Photodegradation of Carbohydrates. Part IV.† Direct Photolysis of D-Glucose in Aqueous Solution

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Radioactive tracer methods have been used to study the degradation of D-glucose on u.v. irradiation in oxygenated aqueous solution. After *ca.* 20% decomposition of the original D-glucose, the main products are gluconic acid, a tetrose, and a reducing product which has $\lambda_{max.}$ 2650 Å. There is considerable secondary decomposition of initial products even in the early stages of the reaction. After 9—10% decomposition of D-glucose, the major decomposition can be accounted for by the arabinose and gluconic acid formed.

D-[1-^{14}C]Glucose and D-[1-^{14}C$_1$]glucono-γ-lactone were used to elucidate the initial site of chemical change. Of the products from D-[1-^{14}C]glucose, only gluconic acid contains ^{14}C; the remaining products, including arabinose, are inactive. The D-glucose which disappears is equivalent to the sum of the gluconolactone and the volatile products produced. When D-[1-^{14}C]glucono-γ-lactone is irradiated, carbon dioxide is released exclusively from C-1. The arabinose and other aldehydic fragments containing fewer carbon atoms are inactive.

The site of radiation action is, therefore, C-1; this supports the view that the ' acetal chromophore ' at C-1 is the primary absorbing site where photochemical reaction subsequently occurs. Filter experiments demonstrate that the spectral region near 2300 Å is the most active in D-glucose irradiations. It appears, therefore, that chemical change results from excitation and dissociation at the lactol ring oxygen.

PHOTODEGRADATION of carbohydrates [1] can be induced in the presence or the absence of a photosensitizer.[2-4] Direct photolysis of D-sorbitol occurs on irradiation in aqueous solution with light from a Hanovia medium pressure lamp. The degradation is a stepwise process starting with oxidation at the terminal primary alcohol groups to give hexoses, which are subsequently degraded to hexonic acid, pentoses, and ultimately to carbon dioxide.[2] Filter experiments demonstrate that 2100—2500 Å radiation is the active portion of the light which initiates degradation, and after a mechanistic study,[3]

† Part III, G. O. Phillips, P. Barber, and T. Rickards, *J. Chem. Soc.*, 1964, 3443.

[1] G. O. Phillips, *Adv. Carbohydrate Chem.*, 1963, **18**, 9.
[2] G. O. Phillips and P. Barber, *J. Chem. Soc.*, 1963, 3984.
[3] G. O. Phillips and P. Barber, *J. Chem. Soc.*, 1963, 3990.
[4] G. O. Phillips, P. Barber, and T. Rickards, *J. Chem. Soc.*, 1964, 3443.

it was concluded that the primary process was excitation of the non-bonding orbitals on the oxygen atom ($n \rightarrow \sigma^*$ transition), which induces dissociation at the primary alcohol group.

Particular practical and theoretical interest centres on the mechanism of direct photolysis of carbohydrate structures containing the lactol ring oxygen atom. Cotton cellulose, for example, undergoes direct photolysis with 2537 Å radiation.[5] While this radiation is sufficiently energetic to rupture C–C, C–O, and C–H bonds in cellulose, there is little or no experimental indication that light in this region is actually absorbed by the cellulose molecule. To overcome this apparent contradiction, it has been postulated that weak chromophoric group, the acetal group at C-1 of the D-glucose residues, is responsible for the absorption near 2650 Å.[6] A subsequent theory of photodegradation of carbohydrates involving alternate hydrolysis and oxidation has been proposed,[7] but it is our view, and that of other workers,[8] that there is at present inadequate experimental evidence in support of this new theory, which is quite at variance with current concepts.

The extremely low absorption of D-glucose at 2537 Å makes it difficult to decide unequivocally by spectroscopic means whether the ' acetal chromophore ' or ($n \rightarrow \sigma^*$) absorption characteristic of hydroxy-groups predominates. A chemical study of the initial products of direct photolysis of D-glucose was therefore undertaken in an attempt to distinguish between these possibilities and thus to establish the primary processes responsible for the photodegradation.

RESULTS

The experimental arrangements and irradiation procedures have been described.[4] When a layer of x cm. of solution is interposed between the lamp and the irradiation vessel, the expression (d + x) cm. will be used, where d is the constant distance of the lamp from the quartz window (*ca.* 2 cm. of air). Unless otherwise stated, irradiations were conducted through 2 cm. of distilled water filter (d + 2) cm.

Paper Chromatography of Irradiated Solution.—An aqueous solution (100 ml.) of D-glucose ($5{\cdot}5 \times 10^{-2}$M) was irradiated in oxygen for 6 hr. It was then concentrated by freeze-drying and chromatographed on paper either in butan-1-ol–acetic acid–water (11 : 5 : 4) [irrigant (i)] or in butan-1-ol–pyridine–water (6 : 4 : 3) [irrigant (ii)]. The chromatographs were developed with alkaline silver nitrate and aniline hydrogen phthalate spray reagents, and three major products (and unchanged D-glucose) were identified, despite considerable streaking [9] (Table 1). Constituent (I) corresponded to a D-glucose control, constituent (II) to gluconic acid (or its lactone), and (III) to arabinose; (IV) showed the characteristics of a tetrose. The acidic nature of (II) was further confirmed by use of a bromophenol blue spray.

Paper chromatographic and radioactive tracer techniques were combined for a more precise examination of the irradiated solution. In a typical experiment, an aqueous solution (100 ml.) of D-glucose ($5{\cdot}5 \times 10^{-2}$M), containing sufficient D-[^{14}C]glucose to give a specific activity of *ca.* 9 μc/mmole, was irradiated for 6½ hr. at (d + 2) cm. water Aliquot portions (0·05 ml.) were removed at various times,

TABLE 1

Constituents in irradiated D-glucose solutions after 6 hr. irradiation

Constituent	(I)	(II)	(III)	(IV)
R_{Glu},* irrigant (i)	1·0	1·1	1·5	2·0
R_{Glu},* irrigant (ii)	1·0	0·3	1·2	1·9
% Activity [a]	80	7	5	6 †
% Activity [b]	77	4	2	

* R_{Glu} indicates movement relative to a control of D-glucose. † Includes the activity associated with ' streaking ' in chromatograms.

[a] By paper chromatography. [b] By isotope dilution analysis.

applied to filter paper (Whatman No. 54) strips (40—45 × 2·5 cm.), and run in irrigants (i) and (ii), and also phenol–water (4 : 1 w/v).[9] The relative amounts of activity lost by D-glucose and transferred to gluconic acid [constituent (II)] are shown in Table 2.

TABLE 2

Formation of gluconic acid by irradiation of D-glucose *

Irradiation time ..... (min.)	0	48	90	116	244	294
Decrease in D-glucose (%)	0	0·6	2·2	6·5	12	24
Appearance of gluconic acid ..... (%)	0	0·5	1·0	2·7	3·0	6·0

* Values determined in each irrigant, and in agreement with the mean values obtained.

After irradiation the solution was passed through Dowex IX-4 anion-exchange resin (acetate form). The neutral components were eluted with water (100 ml.) and the acidic components were subsequently eluted with N-formic acid (150 ml.) until the α-naphthol test showed the absence of carbohydrate material. The typical histogram from a paper chromatographic separation of the acidic fraction in ethyl acetate–acetic acid–formic acid–water (13 : 3 : 1 : 4) indicated that glucono-γ-lactone was the largest single acid component (80%).

Carrier dilution analysis [9] was also used to estimate the products indicated by paper chromatography. A solution (100 ml.) of D-glucose ($5{\cdot}5 \times 10^{-2}$M) containing sufficient D-[^{14}C]glucose to give a specific activity of *ca.* 9 μc/mmole, was irradiated for 6·5 hr., and aliquot portions were removed for carrier dilution analysis as follows.

D-Glucose.—To the irradiated solution (2 ml.) inactive D-glucose (0·90 mmole) was added, and after equilibration for 24 hr. the mixture was freeze-dried, and finally thoroughly dried *in vacuo* (P_2O_5). It was then treated with anhydrous sodium acetate (0·2 g.) and freshly dried acetic anhydride (1·0 ml.), heated at 100° (*ca.* 2—3 hr.), and poured into ice-cold water. The penta-*O*-acetyl-β-D-glucose formed was recrystallised eight times from ethanol–water, to produce chemically and radiochemically pure material with con-

[5] G. O. Phillips and J. C. Arthur, jun., *Textile Res. J.*, 1964, **34**, 397, 572.
[6] A. Beelik and J. K. Hamilton, *Das Papier*, 1959, **13**, 77.
[7] B. C. Bera and P. N. Agarwal, LAB-DEV (Kapur, India), 1964, **2**, 105.
[8] A. Beelik and J. K. Hamilton, *Chem. and Ind.*, 1965, 1341.
[9] G. O. Phillips and K. W. Davies, *J. Chem. Soc.*, 1964, 205.

stant specific activity 0·70 μc/mmole, m.p. 131°, equivalent to D-glucose (4·35 mmoles), indicating 30% decomposition.

D-Arabinose.—To the irradiated solution (5 ml.), carrier D-arabinose (1·0 mmole), acetic acid (1·5 ml.), and phenyl hydrazine (2 ml.) were added, and the mixture was boiled for 15 min. The product required nine recrystallisations from ethanol–water to give pure arabinosazone, m.p. 159° of constant specific activity 0·048 μc/mmole, which indicates a total yield of 0·11 mmole of D-arabinose.

D-Gluconic Acid.—The irradiated solution (5 ml.) was treated with carrier D-gluconolactone (1·0 mmole), and excess of calcium carbonate. The solution was filtered after 48 hr., and the gluconate was precipitated with ethanol. Subsequently, *ca.* eleven reprecipitations were required to give calcium gluconate of constant specific activity 0·102

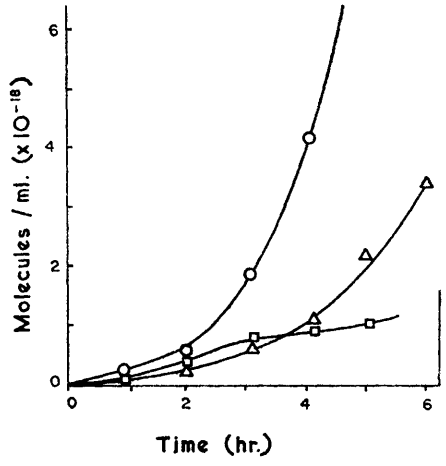

FIGURE 1 Product formation during irradiation of D-glucose solution (0·055M); (—○—) acid, (—△—) carbon dioxide, (—□—) hydrogen peroxide

μc/mmole, which indicates a total yield of 0·22 mmole of D-gluconic acid.

Glyoxal.—The irradiated solution (5 ml.) was treated with 2,4-dinitrophenyl hydrazine (1·5 ml.) and glacial acetic acid (1·0 ml.), after addition of carrier glyoxal (2·0 mmoles). Glyoxal bisphenylhydrazone was obtained after seven recrystallisations from benzene, m.p. 170°, and constant specific activity 0·018 μc/mmole, which indicates a total yield of 0·26 mmole of two-carbon aldehydic fragments.

Glucuronic Acid.—Carrier D-glucuronolactone (1·0 mmole) was added to the irradiated solution (5 ml.), and after equilibration, the solution was freeze-dried. D-Glucuronolactone, after six recrystallisations from glacial acetic acid, had m.p. 175°. Insufficient product remained to check the specific activity, 0·005 μc/mmole, by subsequent recrystallisations. This figure indicates a total yield of 0·03 mmoles of glucuronolactone.

Dihydroxyacetone.—Carrier dihydroxyacetone (2·0 mmoles), acetic acid (1·0 ml.), and phenylhydrazine (1·5 ml.) were added to the irradiated solution (5 ml.) and the mixture was boiled for 5 min. Seven recrystallisations from benzene gave pure glycerol osazone, m.p. 129°, constant specific activity 0·004 μc/mmole, which indicates a total yield of 0·02 mmole of three-carbon aldehydic fragments.

Carbon Dioxide Evolution.—The oxygen stream passing through the irradiated D-glucose solution was passed through a series of traps containing saturated barium hydroxide kept at 100°. The barium carbonate produced was estimated gravimetrically for solutions of D-glucose irradiated for up to 6 hr. (Figure 1).

Hydrogen Peroxide and Acid Formation.—The formation of hydrogen peroxide, estimated by the spectrophotometric

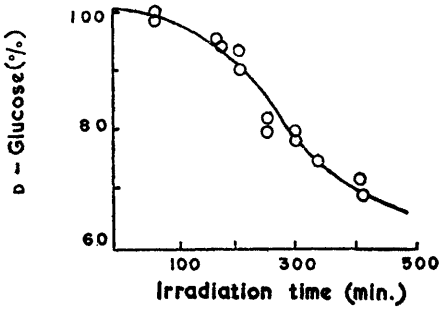

FIGURE 2 Decomposition of D-glucose on irradiation

method [2] of Eisenburg, is shown in Figure 1. Acid yields, estimated by potentiometric titration with 0·005M-sodium hydroxide, are also shown in Figure 1 (based on the assumption that the acid produced is monobasic).

Rate of Product Formation.—By isotope dilution analysis and radiochromatography as already described, initial rates of appearance of gluconolactone and arabinose were measured during the irradiation (7·5 hr.) of an aqueous solution (50 ml.) of D-glucose ($5{\cdot}5 \times 10^{-2}$M) containing sufficient D-[^{14}C]glucose to give a specific activity of *ca.* 18 μc/mmole. The rate of decomposition of D-glucose as estimated by radiochromatography is shown in Figure 2;

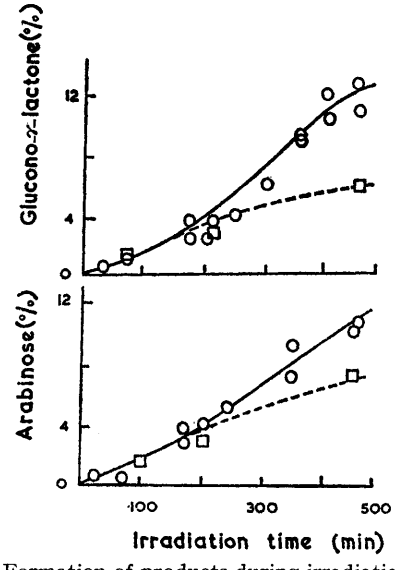

FIGURE 3 Formation of products during irradiation of D-[^{14}C]-glucose; (—○—) radiochromatographically, (—□—) isotope dilution analysis

the product of glucono-γ-lactone and the formation of arabinose are shown in Figure 3. Data obtained by use of isotope dilution analysis are shown in Figure 3.

Irradiation of D-[1-^{14}C]Glucose Solutions.—A solution (50 ml.) of D-glucose ($5{\cdot}5 \times 10^{-2}$M) containing sufficient D-[1-^{14}C]glucose to give a final specific activity of *ca.* 7 μc/mmole was irradiated for 6 hr. Aliquot portions (400

ml.) were applied to filter paper strips, and chromatographed in irrigants (i) and (ii). A comparison of the histograms for irradiated D-[1-^{14}C]-glucose and D-[^{14}C]-glucose (generally labelled) solutions is shown in Figure 4. The major constituent, apart from unchanged D-glucose, corresponded to (I) (Table 1), which was chromatographically identical with a D-gluconic acid control. No further significant amounts of active products were found.

The loss in activity during irradiation due to the formation of volatile products was measured (i) by combustion of aliquot portions (0·1 ml.) of the solution with inactive carrier, and direct measurement of the activities as 'infinitely thick' barium carbonate discs, and (ii) by applying

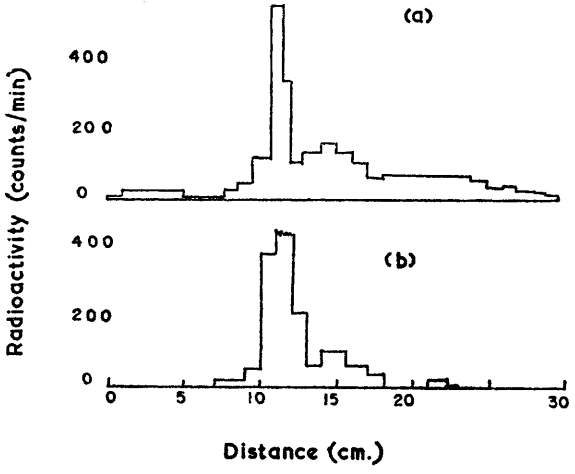

FIGURE 4 Distribution of radioactivity along paper chromatograms for (a) D-[^{14}C]glucose and (b) D-[1-^{14}C$_1$]glucose

aliquot portions (400 μl.) to paper chromatograms, and observing the loss in activity by direct end-window counting of the paper strip. The results are shown in Table 3.

TABLE 3

Volatile products released during irradiation of D-[^{14}C]-glucose

Irradiation time ..... (min.)		65	175	337
Volatile products ..... (%)	(i)	5	17	27
	(ii)	6	12	33

(i) Direct radioactivity measurements. (ii) Radiochromatographically.

The decomposition of D-[1-^{14}C]glucose and the production of gluconolactone were also measured by carrier dilution analysis and paper chromatography. The individual results are shown in Figure 5.

Irradiation of Glucono-γ-lactone Solutions.—Irradiation of glucono-γ-lactone solutions (2·8 × 10^{-2}M) for 50 min. leads to a change in its absorption spectrum as shown in Figure 6. Addition of alkali to the irradiated solution produced a distinct absorption maximum at 2650 Å. After concentration of the irradiated solution by freeze-drying, it was analysed by paper chromatography in the acidic (i) and basic (ii) irrigants used before. Two major products and unchanged glucono-γ-lactone (as the γ-lactone or gluconic acid) were identified (Table 4). Constituent (I) was identical with a control of gluconolactone; (II) corresponded to arabinose, and (III) to a tetrose. In certain experiments it was found that a reducing material ran with gluconolactone in irrigant (ii).

Irradiation of [1-^{14}C]*Glucono-γ-lactone Solution.*—A solution (50 ml.) of gluconolactone (2·9 × 10^{-2}M) was treated with [1-^{14}C]gluconolactone to give a final specific activity of

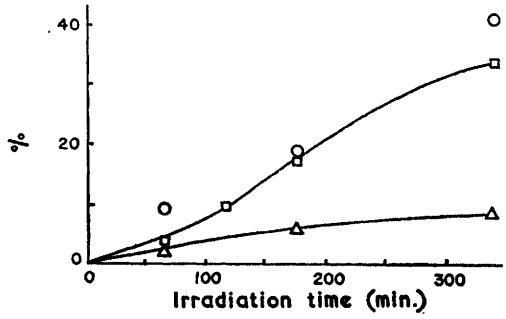

FIGURE 5 Disappearance of D-glucose and formation of gluconolactone during irradiation of D-[1-^{14}C]glucose; (—○—) D-glucose by isotope dilution analysis, (—□—) D-glucose by radiochromatography, (—△—) gluconolactone by radiochromatography

TABLE 4

Constituents in gluconolactone solution (2·8 × 10^{-2}M) after irradiation (50 min.)

Constituent	(I)	(II)	(III)
R_{Glu}, irrigant (i)	1·1	1·5	2·0
R_{Glu}, irrigant (ii)	0·3	1·3	2·0

ca. 9 μc/mmole. Radiochromatographic analysis showed no radioactive constituents (apart from unchanged [1-^{14}C]-gluconolactone). By use of the methods already described to analyse the products from irradiation of D-glucose solutions, the following were obtained: (i) the decrease in activity of the solution during irradiation (Figure 7) and (ii) the gluconolactone lost during irradiation (Table 5).

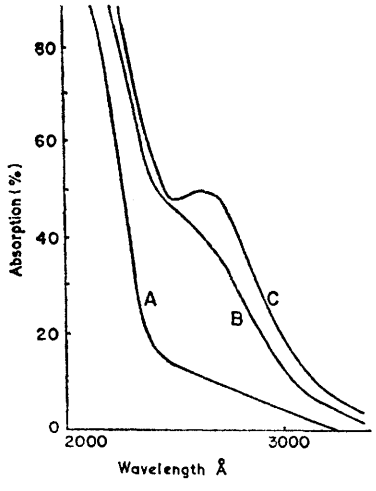

FIGURE 6 Absorption spectrum of irradiated gluconolactone solutions (2·8 × 10^{-2}M); (A) pH 4, (B) pH 4 after irradiation, (C) pH 10 after irradiation

Estimation of Carbon Dioxide and Volatile Products during Irradiations of Gluconolactone Solutions.—A series of experiments with inactive gluconolactone solutions duplicated those with active gluconolactone. The major volatile materials produced (after 80 min.) were (i) carbon dioxide, measured gravimetrically as barium carbonate,

which if evolved from C-1 accounted for a decomposition of 25% of the gluconolactone; (ii) volatile acid (8% decomposition of the gluconolactone if all of the acid was obtained from C-1); and (iii) formaldehyde (6% decomposition of gluconolactone), estimated by carrier dilution analysis on

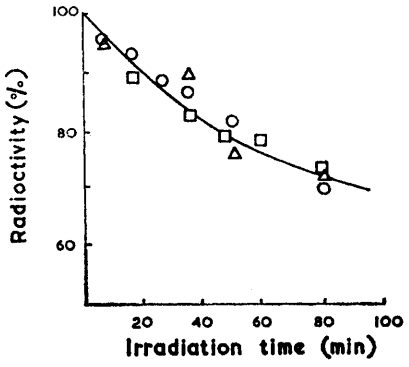

FIGURE 7 Loss of activity during irradiation of D-[1-^{14}C]-gluconolactone solution ($2{\cdot}9 \times 10^{-2}$M); (—○—) total activity before chromatography, (—△—) gluconolactone, (—□—) total activity after chromatography

TABLE 5

Degradation of gluconolactone ($2{\cdot}9 \times 10^{-2}$M) irradiation in aqueous solution (50 ml.) Carrier dilution analysis

Irradiation time (min.)	0	16·5	60·0	80·0
Gluconolactone * (counts per min. $\times 10^{-3}$)	8·8	8·1	6·6	6·1
Specific activity (μc/mmole)	0·87	0·80	0·67	0·61
Yield (mmoles)	1·45	1·34	1·11	1·03
% Original activity	100	92·0	76·8	70·8

* Aliquot portion (3 ml.) treated with inactive carrier (1 mmole), and characterised as calcium salt.

irradiated [1-^{14}C]gluconolactone. The volatile acid was measured, after distillation of the irradiated solution under vacuum into a trap at liquid air temperature, by potentiometric titration of the distillate against 0·005M-sodium hydroxide. To estimate the formaldehyde, an aliquot portion (5 ml.) from the solution of [1-^{14}C]gluconolactone (50 ml.; $2{\cdot}9 \times 10^{-2}$M; 9 μc/mmole) was treated with inactive formaldehyde (1 mmole) (from AnalaR 'formalin' solution) and 10% dimedone solution (in 50% ethanol–water). After gentle warming, the dimedone complex precipitated, and was recrystallised five times from ethanol–water to give a pure substance, m.p. 189°, constant specific activity 0·090 μc/mmole, on combustion (6% decomposition of gluconolactone).

Irradiation of Arabinose Solutions.—A solution (100 ml.) of arabinose ($2{\cdot}7 \times 10^{-2}$M) was irradiated for 4 hr. and the resulting solution was chromatographed on paper in irrigants (i) and (ii). The major product was a material with R_{Glu} 2·0 in both irrigants, identical with constituent (III) (Table 4). Considerable streaking due to acidic products occurred (R_{Glu} 0—1·5) in both irrigants. The rate of appearance of (monobasic) acid, measured potentiometrically, is shown in Table 6.

TABLE 6

Formation of acid by irradiation of arabinose

Irradiation time (min.)	6	13	22	37	52	81	119
Acid (molecules/ml. $\times 10^{-18}$)	0·3	0·2	0·2	0·5	0·8	0·9	2·0

Irradiation Product Absorbing at 2650 Å *in Alkaline Solution.*—Irradiation of solutions of D-glucose, gluconolactone, and arabinose, gave a product with an absorption maximum at 2650 Å if alkali was added to the irradiated solution.

Solutions (3 ml.) of D-glucose ($5{\cdot}5 \times 10^{-2}$M) and gluconolactone ($2{\cdot}7 \times 10^{-3}$M) were irradiated under standardised conditions in spectrophotometer cells, and 0·2M-sodium carbonate was added. The rate of appearance of the product with absorption maximum at 2650 Å was measured spectrophotometrically (Table 7).

TABLE 7

Appearance of material absorbing at 2650 Å during irradiation of D-glucose and gluconolactone solutions

Irradiation time (min.)	10	20	30	40	50	60	140	220
Optical density: (a) D-Glucose ($5{\cdot}5 \times 10^{-2}$M)		0·01		0·025		0·04	0·13	0·126
(b) Gluconolactone ($2{\cdot}7 \times 10^{-2}$M)	0·04	0·07	0·08	0·110	0·13	0·15		

The product was further investigated by paper chromatography. A solution (100 ml.) of gluconolactone ($2{\cdot}7 \times 10^{-2}$M), after irradiation, was concentrated by freeze-drying and chromatographed on paper in irrigant (ii). Elution of 1 cm. strips showed that the product with $\lambda_{max.}$ 2650 Å had R_{Glu} 0·3—0·5 and ran in the same position as gluconolactone. The product was, however, reducing, and gave a brown colour with aniline phthalate spray.

Determination of the Wavelengths Active in the Degradation of D-*Glucose and Gluconolactone.*—Solutions (100 ml.) of D-glucose (0·84M) and gluconolactone (0·028M) were irradiated at (d + 1) cm. of various filter solutions, and rates of

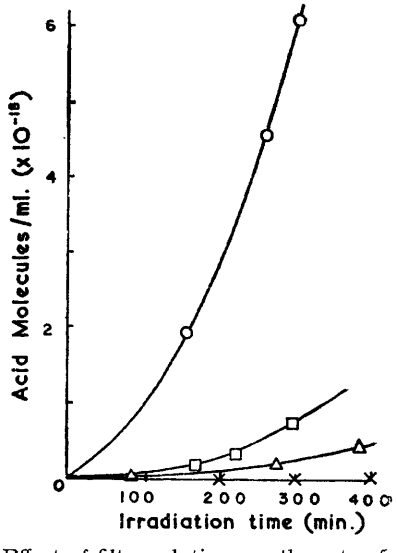

FIGURE 8 Effect of filter solutions on the rate of photolysis of D-glucose solutions ($7{\cdot}4 \times 10^{-2}$M); (—○—) water, (—□—) 0·1M-acetate, (—△—) 2·0M-acetate, (—×—) sodium benzoate

appearance of acidic products from D-glucose, and reducing products from gluconolactone, were measured. Results (Figures 8 and 9) show that the plots of appearance of

products are not linear. Several methods of comparing extents of degradation are available: (i) comparison of tangents drawn to the curves at the initial portions or irradiation; (ii) measurement of products formed at a particular time of irradiation; or (iii) comparison of the areas under the yield–dose curves at a particular time of irradiation. The three methods were used, and the mean values are given in Table 8. From spectra of the filter

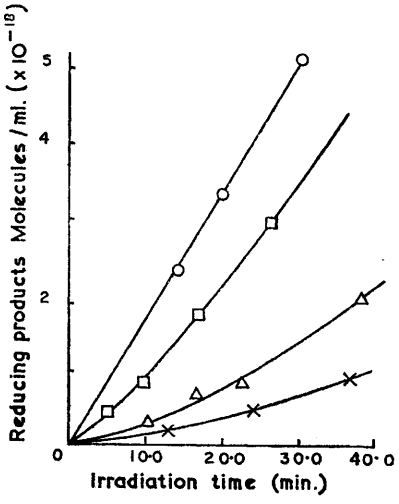

FIGURE 9 Effect of filter solutions on the rate of photolysis of gluconolactone solutions; (—○—) water, (—□—) 0·1M-acetate, (—△—) 1·0M-acetate, (—×—) 2·0M-acetate

solutions before and after irradiation the variations in incident light with different filters at wavelengths causing degradation were obtained (Table 8).

TABLE 8

Effect of filter solutions on the photolysis of gluconolactone and D-glucose

	Product variation (%) *		Radiation variation (%) †		
	(a)	(b)			
Filter solution	Gluconic acid	D-Glucose	(a) 2300 Å	(b) 2400 Å	(c) 2500 Å
2M-Acetate	6—8	4—5	1	33	84
1·0M-Acetate	11—23		3	63	93
0·1M-Acetate	54—64	9—15	89	99	99
Distilled water	100	100	100	100	100

* Expressed as decomposition in relation to a 1 cm. distilled filter. † Expressed as % incident radiation with 1 cm. distilled water filter.

Spectra in the vacuum u.v. region were obtained for D-glucose and various related sugars with a Beckman DK2A Far Ultraviolet Spectrophotometer. While scanning in the region below 2000 Å, the instrument was purged with white spot nitrogen. Traces were corrected for stray radiation, and absorbances for sample and reference were calculated for a number of wavelengths. The absorbance of the solute was then obtained by difference. The values calculated for the region 1850—1775 Å are given in Table 9.

Quantum Yields for the Degradation of D-*Glucose and Gluconolactone.*—Incident radiation was calculated by use of a ferrioxalate actinometer as employed by Hatchard and Parker.[10] Absolute values of rates of decomposition of

TABLE 9

Extinction coefficients of carbohydrates in the vacuum u.v. (l. mole^{-1} cm.$^{-1}$)

	Wavelengths (Å)			
	1850	1825	1800	1775
D-Sorbitol	290	440	650	900
D-Glucose *	225	360	560	780
D-Fructose	285	540	735	1000
Sucrose	340	560	815	1275
Maltose	340	580	880	1365
Xylose	205	360	575	835

* At active wavelengths 2100, 2300, and 2500 Å, extinction coefficients 0·14, 0·08, and 0·05, respectively.

D-glucose and gluconolactone were obtained only from irradiations of ^{14}C-labelled compounds. From the extinction coefficients of D-glucose (Table 9), approximate values of quantum yields of the primary processes were calculated. D-Glucose and glucono-γ-lactone, on direct photolysis, are both degraded with a quantum efficiency of *ca.* 10^{-2}.

DISCUSSION

It has been shown that D-glucose undergoes extensive degradation on irradiation in aqueous solution with unfiltered light from a Hanovia medium pressure 220 W lamp.[11] Two- and three-carbon fragments, formaldehyde, and formic acid were detected when *ca.* 50% or more decomposition of the original D-glucose had occurred. In view of the extensive secondary decomposition of the initial products which would have taken place under these conditions, no conclusions about the nature of the primary absorption process were possible. Attention was directed, therefore, in the present investigation to the initial course of the photodegradation.

Irradiations were carried out at (d + 2) cm. water to ensure that any 1850 Å radiation which might initiate photolysis of water[1,12] was removed. After *ca.* 20% decomposition of the D-glucose had occurred, gluconic acid, arabinose, and a lower molecular weight fragment showing the characteristics of a tetrose were the main products detectable by paper chromatography. Secondary decomposition of these initial products is significant before 20% decomposition of the original D-glucose. This behaviour is probably due to the formation of products which have a more pronounced absorption than D-glucose. Glucono-γ-lactone and the reducing product [product (A)] with the same R_{Glu} and $\lambda_{max.}$ 2650 Å could partly account for this effect. Products with $\lambda_{max.}$ 2650 Å, which is greatly enhanced by alkali, are formed when a variety of carbohydrates are irradiated with u.v. or ionising radiations.[1,13] The divergence between isotope dilution and radiochromatographic estimations of gluconic acid (Figure 3) further point to

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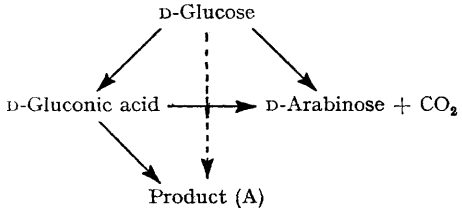
the significant production of this material. Additional agents initiating secondary decomposition are probably hydroxyl radicals produced by photolysis of the hydrogen peroxide formed.

In view of this extensive secondary degradation it was necessary to confine attention to the initial section of the yield–dose curves to provide reliable information about the primary processes. Up to *ca.* 10% decomposition of D-glucose, arabinose, gluconic acid, and product (A) were again the major products. While this provides strong indications that C-1 is the initial site of attack, experiments with D-[1-^{14}C]glucose and D-[1-^{14}C]glucono-γ-lactone were necessary to confirm this.

During irradiation of D-[1-^{14}C]glucose solutions there is loss of radioactivity due to the formation of volatile products, as would be anticipated if carbon dioxide were produced. Only the main acid product contains ^{14}C, and the remaining products, including arabinose, are inactive. There is good agreement between the rates of glucose degradation measured radiochromatographically and by isotope dilution analysis, and the glucose removed in the early stages of the reaction is approximately equivalent to the sum of the gluconolactone and arabinose produced. The divergence between the isotope dilution estimations of gluconic acid and by radiochromatography shown in Figure 3 indicates that product (A) is a secondary product, but it is not possible to be unequivocal about this. Together the results support the initial degradation Scheme shown.



SCHEME

The Scheme is supported by the results for D-[1-^{14}C]-glucono-γ-lactone irradiations, which indicate that the carbon dioxide is produced almost exclusively from C-1. Furthermore, the arabinose and other aldehydic fragments containing fewer carbon atoms are inactive. Indeed, product (A) is the only one which contains 1-^{14}C. Figure 7 further demonstrates that the decomposition of D-[1-^{14}C]glucono-γ-lactone leads only to inactive products. The loss of gluconolactone, estimated by isotope dilution analysis, is equivalent to the radioactivity loss from the solutions as a whole, when measured by a variety of methods. Carbon-1, therefore, is clearly the site of initial chemical change induced by irradiation. The greater decomposition rate for D-[1-^{14}C]glucono-γ-lactone, compared with that for D-glucose, is consistent with its higher extinction coefficient in the active wavelength region and with the pronounced secondary decomposition observed during the initial stages of D-glucose photolysis.

The results, therefore, support the existence of the 'acetal chromophore' at C-1 proposed by Beelik and Hamilton [8] and demonstrate that photochemical reaction occurs initially at this site. The behaviour of arabinose is similar; irradiation yields a tetrose which can undergo further degradation to give lower-carbon fragments. The step-wise degradation postulated for D-sorbitol irradiations [3] is thus confirmed by the present results. The final product is carbon dioxide and thus photodegradation of carbohydrates is, in this respect, a reversal of photosynthesis.

The photodissociation may thus be attributed to excitation to antibonding orbitals of the non-bonding electrons on the lactol oxygen atom. There is no obvious difference between the absorption spectra of D-glucose and D-sorbitol. In the latter compound an $n \rightarrow \sigma^*$ transition, as in aliphatic alcohols, was considered to give rise to absorption which increases as a continuum at wavelengths approaching 2000 Å. For the aliphatic alcohols, absorption becomes significant at about 1961 Å, and maxima are found in the 1835—1808 Å region.[14-16] Despite a careful search we have been unable to detect an absorption maximum for the sugars in this region. Nevertheless, the similarity in the behaviour of D-glucose, D-fructose, maltose, and D-sorbitol (Table 9) suggests that the lactol oxygen atom behaves more like that in alcohols and ethers from a spectroscopic point of view than like the oxygen of an aldehydo-group.

By the use of filter solutions, the limits of wavelength necessary for photolysis can be narrowed, and the results support the conclusion that it is an $n \rightarrow \sigma^*$ transition at the lactol oxygen which leads to the repulsive dissociative excited state. For D-glucose solutions the reductions in rate caused by 0·1M- (9—15%) and 2M-sodium acetate (4—5%) indicate that the most effective wavelength is near 2300 Å. Some degradation can be initiated by light of higher wavelength, but the negative reaction found when a sodium benzoate filter ($3{\cdot}5 \times 10^{-3}$M) is used suggest that *ca.* 2900 Å is the upper limit for light which is able to initiate direct photolysis. The corresponding experiments on irradiation of gluconic acid through various filters indicate that for this compound light of a slightly higher wavelength, in the spectral region 2300—2500 Å, is most active. This observation is consistent with the relative absorption spectra of these compounds.

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