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RADIATION STUDIES OF ARYL GLYCOSIDES

PART II. MECHANISM OF RADIOLYSIS OF PHENYL β-D-GLUCOPYRANOSIDE IN AQUEOUS SOLUTION

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ABSTRACT

The effect of ionising radiations on aqueous solutions of phenyl β-D-glucopyranoside leads to glycosidic scission to yield equivalent amounts of D-glucose and phenol, and to hydroxylation of the aglycon. G(glycosidic scission) increases from 0.4 at mM to 1.0 at 10mM solute concentration. Pulse radiolysis studies indicate that the first intermediate is the hydroxycyclohexadienyl radical (**1**, λ_{max} 235 and 320 nm) derived from the glucoside; the molar extinction of **1** at 320 nm is 3,560. Two alternative reaction-paths for **1** have been kinetically distinguished. One is a second-order process leading to hydroxylation of the aglycon and dimerisation with $k_2 = 1.7 \times 10^8$ $M^{-1}.sec^{-1}$. The alternative is a pH-dependent, first-order process ($k_1 \sim 10^3$ sec^{-1}), leading to a carbonium ion which gives D-glucose and the phenoxyl radical (λ_{max} 400 nm) which yields phenol. The variation of G(phenoxyl) and G(glycosidic scission) with pH demonstrates that the unimolecular, heterolytic scission of the glucosyl–O bond in **1** is catalysed by acid and alkali.

Rate constants for the reaction of water radiolysis products with the glucoside were found to be 4×10^9 $M^{-1}.sec^{-1}$ with OH radicals, 7×10^7 $M^{-1}.sec^{-1}$ with e^-_{aq}, and 3.5×10^7 $M^{-1}.sec^{-1}$ with H atoms. Spectra of the transients formed by reaction of e^-_{aq} and H atoms with the glucoside were determined.

INTRODUCTION

The glycosidic linkage is fundamental to the structure of most naturally occurring carbohydrates and invariably is the bond which is most labile during the degradation of disaccharides[1,2], higher saccharides[3], and naturally occurring polysaccharides[4–6] by ionising radiations. Hydrolytic cleavage of the glycosidic bond also occurs when one of the D-glucose residues in a disaccharide is replaced by an alkyl[7] or aryl[8] aglycon. Whereas the mechanism of hydrolysis of glycosides by acids[9,10] alkali[11,12], and enzymes[13] has been extensively studied, the information about the mechanism of radiation-induced hydrolysis is meagre. Kochetkov *et al.*[8] found the

characteristic, hydrolytic products after γ-radiolysis of aqueous solutions of methyl, phenyl, and benzyl glucosides, and, on this basis, suggested that the solvated electron was the main product of water radiolysis which caused hydrolysis. This, however, is not our conclusion. We have selected phenyl β-D-glucopyranoside for detailed study, since the aromatic aglycon allows a distinction to be made between the possible scission processes at the glycosidic oxygen atom, in a way which would not be possible for a disaccharide. Moreover, the mechanisms of hydrolysis of aryl glycosides by other agents[9–13] are now becoming well established. However, we anticipate that the results would also have some validity to the general problem of radiation-induced glycosidic scission in more-complex, naturally occurring carbohydrates.

EXPERIMENTAL AND RESULTS

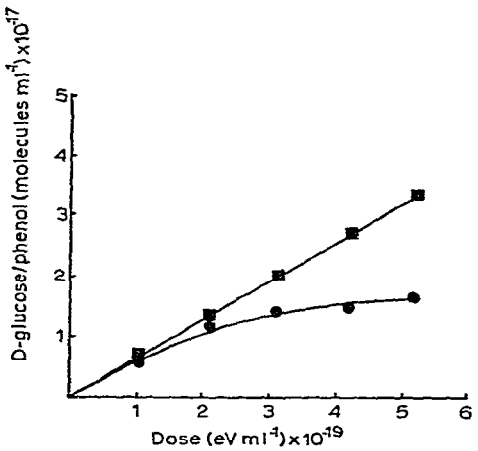
The γ-irradiation source was a N.E.L.9, 10,000-curie ^{60}Co unit built by Nuclear Engineering Ltd. in the Nuclear Science Building, University of Salford. Irradiations were carried out in glass vessels, in which sintered bubblers were fitted to allow the equilibrating gas to be varied. Dosimetry, carried out by using a slight modification[14] of the Fricke procedure, established the dose to be 1.9×10^{17} eV.min^{-1}.ml^{-1}. Phenyl β-D-glucopyranoside was obtained as previously described[15].

Irradiation products. — A solution (15 ml, 50mM) of phenyl β-D-glucopyranoside was irradiated in argon to a dose of 5×10^{19} eV.ml^{-1}, and freeze-dried, and the remaining solid was dried *in vacuo* over fresh silica gel for 72 h. Aromatic products were removed by repeated ether extraction. The material remaining was examined by thin-layer chromatography (t.l.c.) in butyl alcohol–acetic acid–ether–water[16] (9:6:3:1). Alkaline permanganate revealed, in addition to unchanged phenyl β-D-glucopyranoside, one mobile carbohydrate component (R_F 0.49) which behaved identically with control D-glucose. An additional carbohydrate component remained immobile. Phenolic products were detected in the ether extract by t.l.c. in benzene–methanol–acetic acid[17] (45:8:4). *p*-Nitrobenzenediazonium fluoborate spray revealed the presence of phenol (R_F 0.91, pink), resorcinol (R_F 0.59, orange), and hydroquinone (R_F 0.48, pink). The presence of catechol could not be found by using the specific Doty[18] spray reagent. From the intensity of the spots, phenol was clearly the preponderant product.

In a duplicate experiment, the freeze-dried irradiated phenyl β-D-glucopyranoside was trimethylsilylated[19] and examined by using a Perkin–Elmer 880 gas chromatograph fitted with a field ionization detector. The matched pair of columns were packed with SE 52 on Chromosorb W with hexamethyldisilazane purchased from the Perkin–Elmer Co. At a nitrogen flow of 30 ml.min^{-1} and a column temperature of 195°, products having retention times identical with the following controls were observed: α-D-glucose (2.75 min), β-D-glucose (3.75 min), *p*-hydroxyphenyl β-D-glucoside (42 min). Two additional products were observed with retention times (23 and 37 min) characteristic of disaccharides or other hydroxyphenyl β-D-glucosides[20].

Yield–dose curves for glycosidic scission. — The Somogyi method[21] was used to estimate D-glucose. To estimate phenol, the method of Emerson[22] was modified

to take into account the findings of Svobodova and Gasparic[23]. The irradiated solution (5 ml) was first buffered to pH 9.5–10, where maximal colour stabilisation and intensity is obtained, by using boric acid, potassium chloride, and sodium hydroxide. 4-Aminoantipyrine (1 ml, 3% solution) was added with shaking, the mixture was treated with potassium ferricyanide (0.5 ml, 10% solution), and the absorbance was measured at 510 nm. It was established that the presence of the other identified products had no effect on the phenol estimation. A typical yield–dose curve for D-glucose and phenol production in γ-irradiated phenyl β-D-glucoside solutions is shown in Fig. 1. Initial G values were calculated from curves of this type, and Table I summarises the effect of solute concentration on initial G-values.



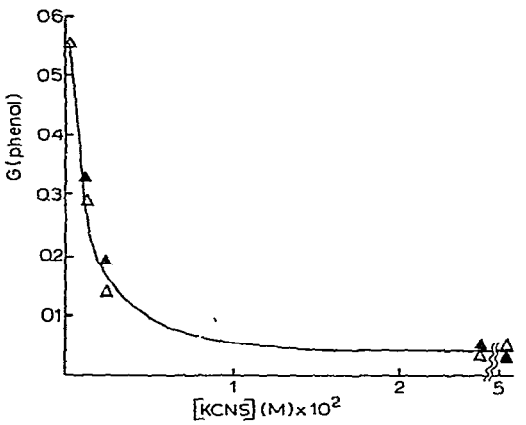


Fig. 1. Formation of D-glucose (■) and phenol (●) during γ-irradiation of aqueous phenyl β-D-glucopyranoside (5mM) in argon.

Fig. 2. Effect of KCNS on G(glycosidic scission) for γ-irradiated solutions of phenyl β-D-glucopyranoside (5mM) in argon. ▲ calculated; △ experimental value.

TABLE I

DEPENDENCE OF INITIAL G-VALUES FOR FORMATION OF D-GLUCOSE AND PHENOL ON CONCENTRATION OF PHENYL β-D-GLUCOPYRANOSIDE (PhGlc) IN ARGON

[*PhGlc*], mM	*1*	*5*	*10*	*25*	*50*	*75*	*100*
Phenol	0.43	0.56	0.63	0.61	0.76	0.73	0.76
D-Glucose	0.43	0.56	0.73	0.90	1.04	0.94	1.00

Effects of radical scavengers. — The effect of varying the equilibrating gas from argon to N_2O was examined over the pH range 0.9–12.7 for a solution initially 5mM in phenyl β-D-glucopyranoside. As in the typical yield–dose curve shown in Fig. 1, the D-glucose production is linear with dose, whereas G(phenol) decreases as irradiation proceeds. The initial G values obtained from the yield–dose curves are

summarised in Table II. Adding potassium thiocyanate (KCNS) to the irradiated solution reduces G(glycosidic scission). This behaviour is shown in Fig. 2 for solutions containing initially 5mM phenyl β-D-glucopyranoside (PhGlc) and KCNS from 1.35–53mM in argon-equilibrated solutions. Adopting the assumption that OH radicals are responsible for glycosidic scission and that this species is scavenged by KCNS, we have calculated the value of G(scission) which would be anticipated at any particular KCNS concentration, using the expression

$$G(\text{scission}) = G_0\left[1 - \frac{k_1[\text{CNS}^-]}{k_1[\text{CNS}^-] + k_2[\text{PhGlc}]}\right],$$

where G_0 is the initial G(scission) in the absence of KCNS, and G(scission) the value at a particular CNS^- and PhGlc concentration. The value of k_2 ($3.5 \times 10^9\,M^{-1}.sec^{-1}$) for the reaction of OH with PhGlc is that calculated by the KCNS competition method[24], using $k_1 = 6.6 \times 10^9\,M^{-1}.sec^{-1}$ for $OH + CNS^-$. Despite the doubt cast on the absolute value of this rate, the value relative to that of CNS^- will remain valid for this purpose. The results are shown in Fig. 2, together with the actual G(scission) obtained experimentally at each particular KCNS concentration. A similar correlation between experimental and calculated values was also found in N_2O. For example, G(scission) is 0.04 at 114mM KCNS, compared with a value of 0.03 calculated on the basis of simple competition. Individual yield–dose curves corresponding to the results are recorded elsewhere[25].

TABLE II

EFFECT OF RADICAL SCAVENGERS AND pH ON INITIAL G-VALUES FOR D-GLUCOSE AND PHENOL PRODUCTION. 5 mM PHENYL β-D-GLUCOPYRANOSIDE

pH	*0.9*	*1.2*	*3.6*	*7*	*9.2*	*11.0*	*12.7*
Argon							
D-Glucose		2.2	1.5	0.6		0.8	
Phenol		2.0	1.4	0.6		0.8	
N_2O							
D-Glucose	4.0		2.6	1.2	1.4		1.4
Phenol	3.9		2.5	1.2	1.4		1.4

Pulse radiolysis. — Details of the pulse-radiolysis equipment and techniques have been described[26]. The experiments were conducted by using two experimental conditions. For the experiments with doses up to ~2000 rads, a fused silica cell, having an optical path-length of 2.5 cm, was employed. For the experiments at low dose (~40 rads), a spiral cell arrangement[27], having an effective path-length of 64 cm, was employed. Doses were measured by using a secondary emission chamber, which was calibrated directly from the hydrated electron absorption at 720 nm in triply distilled water. The values of $G(e_{aq}^-) = 2.6$ and $\varepsilon = 18{,}400$ at 720 nm were used.

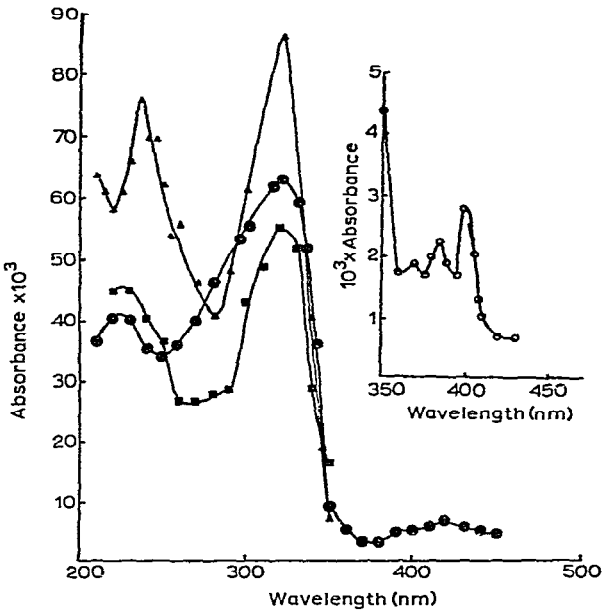


Fig. 3. Transient spectra immediately following pulse radiolysis of aqueous phenyl β-D-glucopyranoside (mM). ▲ pH 6.8 in N_2O; ■ pH 6.8 in argon; ● pH 2 in argon. Inset; pH 2 in argon 230 μsec after the end of the pulse.

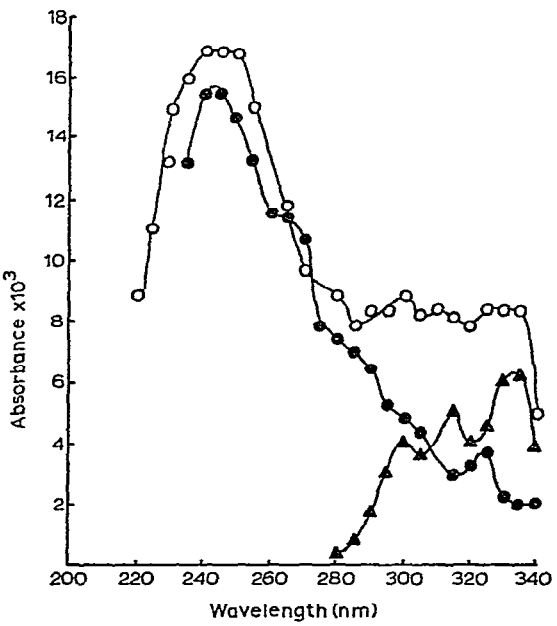


Fig. 4. Evaluation of spectrum of the transient formed by reaction of H atoms with phenyl β-D-glucopyranoside. ○ Phenyl β-D-glucopyranoside (mM)/butan-2-ol (0.22M) in argon, pH 1; ● butan-3-ol (0.22M) in argon, pH 1; ▲ difference spectrum.

A suitable Algol computer programme was written, and the kinetic results were analysed by using the English Electric KDF 9 computer at the University of Salford.

Pulsed irradiations using 1000–2000 rads. — *Transient spectra.* Fig. 3 shows the transient spectrum produced immediately after the pulse of mM phenyl β-D-glucopyranoside solution, irradiated in argon at pH 6.8 and pH 2 and in N_2O at pH 6.8. An additional species showing some hyperfine structure can be observed 230 μsec after the pulse, and the inset to Fig. 3 shows such a spectrum observed in argon at pH 2. An attempt was made to observe the spectrum of the reaction product of H atoms with phenyl β-D-glucopyranoside from the difference spectrum between pulsed irradiation of 0.22M butan-2-ol in argon at pH 1 and the same solution containing mM glucoside (Fig. 4). The spectrum of the e^-_{aq} reaction-product was similarly obtained by using the same solutions at pH 6.8 (Fig. 5). The effects of scavengers on the appearance and yields of the three maxima observed near 235, 320, and 430 nm are shown in Table III.

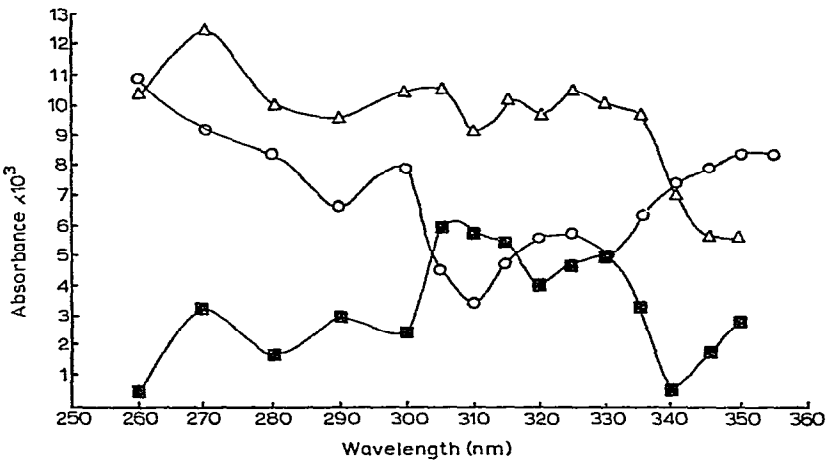


Fig. 5. Evaluation of spectrum of the transient formed by reaction of e^-_{aq} with phenyl β-D-glucopyranoside. △ Phenyl β-D-glucopyranoside (mM), butan-2-ol (0.22M), pH 6.8; ○ butan-2-ol (0.22M), pH 6 8; ■ difference spectrum.

From the transient spectra in N_2O, assuming complete scavenging of OH radicals ($G = 5.25$), we find a mean value of 3,570 for the extinction coefficient (ε) of the OH adduct. From the rough approximation that the electron adduct represents the difference in absorbance at 320 nm in argon and that calculated is due to the OH-.adical adduct, $\varepsilon \sim 4000$ is calculated for the electron adduct. Amphlett[28] found that the electron addition product to salicylic acid had $\varepsilon \sim 4000$.

Kinetics of formation and decay of transients. — From the rate of formation of the transient absorption at 320 nm after pulsed irradiation in N_2O at pH 6.8 (Fig. 6), the rate constant for the reaction OH + PhGlc can be determined. The rates, measured at several concentrations of glucoside, gave $k_2 = 4.4 \times 10^9\ M^{-1}.sec^{-1}$ for the addition of OH radicals. Using the CNS^- competition method[24], another measurement of this rate constant was found. Fig. 7 shows the competition plot, which gives a ratio

of $k_2(OH+PhGlc)/k_2(OH+CNS^-) = 0.53\times10^3$. On the basis of $k_2 = 6.6\times10^9$ $M^{-1}.sec^{-1}$ for $OH+CNS^-$, $k_2(OH+PhGlc) = 3.5\times10^9\,M^{-1}.sec^{-1}$.

TABLE III

EFFECTS OF SCAVENGERS ON THE APPEARANCE AND YIELDS OF TRANSIENT SPECIES

Concentration of phenyl β-D-glucopyranoside (mM)	*0.2*	*0.4*	*1*	*2*	*4*	*20*
Argon (pH 6.8)						
$10^3\times$Absorbance (320 nm)	47	55	60	70	77	97
λ_{max} obs. (nm)			235	320		
N_2O (pH 6.8)						
$10^3\times$ Absorbance (320 nm)	84	84	85	88	91	93
λ_{max} obs. (nm)			235	320	430 (weak)	
Absorbance (N_2O)/Absorbance (Argon)	1.7	1.5		1.3	1.2	1.0
Argon (pH 2)						
$10^3\times$ Absorbance (320 nm)			83			
λ_{max} obs. (nm)			235	320	430	
λ_{max} obs. (nm)						
N_2O+butan-2-ol				320 (weak)		
Argon+butan-2-ol				320 (weak)		

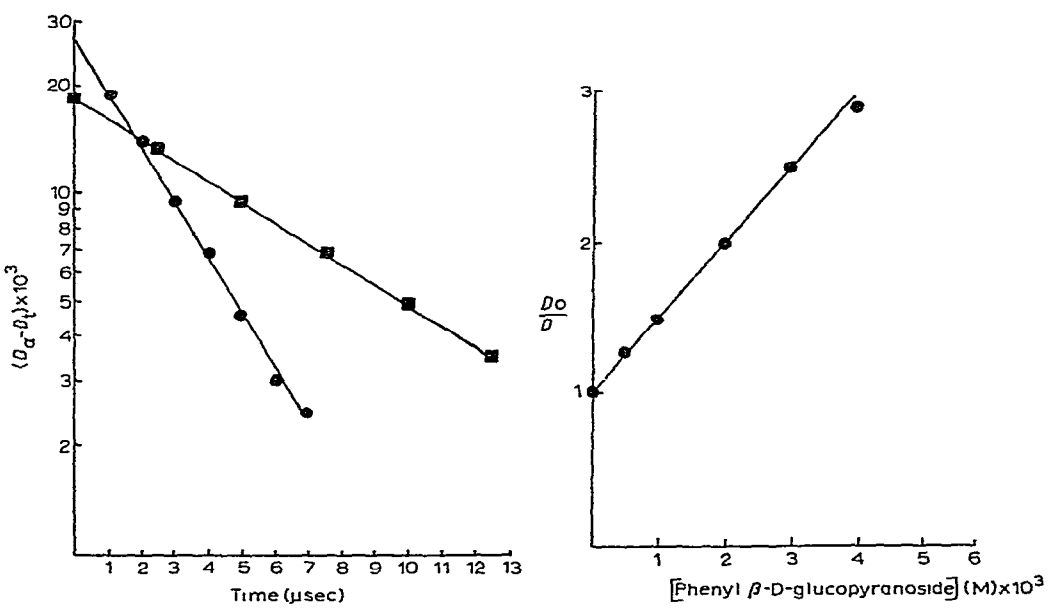


Fig. 6. Rate of formation of the transient absorption at 320 nm after pulse irradiation of phenyl β-D-glucopyranoside in N_2O at pH 6.8. Phenyl β-D-glucopyranoside· ● 50 μM; ■ 10 μM.

Fig. 7. Estimation of the rate constant of OH radicals with phenyl β-D-glucopyranoside, using the CNS^- competition method. $[CNS^-]$ = mM.

The decay of the OH adduct (λ_{max} 320 nm) strictly obeys second-order kinetics over a range of glucoside concentrations in argon and N_2O (Fig. 8). The decay of the species with λ_{max} = 235 nm is also second-order in argon and N_2O at pH 2 and 6.8 (Fig. 9). The rate constants (expressed as $2k/\varepsilon$) are summarised in Table IV for the experiments given in Fig. 8 and utilising other variants, when we also found second-order decays.

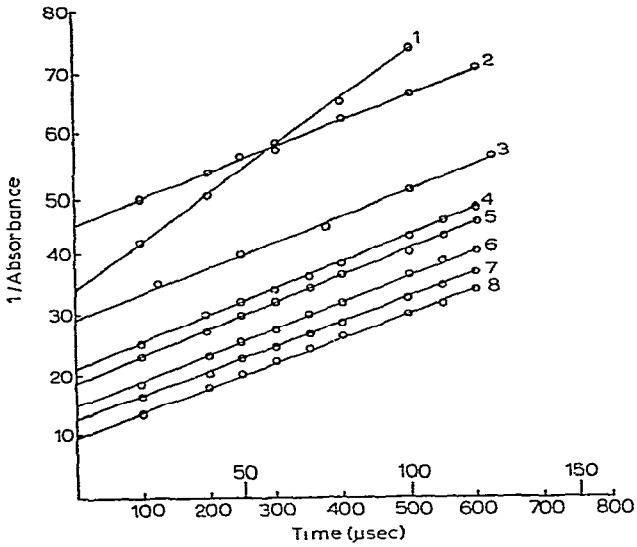


Fig. 8. Second-order decay of transients formed by pulse radiolysis of aqueous phenyl β-D-glucopyranoside. Transients with λ_{max} 235 nm (dose, 600 rads). Phenyl β-D-glucopyranoside (mM); abscissa 0–150 μsec. *1* pH 2 in argon; *2* pH 6.8 in argon; *3* pH 6.8 in N_2O. Transients with λ_{max} 320 nm (dose, 1830 rads); abscissa 0–800 μsec. Phenyl β-D-glucopyranoside: *4* 0.2mM; *5* 0.4mM; *6* 2mM; *7* 4mM; *8* 20mM.

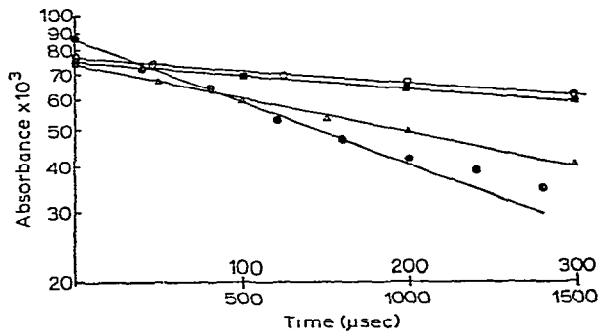


Fig. 9. First-order decay of OH adduct in phenyl β-D-glucopyranoside (mM) in N_2O, following a dose of 38 rads. ○ pH 0.8; ■ pH 1.3; △ pH 2.1 (abscissa 0–300 μsec); • pH 3.1 (abscissa 0–1500 μsec).

An estimate of the rate constant for the formation of the transient arising from the reaction of H atoms was obtained by analysis of the behaviour of the species absorbing at 320 nm when mM phenyl β-D-glucopyranoside was pulsed in N_2O and

TABLE IV

SECOND-ORDER RATE CONSTANTS FOR THE DECAY OF TRANSIENT SPECIES WITH λ_{max} AT 235 AND 320 NM.

(*i*) *Transient* λ_{max} *320 nm.*

Concentration of phenyl β-D-glucopyranoside (mM))	*0.2*	*0.4*	*1*	*2*	*4*	*20*
Argon ($2k/\varepsilon \times 10^5$)						
pH 6.8	1.1	1.1	1.0	1.1	1.2	1.1
pH 2.0			1.9			
N_2O (pH 6.8)						
$2k/\varepsilon \times 10^5$	1.0	0.9	1.1	1.0	1.0	1.0
$2k^a \times 10^8$ ($M^{-1}.sec^{-1}$)	3.4	3.3		3.5	3.4	3.5

(*ii*) *Transient* λ_{max} *235 nm*

Concentration of phenyl β-D-glucopyranoside (mM)	*0.2*	*0.4*	*1*	*2*	*4*	*20*
Argon ($2k/\varepsilon \times 10^5$)						
pH 6.8			1.1			
pH 2.0			1.9			
N_2O (pH 6.8)			1.1			

[a]Calculated by using 3560 for ε.

argon solutions containing 0.22M butan-2-ol at pH 1. The absorption due to 0.22M butan-2-ol in N_2O alone at 320 nm was subtracted to give the absorption of the H-atom reaction-product alone. From the pseudo-first-order production of this transient, $k_2 = 3.5 \times 10^7\ M^{-1}.sec^{-1}$ for the reaction H+PhGlc.

The rate of disappearance of e_{aq}^- in phenyl β-D-glucopyranoside solutions can be employed to calculate the rate constant for the reaction e_{aq}^- +PhGlc. From the pseudo-first-order plot[29] for e_{aq}^- disappearance, for example, in 5mM phenyl β-D-glucopyranoside, $t_{\frac{1}{2}} = 2.0$ μsec, which corresponds to $k_2 = 7 \times 10^7 M^{-1}.sec^{-1}$; the results at other PhGlc concentrations confirm this value.

Pulse irradiations using <40 rads. — The absorption near 400 nm was found to increase in intensity over ~500 μsec after the pulse, and this is attributed to the production of the phenoxyl radical[30]. The spectrum of this species[30] was observed ~230 μsec after the pulse in the experiments using 1000–2000 rads (Fig. 3).

Yields of transient species. — *G*-values for the OH-radical adduct (λ_{max} 320 nm) and the phenoxyl radical (λ_{max} 400 nm) were calculated from the transient absorption in mM phenyl β-D-glucopyranoside in N_2O from pH 0.8–12.5. Table V shows the results and the variation of the maximal absorbance of the species with $\lambda_{max} = 430$ nm with pH.

Kinetics. — (*i*) *Decay of OH adduct.* A change from second- to first-order kinetics is found for the decay of the OH adduct at low pH. Fig. 9 shows the first-

TABLE V

YIELDS OF OH ADDUCT (λ_{max} 320 nm), PHENOXYL (λ_{max} 400 nm), AND TRANSIENT WITH λ_{max} 430 nm. DOSE ~38 rads

pH	*0.8*	*1.3*	*2.1*	*3.1*	*5.1*	*6.8*	*9.0*	*10.9*	*12.5*
OH adduct (*G* value)[a]	4.0	3.2	4.3	4.5	5.7	5.3	5.6	5.2	4.2
Phenoxyl (*G* value)[b]	4.3	4.4	4.2	3.0	1.1	1.4	1.7	1.6	2.3
Transient λ_{max} 430 nm ($10^3 \times$ absorbance)	69	22	16	10	3	4	6	—	—

[a]Calculated by using $\varepsilon = 3560$. [b]Calculated by using $\varepsilon = 2200$.

order plot for the OH adduct in mM phenyl β-D-glucopyranoside solution in N_2O after a dose of 38 rads at pH 0.8, 1.3, and 2.1. The corresponding second-order plot of $1/D$ against time shows no such linear dependence. At pH 3.1, mixed kinetics are encountered. An initial, fast, first-order decay is followed by a slower second-order decay. At higher pH values, the initial first-order decay cannot be observed, and the second-order process is dominant. First-order rate constants for the initial reaction are given in Table VI.

TABLE VI

FIRST-ORDER RATE CONSTANTS (k_1.sec^{-1}) FOR THE DECAY AND FORMATION OF TRANSIENTS IN ACID

pH	*0.8*	*1.3*	*2.1*	*3.1*
OH adduct (decay)	8×10^2	7×10^2	2×10^3	8×10^2
Phenoxyl (formation)		$\sim 10^5$	$\sim 10^4$	$\sim 10^3$
Transient, λ_{max} 430 nm	8×10^6	8×10^6	4×10^3	[a]

[a] Becomes second-order with $2k/\varepsilon = 9 \times 10^5$.

(*ii*) *Phenoxyl radical.* Formation of the absorption at 400 nm due to phenoxyl continues after the pulse. This process is best described by first- rather than second-order kinetics from pH 0.8–2.1. Good first-order kinetics are only observed up to ~500 μsec after the pulse at pH 3.1. The phenoxyl radical is stable over at least 500 μsec at pH 0.8–2.1, and its formation is essentially, although not strictly, first-order. At higher pH, the oscilloscope traces show that at least one other process leads to the production of an additional product absorbing near 400 nm.

(*iii*) *Transient with λ_{max} 430 nm.* The decay of this species is strictly first-order at pH 0.8, 1.3, and 2.1, but obeys mixed kinetics at pH 3.1. The results are summarised in Table VI.

DISCUSSION

From the nature of the reaction products, two processes can be recognised during γ-radiolysis of aqueous phenyl β-D-glucopyranoside solutions. One leads to glycosidic scission, giving D-glucose and phenol, and the second to hydroxylation

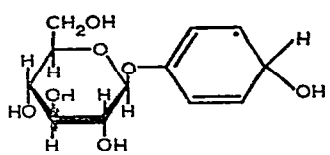
of the aromatic aglycon, giving *p*-hydroxyphenyl β-D-glucopyranoside and probably another monohydroxy isomer. It is evident, from the yield–dose curves for phenol production, that secondary reactions rapidly remove phenol after it is produced, and it is probable that the 1,3- and 1,4-dihydroxybenzenes identified arise in this manner. Such reactions would be compatible with the high reactivity[30] of OH radicals towards phenol ($k_2 = 1.4 \times 10^{10} M^{-1}.sec^{-1}$). During radiolysis of aqueous nitrobenzene[31] and anisole[32] solutions, cleavage of the substituent group is also observed to accompany the hydroxylation of the aromatic nucleus. From nitrobenzene, the products were nitrate ($G = 0.5$) and nitrophenol ($G = 1.6$), and from anisole, phenol ($G = 0.46$) and methoxyphenol ($G = 0.07$). In the glycosidic scission which we have studied quantitatively (Table I), equivalent amounts of phenol and D-glucose are initially formed. Rapid, secondary degradation of phenol occurs and is probably the explanation for the discrepancy between the G values of the two products at the higher solute (~10mM) concentrations. Scavenging of the reactive species responsible for glycosidic scission is not complete until a solute concentration of ~50mM (Table I), an observation which is paralleled by the concentration dependence during the formation of transient products during pulse radiolysis On the basis of simple competition, it is calculated that all OH radicals should be scavenged by mM glucoside. Thus, the inadequacy of this simple model to account for the effect of increasing solute concentration is again demonstrated here as in other aqueous systems containing organic solutes[33]. It has been suggested that scavenging from the spurs, before appreciable diffusion occurs, is possible when $k_{RS}\, c_S > 10^7 sec^{-1}$, where c_S is the solute concentration and k_{RS} is the rate constant for the reaction with water radiolysis products[34]. For 10mM phenyl β-D-glucopyranoside, the product $k_{RS}\, c_S$ for reaction with OH radicals is $\sim 10^8$ sec^{-1}, and an enhanced yield from this source could, therefore, be one possibility. However, the behaviour of concentrated solutions is worthy of closer investigation.

From the results of our scavenging experiments, it is clear that OH radicals are the main product of water radiolysis responsible for glycosidic scission. Scission is approximately doubled in N_2O, when $e_{aq}^- + N_2O \rightarrow N_2 + OH + OH^-$ and, therefore, the OH yield is effectively doubled[35]. Moreover, the predicted scavenging by KCNS, based on rate-constant data for OH radical reactions, is observed (Fig. 2) In this respect, therefore, we are at variance with Kochetkov *et al.*[8] who proposed that a dissociative electron-capture, following reaction with e_{aq}^-, was responsible for glycosidic scission in aryl glycosides on γ-irradiation in aqueous solution. For this mechanism, N_2O would be expected to inhibit, rather than enhance, glycosidic scission. Moreover, the rate constant for the reaction $e_{aq}^- + PhGlc$ is low ($7 \times 10^7 M^{-1}.sec^{-1}$) and contributes only marginally to the spectra of the transient radiolysis products (Fig. 5). The low yield of H atoms at pH 6.8 ($G = 0.55$), the low rate-constant for reaction H+PhGlc ($\sim 10^7 M^{-1}.sec^{-1}$), and the small contribution which this species makes to the transient spectra (Fig. 4) all support the view that H atoms cannot be the dominant species promoting glycosidic scission in neutral solution. On the other hand, during γ-irradiation of aryl glycosides in the solid state, cyclohexadienyl type

radicals, formed by addition of H atoms to the aromatic aglycon, are[15] the predominant species observed by e.s.r.

The major participation of OH radicals in both glycosidic scission and hydroxylation of the aglycon is fully supported by the pulse-radiolysis results ($k_2 = 4 \times 10^9$ $M^{-1}.sec^{-1}$ for OH+PhGlc). Three maxima can be observed on pulse irradiation of phenyl β-D-glucopyranoside solutions at 320, 235 (of lower intensity), and 430 nm. The latter species is most apparent at low pH and also in N_2O. Since there is no change in the maxima and no significant difference in intensity of transients at 235 and 320 nm at low pH in argon when e^-_{aq} are converted into H atoms, and in neutral N_2O solution when e^-_{aq} are converted into OH radicals, we conclude that the products of reaction of H and OH radicals have similar spectra. We also find that, when conditions are changed, there is a parallel behaviour of the maxima at 235 and 320 nm, which leads us to the view that these maxima represent mainly two bands of the same, rather than different, transient species. One additional feature of the transient spectra which merits comment is the enhancement of the absorption and the structure which appears near 400 nm during a time of 230 μsec after a pulse of 350 rads to mM phenyl β-D-glucopyranoside solution at pH 2 (Fig. 3). This absorption persists for at least 1500 μsec and is, in our view, due to an additional species, which is probably the phenoxyl radical, for reasons similar to those suggested elsewhere[30,36].

The main species absorbing at 235 and 320 nm is due to the OH addition product to the aromatic aglycon (**1**). The influence of OH-radical scavengers and N_2O support this assignment. A minor portion of the same absorptions could be associated with e^-_{aq} and H-atom adducts, and by using conditions which eliminate the contribution due to OH radicals at pH 6.8 and pH 1, an approximate spectrum of these species was obtained (Figs. 4 and 5). Radicals similar to **1** have been found during irradiation of other aromatic solutes in aqueous solution[37]. The additional



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maximum observed at 235 nm is adequately accounted for by the presence of the sugar moiety. The value of ε found for the OH adduct to PhGlc is based on the assumption that all the OH radicals react by addition to the aglycon. The good agreement with other such species shown in Table VII indicates that this assumption is valid, and in turn demonstrates that no significant attack occurs at the D-glucose moiety, which explains the lack of products derived from this source. Substitution of the rate constant of the reaction e^-_{aq}+PhGlc into the Hammett correlation established by Anbar and Hart[38] gave a σ_{para} value of +0.12. The glucosyl group, therefore, exercises definite electron-attracting properties which could reduce the electron density marginally at the *o*- and *p*-positions and, therefore, make the *m*-position also a

TABLE VII

PROPERTIES OF SUBSTITUTED HYDROXYCYCLOHEXADIENYL RADICALS[37]

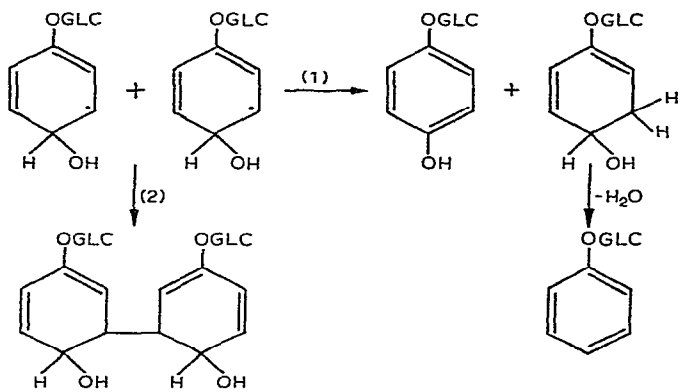
Compound	C_6H_5COOH	$C_6H_5COO^-$	*PhGlc*	C_6H_5OH	C_6H_6	$C_6H_5OCH_3$
λ_{max} (nm)	347	330	320	330	313	330
$k_2(10^9 M^{-1}\cdot sec^{-1})$	4.3	6.0	4.0	14	7.8	12
ε (at λ_{max})	3600		3560	4400	3500	

possible site for attack by the electrophilic OH-radicals. Our product studies definitely show hydroxylation at the para position, and it is possible, therefore, that the unidentified hydroxylated product could be the *m*-isomer. By using the Chutny Rule[39], the absorption of the OH adduct of phenyl β-D-glucopyranoside would be predicted at 398 and not at 320 nm as we have found. A similar deviation from the rule was found by Sangster[40] for OH-radical transients from benzoic acids.

We are unable to assign the broad absorption at λ_{max} 430 nm unambiguously. Its yield in N_2O solutions is strongly dependent on pH and it could, therefore, be due to a form protonated either at the glycosidic or lactol oxygen atom of the species already considered.

On the basis of our observations on the products and transient spectra, we suggest the following mechanism to account for the glycosidic scission and hydroxylation of the aromatic aglycon which accompanies the action of ionising radiations on aqueous solutions of phenyl β-D-glucopyranoside. The principal intermediate is the hydroxycyclohexadienyl radical, shown in **1** for the para position, although other isomers can be produced also. Alternative paths for the reaction of this transient are evident.

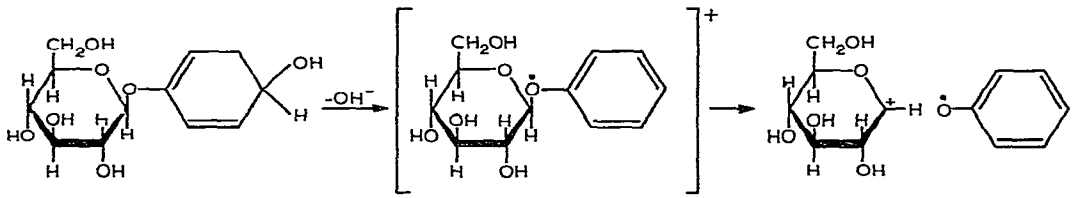
(*a*) *Bimolecular reactions.* — At doses of 1000–2000 rads, the decay of the OH transient is strictly second-order with $k_2 = 1.7 \times 10^8$ $M^{-1}.sec^{-1}$. Production of the hydroxylated product and accompanying regeneration of phenyl β-D-glucopyranoside



Scheme 1

is similar to that proposed by Schulte-Frohlinde *et al.*[41] to account for the low hydroxylation-yields in substituted benzoic acids. We have found indications also of the dimerisation process, which could be the path to production of polymeric material which cannot be volatilised in g.l.c. experiments and is immobile on t.l.c. Although the presence of an electron-attracting group would be expected to favour path **1** (Scheme 1), Cercek[42] has found that even nitrocyclohexadienyl radicals only undergo ~25% disproportionation. Path **2** would, therefore, be expected to be significant for the hydroxycyclohexadienyl radicals derived from the glucoside.

(*b*) *Unimolecular reaction.* — At low doses, Land and Ebert[30] found a first-order elimination reaction for the OH-adduct of phenol, which was acid-catalysed, to yield the phenoxy radical: $Ph(OH)_2^{\cdot} \rightarrow PhO^{\cdot} + H_2O$. Their proposed mechanism (Scheme 2), when applied to the phenyl β-D-glucopyranoside–OH adduct, satisfactorily accounts



Scheme 2

for our observations. The intermediate sugar carbonium ion has been encountered in other reactions at the lactol carbon atom[43], and it reacts rapidly with water to give D-glucose. Equimolecular amounts of D-glucose and phenol would be produced if the phenoxyl radical yielded phenol, possibly by H-abstraction from the sugar. Experimental observations which support the unimolecular pathway are the first-order kinetics of decay of **1** found at low pH and the accompanying first-order production of phenoxyl under the same conditions. At pH >3.1, the second-order process is predominant, but the importance of the acid catalysis in phenoxyl production is clear from the enhanced *G*-values of this radical at low pH (Table V). There are indications from the dependence of *G*(phenoxyl) on pH that heterolytic scission of the hexose–O bond in **1** is also catalysed by alkali. Such acid and alkaline catalysis of glycosidic bond-cleavage is, of course, well established[9–12] for aryl glycosides, where O–aryl bond-scission is unknown. It is significant also that *G*(glycosidic scission) under steady-state conditions varies with pH as does *G*(phenoxyl), which further illustrates their interdependence. Over a limited region of pH (near 3), the first-order disappearance of **1** approaches the first-order production of phenoxyl ($k_1 \sim 10^3$ sec^{-1}). Production of phenoxy radicals from OH adducts of substituted aromatic molecules is also favoured[44] at pH ~3.

The steady-state ^{60}Co γ-radiation experiments are more closely related to the low-dose experiments in pulse radiolysis, which may account for the predominance of glycosidic scission over hydroxylation of the aglycon found under these conditions.

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REFERENCES

1 G. O. PHILLIPS AND G. J. MOODY, *J. Chem. Soc.*, (1960) 762.
2 G. O. PHILLIPS AND K. W. DAVIES, *J. Chem Soc*, (1964) 205.
3 G. O. PHILLIPS AND M. D. YOUNG, *J. Chem Soc. (A)*, (1966) 383.
4 G. O. PHILLIPS AND G. J. MOODY, *J. Chem. Soc.*, (1958) 3534.
5 G. O. PHILLIPS, *Advan. Carbohyd. Chem.*, 16 (1961) 13.
6 G. O PHILLIPS AND M. D. YOUNG, *J. Chem Soc. (A)*, (1968) 1240.
7 M. L. WOLFROM, W. W. BINKLEY, AND L J. MCCABE, *J. Amer. Chem Soc.*, 81 (1959) 1442.
8 N. K. KOCHETKOV, L. I. KUDRYASHOV, AND M. A. CHLENOV, *Zh Obsch. Khim.*, 35 (1965) 897.
9 J. N. BEMILLER, *Advan Carbohyd. Chem.*, 22 (1967) 25.
10 P. NUHN AND G. WAGNER, *Pharmazie*, 21 (1966) 261.
11 C. E. BALLOU, *Advan. Carbohyd. Chem.*, 9 (1954) 59.
12 R. C. GASMAN AND D. C. JOHNSON, *J. Org. Chem.*, 31 (1966) 1830.
13 S. M. HOPKINSON, *Quart. Rev.* (London), 23 (1969) 98.
14 J. J. WEISS, A. O ALLEN, AND H. A SWARZ, *Proc. Intern Conf. Peaceful Uses At. Energy*, New York, 1956, Vol. 14, p. 179.
15 J. S MOORE AND G. O. PHILLIPS, *Carbohyd. Res.*, 16 (1971) 79.
16 G. W. HAY, B. A LEWIS, AND F. SMITH, *J. Chromatogr.*, 11 (1963) 479.
17 L. CHAFETZ, A. I. SCHRIFTMAN, AND H. KAY, *J. Chromatogr.*, 35 (1968) 567.
18 J. R. DOTY, *Anal. Chem.*, 20 (1948) 1166.
19 C C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
20 R BENTLEY, C. C SWEELEY, M. MAKITA, AND W. W. WELLS, *Biochem. Biophys. Res. Commun.*, 11 (1963) 14,
21 M. J. SOMOGYI, *J. Biol. Chem.*, 22 (1952) 22.
22 E. EMERSON AND K. KELLY, *J. Org. Chem*, 13 (1948) 532.
23 D. SVOBODOVA AND J. GASPARIC, *Coll. Czech. Chem. Commun.*, 33 (1968) 42.
24 G. E ADAMS AND B. D. MICHAEL, IN J. H. BAXENDALE, M. EBERT, J. P. KEENE, AND A. J SWALLOW, (Eds.), *Pulse Radiolysis*, Academic Press, New York, 1965.
25 G. FILBY, Ph.D. Thesis, University of Salford, 1970.
26 J P. KEENE, *J. Sci Inst.*, 41 (1963) 493.
27 J. P. KEENE, in J. H. BAXENDALE, M. EBERT, J. P. KEENE, AND A. J. SWALLOW (Eds), *Pulse Radiolysis*, Academic Press, New York, 1965.
28 C. A. AMPHLETT, G. E. ADAMS, AND B. D. MICHAEL, *Advan. Chem. Ser.*, 81 (1968) 231.
29 E. A. BALAZS, J. V. DAVIES, G O. PHILLIPS, AND D. S. SCHEUFELE, *J. Chem. Soc. (C)*, (1968) 1429.
30 E. J. LAND AND M. EBERT, *Trans. Faraday Soc.*, 63 (1967) 1181.
31 J. H. FENDLER AND G K. GASOWSKI, *J. Org. Chem.*, 33 (1968) 1865.
32 J. H. FENDLER AND G. L. GASOWSKI, *J. Org. Chem.*, 33 (1968) 2755.
33 J. V. DAVIES, W. GRIFFITHS, AND G. O. PHILLIPS, IN J. H. BAXENDALE, M. EBERT, J. P. KEENE, AND A. J. SWALLOW (Eds.), *Pulse Radiolysis*, Academic Press, New York, 1965.
34 E. HAYON, *Trans. Faraday Soc.*, 61 (1965) 723.
35 F. S DAINTON AND W. S. WATT, *Proc. Roy. Soc.* (London), *Ser. A*, 275 (1963) 447.
36 E. J. LAND, G. PORTER, AND E. STRACHAN, *Trans. Faraday Soc.*, 57 (1961) 1885.
37 P. NETA AND L. M. DORFMAN, *Advan. Chem. Ser.*, 81 (1968) 222.
38 M. ANBAR AND E. HART, *J. Amer. Chem. Soc.*, 86 (1964) 5633.
39 B. CHUTNY, *Nature*, 213 (1967) 593.
40 D. F. SANGSTER, *J. Phys. Chem.*, 70 (1966) 1712.
41 D. GRASSLIN, F. MERGER, D. SCHULTE-FROHLINDE, AND C. VOLKERT, *Ber.*, 100 (1967) 3077.
42 K. D. ASMUS, B. CERCEK, M. EBERT, AND W. WIGGER, *Trans. Faraday Soc.*, 63 (1967) 2435.
43 F. H. NEWTH AND G. O. PHILLIPS, *J. Chem. Soc.*, (1953) 2896, 2900, 2904
44 C. R. E. JEFCOATE AND R. O. C. NORMAN, *J. Chem. Soc. (B)*, (1968) 48.