: the only radical which could be formed in common is the C(3)-derived species, shown in both $^4C_1$ and $^1C_4$ forms (20) and (21), respectively.

Although this strongly suggests that the C(3)-derived radical is responsible for the spectrum with *a*(3H) 0.075 mT it is by no means clear how such an unexpectedly small overall signal width and splittings may be explained. Two possibilities may be suggested. The first is that the radical exists mainly in the $^1C_4$ form * with three small couplings from, *e.g.* the α-OH proton and the two equatorial β-protons, with these held close to the nodal plane of the *p*-orbital of the unpaired electron. The interaction between the radical centre and the β-C–O bond in radicals of this type, which is responsible for conformational locking in analogous radicals, *e.g.* ·CH(OH)CH_2OH,[17] may provide not only stabilisation of (21) relative to its conformer (20) but also sufficient distortion to reduce the equatorial β-proton hyperfine splittings to near zero. Alternatively, the radical could be flipping at rates in the intermediate exchange region between conformers (20) and (21) with the result that a particularly broadened spectrum results: since (20) and (21) would differ in the magnitude of their β-hydrogen splittings [a large triplet is expected for (20), a much smaller one for (21)] the overall result would be broadening of the *outer* lines of the anticipated triplets, leaving a *relatively* sharp central peak (with residual coupling from, *e.g.* α- and β-OH groups). Although variable temperature studies over a wide temperature range should enable this suggestion to be verified, it proved impossible for us to generate this species over a wide enough range to detect the outside lines, if indeed they are present.

When the pH was lowered in experiments with D-xylose (both before and after mutarotation) signals from all radicals except those from the C(5) species (17) and (19) disappeared, to be replaced by carbonyl-conjugated radicals, as expected for αβ-dihydroxy-substituted radicals. The spectra which can be clearly analysed are assigned to (22), derived from the C(2)-(α) radical (16), and (23), derived from the C(3)-radical [(20) and/or (21)].

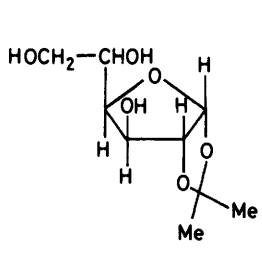
We have previously described [1] the reactions of ribose, xylose, and other sugars at high pH with ·OH; base-catalysed rearrangements of the C(2)- and C(5)-derived radicals from the pentoses lead to the production of ring-opened semidiones $HOCH_2CH(OH)CH(OH)C(O^-)=CH(O\cdot)$ and $CH(O^-)=CH-(O\cdot)$.

(c) *Glucofuranose Derivatives.*—The locked furanose derivatives (24) [1,2-*O*-isopropylidene-D-glucofuranose] and (25) [1,2:5,6-di-*O*-isopropylidene-D-glucofuranose] were next chosen for study in view of their acceptable level of solubility in water [in addition (25) proved sufficiently soluble in di-t-butyl peroxide–dichloromethane for parallel photolytic experiments to be carried out].

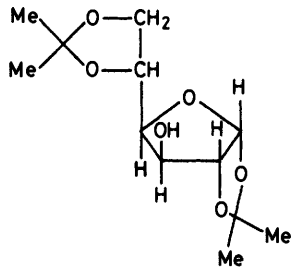
When a solution of (24) (*ca.* 0.05 mol dm^{-3}) was oxidised in the flow system with ·OH at pH *ca.* 4, a mixture of two signals was obtained (see Figure 2); the first had *a*(1H) 1.780, *a*(1H) 0.825, and a multiplet with a line separation of 0.045 mT and at least seven lines, and *g* 2.0032, and the second, present in somewhat higher concentration, had *a*(1H) 1.075, *a*(1H) 0.940, *a*(1H) 0.095 mT, with *g* 2.0031. Our assignments are based in part on our understanding of the steric and electronic factors which govern the magnitude of splittings in oxygen-conjugated cyclic and alicyclic radicals and also upon the results for (25) described below.

The first of the two spectra is attributed to the radical (26) formed by abstraction of hydrogen from the methylene

* It is conceivable that removal of the hydrogen at C(3) encourages the preferential adoption of a $^1C_4$ geometry; it is also perhaps relevant that, at least in β-ribose, the $^4C_1$ and $^1C_4$ forms are similar in energy.[2]



(24)



(25)

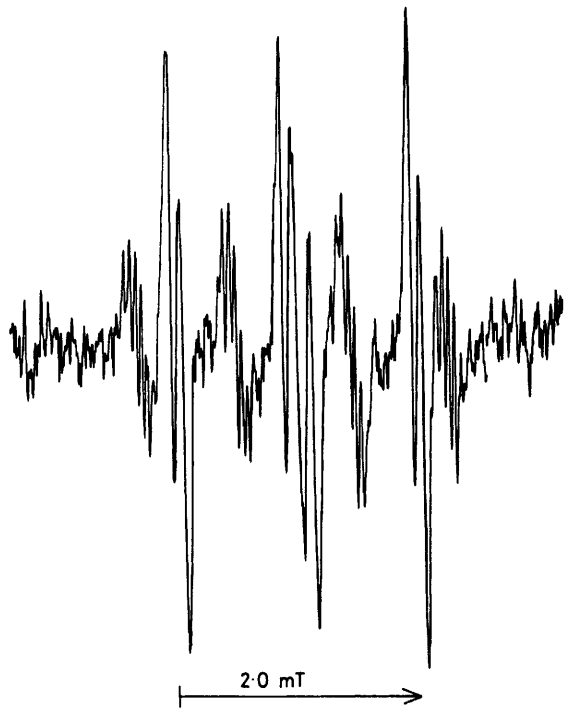
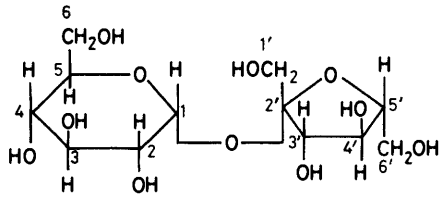


Figure 2. E.s.r. spectra of radicals (26) and (27) derived from the reaction of ·OH with 1,2-*O*-isopropylidene-D-glucofuranose (24) at pH 4

carbon C(6) on the basis of the splitting of 1.780 mT, typical of an α-proton splitting in a hydroxy-conjugated radical, and the splitting (0.825 mT) characteristic of a β-proton in αβ-dioxygen-substituted radicals (which typically possess an eclipsed geometry and concomitant low β-H splittings). The further coupling which can be seen in Figure 2 is analysed in terms of interaction with four protons [*a*(2H) 0.045, *a*(2H) 0.090 mT], which presumably include the α- and β-hydroxy-protons and the γ-proton (see later). Further evidence to support assignment to (26) derives from the observation that as the pH was lowered the signal disappeared by pH 2, to be replaced by a species with *g* 2.0045 [which is typical of carbonyl-conjugated radicals (Ċ–CO)]. This is characteristic of acid-catalysed dehydration of a first-formed αβ-di*hydroxy*-alkyl radical.

The second, major radical is clearly oxygen-conjugated, with two β-proton splittings: radicals formed by hydrogen abstraction from C(2), C(3), or C(4) are clearly candidates. Although the radicals from C(2) and C(3) cannot be ruled out at this stage as being responsible for this spectrum, the C(4)-derived radical (27) clearly possesses the appropriate structural features [*cf.* for example, typical *a*(β-H) values for



(31)

·CMe(OH)CH(OH)Me [18] and 3-hydroxytetrahydrofuran-2-yl [19]].

These assignments are entirely consistent with findings for (25). Reaction with ·OH led to the detection of three radicals, two of which were also clearly present in the reaction with Bu^{t}O·. The splittings for these two radicals varied slightly with conditions and the following refer to the somewhat better resolved spectra obtained from Bu^{t}O· at *ca.* −20 °C. The first, prominent, radical had *a*(1H) 2.670, *a*(1H) 1.235, *a*(1H) 0.075 mT, *g* 2.0032; this is assigned to the radical (28), obtained by abstraction from C(6), on the basis of the two large splittings, typical of a β- and an α-proton in an oxygen-conjugated radical in a five-membered ring [*cf.* (26)]. The second, with a series of splittings from single hydrogens of 2.645, 2.540, 0.430, 0.150, and 0.075 mT (and *g* 2.0032) is assigned structure (29), obtained by abstraction from C(5): the two large β-proton splittings are diagnostic here. The third radical, considerably more prominent in experiments with ·OH, and with *a*(1H) 1.075, *a*(1H) 0.440 mT (and some further incompletely resolved, small splittings) and *g* 2.0031 is evidently (30) [*i.e.* from abstraction at C(4)]: of the two low β-proton splittings [*cf.* also (27)] the particularly small value of 0.440 mT is probably associated with the C(5) proton which is now held in a locked isopropylidene ring [unlike (27)]. In the experiments with ·OH in water, no evidence for rearrangement of any of these first-formed radicals was obtained. Comparison of the spectra from (24) and (25) confirms that no radicals in common are present in the spectra from the two substrates; this is consistent with attack in the C(4)–C(6) moiety in each case since radicals formed by attack at C(1), C(2), or C(3) would have been expected to show a close correspondence for each compound.

The findings that (25) reacts to give (27)—(30) *via* attack at C(4)—C(6) and that (24) reacts predominantly at C(4) can be understood in terms of the ease of abstraction of C–H bonds adjacent to oxygen in furanose rings. These hydrogens are evidently activated to abstraction by comparison with those in exocyclic –CH(OH)– groups (*cf.* tetrahydrofurfuryl alcohol). In contrast the *lack* of reactivity of C(1)–H and C(2)–H in both substrates, despite the adjacent oxygen atom(s), may be ascribed to the strain which would be produced in the appropriate bridge-head radicals if these were to be formed. These conclusions are entirely in agreement with those made previously from the reactions of Bu^{t}O· with cyclic ethers: [9] of particular note here is that the more reactive hydroxyl radical demonstrates a selectivity similar to that shown by its counterpart.

(d) *Sucrose* (1-O-α-D-*Glucopyranosyl*-β-*fructofuranoside*) (31).—This readily available and water-soluble disaccharide was chosen for study since it provides an internal comparison between the reactivities of hydrogens in pyranose and furanose rings and also serves as a suitable model compound for the reactivity of the deoxyribose ring in DNA.

The e.s.r.spectrum obtained from the reaction between ·OH and sucrose is shown in Figure 3; e.s.r. parameters of the radicals present, and their assignments, are presented in

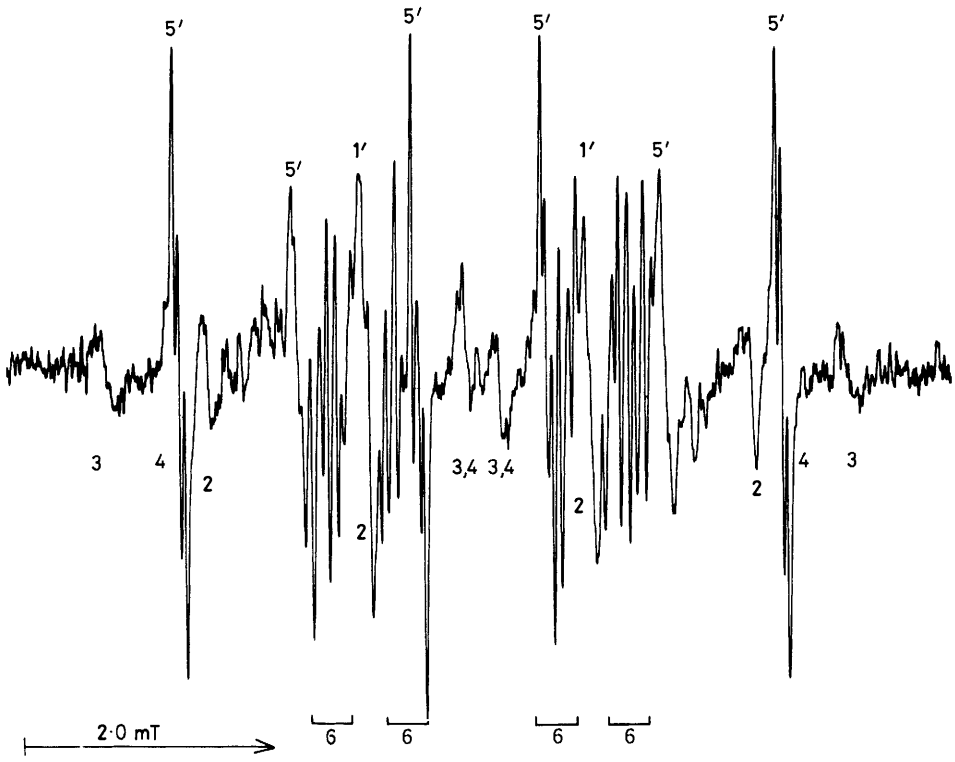


Figure 3. E.s.r. spectra of radicals derived by reaction of ·OH with sucrose at pH 3. Radicals are identified by numbers which refer to the carbon atom from which the hydrogen atom was abstracted [see (31)]

Table 1. One of the most notable features is the close correspondence between the positions and relative intensities of many of the lines and the appropriate lines obtained [7] from α-D-glucopyranose and from the disaccharide trehalose (1-*O*-α-D-glucopyranosyl-α-D-glucopyranoside) and assignments have been made on this basis. Although the radical formed by attack at C(1) cannot be clearly distinguished (as noted previously,[7] α-dioxygen substituted radicals are particularly susceptible to oxidation by, *e.g.*, H_2O_2, (32)—(38) can all be characterized. The spectrum of (32) [from attack at C(2)] contains splittings from a single axial and an equatorial β-proton (*cf.* the identical spectrum from the corresponding radical from trehalose), and both (33) and (34) show large interactions with two *axial* β-protons [our individual assignment is based on that for the C(3)- and C(4)-derived radicals from α-D-glucose [7]]. The C(5)-derived radical (35) has rather broad (and hence weak) lines, and no detailed analysis is possible; (36), as with the appropriate glucose-derived radical, is characterized by typical α- and β-proton splittings together with further long-range coupling (as in the corresponding radical from α-D-glucose, *cf.* ref. 7). Of these radicals, (36) is most prominent, possibly on account of the statistical factor which favours attack in the pyranose moiety at the methylene group, but otherwise, as with glucose and its derivatives, attack is largely unselective.

On the other hand, the dominant lines in the overall spectrum suggest that one radical formed by attack on the furanose ring is especially favoured. This is characterized by a large β-proton coupling (2.950 mT) with two, almost equivalent, smaller β-proton splittings (0.940, 0.965 mT). This we attribute to the radical (37) formed by abstraction of C(5′)-H: the large splitting is typical of a pseudo-axial proton in a five-membered ring, and the two almost equivalent β-protons are typical of a CH_2OH group 'locked' with the β-OH group eclipsing the orbital of the unpaired electron (the β-methylene protons are diastereotopic, though the non-equivalence may also be the result of restriction of rotation about C_α–C_β). Lines from the radical (38), formed by abstraction from the C(1′)-hydroxymethyl group in the furan ring are also present (see Table 1); abstraction from the other hydroxymethyl group in the furanose ring probably also occurs, but the spectra could not be distinguished from those of (36) which is expected to be similar in appearance. There was no clear indication of signals which could be assigned to radicals obtained by hydrogen-abstraction from C(3′), or C(4′) in the furanose ring.

Conclusions.—Our findings for sucrose reveal a striking contrast between the lack of selectivity in attack of ·OH at the pyranose ring positions and the preferential attack at C(5′)-H in the furanose ring. This, which parallels our observations for the pyranoses (xylose and ribose) and the isopropylidene derivatives of glucofuranose, is presumably a further example of a stereoelectronic effect [9] which reflects the relatively small dihedral angle subtended by the α-(C–H) bonds and the lone-pair of electrons on oxygen. What is particularly surprising is that this type of selectivity is found for the very reactive hydroxyl radical. Since it has been suggested [5] that breakage of DNA strands can result from the formation of radicals at the corresponding position [C(4)] in the deoxyribofuranose moiety our findings concerning the reactivity of such positions towards ·OH may assume particular significance.

Experimental

E.s.r. spectra were recorded on a Varian E-104 spectrometer equipped with an X-band klystron and 130 KHz modulation. Hyperfine splittings were measured to within 0.005 mT and

g values to within 0.001, both by comparison with Fremy's salt [a(N) 1.309 mT, g 2.0055 [20]].

Rapid-flow experiments were carried out with an aqueous sample cell and a mixing chamber which allowed three reagent streams to be mixed simultaneously; solutions were pumped through the cell with a Watson–Marlowe MHRE peristaltic pump, such that the dead-time (between mixing and observation) was *ca.* 50 ms. The three reactant solutions contained typically 0.008 mol dm^{-3} titanium(III) [prepared from Fisons technical grade titanium(III) chloride, 12.5% w/v solution], 0.05 mol dm^{-3} hydrogen peroxide (prepared from Fisons 100 volume hydrogen peroxide solution), and 0.05 mol dm^{-3} substrate [except for tetrahydrofurfuryl alcohol (1) and 2-hydroxymethyltetrahydropyran (2) where a concentration of 0.1 mol dm^{-3} was employed]. Adjustment of the pH was made by adding concentrated sulphuric acid or aqueous ammonia solution (d 0.880) to the titanium(III) solution, with edta also being added if a pH >2.5 was required. pH Values were recorded with a Pye–Unicam PW 9410 digital pH meter equipped with a Russell electrode inserted into the effluent stream. All solutions were thoroughly deoxygenated with a nitrogen purge prior to and during use, and where it was necessary to inhibit mutarotation the solutions containing the sugars were made up with ice-cold water.

Photolysis experiments were conducted by continuous irradiation, with an Hanovia 977B-1 1 kW mercury–xenon compact arc, of deoxygenated solutions of the substrate, dissolved where necessary in a minimum amount of dichloromethane, to which a few drops of di-t-butyl peroxide had been added. Temperature control was achieved with a Varian variable temperature accessory; the temperature was monitored using a Comark 3015 Cr–Al digital thermometer.

The configuration of the D-xylose sample employed was established by recording the specific rotation of a freshly prepared solution using a Perkin-Elmer 141 polarimeter; the value of $[\alpha]_D^{20}$ +93.6° obtained confirms that the sugar is in its α-pyranose form.[21]

All substrates were obtained commercially, the carbohydrates (all at least 99%) from Sigma Ltd. and both tetrahydrofurfuryl alcohol (99%) and 2-hydroxymethyltetrahydropyran (98%) from Aldrich Chemical Co. Ltd.

Acknowledgements

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