Radiation Effects on Aryl Glycosides. Part VII.† Radiolysis of Aqueous Solutions of *p*-Nitrophenyl β-D-Glucopyranoside

By **Kenneth Kemsley, John S. Moore,*** and **Glyn O. Phillips,** Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT, Lancashire

Pulse radiolysis experiments demonstrate that reactions of e_{aq}^- (k_2 = 4 × 10^{10} l mol^{-1}) and OH (k_2 = 2.1 × 10^9 l mol^{-1} s^{-1}) with *p*-nitrophenyl β-D-glucopyranoside (*p*NPG) yield transient species with absorption maxima near 300 nm. The results indicate that reactions occur mainly at the nitro-group. A transient species with absorption maximum at 450 nm in N_2O-saturated and in acidic solution is considered to be the nitrocyclohexadienyl radical. *p*-Nitrophenol, 4-nitrocatechol, glucose, 2-hydroxy-4-nitrophenyl β-D-glucopyranoside, and traces of αβ-unsaturated carbonyl compounds are produced during steady state ^{60}Co γ-irradiation of *p*NPG solutions, as a result of initial reaction of hydroxyl radicals. A mechanism is advanced for the formation of these products.

PREVIOUS studies have established the radiation susceptibility of *O*-glycosidic bands.[1-6] Phenyl β-D-glucopyranoside yields equivalent amounts of glucose and phenol; hydroxylation of the aglycone also occurs.[6] Whereas Phillips *et al.*[6] attribute this glycosidic scission to the OH radical, Kochetkov considers that the hydrated electron (e^-_{aq}) is the principal transient species of water radiolysis which leads to hydrolytic scission of aryl and alkyl glycosides.[4] We have now investigated the effects of the electron-withdrawing nitro-group, which should favour participation by the nucleophilic e^-_{aq}, in an attempt to distinguish between the actions of e^-_{aq} and of OH radicals, and to elucidate the mechanism of the radiation-induced glycosidic bond scission process.

EXPERIMENTAL

Materials.—*p*NPG was obtained from Koch–Light Laboratories Ltd.; *p*-nitrophenol, 4-nitrocatechol, and D-glucose were obtained from B.D.H. Ltd.

Methods.—Pulse radiolysis was carried out with a Febetron 705B 2 MeV accelerator at the University of Salford. Full details are described elsewhere.[7] Dosimetry was carried out with potassium thiocyanate.[8] The dose was varied from 200 to 4 000 rads per pulse. ^{60}Co γ-irradiation was carried out as previously described.[6]

Products were isolated from the irradiated solution by rotary evaporation, freeze-drying, or extraction with ether. Phenolic products were extracted into ether at pH 2 leaving the carbohydrate derivative in the aqueous phase. The phenols were separated and identified by t.l.c. on Antec-

---

† Part VI, P. J. Baugh, K. Kershaw, G. O. Phillips, and M. G. Webber, *Carbohydrate Res.*, 1973, **31**, 199.

[1] G. O. Phillips and G. 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] N. K. Kochetkov, L. I. Kudryashov, and M. A. Chlenov, *Zhur. obshchei Khim.*, 1965, **35**, 897.

[5] M. L. Wolfrom, W. W. Binkley, and L. J. McCabe, *J. Amer. Chem. Soc.*, 1959, **81**, 1442.

[6] G. O. Phillips, W. G. Filby, J. S. Moore, and J. V. Davies, *Carbohydrate Res.*, 1971, **16**, 89.

[7] K. G. Kemsley, Ph.D. Thesis, University of Salford, 1972.

[8] G. E. Adams, J. W. Boag, and B. D. Michael, *Trans. Faraday Soc.*, 1965, **61**, 1674.

polyamide F254 glass plates (1 : 4 ethyl methyl ketone–n-hexane as irrigant). The spots obtained after continuous elution were removed from the plates and the products were extracted with ethanol; they were identified by comparing their polarographic behaviour and absorption spectra with those of pure reference samples.

A modification of the ferricyanide method was used to estimate reducing products by using glucose as standard.[9] Assay was carried out with the solution obtained after neutralising the ethereal extract from the irradiated solution, in order to avoid reduction by nitrophenols.

Deoxy-sugars and materials resembling malonaldehyde were estimated by the method of Waravdekar.[10]

After repeated extraction of the irradiated aqueous solution with ether to remove free phenols, subsequent hydrolysis yielded 4-nitrocatechol, which indicates the production of 2-hydroxy-4-nitrophenyl β-D-glucopyranoside on irradiation.

RESULTS AND DISCUSSION

Reactions of e^-_{aq}.—The second-order rate constant for the reaction of e^-_{aq} with *p*NPG, as determined by the disappearance of its absorption at 650 nm, is 4 (±0.2) × 10^{10} l mol^{-1}. Unsubstituted carbohydrates react extremely slowly with e^-_{aq};[11] consequently reaction occurs at the aromatic aglycone, to give the radical anion (I), $\lambda_{max.}$ 295 nm [16.8 (±2.5) × 10^3 l mol^{-1} cm^{-1} [(Figure 1). The *pK* value of (I), leading to the protonated radical (II) [ε_{295} 10 (±0.2) × 10^3 l mol^{-1} cm^{-1}], was determined from Figure 2 to be 3.9. A similar pattern of reactivity has been observed for reaction with the free *p*-nitrophenol;[12] consequently the proposed reaction scheme is that shown in Scheme 1.

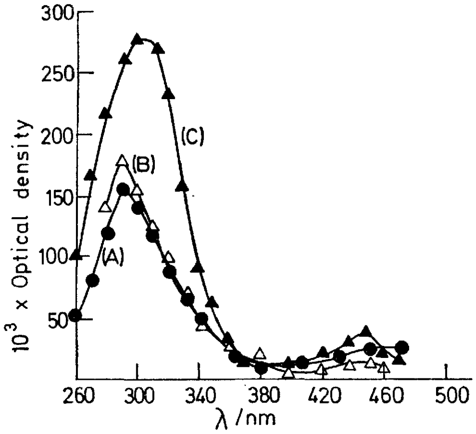


FIGURE 1 Transient spectra observed on pulse radiolysis of aqueous solutions of *p*-nitrophenyl β-D-glucopyranoside (5 × 10^{-5} mol l^{-1}); dose 1 900 rads; (A) deaerated containing t-butyl alcohol (10^{-2} mol l^{-1}), pH 2; (B) deaerated containing t-butyl alcohol (10^{-2} mol l^{-1}); (C) saturated with N_2O

Reactions of OH•.—The rate constant for reaction of OH with *p*NPG was determined to be 2.1 (±0.1) × 10^9 l mol^{-1} s^{-1}, by the thiocyanate competition method, based on $k_2(OH + CNS^-) = 1.08 \times 10^{10}$ l mol^{-1} s^{-1}.[13] The reaction of OH at neutral pH produces a transient species absorbing at $\lambda_{max.}$ 305 nm [ε 10.3 (±0.4) × 10^3 l mol^{-1} cm^{-1}] and a product absorbing at 450 nm (Figure 1). Unsubstituted carbohydrates yield species with absorption at *ca.* 250 nm and low extinction coefficient (*ca.* 10^3 l mol^{-1} cm^{-1});[11,14] consequently the observed absorption is due to reaction at the aglycone. In view of the electrophilic nature of OH•, reaction presumably occurs at the nitro-group[12] to produce the radical (III), which is readily deprotonated (Figure 2) to produce the radical (IV), ε_{295} 9.4 × 10^3 l mol^{-1} cm^{-1}, with p*K* 5.0.

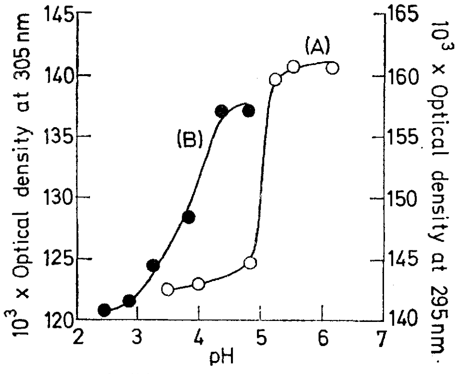
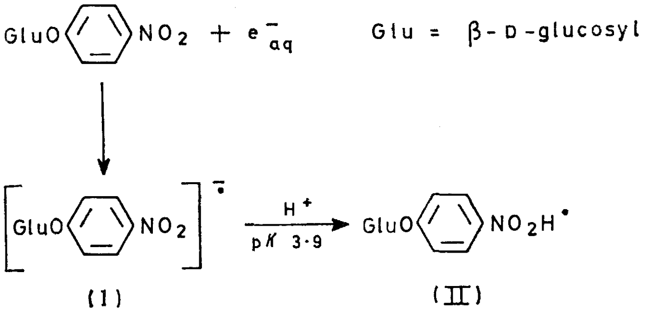


FIGURE 2 Effect of pH on the transient species produced following reaction of hydrated electrons and hydroxyl radicals with *p*-nitrophenyl β-D-glucopyranoside (10^{-5} mol l^{-1}); dose 2 000 rads; (A) saturated with oxygen (left hand ordinate); (B) deaerated containing t-butyl alcohol (10^{-1} mol l^{-1}) (right hand ordinate)

Reactions of H•.—The absorption produced in deaerated solutions containing t-butyl alcohol (10^{-2} mol l^{-1}) at pH 2 has $\lambda_{max.}$ 295 nm [ε 9.8 (±0.5) × 10^3 l mol^{-1} cm^{-1}] and a small absorption at 450 nm. As in the case of OH•, reaction of unsubstituted carbohydrates with H• produces species with $\lambda_{max.}$ *ca.* 250 nm and small extinction coefficients. In view of the electrophilic nature of H•, it is proposed that H• reacts directly at the nitro-group, to produce the radical (II) [the protonated form of (I)].



SCHEME 1

---

[9] J. T. Park and M. J. Johnson, *J. Biol. Chem.*, 1949, **181**, 149.

[10] V. S. Waravdekar and L. D. Saslow, *J. Biol. Chem.*, 1959, **234**, 210.

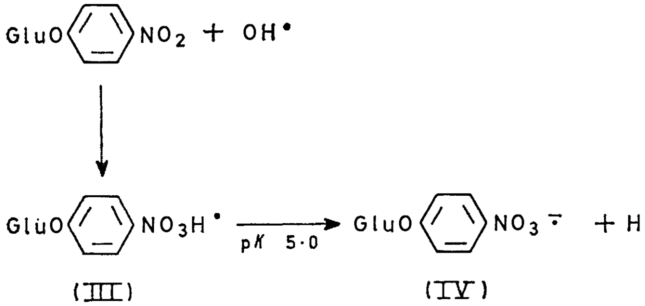
[11] G. O. Phillips, W. Griffiths, and J. V. Davies, *J. Chem. Soc.* (*C*), 1966, 194.

[12] B. Cercek and M. Ebert, A.C.S. Advances in Chemistry Series, No. 81, 1968, p. 210.

[13] D. H. Ellison, G. A. Salmon, and F. Wilkinson, *Proc. Roy. Soc.* 1972, *A*, **328**, 23.

[14] J. S. Moore, G. O. Phillips, J. V. Davies, and K. S. Dodgson, *Carbohydrate Res.*, 1970, **12**, 253.

All the radicals (I)—(IV) absorb at the same wavelength as the parent compound. Consequently, corrections were made for the disappearance of solute when calculating the extinction coefficients, based on $G(e^-_{aq}) + G(OH) + G(H) = 6.0$.[15-17]



SCHEME 2

After ^{60}Co γ-irradiation of solutions of *p*NPG (10^{-3} mol l^{-1}) to a dose of 3.12×10^{18} eV ml^{-1}, t.l.c. of the ethereal extract showed three products which were visible under u.v light; two of these were mobile, and could be further separated by continuous elution for **3** days. The absorption spectra of solutions of these products in alcoholic sodium hydroxide were identical with those of *p*-nitrophenol ($\lambda_{max.}$ 402 nm) and 4-nitrocatechol ($\lambda_{max.}$ 424 nm). Polarography of the products confirmed the presence of *p*-nitrophenol, $E_{\frac{1}{2}}$ — 0.197, and 4-nitrocatechol, —0.90 V. *G* Values for the formation of these products were also determined from Figure 3 (Table), from calibration plots using alkaline ethanol standard solutions. The increase in reducing power, relative to D-glucose, was calculated to be 2.8 or 1.5 for N_2O-saturated and deaerated solutions, respectively, and is independent of dose up to at least 7×10^{16} eV ml^{-1}. Alkaline hydrolysis of the irradiated aqueous solution after extraction with ether, followed by t.l.c., showed the presence of *p*-nitrophenol and 4-nitrocatechol. The former arises as a result of hydrolysis of unchanged *p*NPG and *p*NPG modified only in the sugar unit. The latter arises as a result of hydroxylation of the aglycone. However, the products are the result of initial reaction of OH•.

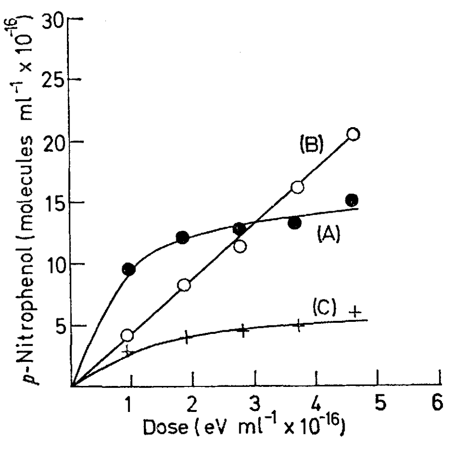


FIGURE 3 Formation of *p*-nitrophenol following ^{60}Co γ-irradiation of solutions of *p*-nitrophenyl β-D-glucopyranoside in the presence of (A) N_2O, (B) nitrogen, (C) t-butyl alcohol and nitrogen

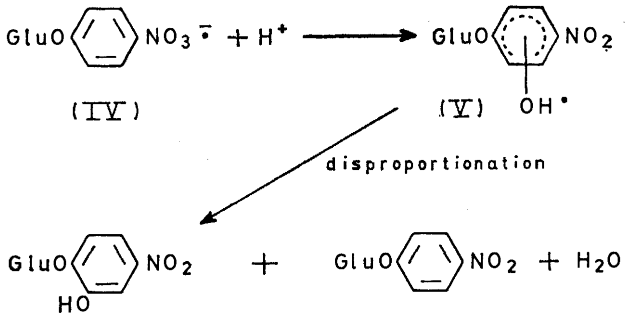
Consideration of the data given in the Table leads to

G values of products from ^{60}Co γ-irradiated *p*-nitrophenyl β-D-glucopyranoside (10^{-3} mol l^{-1})

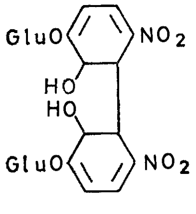
	N_2O	Deaerated	Deaerated + t-butyl alcohol (10^{-1} mol l^{-1})
p-Nitrophenol	0.44 *	0.34	0.13 *
4-Nitrocatechol	0.34 *	0.13	
Reducing compounds	2.7	1.46	
Malonaldehyde	0.04		
2-Hydroxy-4-nitrophenyl β-D-glucopyranoside	0.05		

* Initial *G* values, obtained from non-linear yield-dose curves.

the conclusion that the major changes we have observed are initiated by reaction of OH• with the glycoside and pulse radiolysis data indicate that the radical is (IV) above pH *ca.* 5. The radical (IV) disappears very slowly by first-order kinetics, $k_1 = 36$ s^{-1}, suggesting that an intramolecular rearrangement is occurring,[12] to produce a hydroxycyclohexadienyl radical (V) (Scheme 2), which



may disproportionate by second-order kinetics to produce 2-hydroxy-4-nitrophenyl β-D-glucopyranoside (*G* 0.05) or dimerize to produce the unidentified brown product immobile on t.l.c., *e.g.* (VI).



(VI)

The absorption produced at *ca.* 450 nm during pulse radiolysis of N_2O-saturated solutions of *p*NPG disappears by second-order kinetics (k_2 7.9×10^8 l mol^{-1} s^{-1}) and this may be due to the radical (V) produced as a

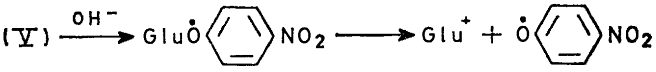
---

[15] A. O. Allen, 'The Radiation Chemistry of Water and Aqueous Solution,' Van Nostrand, New York, Toronto, and London, 1961, pp. 47, 64.

[16] E. Hayon, *Trans. Faraday Soc.*, 1964, **61**, 723.

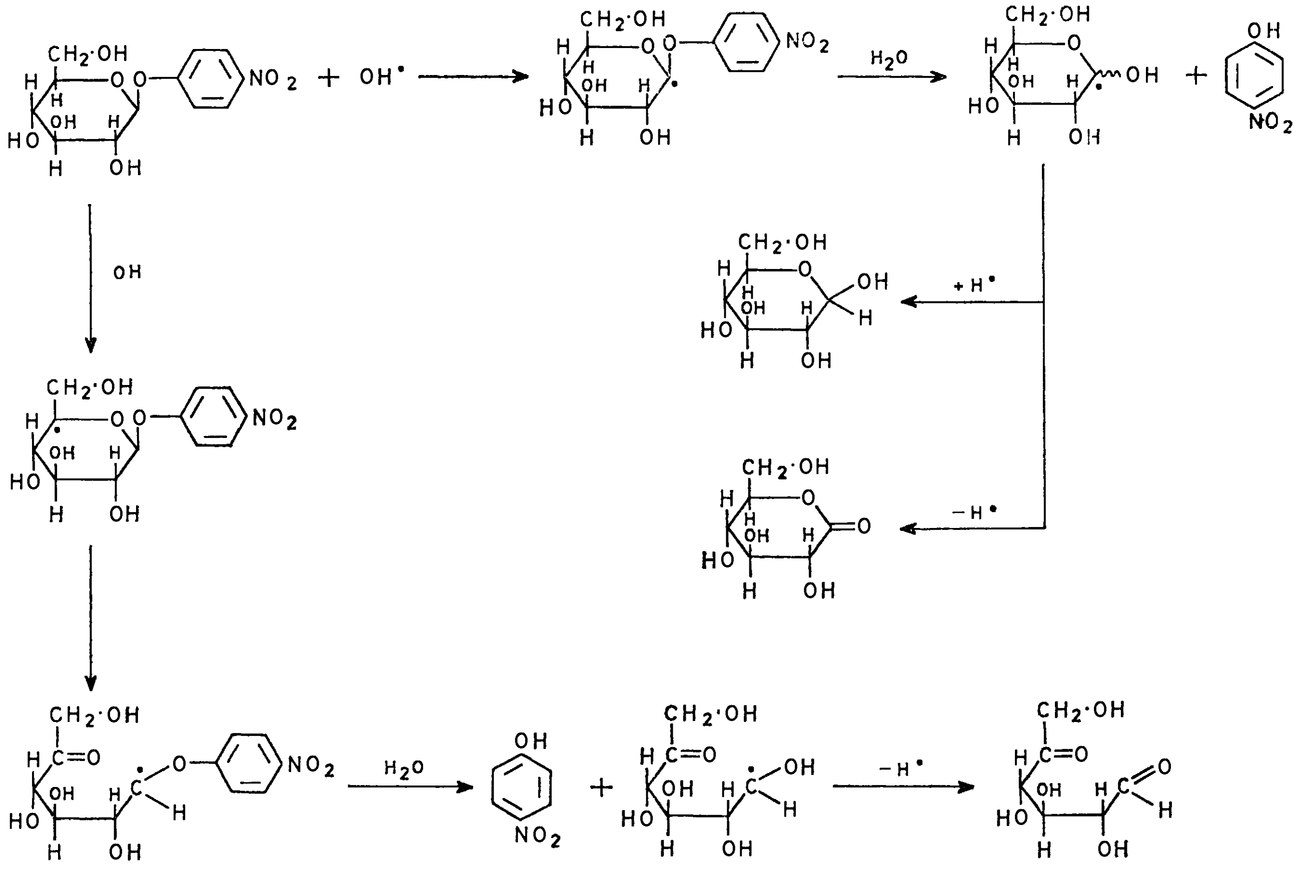
[17] J. Rabani, W. A. Mulac, and M. S. Matheson, *J. Phys. Chem.*, 1965, **69**, 53.

result of direct reaction of OH$\cdot$ with the aromatic ring. The position of $\lambda_{max.}$ is close to that which one would expect on the basis of the bathochromic shift shown by hydroxycyclohexadienyl radicals,[18] in comparison with the parent compound.



Scheme 3

A further consequence of initial reaction of hydroxyl radicals is scission of the glycosidic bond. Based on D-glucose production, G(reducing) = 2.76 for N_2O-saturated solutions, to be compared with 1.5 for deaerated solutions. Under the low dose-rate conditions of ^{60}Co γ-irradiation, the radical (V) may disappear by first-order kinetics, in an analogous manner to that observed for phenyl β-D-glucopyranoside[6] (Scheme 3). Nitrocatechol production is also enhanced in N_2O-saturated solution, and is not found when a solution of *p*NPG containing t-butyl alcohol (0.1 mol l^{-1}) is irradiated. However, though the results of pulse radiolysis suggest that the predominant reaction of OH$\cdot$ is attack at the aglycone, it is possible that glycosidic bond scission may also arise as a result of initial reaction at C-1 and/or C-5 of the sugar, by reactions analogous to those proposed by von Sonntag[19] (Scheme 4). Furthermore, reaction of OH$\cdot$ at C-2, C-3, and C-4 of the carbohydrate would not result in glycosidic bond scission, but produce the αβ-unsaturated keto-sugars found here, with the glycosidic bond intact.



Scheme 4

Scission of the glycosidic bond to produce *p*-nitrophenol (G 0.44 in N_2O-saturated solution) requires *ca.* 20% of the hydroxyl radicals which are reacting at the sugar unit to react at C-1 and/or C-5, in view of the second-order rate constants for reaction of OH$\cdot$ with D-glucose (1.64×10^9 l mol^{-1} s^{-1}), *p*-nitrophenol (3.8×10^9 l mol^{-1} s^{-1}), and *p*NPG (2.4×10^9 l mol^{-1} s^{-1}). This percentage is in reasonable agreement with the value obtained for deoxyribose.[20]

[5/463 *Received, 10th March,* 1975]

---

[18] B. Chutny, *Nature*, 1967, **213**, 593.

[19] C. von Sonntag, personal communication.

[20] V. Hartman, C. von Sonntag, and D. Schulte-Frohlinde, *Z. Naturforsch.*, 1971, **41b**, 2071.