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# RADIATION STUDIES OF ARYL GLYCOSIDES

## PART I. THE EFFECTS OF γ-IRRADIATION ON ARYL GLUCOSIDES IN THE SOLID STATE

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ABSTRACT

γ-Irradiation of crystalline aryl glucosides yields D-glucose and an equivalent amount of the corresponding phenol. The nature of the aglycon influences *G*(glycosidic scission) values, which vary from 1.2 for *p*-cresyl β-D-glucopyranoside to 0.3 for 2-naphthyl β-D-glucopyranoside. The greater radiation stability of aryl glycosides, compared with alkyl glycosides, is attributed to energy transfer, which varies in efficiency according to the nature of the aryl aglycon. Radical yields and the nature of the radical species produced on irradiation are dependent on the aglycon. *G*(glycosidic scission) values are lower at 77°K than at 298°K. The effects of γ-irradiation on glycosides, 1-thioglycosides, and glycosylamines are compared.

## INTRODUCTION

Whereas carbohydrates are extensively degraded on γ-irradiation in the solid state[1–3], aromatic compounds are extremely stable[4]. For the aryl glycosides, preliminary studies demonstrate that the aryl group confers considerable radiation stability to the molecule[5]. Glycosidic scission appears to be the dominant process induced by γ-radiation[5], which thus resembles the effects of ultraviolet radiation[6,7], acids[8–10], alkali[11,12], and certain enzymes[8] on the aryl glycosides. Here we examine the influence of the aryl aglycon on glycosidic scission following γ-irradiation, in an attempt to establish the basis of the pronounced radiation protection which is conferred by the aromatic moiety in these molecules.

## EXPERIMENTAL

Phenyl and *o*- and *p*-nitrophenyl β-D-glucopyranosides were commercial samples and were recrystallised before use; *o*-, *m*-, and *p*-cresyl, 2,4-dimethylphenyl, *p*-methoxyphenyl, and *p*-chlorophenyl β-D-glucopyranosides were prepared from their tetra-acetates[13,14]. Deacetylation was effected by using warm sodium methoxide[15], except for the *p*-chlorophenyl glycoside for which the method of Glaser and Wulwek[16] was employed. This procedure was also used to prepare *m*-nitrophenyl β-D-glucopyranoside. The glucopyranosides of 2-naphthol[17], 9-anthrol[18], and benzyl alcohol[19] were prepared by the literature methods. Phenyl, 4-methylphenyl, and *p*-chlorophenyl 1-thio-β-D-glucopyranosides were prepared by the method of Mont-

gomery, Richtmyer, and Hudson[20]. Ethyl 1-thio-D-glucofuranoside was prepared[21] from D-glucose diethyl dithioacetal[22], and the literature methods were utilised to prepare D-glucosylamine[23], *N*-hexyl-D-glucosylamine[24], and *N*-(*m*-nitrophenyl)-β-D-glucosylamine[25].

The $^{60}Co$ γ-irradiation source used for the irradiations has been previously described[26]. Irradiations were carried out in polythene vessels. The dose rate was varied between $2.1–2.72 \times 10^{17}$ $eV.g^{-1}min^{-1}$ and was measured by using the Fricke dosimeter[27]. Approximately 1 g of solid was generally γ-irradiated. To allow for the difference in electron density between the solid glycosides and the dosimeter solution, a suitable correction was applied. For phenyl β-D-glucopyranoside, this amounted to 4.4%, and was of this order also for the other glycosides.

Electron spin resonance spectra were recorded by using a Varian V4502 spectrometer system operating at 100 kc full modulation. The instrument was operated at the lowest possible, microwave power-level in order to avoid power saturation effects. Radical yield measurements were carried out by using the same settings of radio-frequency power, modulation amplitude, time constant, and sweep speed; only the spectrometer gain was varied. *G*(radical) values were calculated by using γ-irradiated glycine[28] ($G = 4.4$) as a standard.

Reducing sugar measurements were carried out by the method of Somogyi[29] after dissolving the irradiated solid in water (5 ml). Phenol was estimated by a procedure based initially on the method of Folin and Ciocalteu[30], as subsequently modified by Hawk[31] and Phillips[5] and further adapted here as follows. An aqueous solution (10 ml) of the γ-irradiated glycoside was treated with phenol reagent[30] (0.5 ml), which had been mixed with an equal volume of water directly before use, and 20% aqueous sodium carbonate (2 ml). The mixture was shaken and heated for 1 min. After cooling, the absorbance at 454 nm was measured, using a Unicam S.P. 500 spectrophotometer. For calibration, standard phenol solutions were prepared by the method of Ingberman[32]. It was established by using *o*-, *m*-, and *p*-cresol and 2,4-dimethylphenol that the calibrations were linear in the range 0–400 μg of phenol. Molar extinction coefficients at 454 nm were 6,023 (*o*-cresol), 4,302 (*m*-cresol), 3,559 (*p*-cresol), and 5,246 (2,4-dimethylphenol) $mole^{-1}cm^{-1}$. The *m*- and *p*-nitrophenols were estimated by directly calibrating standard solutions of these phenols in buffer at pH 11.7. For these derivatives, the irradiated glycoside was dissolved in the buffer (10 ml), and the solution estimated at 400 nm after 1 h, as for the standard solutions. For *p*-nitrophenol, the calibration was linear over the range 0–7 μg/ml, and for *m*-nitrophenol over the range 0–150 μg/ml. Molar extinction coefficients were 33,275 and 1,500 $mole^{-1}cm^{-1}$, respectively.

Fluorescence and phosphorescence excitation and emission spectra were recorded by using an Aminco-Bowman spectrofluorimeter.

## RESULTS

By using thin-layer chromatography, the presence of phenol and D-glucose was detected after dissolution of the γ-irradiated, solid aryl glycoside in water. No addi-

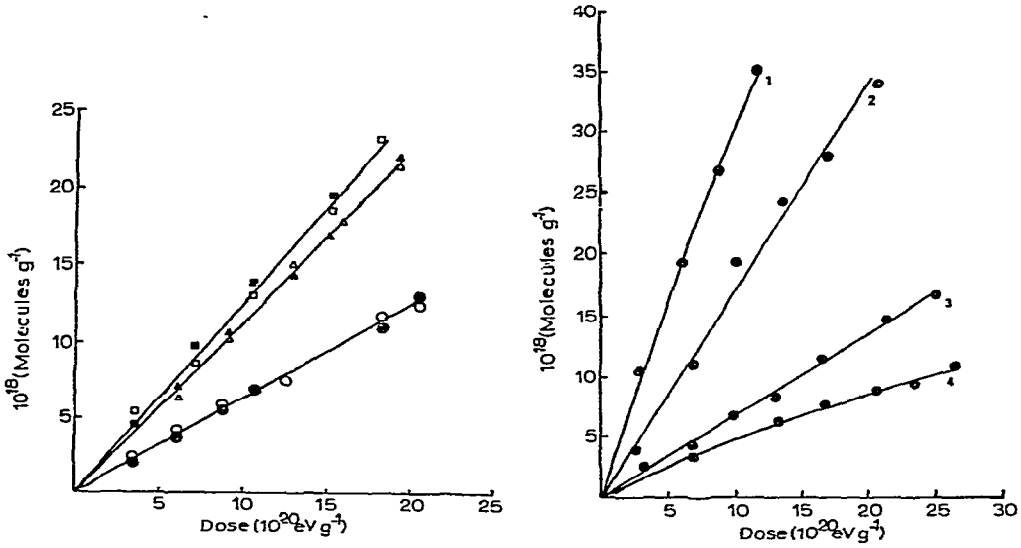



Fig. 1. Formation of D-glucose and the corresponding phenol after $\gamma$-irradiation of aryl glycosides. *p*-Cresyl $\beta$-D-glucopyranoside : ■ *p*-cresol, □ D-glucose; *o*-cresyl $\beta$-D-glucopyranoside : ▲ *o*-cresol, △ D-glucose; phenyl $\beta$-D-glucopyranoside; ● phenol, ○ D-glucose.

Fig. 2. Formation of D-glucose/D-arabinose after $\gamma$-irradiation of aryl glycosides. *1* 9-Anthryl $\beta$-D-glucopyranoside; *2* benzyl $\beta$-D-arabinopyranoside; *3* *m*-nitrophenyl $\beta$-D-glucopyranoside; *4* *p*-methoxyphenyl $\beta$-D-glucopyranoside.

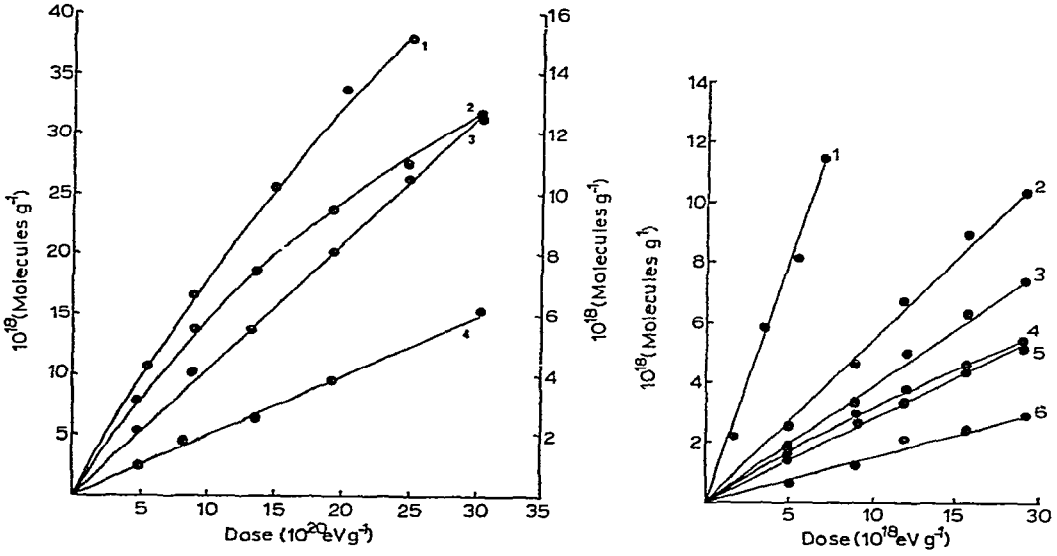



Fig. 3. Formation of D-glucose after $\gamma$-irradiation of 1-thioglycosides. *1* Ethyl 1-thio-D-glucofuranoside and *2* *p*-chlorophenyl 1-thio-$\beta$-D-glucopyranoside (left-hand ordinate); *3* phenyl 1-thio-$\beta$-D-glucopyranoside and *4* 4-methylphenyl 1-thio-$\beta$-D-glucopyranoside (right-hand ordinate).

Fig. 4. Formation of D-glucose after $\gamma$-irradiation of D-glucosides at 77 °K. *1* Methyl $\alpha$-D-glucopyranoside; *2* *o*-cresyl $\beta$-D glucopyranoside; *3* phenyl $\beta$-D-glucopyranoside; *4* phenyl 1-thio-$\beta$-D-glucopyranoside; *5* *p*-nitrophenyl $\beta$-D-glucopyranoside; *6* 4-methylphenyl 1-thio-$\beta$-D-glucopyranoside.

tional products were observed up to the maximal energy inputs employed for the quantitative measurements given below.

*Yield–dose curves for glycosidic scission.* — Equivalent amounts of D-glucose and the corresponding phenol are found in an aqueous solution of the $\gamma$-irradiated, solid glycoside. This general behaviour is illustrated for three glycosides in Fig. 1. Other typical yield–dose curves for the radiation-induced, hydrolytic scission of glycosides and 1-thioglycosides are shown in Figs. 2 and 3. From such yield–dose curves, initial *G*-values for glycosidic scission may be obtained. The glycosidic bond in *m*- and *p*-nitrophenyl $\beta$-D-glucopyranosides is particularly labile to alkali, and the Somogyi method for estimating formation of D-glucose cannot, therefore, be employed. For these two glycosides, *G*(glycosidic scission) was estimated from the production of phenol alone. The results are summarised in Table I.

*Effect of temperature.* — $\gamma$-Irradiation of selected glycosides and 1-thioglycosides was carried out at 77°K by using a dose rate of $0.69 \times 10^{17}$ eV.g$^{-1}$min$^{-1}$. From the yield–dose curves (Fig. 4) for D-glucose production, *G*(glycosidic scission) values were obtained (Table I).

*Radical yields.* — The e.s.r. spectra of $\gamma$-irradiated glycosides are generally not well-resolved. Nevertheless, definite differences in the spectra could be observed for individual glycosides after $\gamma$-irradiation. Two typical spectra are shown in Fig. 5. By double integration of the spectra, with reference to $\gamma$-irradiated glycine as a standard[28], yield–dose curves for radical production were obtained. Typical curves are given in Fig. 6, from which *G*(radical) values were calculated.

*Deactivation processes.* — Fluorescence and phosphorescence emission spectra were recorded in ethanol solution (1 mg/ml) at 298°K and 77°K. The emission spectra were unaffected by varying the excitation wavelength. The results are summarised in Table I.

## DISCUSSION

From the linear yield–dose curves, the equivalence of D-glucose and phenol formation, and the absence of other products, it is probable that glycosidic scission is the primary, chemical consequence of $\gamma$-irradiation of aryl glycosides in the solid state. Initial *G*-values for this process are, moreover, markedly dependent on the nature of the aglycon. For methyl $\alpha$-D-glucopyranosides[5], initial *G*(glycosidic scission) is 2.3, which is close to the values generally found for the radiation-induced scission of $\alpha$-(1→4) bonds in disaccharides and higher saccharides[33]. The presence of the aryl aglycon thus confers a marked radiation stability on the glycosidic bond. Previously, to account for intermolecular and intramolecular energy-transfer effects in carbohydrate systems[34], it was suggested that exciton states of ~4 eV are produced in high yield in crystalline carbohydrates on $\gamma$-irradiation. Such exciton states can be efficiently transferred to suitable acceptor molecules. When aromatic molecules having a first singlet excitation-level ($E_1$) near 4 eV were in association with the carbohydrate, either covalently bound or in the form of inclusion complexes[26,34],

TABLE I

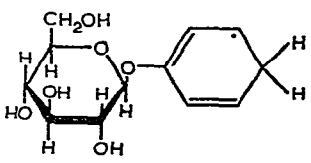
RADIATION-INDUCED GLYCOSIDIC SCISSION IN ARYL GLYCOSIDES AND GLYCOSYLAMINES

| | G(*scission*) *at 298°K* | G(*scission*) *at 77°K* | G(*radical*) | $E_1^{a*}$ (*eV*) | $E_1^f$ (*eV*) | $E_1^t$ (*eV*) |
|---|---|---|---|---|---|---|
| *β*-D-*Glucopyranoside* | | | | | | |
| Phenyl | 0.6 | 0.4 | 0.1 | 4.55 | 4.19 | 3.15 |
| *o*-Cresyl | 0.8 | 0.5 | 0.4 | 4.53 | 4.15 | 3.14 |
| *m*-Cresyl | 0.8 | — | 0.5 | 4.54 | 4.16 | 3.09 |
| *p*-Cresyl | 1.2 | — | 0.8 | 4.43 | 4.08 | 3.24 |
| 2,4-Dimethylphenyl | 1.1 | — | 0.5 | 4.42 | 4.06 | 3.17 |
| *p*-Nitrophenyl | 0.4 | 0.3 | 0.4 | 4.09 | — | 2.98 |
| *m*-Nitrophenyl | 0.7 | — | 0.6 | 4.57 | — | — |
| *o*-Nitrophenyl | — | — | 0.4 | 3.93 | — | — |
| *β*-Naphthyl | 0.3 | — | 0.2 | 3.82 | — | — |
| *p*-Methoxyphenyl | 0.4 | — | — | 4.39 | 3.89 | 3 09 |
| *p*-Chlorophenyl | 0.8 | — | 0.7 | 4 67 | 4 03 | 3.09 |
| 9-Anthryl | 2.9 | — | 2.4 | 3.2 | — | — |
| Methyl *α*-D-glucopyranoside | 2.3[5] | 1.6 | 3.0 | 7 | — | — |
| Benzyl *β*-D-arabinopyranoside | 1.7 | — | 0.6 | 4.81 | 4.32 | 3.26 |
| *1-Thio-β-D-glucoside* | | | | | | |
| Phenyl | 0.7 | 0.3 | 0.1 | 5.05 | — | 2.81 |
| *p*-Cresyl | 0 4 | 0.2 | 0.2 | 4.99 | — | 2.71 |
| *p*-Chlorophenyl | 0.5 | — | 0.2 | 4.79 | — | 2 81 |
| Ethyl 1-thio-D-glucofuranoside | 1.9 | — | — | — | — | — |
| D-*Glucosylamine* | | | | | | |
| *N*-(*m*-nitrophenyl)-*β*- | — | — | 0.5 | — | — | — |
| *N*-(Hexyl)-*β*- | — | — | 4.2 | — | — | — |
| *β*- | — | — | 1.8 | — | — | — |

*$E_1^a$ from absorption spectra; $E_1^f$ from fluorescence spectra; $E_1^t$ from phosphorescence spectra.

chemical protection of glycosidic bonds was observed. A second observation was that, when protection is evident, the radicals produced by *γ*-irradiation are associated with the aromatic molecule or group which confers the radiation stability, rather than with the carbohydrate component. The same features also characterise the behaviour of *γ*-irradiated aryl glycosides.

We consider first the e.s.r. evidence. On *γ*-irradiation of D-glucose, a composite spectrum is obtained consisting of a 1:1 doublet with hyperfine splitting of 20.5 gauss and a 1:2:1 triplet with hyperfine splitting of 29.5 gauss[35]. The radicals are considered to be of the type $\dot{C}-CH_2OH$ with free rotation of the two equivalent protons about the C-5–C-6 bond. The superimposed doublet is due to a radical formed by abstraction of a proton from C-1. *γ*-Irradiation of phenyl *β*-D-glucopyranoside yields a radical



species which is clearly not associated with the D-glucose moiety. The spectrum (Fig. 5A) consists of a basic triplet with each line split symmetrically into a further triplet. The spectrum is consistent with a radical **1** with coupling constants of 48 gauss for the protons of the methylene group, and 11 gauss for the protons ortho to the glycosidic oxygen atom. These constants are in broad agreement with the values[36,37] for similar cyclohexadienyl type radicals formed by $\gamma$-irradiation of substituted benzenes

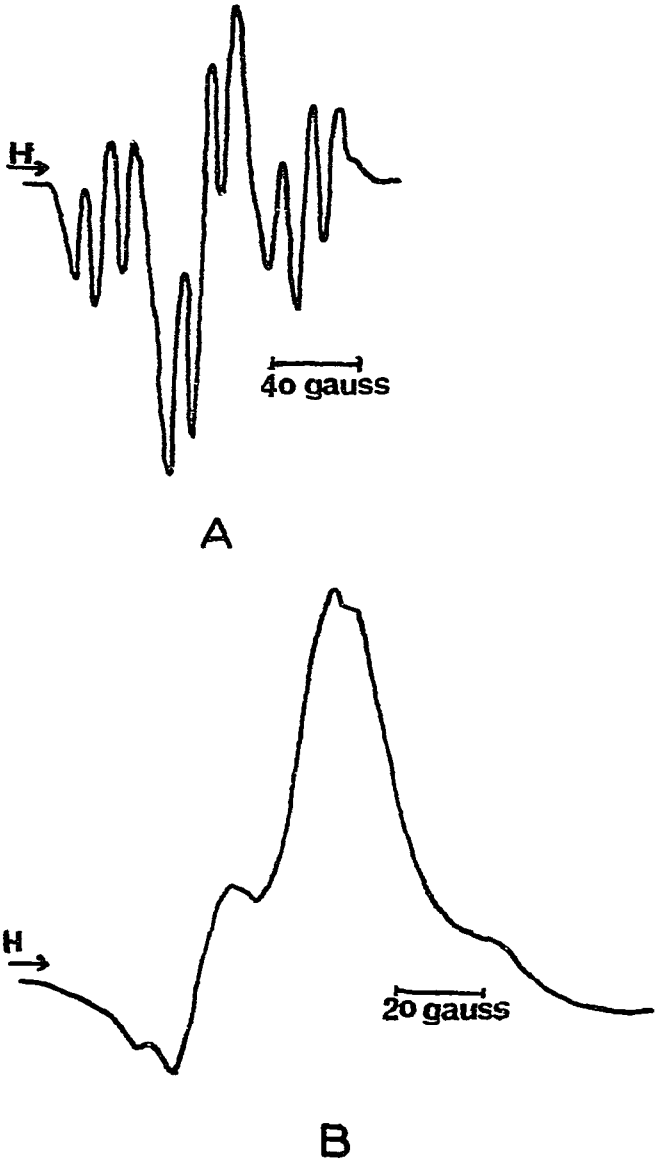



Fig. 5. E.s.r. spectra of $\gamma$-irradiated glycosides. A, phenyl $\beta$-D-glucopyranoside; B, *N*-hexyl-D-glucosylamine.

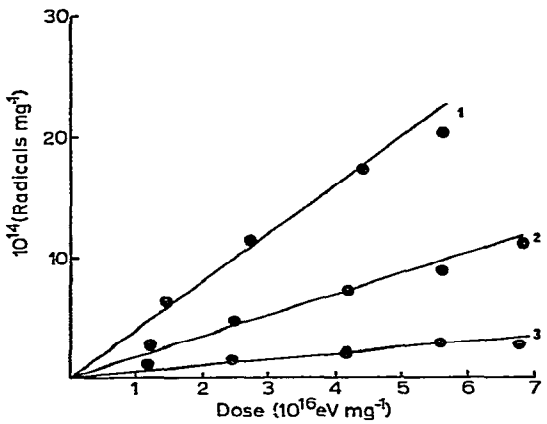



Fig. 6. Radical formation from $\gamma$-irradiated glycosylamines. *1* *N*-hexyl-D-glucosylamine, *2* D-glucosylamine, and *3* *N*-(m-nitrophenyl)-D-glucosylamine.

in the solid state. As required by **1**, where coupling occurs with equivalent protons, we find that the relative intensities of the triplets are 1:2:1. Despite the fact that the activation energy for the addition of H atoms to aromatic molecules is close to zero and the process readily occurs[34,38–41], there are indications that such addition of hydrogen atoms occurs preferentially at ortho and/or para positions[37]. It is, therefore, significant that we find the H-atom addition radical only when the aglycon is unsubstituted. In the e.s.r. spectrum of $\gamma$-irradiated *p*-cresyl $\beta$-D-glucopyranoside, for example, there is no indication of the broad, 50-gauss splitting characteristic of the methylene group. The smaller splittings of 4 and 12 gauss, which we observed, are more characteristic of substituted phenoxy radicals[42]. The previous suggestion by Ueda[43] that $\dot{O}CH_3$ is formed during $\gamma$-irradiation of methyl $\alpha$-D-glucopyranoside additionally supports the view that removal of the p-electron of the glycosidic oxygen atom is an early consequence of radiation action. Lack of resolution and symmetry does not allow the spectra to be positively identified in all instances. Nevertheless, it can be concluded that, for the aryl glycosides with low $G$(glycosidic scission), the radical is associated with the aromatic and not the D-glucose moiety. Where energy transfer was observed for cycloamylose complexes[34], a similar behaviour was found, and $G$(radical) values were considerably lower than for $\gamma$-irradiated cycloamylose ($G = 5$), D-glucose ($G = 4$), and aliphatic glycosides ($G = 3$–5). For $\gamma$-irradiated aryl glycosides, $G$(radical) values are also low and in the range 0.1–0.8. Where no such protection occurs, as in *N*-hexyl-D-glucosylamine with $G$(radical) $\sim 4$, the e.s.r. spectrum (Fig. 5B) shows the characteristics of $\gamma$-irradiated D-glucose already noted. Similar features are also found with the anthrol derivative.

As for cycloamylose complexes, chemical protection of aryl glycosides is dependent on the nature of the aromatic moiety present. Because of the broad transitions observed for these molecules, $E_1$ cannot be accurately calculated from absorption spectral measurements, and for this reason we have attempted, where possible, to estimate $E_1$ also from the fluorescent spectra (Table I). We cannot,

however, find a meaningful correlation between $G$(glycosidic scission) and $E_1$ or the triplet level $E_T$. However, it is clear that the values of $E_1$, even when taken as mean values from absorption and fluorescence measurements, can only be taken as a rough approximation. Nevertheless, it is clear that when $E_1$ is $\sim$4 eV, efficient protection occurs, which is within the limits established for the intermolecular energy transfer found for the cycloamylose complexes. When $E_1$ clearly diverges from 4, as for 9-anthryl $\beta$-D-glucopyranoside, there is no significant protection. Therefore, energy transfer from the carbohydrate to an aromatic aglycon with $E_1$ near 4 eV would appear to be the most satisfactory explanation for the radiation protection. The decrease in $G$(glycosidic scission) found at 77 °K is consistent with this conclusion, arising from a decrease in exciton mobility or in the efficiency of transfer, according to the specific transfer mechanism which is favoured. It is also significant that there is no parallel between $G$(glycosidic scission) and the lability of glycosidic bonds to hydrolysis by acids or alkali. Here, aryl glycosides are generally more easily hydrolysed than aliphatic glycosides[44], which eliminates the possibility that the variations in $G$(scission) we have observed are simply due to a bond-strength effect.

For the 1-thioglycosides studied, $G$(glycosidic scission) values are generally somewhat lower than for the corresponding glycosides. Thus, energy transfer also occurs readily in these 1-thioglycosides and may marginally be more efficient than in the glycosides. The lower $G$(radical) value for $\gamma$-irradiated $N$-($m$-nitrophenyl)-$\beta$-D-glucosylamine compared with the values for the non-aromatic glycosylamines, for example, $N$-hexosyl-D-glucosylamine, demonstrates that aromatic groups confer radiation protection in these compounds also. It would appear, on this basis also, that the -NH bridge in these glycosylamines must allow energy transfer from the sugar moiety.

REFERENCES

1 G. O. PHILLIPS AND P. J. BAUGH, *J. Chem. Soc.* (*A*), (1966) 370, 377, 383, 387.
2 A. EHRENBERG, L. EHRENBERG, AND G. LOFROTH, *Acta Chem. Scand.*, 17 (1963) 53.
3 G. LOFROTH, *Acta Chem. Scand.*, 21 (1967) 1997.
4 J. KROH AND S. KAROLCZAK, *Radiation Res. Rev.*, 1 (1969) 411.
5 G. O. PHILLIPS, F. A. BLOUIN, AND J. C. ARTHUR, *Radiation Res.*, 23 (1964) 527.
6 A. GUILLAUME AND G. TAURET, *Compt. Rend.*, 201 (1935) 1057.
7 L. J. HEIDT, *J. Amer. Chem. Soc.*, 61 (1939) 2981.
8 R. L. NATH AND H. N. RYDON, *Biochem. J.*, 57 (1954) 1.
9 A. N. HALL, S. HOLLINGSHEAD, AND H. N. RYDON, *J. Chem. Soc.*, (1951) 4290.
10 J. N. BEMILLER, *Advan. Carbohyd. Chem.*, 22 (1967) 25.
11 A. DYFVERMAN AND B. LINDBERG, *Acta Chem. Scand.*, 4 (1950) 879.
12 C. TAURET, *Bull. Soc. Chim. France*, 11 (1894) 999.
13 P. G. SCHEURER AND F. SMITH, *J. Amer. Chem. Soc.*, 76 (1954) 3224.
14 B. HELFERICH AND E. SCHMITZ-HELLEBRECHT, *Ber.*, 66 (1933) 378.
15 G. ZEMPLÉN AND E. PACSU, *Ber.*, 62 (1929) 1613.
16 E. GLASER AND W. WULWEK, *Biochem. Z.*, 145 (1924) 516.
17 A. ROBERTSON AND R. B. WATERS, *J. Chem. Soc.*, (1930) 273.
18 J. H. GARDNER AND T. F. MCDONNELL, *J. Amer. Chem. Soc.*, 59 (1937) 857.
19 C. E. BALLOU, *J. Amer. Chem. Soc.*, 79 (1957) 165.
20 E. M. MONTGOMERY, N. K. RICHTMYER, AND C. S. HUDSON, *J. Org. Chem.*, 11 (1946) 301.
21 J. W. GREEN AND E. PACSU, *J. Amer. Chem. Soc.*, 59 (1937) 1205.

22 *Polarimetry, Saccharimetry and The Sugars*, Natl. Bur. Std. (U. S.), Circ. C440, 1942, p. 521.
23 I. E. MUSCAT, *J. Amer. Chem. Soc.*, 56 (1934) 693.
24 W. PIGMAN, E. A. CLEVELAND, D. H. CROUCH, AND J. H. CLEVELAND, *J. Amer. Chem. Soc.*, 73 (1951) 1976.
25 F. WEYGAND, W. PERTIOW, AND P. KUHNER, *Ber.*, 84 (1951) 594.
26 G. O. PHILLIPS AND M. D. YOUNG, *J. Chem. Soc. (A)*, (1968) 1240.
27 H. FRICKE AND E. J. HART, *J. Chem. Phys.*, 3 (1935) 60.
28 T. HENDRIKSEN, *Radiation Res.*, 17 (1962) 158.
29 M. SOMOGYI, *J. Biol. Chem.*, 195 (1952) 19.
30 O. FOLIN AND V. CIOCALTEU, *J. Biol. Chem.*, 73 (1929) 677.
31 P. B. HAWK, B. L. OSLER, AND W. H. SUMMERSON, *Practical Physiological Chemistry*, The Blackiston Co., Philadelphia, 1947, p. 878.
32 A. K. INGBERMAN, *Anal. Chem.*, 30 (1958) 1003.
33 G. O. PHILLIPS AND K. W. DAVIES, *J. Chem. Soc.*, (1964) 205.
34 G. O. PHILLIPS AND M. D. YOUNG, *J. Chem. Soc. (A)*, (1966) 393.
35 P. J. BAUGH, K. KERSHAW, AND G. O. PHILLIPS, *Nature*, 221 (1969) 1138.
36 R. W. FESSENDEN AND R. H. SCHULER, *J. Chem. Phys.*, 39 (1963) 2147.
37 D. CAMPBELL AND D. T. TURNER, *Can. J. Chem.*, 45 (1967) 881.
38 I. I. CHKHEIDZE, V. I. TROFIMOV, AND N. YA. BUBEN, *Zh. Strukt. Khim.*, 5 (1964) 624.
39 S. OHNISHI, T. TANEI, AND I. NITTA, *J. Chem. Phys.*, 37 (1962) 2402.
40 L. A. WALL AND R. B. INGALLIS, *J. Chem. Phys.*, 41 (1964) 1112.
41 K. ARAKI, D. CAMPBELL, AND D. T. TURNER, *Polymer Lett.*, 3 (1965) 993.
42 T. J. STONE AND W. A. WATERS, *J. Chem. Soc.*, (1964) 213.
43 H. UEDA, *J. Phys. Chem.*, 67 (1963) 966.
44 C. A. BUNTON, T. A. LEWIS, D. R. LLEWELLYN, AND C. A. VERNON, *J. Chem. Soc.*, (1955) 4419.