679. *Radiation Chemistry of Carbohydrates. Part V.* The Effect of Ultraviolet Light on Aqueous Solutions of* D-*Glucose in Oxygen.*

By G. O. Phillips and G. J. Moody.

D-Glucose is degraded when irradiated in aqueous solution with ultraviolet light. The main products, revealed by paper chromatography and estimated quantitatively by isotope dilution analysis, are arabinose, three- and two-carbon aldehydic fragments, formaldehyde, formic acid, carbon dioxide, and possibly glucosone. The degradation is examined by measuring the changes in acidity, optical rotation, and ultraviolet absorption spectra. The rates of formation of the main products with dose were measured. Hydrogen peroxide is formed continuously, and there is evidence for a post-irradiation process.

The nature of the products and the quantitative measurements indicate that the degradation by ultraviolet light is different from the changes induced by gamma-radiation under comparable conditions.

The numerous investigations of the effect of ultraviolet light on carbohydrate solutions have provided little information about the chemical changes induced. Attention has been focused mainly on the behaviour of photosensitisers,[1,2] the formation of acid,[1,3,4] of hydrogen,[3,4,5] and of carbon dioxide,[3,4,6,7] changes in optical rotation,[8] and the influence of air.[2,6] Holtz [9] observed that monosaccharides on irradiation give compounds with strongly negative oxidation–reduction potentials and absorption maxima at 265—290 mμ. More recently Laurent [10] noticed similar changes, and demonstrated, by using glass filters, that the most active part of the light is below 280 mμ. Whelan and Peat [11] have shown that when dilute aqueous solutions of amylose, maltose, and glucose are exposed to ultraviolet light there is complete conversion into carbon dioxide, and that the process is accelerated in the presence of zinc oxide. Later, several other products were identified by paper chromatography.[12]

Previously we studied the action of ionising radiations on D-glucose solutions and elucidated the degradation pattern by identifying the products.[13] This study is now extended to ultraviolet light: our results indicate important differences between the two.

Results

Irradiation.—Glucose solutions (500 ml.) were irradiated in quartz flasks with unfiltered light from a Hanovia 220 w lamp which had strong emission lines at 256, 296, 305, 315, and 370 mμ, the lamp being 1 cm. from the quartz flask in a reproducible position. The light was roughly collimated by means of a polished aluminium cylinder. There was no fluctuation in the lamp output as measured with a potassium ferrioxalate actinometer. A fine stream of purified oxygen was passed continuously through the solutions during irradiation, and loss by evaporation was prevented by a reflux condenser.

Chromatographic Separation of Irradiation Products.—The constituents present in irradiated D-glucose solutions were separated by the methods described previously.[13] A solution (500 ml.)

* Part IV, *J.*, 1960, 762.

[1] Neuberg, *Biochem. Z.*, 1912, **39**, 158.
[2] Palit and Dhar, *J. Phys. Chem.*, 1928, **32**, 1263.
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[4] Bernoulli and Cantieni, *Helv. Chim. Acta*, 1932, **15**, 119.
[5] Bethollet and Gaudechon, *Compt. rend.*, 1913, **156**, 707.
[6] Bierry, Henri, and Ranc, *Compt. rend.*, 1910, **151**, 316.
[7] Bierry and Ranc, *Bull. Soc. chim. France*, 1929, **35**, 771.
[8] Dillman, *J. Lab. Clin. Med.*, 1931, **17**, 236.
[9] Holtz, *Arch. Exp. Path. Pharmak.*, 1936, **182**, 141.
[10] Laurent, *J. Amer. Chem. Soc.*, 1956, **78**, 1875.
[11] Whelan and Peat, *Symp. J. Soc. Dyers Colourists*, 1949, **65**, 748.
[12] Evans, Ph.D. Thesis, Wales, 1951.
[13] Phillips, Moody, and Mattok, *J.*, 1958, 3522.

of D-[^{14}C]glucose (27·7 millimoles with specific activity 1·22 μc/millimole) was irradiated for 171 hr. The temperature of the solution rose to 38° after about 1 hr., and thereafter remained constant. The irradiated solution was chromatographed in butan-1-ol–ethanol–water (4 : 1 : 5), and spraying with *p*-anisidine revealed five spots: pink, R_F 0·02; brown, R_F 0·09; pink, R_F 0·12; pink, R_F 0·15, and pink, R_F 0·18. Only two of these constituents were readily identified, namely, R_F 0·09, glucose, and R_F 0·12, arabinose. In butan-1-ol–acetic acid–water (4 : 1 : 5) a similar pattern was observed: pink, R_F 0·1—0·14; brown, R_F 0·18, glucose; pink, R_F 0·22, arabinose; pink, R_F 0·25; and pink, R_F 0·29. In addition, streaking was observed on all the chromatograms.

Volatile Acid.—Formic acid was detected as follows. The distillate from the irradiated solution obtained under reduced pressure (<35°) was frozen in liquid air, and concentrated

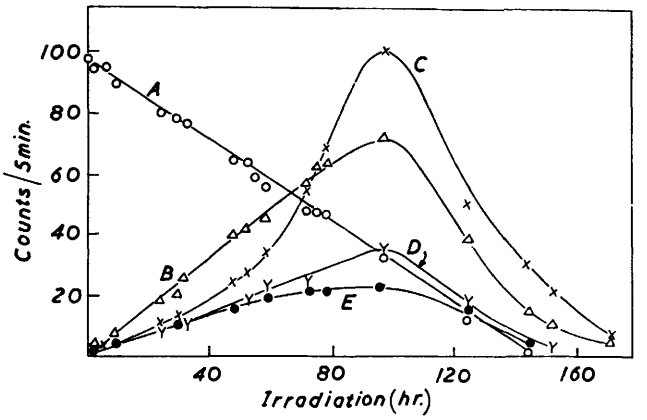


FIG. 1. *Rate of formation of products during irradiation of aqueous* D-*glucose solutions with ultraviolet light.*

A, D-*Glucose* (× 10^{-2}), R_F 0·09, *brown.*
B, D-*Arabinose*, R_F 0·12, *pink.*
C, *Product of* R_F 0·02, *pink.*
D, *Product of* R_F 0·15, *pink.*
E, *Product of* R_F 0·18, *pink.*

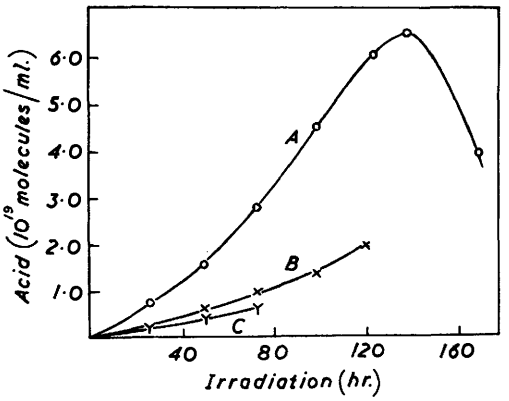


FIG. 2. *Rate of formation of acid during irradiation of aqueous* D-*glucose.*

A, 0·55 × 10^{-2}M; *B*, 2·5 × 10^{-2}M; *C*, 5·5 × 10^{-3}M.

ammonia solution was added. The water and excess of ammonia were removed by a further distillation under reduced pressure, and the white solid remaining was dissolved in water (0·2 ml.). This solution was chromatographed in ethanol–ammonia (*d* 0·88) (100 : 1) for 8 hr.[14] After drying, spraying with Bromocresol Green revealed one blue spot (R_F 0·4) on a yellow background, corresponding to a control of ammonium formate.

Rate of Formation of Products.—Accurately known amounts (0·05 ml.) of aqueous D-[^{14}C]glucose (27·7 millimoles with specific activity 1·22 μc/millimole in 500 ml.) which had been exposed to progressively increasing radiation doses were chromatographed in butan-1-ol–ethanol–water, and the radioactivity of the spots was measured. There is a linear decrease in the glucose concentration with time of irradiation. The products increase in concentration for 160 hr. and thereafter there is a sharp fall. The results are shown in Fig. 1. Paper chromatography of the solution after irradiation for 160 hr. indicates that no detectable amounts of the products remain. During this period 0·65 g. of carbon dioxide was liberated.

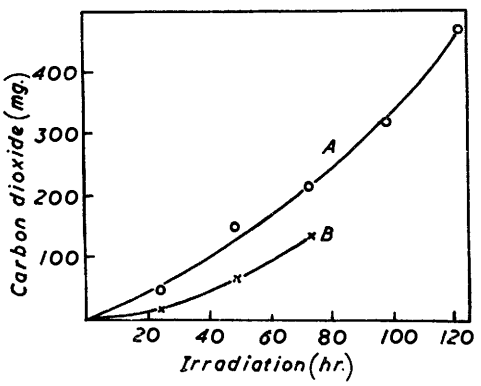
[14] Kennedy and Barker, *Analyt. Chem.*, 1951, **23**, 1033.

Changes Produced by Irradiation.—Irradiation of glucose solutions in oxygen leads to a fall in pH. The amount of acid increases with time of irradiation and depends on glucose concentration within the range 5×10^{-2} to 5×10^{-3}M (Fig. 2). The amount of acid present decreases markedly after 140 hours' irradiation. Paper chromatography after 45 hr. indicates the presence of similar degradation products at the three concentrations studied.

During irradiation, carbon dioxide is evolved continuously, and the rate increases with time of irradiation; the amount of carbon dioxide formed depends on the glucose concentration (Fig. 3).

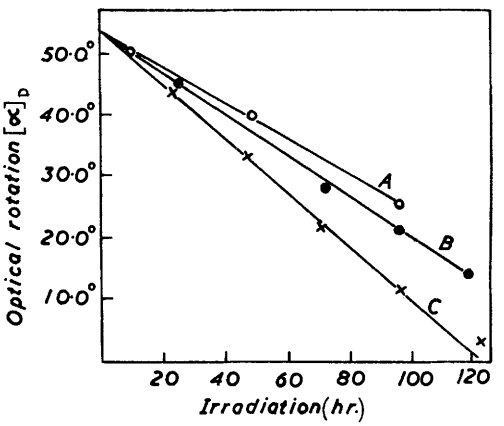
The optical rotation of the solution decreases steadily during irradiation, and the rate is slower at lower glucose concentrations (Fig. 4).

FIG. 3. *Rate of formation of carbon dioxide in irradiated* D-*glucose solutions.*



A, $5{\cdot}5 \times 10^{-2}$M; *B*, $5{\cdot}5 \times 10^{-3}$M.

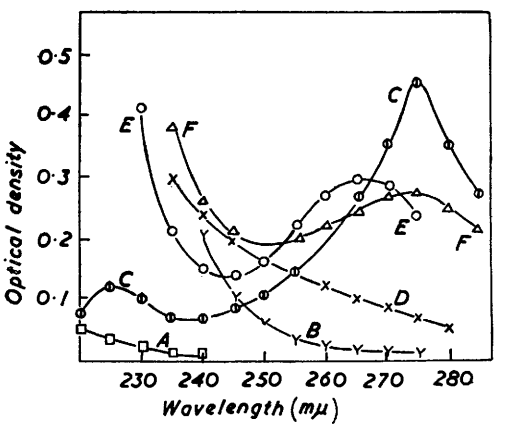
FIG. 4. *Change in optical rotation of aqueous* D-*glucose solutions during irradiation.*



A, $5{\cdot}5 \times 10^{-2}$M; *B*, $2{\cdot}5 \times 10^{-2}$M; *C*, $5{\cdot}5 \times 10^{-3}$M.

FIG. 5. *Ultraviolet absorption spectra of* D-*glucose solutions after irradiation with ultraviolet light.*

A, $5{\cdot}5 \times 10^{-3}$M *after* 97 *hr.*
B, $5{\cdot}5 \times 10^{-3}$M *irradiated for* 97 *hr. with added* $KHCO_3$.
C, *Furfuraldehyde* (3×10^{-5}M).
D, $2{\cdot}5 \times 10^{-2}$M *after* 97 *hr.*
E, $2{\cdot}5 \times 10^{-2}$M *irradiated for* 97 *hr. with added* $KHCO_3$.
F, *Furfuraldehyde and irradiated glucose solution* ($2{\cdot}5 \times 10^{-2}$M).



Irradiated neutral glucose solutions do not show an ultraviolet absorption maximum (Fig. 5). Addition of potassium hydrogen carbonate, however, to a solution ($2{\cdot}5 \times 10^{-2}$M) which had been irradiated for 97 hr. leads to the appearance of an absorption maximum at 265 mμ, also characteristic of glucose solutions irradiated with gamma-radiation. At lower concentrations (5×10^{-3}M), after similar treatment, this characteristic absorption is not detectable. It has been suggested [12] that under acidic conditions furfuraldehyde is present in the irradiated solution. The ultraviolet absorption spectrum of furfuraldehyde (3×10^{-5}M) was measured; its maximum occurs at 278 mμ (ε 14,800). Owing to its high extinction coefficient, small quantities present in the irradiated glucose solution could be easily detectable. It is improbable, therefore, that furfuraldehyde is present in irradiated glucose solutions in quantity.

Irradiated glucose solutions give a yellow colour with titanium sulphate reagent, indicating the presence of peroxides. The amount increases with time of irradiation and gradually decreases after the irradiation. This post-irradiation effect is greater at the higher glucose concentrations.

There is evidence that after about 160 hours' irradiation the solution is entirely free from organic solutes. The amounts of glucose and other products as measured on paper chromatograms (Fig. 1) are negligible, the solution has zero optical rotation, and even at the highest glucose concentrations used, after 270 hours' irradiation, addition of potassium hydrogen carbonate does not produce the characteristic absorption at 265 mμ.

Estimation of the Products by Isotope Dilution.—Isotope dilution was applied directly to the irradiated mixture. For this purpose an aqueous solution (500 ml.) of glucose (27·7 millimoles) and specific activity 1·22 μc/millimole was irradiated for 72 hr. in oxygen, and the individual products were estimated by applying the isotope dilution method directly to the untreated irradiated solution.

D-*Glucose.*—(a) *Penta-O-acetylglucose.* The irradiated solution (20 ml.) was freeze-dried, treated with carrier glucose (1·9 millimoles), acetic anhydride (1·2 ml.), and sodium acetate (0·25 g.), and kept at 100° for 2 hr. The resulting penta-acetate, after ten recrystallisations from ethanol, had m. p. 135° and constant specific activity (0·28 μc/millimole).

(b) *Glucosazone.* The irradiated solution (10 ml.) was boiled with phenylhydrazine (2 ml.), acetic acid (1·5 ml.), and carrier glucose (1·49 millimoles) for 30 min. The osazone was filtered off and after eight recrystallisations from ethanol had m. p. 201° and constant specific activity (0·207 μc/millimole).

(c) D-*Gluconic acid.* The solution (5 ml.) was treated with carrier D-gluconolactone (0·97 millimole) and excess of calcium carbonate and filtered after 3 days. The calcium gluconate was precipitated with ethanol and separated. Eight successive precipitations from solution gave the pure gluconate with constant specific activity ($1·8 \times 10^{-3}$ μc/millimole).

(d) D-*Glucuronic acid.* The irradiated solution (10 ml.) was treated with carrier glucurone (0·82 millimole); the solid remaining after freeze-drying gave, on recrystallisation seven times from hot water, pure D-glucurone with m. p. 175° and constant specific activity of $2·7 \times 10^{-3}$ μc/millimole.

(e) D-*Glucosaccharic acid.* Saccharic acid carrier (0·42 millimole) was added to the irradiated solution (5 ml.). The solid remaining after freeze-drying and six recrystallisations from hot water had m. p. 125° and specific activity of 9×10^{-4} μc/millimole.

(f) D-*Arabinose.* The irradiated solution (20 ml.) with carrier arabinose (0·77 millimole) was boiled with phenylhydrazine (1 ml.) and acetic acid (0·5 ml.) for 20 min. The osazone, recrystallised seven times from benzene, gave arabinosazone, m. p. 159°, constant specific activity of $8·4 \times 10^{-2}$ μc/millimole.

(g) D-*Xylose.* The irradiated solution (10 ml.) with carrier xylose (1·58 millimoles) was freeze-dried and the residue was heated with acetic anhydride (1 ml.) and sodium acetate (0·15 g.) at 100° for 2 hr. The resulting tetra-*O*-acetylxylose, after seven recrystallisations from ethanol, had m. p. 124° and specific activity 7×10^{-4} μc/millimole.

(h) D-*Ribose.* The irradiated solution (20 ml.) with carrier ribose (1·44 millimoles) was freeze-dried, and acetic anhydride (1·2 ml.) and pyridine (1·5 ml.) were added. After 24 hr. at about 4° the acetate was precipitated with ice-water and after eight recrystallisations from ethanol had m. p. 110° and specific activity 10^{-3} μc/millimole.

(i) *Three-carbon aldehydic fragments.* The irradiated solution (5 ml.) with carrier dihydroxyacetone (1·84 millimoles) was boiled with phenylhydrazine (1 ml.) and acetic acid (0·5 ml.) for 10 min. The osazone was recrystallised eight times from benzene, to give pure glycerosazone, m. p. 129° and specific activity $3·7 \times 10^{-3}$ μc/millimole.

(j) *Two-carbon aldehydic fragments.* Carrier glyoxal (2·42 millimoles), phenylhydrazine (2 ml.), and acetic acid (1 ml.) were added to the irradiated solution (10 ml.). The precipitate, after ten crystallisations from benzene, had m. p. 170° and constant specific activity ($6·9 \times 10^{-3}$ μc/millimole).

(k) *Oxalic acid.* The irradiated solution (5 ml.), after treatment with carrier oxalic acid (2·61 millimoles), was distilled under reduced pressure (<35°) to a syrup; the distillate, frozen in liquid air, was used for determination of formaldehyde. The solid which eventually separated was recrystallised seven times from hot water, to give oxalic acid, m. p. 101°, constant specific activity 2×10^{-4} μc/millimole.

(l) *Formaldehyde.* The above distillate was treated with carrier formaldehyde (0·233 millimole) and 5% ethanolic dimedone (10 ml.). The solid which separated after 48 hr. was recrystallised twice from ethanol; it had m. p. 189° and specific activity $2·3 \times 10^{-2}$ μc/millimole.

The results for the isotope dilution analysis are tabulated.

Products formed when an aqueous solution of D-*glucose is irradiated with ultraviolet radiation for 72 hr. in oxygen.*

Initial D-Glucose = 27·7 millimole

Products	Carrier (millimole)	Sp. activity (μc/millimole)	Yield (millimole)
D-Glucose			
penta-acetate	1·9	0·28	14·18
glucosazone	1·49	0·207	15·20
D-Gluconic acid	0·97	$1·8 \times 10^{-3}$	0·07
D-Glucuronic acid	0·82	$2·7 \times 10^{-3}$	0·09
D-Glucosaccharic acid	0·42	9×10^{-4}	0·03
D-Arabinose	0·77	$8·4 \times 10^{-2}$	1·74
D-Xylose	1·58	7×10^{-4}	0·05
D-Ribose	1·44	1×10^{-3}	0·03
Three-carbon aldehydic fragments	1·84	$3·7 \times 10^{-3}$	1·11
Two-carbon aldehydic fragments	2·42	$6·9 \times 10^{-3}$	2·09
Formaldehyde	0·233	$2·3 \times 10^{-2}$	2·95
Oxalic acid	2·61	2×10^{-4}	0·10

Discussion

The degradation of D-glucose when irradiated in dilute aqueous solution with ultraviolet radiation shows certain similarities with the degradation by gamma-radiation. Acid is formed, the optical rotation of the solution decreases, and in alkaline solution an absorption band appears at 265 mμ. Bothner-by and Balazs [15] noted changes of this nature in glucose solutions irradiated with *X*-rays and electrons at doses ranging from 1 to 10^7 rads, and compared their observations with those of Laurent,[10] who studied the absorption spectra of alkaline glucose solutions irradiated with ultraviolet radiation. From this comparison Bothner-by and Balazs [15] conclude that the same pattern of degradation prevails in both cases. Our results, however, indicate that there are also significant differences in the behaviour of the two types of radiation.

In the solution irradiated with ultraviolet light, apart from unchanged glucose, four other constituents were detected by paper chromatography. The pink spot at R_F 0·1—0·14 in butan-1-ol–acetic acid–water indicated initially the presence of glucuronic acid, but isotope dilution analysis (Table) proves that the amount of this acid is small (0·09 millimole). However, the radioactive count of the spot at R_F 0·1—0·14 after 72 hours' irradiation (Fig. 1) indicates that the product or products responsible are present in comparable amounts to that of arabinose (R_F 0·22), shown by isotope dilution to be 1·7 millimoles. Therefore glucuronic acid cannot be the main product responsible for the spot at R_F 0·11—0·14; this view is supported by the observation that no glucurone was detected at R_F 0·34. From our results there is also no evidence that gluconic acid is a major product during ultraviolet irradiations although this acid and glucuronic acid are primary products of gamma-radiation of glucose solutions.[13]

We are unable to identify the products responsible for the pink spots with R_F 0·25 and 0·29 (in butan-1-ol–acetic acid–water). These are formed more slowly (Fig. 1) than arabinose, indicating that they are lower degradation products. The pink spot of R_F 0·29 corresponds closely with ribose in its chromatographic behaviour, but the large radioactivity count at R_F 0·29 shows no correlation with the very low assay of ribose as tetra-acetate by isotope dilution analysis; it is also difficult to conceive the formation of ribose on configurational grounds.[12] Xylose is present only in negligible amounts and therefore

[15] Bothner-By and Balazs, *Radiation Res.*, 1957, **6**, 302.

we conclude that arabinose is the main pentose present. The yield–dose curve (Fig. 1) further indicates that arabinose is a primary product.

Our results support the view [12] that glucosone is present in the irradiated solution. Isotope dilution estimations of glucose as penta-*O*-acetylglucose and glucosazone show considerable divergence, as is to be expected if glucosone is present. This compound would contribute to the latter but not to the former assay. A similar divergence between the two methods resulting from the presence of glucosone was observed for irradiated fructose solutions.[16]

Isotope dilution analysis with carrier dihydroxyacetone and assay as glycerosazone indicate that appreciable amounts of three-carbon aldehyde fragments are formed. Similar three-carbon fragments were observed during gamma-irradiation of glucose solution, and the results indicated that the characteristic absorption at 265 mμ is due to isomerisation of glyceraldehyde to dihydroxyacetone. Glucose solutions subjected to ultraviolet irradiation, however, only show the absorption maximum at 265 mμ when alkali is added to the solution (Fig. 5). Since pure dihydroxyacetone in alkaline solution shows an absorption maximum at 294 mμ, it is probable that other enediol structures are present which make a more important contribution to the absorption at 265 mμ than does dihydroxyacetone. Markham *et al.*[17] concluded from similar absorption-spectra evidence that glyceraldehyde and/or dihydroxyacetone are formed during the ultraviolet irradiation of glycerol catalysed with zinc oxide. Isotope dilution analysis with carrier glyoxal and assay as glyoxal bisphenylhydrazone confirm that two-carbon aldehydic fragments are present among the lower degradation products (Table). The final degradation products are formaldehyde, formic acid, and carbon dioxide. The yield–dose curve for carbon dioxide supports this since the rate of formation increases sharply during the later stages of the degradation. After 72 hours' irradiation formic acid accounts for most of the acid present in the irradiated solution.

The yields of acid and carbon dioxide, and the fall in optical rotation, on ultraviolet radiation depend on glucose concentration, whereas for gamma-radiation the yields were independent of it within a ten-fold range. This indicates that direct absorption of radiation rather than indirect action is the dominant process responsible for degradation by ultraviolet light. Heidt [18] observed similar direct absorption of light by solutions of glycosides, leading to changes in optical activity due to hydrolysis of the glycosidic link. The reaction is considered to arise from an intramolecular transfer of absorbed energy from the aglycone to the hemiacetal oxygen bridge which is the reactive centre. The degradation of glucose in solution by gamma-radiation is, however, an indirect process, the radiation energy being absorbed by the water and degradation caused by reactive species formed by radiolysis of water.[13]

Thus, from our quantitative studies and the nature of the products identified, it is clear that although there are similarities there are also important differences between the action of gamma-radiation and of ultraviolet light. Particularly noticeable is the negligible formation of gluconic and glucuronic acid by ultraviolet light. Whelan and Peat [11] also concluded that glucuronic acid is not an intermediate product in the photo-oxidation of glucose. A stepwise degradation of the hexose molecule was postulated by these workers to account for the photodecomposition of starch, where the only intermediate products identified were formaldehyde, formic acid, and carbon dioxide. A similar degradation pattern would account for our main observations. Experiments are in progress to examine this possibility further.

However, there are clearly other types of attack occurring on glucose. The presence of glucosone would indicate oxidation at $C_{(2)}$ leading to 2-oxogluconic acid which may account for the spot at R_F 0·1—0·14. The unknown spots at R_F 0·25 and 0·29 may be due

[16] Phillips and Moody, *J.*, 1960, 754.
[17] Markham, Hawnan, Paternaste, and Rose, *J. Amer. Chem. Soc.*, 1958, **80**, 5394.
[18] Heidt, *J. Franklin Inst.*, 1942, **234**, 473.

to keto-sugars formed from arabinose. After 160 hours' irradiation the carbohydrate content of the solution is negligible and glucose is almost entirely converted into carbon dioxide, formaldehyde, and formic acid. Therefore, although several products remain unidentified, it is clear that the pattern of degradation by ultraviolet radiation is substantially different from that of gamma-radiation.

With respect to this and the following paper, the authors thank Dr. W. Wild for valuable discussions, Professor A. G. Evans for his interest, and the United Kingdom Atomic Energy Authority (Research Group) for financial support. A maintenance grant (to G. J. M.) from U.K.A.E.A. (Research Group) is gratefully acknowledged.

UNIVERSITY COLLEGE, CARDIFF. [*Received, February 18th,* 1960.]