757. *Photo-degradation of Carbohydrates. Part I. Products from the Direct Photolysis of Aqueous Solutions of* D-*Sorbitol.*

By G. O. Phillips and W. J. Criddle.

Irradiation of aqueous D-sorbitol solutions in oxygen with unfiltered light from a Hanovia medium-pressure mercury lamp leads to the formation of hexoses, hexonic acids, pentoses, and aldehydic products containing smaller numbers of carbon atoms. Ultimately, the D-sorbitol is converted completely into carbon dioxide. Yield–dose curves, obtained by isotope dilution analysis, indicate that D-sorbitol is converted into smaller fragments by stepwise degradation.

Studies of ultraviolet irradiation of aqueous solutions of carbohydrates have revealed little about the nature of the products or about the processes which initiate degradation. Acid formation,[1] changes in optical rotation,[2] liberation of gas,[1,3] and production of compounds with ultraviolet absorption maxima near 265 mμ [4] have been shown to occur but there is lack of agreement about the conditions necessary to initiate photo-degradation.[1,5] Scant attention has been given to the active wavelength of the light, and factors such as pH, degree of oxygenation, presence of photo-sensitiser, etc., which may influence the nature of primary absorbing species. From a review [6] it seems that studies of compounds related to cellulose have provided the most reliable data on the effect of ultraviolet light on carbohydrates. Here, two types of photo-degradation have been recognised, direct photolysis [7] and photo-sensitised degradation.[8]

In the present paper, the products formed when oxygenated aqueous D-sorbitol solutions are irradiated with ultraviolet light from a medium-pressure mercury lamp in the absence of photo-sensitiser are investigated. In the following paper, consideration is given to the mechanism of direct photolysis.

Previously,[9] it was shown that arabinose, two- and three-carbon aldehydic fragments, formaldehyde, formic acid, and possibly glucosone are formed when D-glucose is exposed to the unfiltered light from a mercury lamp. If irradiation is sufficiently prolonged, no detectable product remains and the initial D-glucose is converted completely into carbon dioxide. Beelik and Hamilton [7] studied the ultraviolet (2200—4000 Å) irradiation of cellobiose, cellopentaose, methyl β-cellobioside, cellobiitol, and cellopentaitol as model compounds related to cellulose. The effects of irradiation were very similar. All the compounds were fragmented, yielding comparable amounts of acidic compounds and products with absorption maxima near 2600 Å.

D-Sorbitol was selected for the present study because it does not contain the lactol ring-oxygen atom present in hexoses, giving rise to the proposed " acetal chromophore " [7] which it has been suggested is responsible for the primary absorption of light near 2650 Å to initiate degradation. As far as we are aware, no previous study of the ultraviolet irradiation of D-sorbitol has been reported.

[1] Euler and Lindberg, *Biochem. Z.*, 1912, **39**, 410; *J.*, 1912, **102**, 407; Cantieni, *Helv. Chim. Acta*, 1934, **17**, 1528; 1935, **18**, 473, 933, 1420.
[2] Bernoulli and Cantiene, *Helv. Chim. Acta*, 1932, **15**, 119.
[3] Berthelot and Gandechon, *Compt. rend.*, 1912, **155**, 401, 1153; Bernoulli and Cantieni, *Helv. Chim. Acta*, 1932, **15**, 119.
[4] Laurent and Wertheim, *Acta Chem. Scand.*, 1952, **6**, 678; *J. Amer. Chem. Soc.*, 1956, **78**, 1875; Holtz, *Arch. exp. Path. Pharmak.*, 1936, **182**, 141, 160.
[5] Bertholet and Gandechon, *Compt. rend.*, 1912, **155**, 831; Neuberg, *Biochem. Z.*, 1912, **39**, 158; Dillman, *J. Lab. Clin. Med.*, 1931, **17**, 236.
[6] Phillips, *Adv. Carbohydrate Chem.*, 1963, **18**, Ch. 2.
[7] Egerton, *J. Soc. Dyers Colourists*, 1949, **65**, 764; Launer and Wilson, *J. Amer. Chem. Soc.*, 1949, **71**, 958; Flynn, *J. Polymer Sci.*, 1958, **27**, 83; Beelik and Hamilton, *Das Papier*, 1959, **13**, 77; *J. Org. Chem.*, 1961, **26**, 5074.
[8] Egerton, *J. Soc. Dyers Colourists*, 1947, **63**, 161; 1959, **64**, 659; *Textile Res. J.*, 1948, **18**, 659; *J. Textile Inst.*, 1948, **39**, 293.
[9] Phillips and Moody, *J.*, 1960, 3398.

Results and Experimental

Irradiations were carried out with unfiltered light from a Hanovia 220 w medium-pressure mercury lamp. Silica flasks were used for irradiations and the lamp was maintained at a reproducible distance from the flask. By using a potassium ferrioxalate actinometer it was ascertained that no appreciable change in the power output of the lamp occurred during the experiments.

Chromatographic Analysis of the Irradiated Solution.—A solution (500 ml.) of D-sorbitol (27·5 mmoles) was irradiated for 12 hr. and subsequently examined by paper chromatography in two irrigants, (i) butan-1-ol–acetic acid–water (4 : 1 : 5) and (ii) butan-1-ol–pyridine–water (10 : 3 : 3). The organic constituents detected by spraying with *p*-anisidine and alkaline

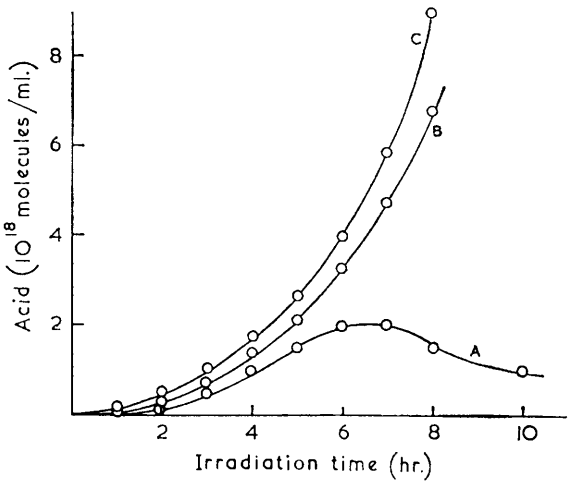


Fig. 1. Acid formation during the ultraviolet irradiation of oxygenated D-sorbitol solutions.
A, 5×10^{-3}M. B, 5×10^{-2}M. C, 5×10^{-1}M.

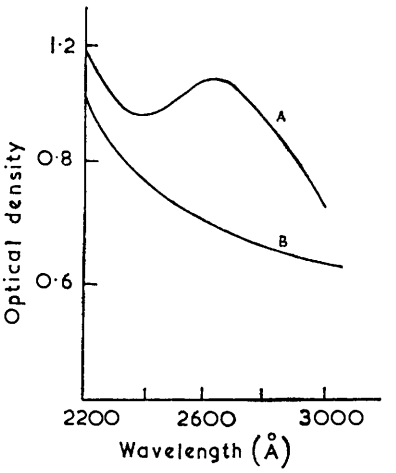


Fig. 2. Absorption spectra of D-sorbitol solutions after ultraviolet irradiation.
A, With added $KHCO_3$.
B, Without added $KHCO_3$.

sodium hydroxide–silver nitrate are shown in Table 1. Streaking along the chromatogram necessitates running many paper chromatograms.

Table 1.

Products after irradiation of D-sorbitol solution in oxygen with ultraviolet light.

(i) Butan-1-ol–pyridine–water (10 : 3 : 3)

Products	1	2	3	4	5	6	7	8
R_F	0·02	0·05	0·09	0·18	0·23	0·26	0·29	0·46
Colour with *p*-anisidine	Pink	—	—	Brown	Brown	Pink	Pink	Yellow
Constituent	Acid	Acid	Acid	Glucose	Gulose	Arabinose	Xylose	Tetrose

(ii) Butan-1-ol–acetic acid–water (4 : 1 : 5)

Product	4	5	6	7	8
R_F	0·18	0·22	0·24	0·27	0·44
Colour with *p*-anisidine	Brown	Brown	Pink	Pink	Yellow
Constituent	Glucose + Sorbitol	Gulose	Arabinose	Xylose	Tetrose

Electrophoresis in phosphate buffer (pH 7·4) for 3 hr. at 360 v and subsequent spraying with alkaline silver nitrate revealed two acidic components with relative mobilities 74 : 106. No evidence for uronic acid formation was obtained by this method.

Acid Formation.—Acid formation (Fig. 1) is a secondary process which is dependent on D-sorbitol concentration within the range 5×10^{-3} to 5×10^{-1}M. *In vacuo*, the amount of acid formed is extremely small.

Ultraviolet Spectra of Irradiated Solutions.—The ultraviolet spectrum of D-sorbitol solutions irradiated in oxygen is shown in Fig. 2. No characteristic absorption maximum is developed

during irradiation, although during irradiation the solution exhibits an overall increase in absorption in the range 220—300 mμ. Table 2 demonstrates this effect and shows the increase in optical density at 265 mμ with increasing dose. On addition of potassium hydrogen carbonate to the irradiated solution an absorption maximum develops at 265 mμ; the peak height increases proportionally with dose (Table 2).

Estimation of Products by Isotope Dilution Analysis.—A solution (500 ml.) of D-sorbitol (27·5 mmoles) and specific activity 3·6 μc/mmole was irradiated for 30 hr. and the products were estimated by application of isotope dilution analysis directly to the untreated solution as follows:

D-*Sorbitol.* The irradiated solution (5 ml.) was freeze-dried and treated with carrier D-sorbitol (1·0 mmole), anhydrous sodium acetate (0·2 g.), and acetic anhydride (1 ml.), and the

TABLE 2.

Change in optical density at 265 mμ with dose during irradiation of aqueous D-sorbitol in oxygen.

(1) No alkali present						
Time of irradiation (hr.)	0	2	4	6	8	10
Optical density ($l = 1$)						
(*a*) 5×10^{-1}M	0·315	0·41	0·54	0·66	0·77	0·91
(*b*) 5×10^{-2}M	0·21	0·29	0·39	0·43	0·54	0·62
(*c*) 5×10^{-3}M	0·13	0·18	0·24	0·32	0·33	0·31
(2) With added $KHCO_3$						
Time of irradiation (hr.)	0	2	4	6	8	10
Optical density ($l = 1$)						
(*a*) 5×10^{-1}M	0·31	0·60	0·94	1·12	1·41	1·68
(*b*) 5×10^{-2}M	0·22	0·36	0·68	0·81	0·97	1·10
(*c*) 5×10^{-3}M	0·11	0·24	0·40	0·57	0·62	0·63

mixture was refluxed for 30 min. The solid which separated on the addition of ice–water gave, after seven recrystallisations from ethanol, pure hexa-*O*-acetyl-D-sorbitol with m. p. 99° and constant specific activity 0·07 μc/mmole.

D-*Glucose.* The irradiated solution (5 ml.) was freeze-dried and treated with carrier D-glucose (0·8 mmole), anhydrous sodium acetate (0·2 g.), and freshly distilled acetic anhydride (1 ml.) and heated at 100° for 1 hr. The solid which separated on addition of ice–water gave pure penta-*O*-acetyl-β-D-glucose with m. p. 131° and constant specific activity 0·089 μc/mmole.

D-*Arabinose.* Carrier D-arabinose (1·02 mmoles) was added to the irradiated solution (5 ml.), followed by ethanol (15 ml.) and diphenylhydrazine (0·5 ml.). The mixture, after being heated at 100° for 1 hr., was cooled and the solid which separated was recrystallised six times from ethanol, to give pure D-arabinose diphenylhydrazone with m. p. 196° and constant specific activity 0·044 μc/mmole.

L-*Xylose.* The irradiated solution (5 ml.) was freeze-dried, treated with carrier L-xylose (1·04 mmoles), anhydrous sodium acetate (0·29), and freshly distilled acetic anhydride (1 ml.) and heated at 100° for 1 hr. On cooling, a solid separated which, recrystallised seven times from ethanol, gave tetra-*O*-acetyl-β-L-xylose with m. p. 126° and constant specific activity 0·046 μc/mmole.

D-*Glucuronic acid.* The irradiated solution was treated with carrier D-glucurone (1·2 mmoles) and allowed to equilibrate for 24 hr. The lactone was recovered, and after seven recrystallisations from water was obtained pure with m. p. 175° and constant specific activity 0·020 μc/mmole.

D-*Gluconic acid.* The irradiated solution (5 ml.) was freeze-dried and carrier D-glucono-γ-lactone (1·0 mmole) was added. After addition of ethanol (25 ml.) and hydrazine hydrate (0·5 ml. of a 95% solution in water) a solid separated which gave pure D-gluconic acid hydrazide with m. p. 143° and constant specific activity 0·058 μc/mmole after six recrystallisations from ethanol.

Three-carbon aldehydic fragments. The irradiated solution (5 ml.) was treated with 1,3-dihydroxyacetone (1·0 mmole), acetic acid (1 ml.), and phenylhydrazine (1·5 ml.), and heated for 15 min. at 100°. The solid which separated was recrystallised eight times from benzene to give pure glycerosazone with m. p. 127° and constant specific activity 0·018 μc/mmole.

TABLE 3.

Products formed during the irradiation of aqueous D-sorbitol solutions with ultraviolet light for 30 hr.

Initial D-Sorbitol, 27·5 mmoles

Product	D-Sorbitol	D-Glucose	D-Arabinose	L-Xylose
Carrier (mmoles)	1·0	0·8	1·02	1·03
Specific activity (μc/mmole)...	0·07	0·089	0·044	0·046
Yield (mmoles)	2·0	2·0	1·5	1·6

Product	D-Glucuronic acid	D-Gluconic acid	Three-carbon fragments	Two-carbon fragments	Oxalic acid
Carrier (mmoles)	1·2	1·0	1·0	2·0	1·65
Specific activity (μc/mmole)...	0·02	0·058	0·018	0·0007	0·0036
Yield (mmoles)	0·7	1·6	1·0	0·6	0·1

D-Erythrose, estimated by paper chromatography, amounted to 1·37 mmoles.

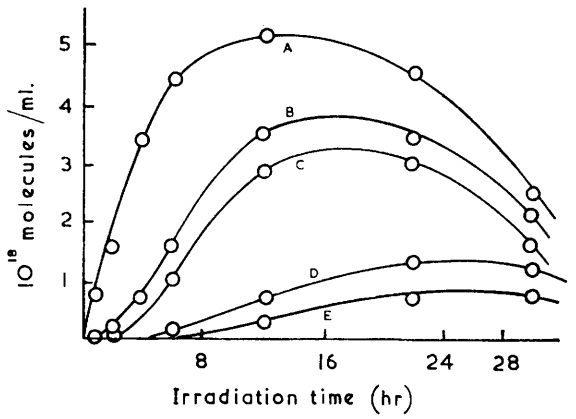


FIG. 3. Products formed during the ultraviolet irradiation of D-sorbitol solutions, estimated by isotope dilution analysis.

A, D-Glucose. B, D-Gluconic acid. C, D-Arabinose. D, Three-carbon aldehydic fragments. E, Two-carbon aldehydic fragments.

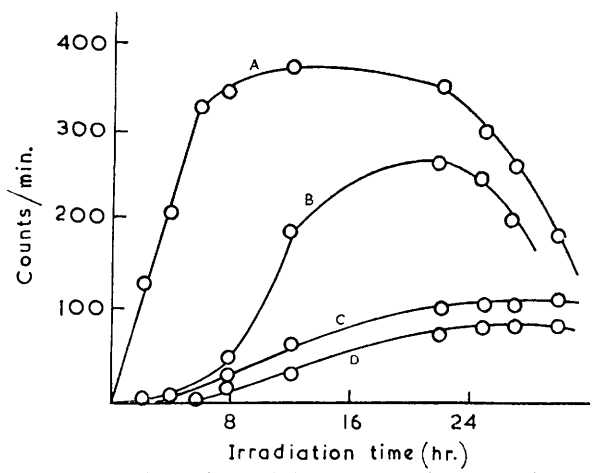


FIG. 4. Products formed during ultraviolet irradiation of D-sorbitol solutions, estimated by paper chromatography.

A, Hexose. B, Pentose. C, Product 1. D, Products 2 and 3.

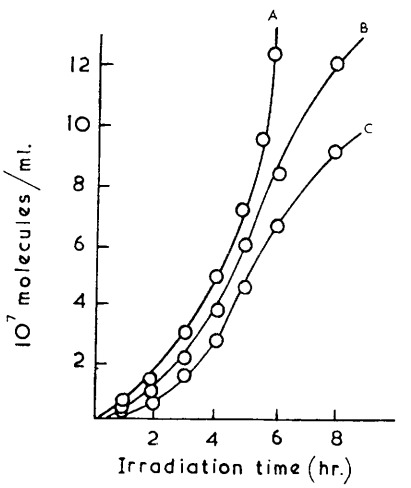


FIG. 5. Formation of carbon dioxide during the ultraviolet irradiation of oxygenated D-sorbitol solutions.

A, 5×10^{-1}M. B, 5×10^{-2}M. C, 5×10^{-3}M.

Two-carbon aldehydic fragments. The irradiated solution (5 ml.) was treated with carrier glyoxal (2·0 mmoles), acetic acid (1 ml.), and phenylhydrazine (1·5 ml.). The solid which separated was recrystallised eight times from benzene, to give pure glyoxal bisphenylhydrazone with m. p. 170° and constant specific activity 0·0036 μc/mmole.

Oxalic acid. The irradiated solution was treated with carrier oxalic acid (1·65 mmoles), and pure oxalic acid dihydrate with m. p. 99° and constant specific activity 0·0007 μc/mmole was recovered. The results are shown in Table 3.

Rate of Formation of Products.—To establish yield–dose curves for the main products, isotope dilution analysis was used, after increasing irradiation doses had been applied to the solution, as follows: A solution (500 ml.) of D-sorbitol (27·5 mmoles) and specific activity 3·6 μc/mmole was irradiated as described above and samples (30 ml.) were withdrawn at periods up to 30 hr. As described above, D-sorbitol, D-glucose, D-gluconic acid, and two- and three-carbon fragments were estimated and the results are shown in Fig. 3. Table 4 shows the rate of degradation of D-sorbitol with increasing dose.

Yield–dose curves were also obtained by paper chromatography for the pentose and unidentified products 1, 2, and 3 (Table 1). Accurately known volumes (0·05 ml.) of the

TABLE 4.

Rate of degradation of D-sorbitol during irradiation with ultraviolet light.

Time (hr.)	0	2	4	6	8	12	20	30
D-Sorbitol (10^{18} molecules/ml.)...	33·0	30·2	25·8	21·8	18·4	16·3	8·2	2·4

irradiated solution, after successively increasing doses, were applied to paper strips and irrigated with butan-1-ol–pyridine–water (10 : 3 : 3); the radioactivity of the spots, was measured (Fig. 4).

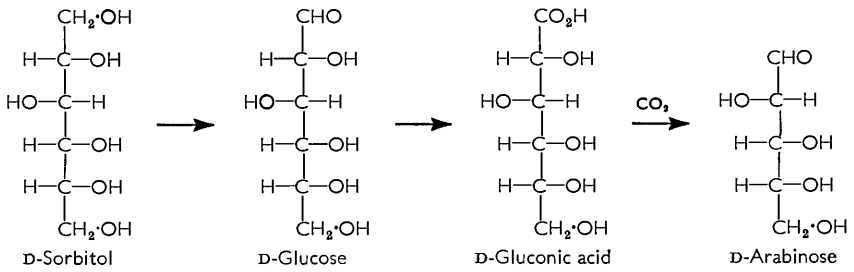
Carbon dioxide formation. The oxygen stream from the irradiated solution was passed into a series of barium hydroxide traps, and the amount of carbon dioxide produced was measured gravimetrically as carbonate. The formation of carbon dioxide during irradiation of 5×10^{-3} to 5×10^{-1}M-D-sorbitol solutions are shown in Fig. 5.

Effect of Ozone on Aqueous D-Sorbitol Solutions.—When a solution (250 ml.) of D-sorbitol (13·75 mmoles) was treated with ozonised oxygen (*ca.* 5% ozone) for 30 hr., the amount of chemical change, as indicated by paper chromatography and carbon dioxide evolution, was insignificant.

DISCUSSION

Unfiltered light from a Hanovia 220 w medium-pressure mercury lamp initiates degradation of D-sorbitol in oxygenated solution in the absence of a photo-sensitiser. The degradation is rapid, as indicated by the continuous liberation of carbon dioxide, and the numerous oxidation and degradation products revealed by paper chromatography. Hexoses, hexonic acids, pentoses, and tetrose were identified (Table 1), and isotope dilution analysis confirms these observations (Table 3). D-Glucose and D-arabinose are formed by oxidation at one extremity of the molecule; the second pentose present is L-xylose. Therefore, on configurational grounds, it may be inferred that gulose, identified by paper chromatography, is present as the L-isomer.

Yield–dose curves indicate that the hexoses are initial products of the degradation. Thereafter, from the results, it is probable that degradation proceeds by way of the hexonic acid to the pentose. Hexonic acid formation proceeds faster than pentose formation, but slower than the initial formation of hexose. A slow initial production of hexonic acid occurring simultaneously with hexose production is not precluded by the results, but as the irradiation progresses, there is an increased rate of hexonic acid production by a secondary process. For irradiation periods up to *ca.* 6 hr. the D-sorbitol destroyed may be



accounted for in terms of the amounts of hexoses, hexonic acids, and pentoses formed. The equivalence of carbon dioxide liberation with total pentoses formed in up to *ca.* 6 hours' irradiation indicates that the pentoses arise by decarboxylation of the hexonic acids. The initial stages of the degradation may therefore proceed as annexed. From the other extremity of the molecule, the corresponding L-series of products may be formed.

The increased number of products present after prolonged irradiation points to alternative secondary degradative paths from the hexoses. The results indicate that the secondary processes lead to fragments containing smaller numbers of carbon atoms than the six of hexoses. From the yield–dose curves, successively lower fragments appear to be formed by stepwise degradation. Previously, a similar route was envisaged in the photochemical degradation of starch.[10] The ultimate product is carbon dioxide, and if irradiations are sufficiently prolonged the solution becomes entirely depleted of D-sorbitol. In this respect, therefore, the photo-degradation may be regarded as a reversal of photosynthesis.

The pattern of photo-degradation of D-sorbitol differs somewhat from the γ-ray degradation of D-sorbitol in aqueous solution, although there are similarities in the products formed by the two types of radiation. It is significant, also, that the amount of carbon dioxide formed during γ-irradiation is very small. A common feature, however, is the characteristic absorption maximum at 265 mμ.

In the present investigation, unfiltered light was used, and the solutions became hot during irradiation. Secondary degradation by purely thermal reactions may thus occur. In this connection we have examined the effect of ozone on aqueous D-sorbitol solutions, and ascertained that negligible degradation arises by this route.

Primary processes which initiate the photo-degradation of D-sorbitol are discussed in 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 from U.K.A.E.A. (to W. J. C.) is gratefully acknowledged.

UNIVERSITY COLLEGE, CATHAYS PARK, CARDIFF. [*Received, January 21st*, 1963.]

[10] Whelan and Peat, *J. Soc. Dyers Colourists*, 1949, **65**, 165.