Carbohydrate Research, 31 (1973) 199–209
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RADIATION STUDIES OF ARYL GLYCOSIDES

PART VI. RADICALS DERIVED FROM PHENYL β-D-GLUCOPYRANOSIDES

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(Received March 23rd, 1973; accepted for publication, June 6th, 1973)

ABSTRACT

Radicals formed by γ-irradiation of *o*-, *m*-, and *p*-substituted-phenyl β-D-glucopyranosides have been studied in the polycrystalline state, in glassy methanol, and in frozen, aqueous solution. Substituted cyclohexadienyl radicals and radicals derived from the D-glucopyranosyl group are evident after irradiation of the compounds in the solid state and in frozen, aqueous solutions. Cyclohexadienyl radicals are the more stable during thermal annealing and are present in 2–3 times greater yield than the sugar radicals. *p*-Hydroxy- and methoxy-phenyl β-D-glucopyranosides yield phenoxy radicals, which can be transformed into substituted cyclohexadienyl radicals by thermal annealing. Hydrogen abstraction and inter- and intra-molecular hydrogen-transfer are the most likely processes leading to the radicals which have been identified.

INTRODUCTION

Glycosidic scission is the main, chemical consequence of the effect of γ-irradiation on solid aryl glycosides[1]. The extent to which scission occurs, however, is considerably less than is found when alkyl glycosides are irradiated[1]. This protective effect varies with the nature of the aglycon, and this behaviour was interpreted in terms of intramolecular energy transfer[2a]. On irradiation, cyclohexadienyl-type radicals are produced from the aryl glycosides that are most stable to radiation, and radical yields generally parallel the chemical yields[2a]. Similar radiation effects have been demonstrated for cycloamylose complexes with aromatic molecules[2b]. More generally, aromatic compounds can be divided into two groups, according to whether or not cyclohexadienyl radicals are formed on γ-irradation[3]. Moreover, hydrogen-atom bombardment and γ-irradiation for a series of aromatic carboxylic acids do not necessarily lead to the hydrogen-atom adduct in the same compounds[4]. Here, we examine radical production from a series of substituted-phenyl β-D-glucopyranosides when γ-irradiated in the solid state, in frozen, aqueous solution, and in methanolic glasses. Our objective is to obtain a clearer understanding of the factors leading to radical production, which is intermediary in glycosidic scission.

EXPERIMENTAL

Phenyl β-D-glucopyranoside and the nitrophenyl β-D-glucopyranosides were "AnalaR" grade and were recrystallised from ethanol before use. The other aryl D-glucosides used were prepared by standard methods[2,5]. Methanol was initially "AnalaR" grade, and was further purified by distillation from an acidified solution to which 2,4-dinitrophenylhydrazine had been added. Irradiation techniques and dosimetry have been described previously[5]. Irradiations were conducted in Spectrosil, quartz e.s.r. tubes which had been annealed. Methanolic and aqueous solutions of phenyl β-D-glucopyranosides were frozen in the form of spheres (diameter ~3 mm) by dropping the solutions from a fine capillary into liquid nitrogen. The spheres were subsequently sieved to a standard size to ensure that the packing was uniform and reproducible in the e.s.r. Dewar "tip". Nitrogen was bubbled through the solutions for 1 h prior to freezing, in order to eliminate oxygen. Concentrations of the aryl D-glucosides employed were 0.25–1M. The spheres were irradiated to a total dose of $\sim 2 \times 10^4$ J.kg^{-1} in a vacuum Dewar containing liquid nitrogen.

E.s.r. spectra were recorded on a Varian V4502 spectrometer, using 100-kHz field modulation. The field was calibrated by using a proton-resonance probe coupled to an "AEG" gaussmeter and Hewlett–Packard frequency counter. For e.s.r. measurements at 77K, the Dewar "tip" was used. The variable-temperature control unit was employed for thermally annealing the γ-irradiated solids and frozen solutions in the temperature range 110–363K.

RESULTS AND DISCUSSION

Polycrystalline state

(*a*) *Phenyl β-D-glucopyranoside.* — E.s.r. power-saturation studies on γ-irradiated phenyl β-D-glucopyranoside revealed the presence of two radicals, a doublet spectrum (hyperfine splitting 19.6 ±1 gauss) and a more-complex spectrum of nine lines (Figs. 1A and B). Annealing the irradiated sample to 363K led to preferential decay of the radical responsible for the doublet, leaving only the nonet spectrum (Fig. 1C). Irradiation and e.s.r. recording at 77K showed the presence of the same two radicals, but not as distinctly. However, on warming to room temperature, the spectra obtained were identical with those obtained after irradiation at room temperature.

By carefully subtracting the derivative absorption spectrum (after single integration) which remains after warming to 363K from the spectrum observed at room temperature, the spectra associated with each of the two radicals can be distinguished (Figs. 1D, E, and F). The nonet spectrum consists of a triplet (1:2:1, $a_H = 44.1$ ±1 gauss), each line split into a further triplet (1:2:1, $a_H = 10.5$ ±0.5 gauss). Such a spectrum was previously identified with a cyclohexadienyl-type radical formed by hydrogen-atom addition to the aromatic ring of the aryl glucoside[2]. Possible structures of this radical are **1** and **2**.

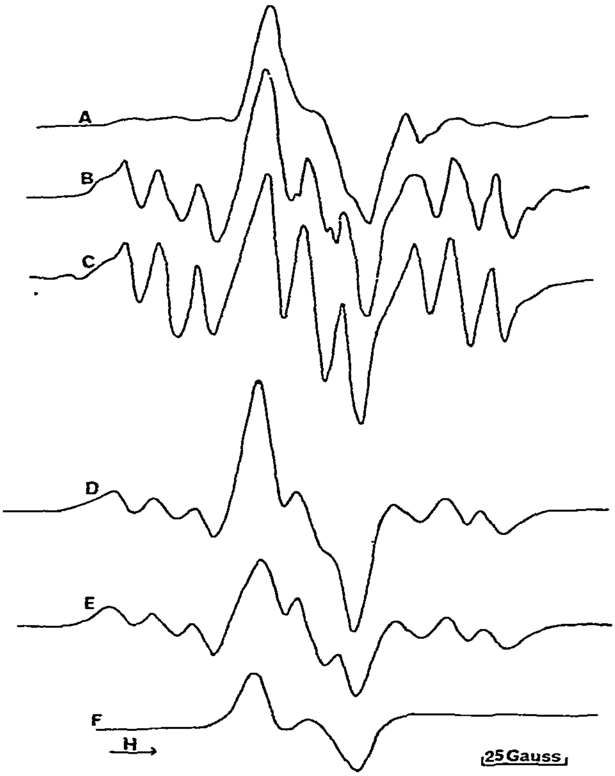
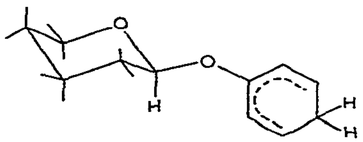


Fig. 1. Effects of power saturation and thermal annealing on the e.s.r. spectra of γ-irradiated phenyl β-D-glucopyranoside. Power saturation: A, 6dB, B, 40 dB, both recorded at 293K; C, 40dB recorded at 363K. Thermal annealing: D, spectrum at 293K; E, spectrum of radicals remaining at 363K; F, spectrum of radicals thermally bleached out between 293 and 363K.

The large triplet-splitting is due to interaction with the two methylene protons and the small triplet-splitting with the protons, ortho–ortho (1) or ortho–para (2) in relation to the site of hydrogen addition. There are, however, small differences in the coupling of protons in the ortho and para positions[6,7] ,but they are too small to be resolved under these conditions. Such differences could, nevertheless, be responsible for the deviations we observe from the theoretical intensities for the hyperfine lines. Small differences may also exist in the couplings for the two methylene protons[8]. The doublet spectrum, which disappears on annealing to 363K, can be attributed to a radical in the D-glucopyranosyl moiety (3). Loss of a hydrogen atom probably



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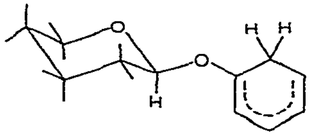

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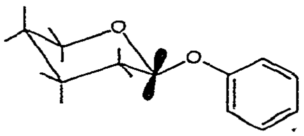


3

occurs from C-1, as the splitting is near to that observed for a similar radical in frozen, aqueous solutions of D-glucose after γ-irradiation[9]. The yield of **3** is 2–3 times less than the yield of the cyclohexadienyl radical.

(*b*) *Methylated phenyl β-*D*-glucopyranosides.* — The coupling constants for the radicals derived from the phenyl D-glucosides studied are shown in Table I. Apart from 2,5-xylyl β-D-glucopyranoside, all the D-glucosides form cyclohexadienyl-type radicals after γ-irradiation (see Figs. 2A–F). A D-glucopyranosyl radical is also evident in every instance.

TABLE I

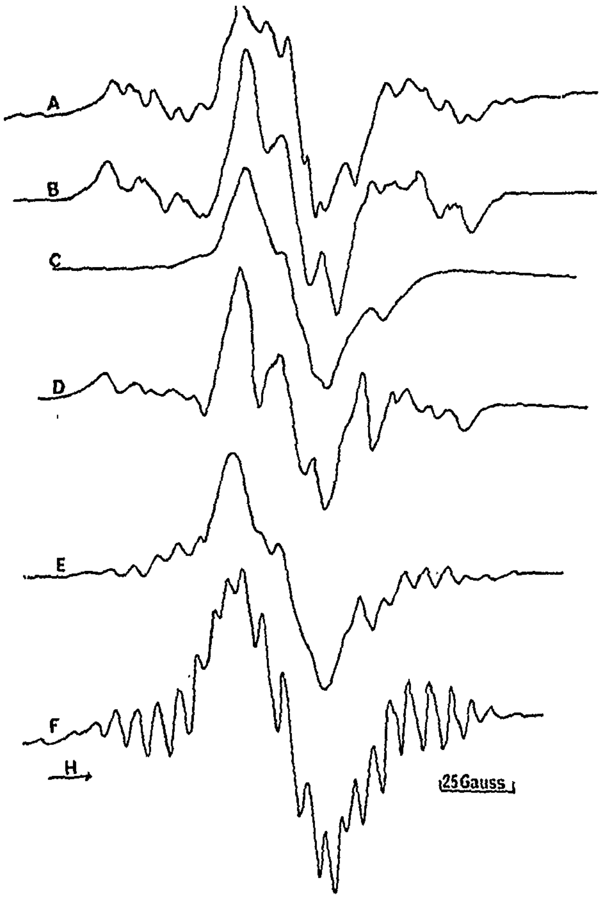
PROTON COUPLING CONSTANTS FOR CYCLOHEXADIENYL RADICALS TRAPPED IN γ-IRRADIATED, POLYCRYSTALLINE, SUBSTITUTED-PHENYL β-D-GLUCOPYRANOSIDES

Substituent	*Coupling constant*[a] (*gauss*)		
	a_H (CH_2) (± 1)	a_H (o,p) (± 0.5)	a_H (CH_3) (± 0.3)
–H	44.1	10.5	—
p–OH	42.4	10.8	—
p–OCH_3	43.1	11.1	—
p–CH_3	41.9	11.4	7.1
o–CH_3	42.0	11.5	7.0
m–CH_3	43.0	Not resolved	Not resolved
2,4–di–CH_3	41.9	11.4	7.1
2,5–di–CH_3	No cyclohexadienyl radical formed		
3,4–di–CH_3	42.0	11.5	7.0
2,4,6–tri–CH_3	41.0	—	7.0

[a] a_H (*m*) not resolved.

A notable feature of the spectra of these radicals is the additional hyperfine lines, which are particularly well-defined with mesityl β-D-glucopyranoside (Figs. 2E and F). These additional lines are due to a contribution from protons of the methyl groups at positions 2, 4, and 6 in the aromatic ring ($a^{2,4,6}_{CH_3} = 7 \pm 0.3$ gauss). In most instances, hydrogen addition occurs at different unsubstituted positions. For example, with *p*-tolyl β-D-glucopyranoside, hydrogen-atom addition occurs at all four unsubstituted positions in the ring. However, for *m*-tolyl β-D-glucopyranoside, addition occurs only at the 2-position. The yield of cyclohexadienyl radicals derived from *m*-tolyl β-D-glucopyranoside is particularly low. Other investigators have also found that the yield of these radicals in irradiated, 1,3-disubstituted, aromatic compounds is always lower[4,10].

(*c*) p-*Hydroxy- and* p-*methoxy-phenyl β-*D*-glucopyranosides.* — These two compounds, when γ-irradiated at room temperature, behave similarly to the parent phenyl D-glucoside, giving cyclohexadienyl and D-glucopyranosyl radicals. However, if irradiation is carried out at 77K and e.s.r. observations are made at this temperature there is no evidence of cyclohexadienyl radicals. The spectra (Figs. 3A and B) are



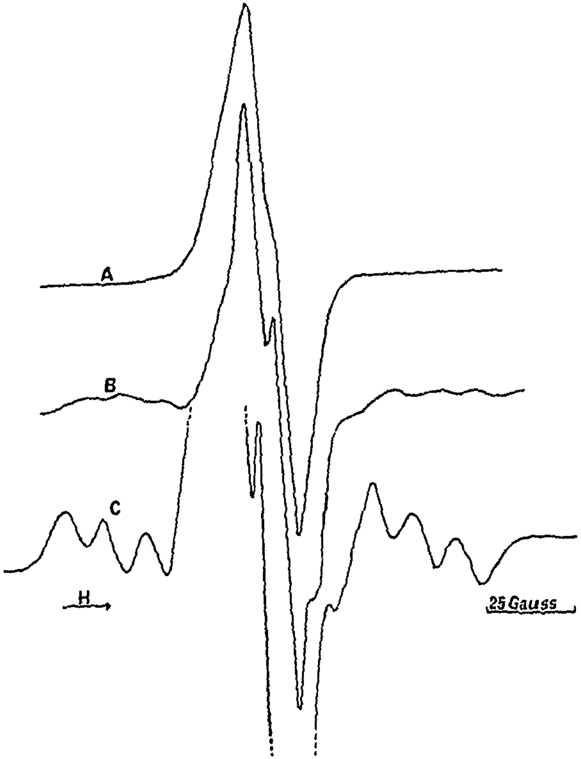


Fig. 2. E.s.r. spectra of γ-irradiated substituted-phenyl β-D-glucopyranosides. Power level, 40 dB: A, *p*-tolyl; B, *o*-tolyl; C, 2,5-xylyl; D, 2,4-xylyl; E and F, mesityl at 293 and 363K.

Fig. 3. E.s.r. spectra of *p*-hydroxyphenyl β-D-glucopyranoside γ-irradiated at 77K. A, 77K; B, 293K, power level 25 dB; C, 293K, signal level × 3.

characterised by a singlet ($\Delta H = 11.7 \pm 0.3$ gauss). With the irradiated *p*-hydroxyphenyl derivative, annealing to 293K is accompanied by some decay of the singlet, and the simultaneous appearance of hyperfine lines characteristic of cyclohexadienyl radicals (Fig. 3C) in low yield. However, if the irradiated *p*-methoxyphenyl derivative is warmed to 293K, the singlet spectrum disappears almost completely and is replaced by a definite spectrum for the cyclohexadienyl radical.

For irradiated *p*-hydroxy- and *p*-methoxy-phenyl D-glucosides, therefore, the radical trapped at low temperature is transformed to a cyclohexadienyl radical on thermal annealing. The singlet spectrum could be due to a substituted-phenyl radical formed by loss of a ring proton. The latter possibility can be excluded, as the observed line-width of 11.7 ± 0.3 gauss is widely different from the reported coupling-constant[11] for the unsubstituted phenyl radical, namely a_H(ortho) = 18, a_H(meta) = 6.5, and a_H(para) = 0 gauss. Our observations are consistent with the formation of phenoxy radicals. Atherton and Harding[12] have irradiated single crystals of 2,6- and 2,4-di-*tert*-butyphenol and found substituted phenoxy radicals having two sets of equivalent protons [a_H(ortho) = 8 gauss; a_H(meta) = 2.5 gauss].

(*d*) *Nitrophenyl β-D-glucopyranosides.* — Irradiation of *o*-, *m*-, and *p*-nitrophenyl β-D-glucopyranosides, both at 77K and 293K, also leads to the formation of two radicals: an asymmetric triplet associated with the nitro group, due[2,13] to the formation of RNO_2H, and a D-glucopyranosyl radical.

Methanol glasses

After γ-irradiation of phenyl β-D-glucopyranoside (0.25M) in a methanol glass at 77K, trapped electrons (e_t^-) and hydroxymethyl radicals ($\cdot CH_2OH$) can be

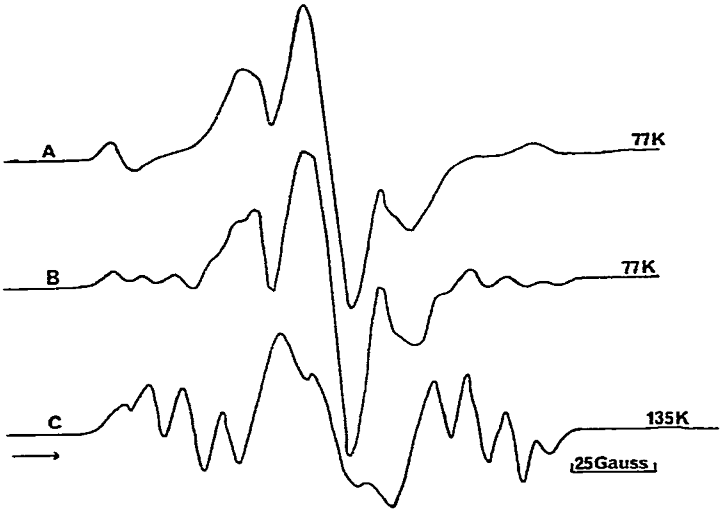


Fig. 4. E.s.r. spectra of phenyl β-D-glucopyranoside in γ-irradiated, methanol glass at 77K. A, Methanol glass (5.3% H_2O) at 77K; B and C, phenyl β-D-glucopyranoside (0.25M) in methanol glass (5.3% H_2O) at 77 and 135K.

detected (Fig. 4). The presence of cyclohexadienyl radicals can also be deduced from the outer sextet of lines. Both e_t^- and $\cdot CH_2OH$ disappear on bleaching with light (>530 nm) and also on thermally annealing to 160K, leaving a triplet of triplets ($a_H = 43.7 \pm 1$ gauss and 11.6 ± 0.5 gauss) arising a cyclohexadienyl-type radical (Fig. 4C). All the aryl D-glucosides, apart from the nitrophenyl β-D-glucopyranosides, when irradiated in glassy methanol, give cyclohexadienyl radicals (see Table II for summary of coupling constants). The methylene-proton couplings ($a_H^{CH_2}$) observed are considerably smaller than for the unsubstituted cyclohexadienyl radical. After irradiation of glassy methanol solutions of nitrophenyl glucosides, $R\dot{N}O_2H$ type radicals can be detected, but no e_t^- spectrum is evident in these irradiated glasses.

TABLE II

PROTON COUPLING CONSTANTS FOR CYCLOHEXADIENYL RADICALS TRAPPED AT 77K IN γ-IRRADIATED, METHANOLIC GLASSES CONTAINING SUBSTITUTED-PHENYL β-D-GLUCOPYRANOSIDES

Substituent	*Coupling constant*[a] *(gauss)*		
	a_H (CH_2) (±*1*)	a_H (o,p) (±*0.5*)	a_H (CH_3)
-H	43.7	11.6	
p-OH	42.5	11.3	
p-OMe	41.9	11.2	
p-CH_3	41.9	11.4	Not resolved
o-CH_3	42.0	11.4	Not resolved
m-CH_3	42.2	11.3	Not resolved
2,4-di-CH_3	41.9	11.3	7.1 ±0.5
2,5-di-CH_3	42.2	Not resolved	Not resolved
2,6-di-CH_3	42.0	11.5	7.0 ±0.5
3,4-di-CH_3	42.0	11.5	Not resolved
2,4,6-tri-CH_3	41.0	—	7.0 ±0.1
Benzene	48.3	10.7	—

[a] a_H (*m*) not resolved.

Frozen, aqueous solutions

Irradiation of a frozen, aqueous solution containing phenyl β-D-glucopyranoside (0.2M) at 77K leads to formation of OH and cyclohexadienyl radicals. The latter can be distinguished only at high field by the characteristic triplet ($M_I = -1$ for the methylene protons) due to the ring protons (Figs. 5A and B). On warming to 160K, the OH radicals disappear, leaving only the solute radicals. Figs. 5C, D, and E show the spectra from phenyl β-D-glucopyranoside irradiated in H_2O and D_2O at 77K, after thermal annealing of the frozen matrix to 293K. It is simpler to interpret the spectra in D_2O. Here, radicals formed by addition of deuterium atoms to the aromatic ring and D-glucopyranosyl radicals are evident. The hyperfine coupling-constants for the deuteriocyclohexadienyl radical are $a_H^{CHD} = 38 \pm 1$, $a_D^{CHD} = 8 \pm 0.5$, and a_H (*o* or *p*) $= 10 \pm 0.5$ gauss. The glucopyranosyl radical gives rise to a doublet of 19 ± 1 gauss. There is, therefore, a definite similarity between radicals produced in

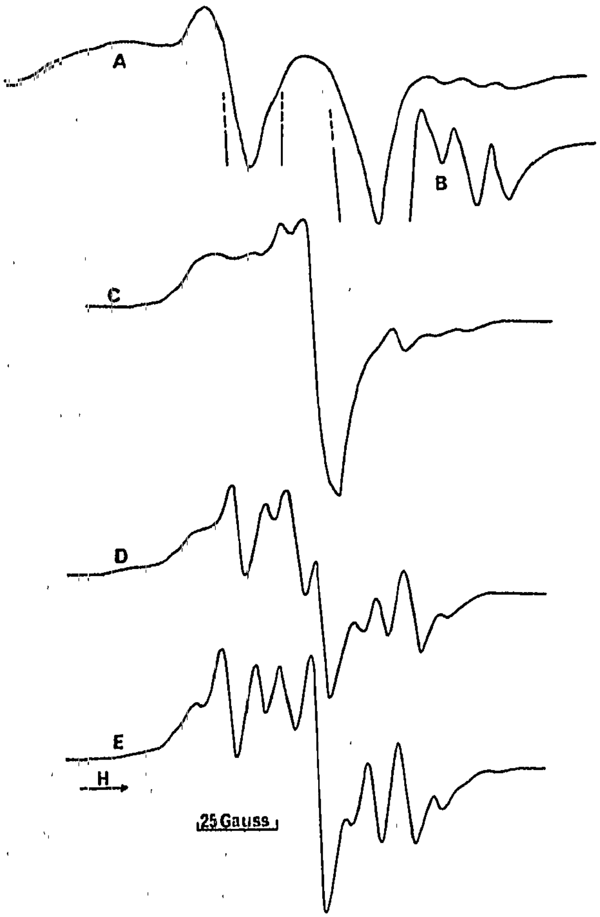


Fig. 5. E.s.r. spectra of frozen solutions of phenyl β-D-glucopyranoside γ-irradiated at 77K. A, 77K signal level × 1; B, 77K signal level × 5; C, 183K in H_2O; D, 183K in D_2O; E, 153K in D_2O.

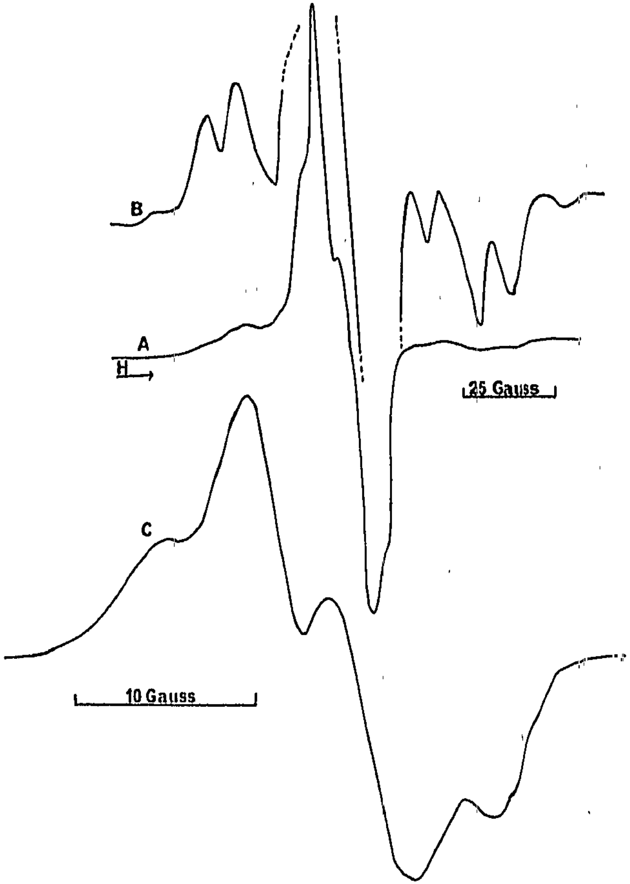


Fig. 6. E.s.r. spectra of p-hydroxyphenyl β-D-glucopyranoside in γ-irradiated deuterium oxide at 77K, after thermal annealing. A, 213K signal level × 1; B, 213K signal level × 10; C, centre part of spectrum at 220K.

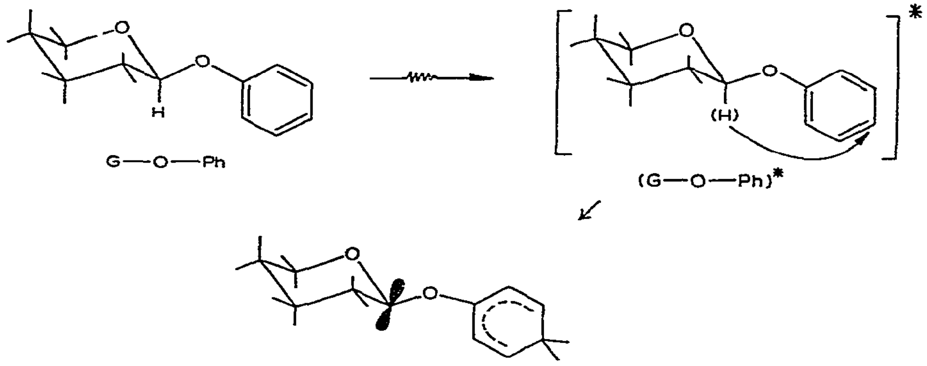
frozen solutions and in the solid state; the yields in frozen solution, however, are considerably lower than in irradiated solids.

For *p*-hydroxy- and *p*-methoxy-phenyl D-glucosides irradiated in frozen, aqueous matrices at 77K, the e.s.r. spectra show a large, central resonance, which is unaffected when D_2O is substituted for H_2O. After OH or OD radicals have been removed by annealing, features of the cyclohexadienyl radical can be observed. The large, central resonance does not commence to decrease until ~220K, and at this temperature some hyperfine splitting is evident (Fig. 6), which is characteristic of the phenoxy radical. Irradiated, frozen solutions of phenol and resorcinol exhibit similar features.

Irradiation of frozen, aqueous solutions containing nitrophenyl β-D-glucopyranosides (0.1M) at 77K yields the hydrogen-adduct radical, $R\dot{N}O_2H$, as in the solid state and in glassy methanol. Higher yields of these radicals are found, compared with other aryl D-glucosides, due to the efficient scavenging of electrons by the substituent nitro-group.

Mechanism of radical formation

(*a*) *Polycrystalline state.* — Hydrogen-adduct radicals can arise by intra- and inter-molecular transfer of a hydrogen atom. Abstraction or ejection of a hydrogen atom from C-1 of the D-glucopyranosyl group and transfer to the phenyl group of the same molecule would occur, as described in Scheme 1.



Scheme 1.

A similar mechanism for H-atom transfer, involving the primary molecular ion, has been postulated to account for the mass-spectral fragmentation of phenyl β-D-glucopyranoside[5,14]. Intermolecular H-atom transfer could also arise by addition to a phenyl group of a hydrogen atom ejected from a neighbouring molecule, as in Scheme 2.

$$\text{G–O–Ph} \rightarrow (\text{G–O–Ph})^{*} \rightarrow \cdot\text{G}'\text{–O–Ph} + \text{H}\cdot \qquad (1)$$

$$\text{G–O–Ph} + \text{H}\cdot \rightarrow \text{G–O–Ph H}\cdot \qquad (2a)$$

$$\searrow \cdot\text{G}'\text{–O–Ph} + \text{H}_2 \qquad (2b)$$

$\cdot$G′ = D-Glucosyl—H

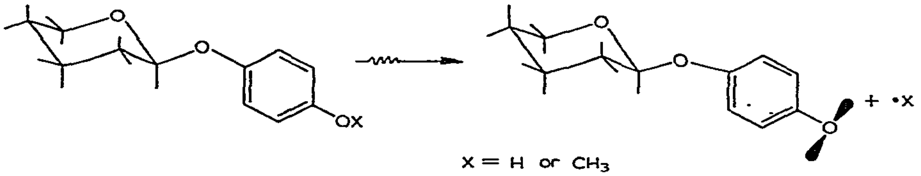
Scheme 2.

Alternatively, abstraction of a hydrogen atom from a neighbouring molecule by an excited molecule could occur as follows:

$$\text{G–O–Ph} + (\text{G–O–Ph})^{*} \rightarrow \cdot\text{G}'\text{–O–Ph} + \text{G–O–Ph H}\cdot$$

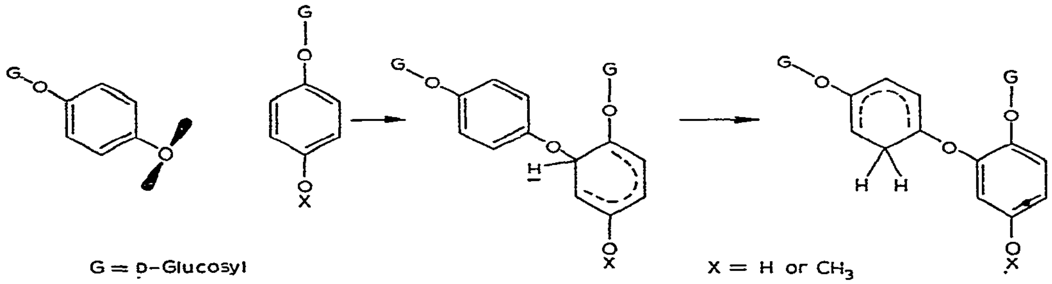
Hydrogen atoms thus formed can then react with the D-glucopyranosyl group to give the carbohydrate radical (reaction *2b*), but addition of a hydrogen atom to the aromatic nucleus would be more favourable (reaction *2a*) because of the negligible activation energy required[15].

Different mechanisms must be operative after γ-irradiation of *p*-hydroxy- and *p*-methoxy-phenyl D-glucosides. No hydrogen-adduct radicals are found at 77K, which is consistent with other work[3], and it is probable that phenoxy radicals are produced directly (Scheme 3).



Scheme 3.

Substituted cyclohexadienyl radicals produced on warming to room temperature could then arise by reaction of the phenoxy radical with a neighbouring molecule of aryl D-glucoside, as illustrated in Scheme 4.



Scheme 4.

A similar mechanism was proposed by Campbell and Symons[8] to account for the appearance of substituted cyclohexadienyl radicals in γ-irradiated resorcinol.

(*b*) *Glassy methanol.* — The hydrogen-adduct radicals formed by irradiation in glassy methanol are identical with those produced in the solid-state irradiations. Their formation is probably due to reaction of the aryl D-glucoside with mobile electrons (e_m^-), followed by protonation of the anion by the solvent[16]:

$$\text{G–O–Ph} + e_m^- \rightarrow (\text{G–O–Ph})^- \xrightarrow{(+\text{CH}_3\text{OH})} \text{G–O–Ph H}\cdot + \text{CH}_3\text{O}^-$$

(*c*) *Frozen, aqueous solution.* — Here, direct and indirect action can lead to the radicals observed. On thermal annealing, radicals can arise by addition of H and OH radicals to the aromatic moiety, which would account for the low yields in comparison with solid-state and methanol-glass irradiations. The direct-action process would parallel the solid-state irradiations and lead to similar products.

For the *p*-hydroxy- and *p*-methoxy-phenyl β-D-glucopyranosides, the situation is different. No cyclohexadienyl radicals are observable at 77K, and the OH-radical yield, although decreased, is more pronounced for the *p*-hydroxyphenyl derivative than for the *p*-methoxyphenyl D-glucoside. The solute, therefore, may react with OH radicals to give substituted-phenoxy radicals in high yield.

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