The Photochemistry of Phosphorus Compounds. Part 11.[1] Photolysis of Dipotassium α-D-Glucose 1-Phosphate in Aqueous Solution under Argon and Oxygen

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The stable products of the photolysis of dipotassium α-D-glucose 1-phosphate at 254 nm were determined in either anoxic (under argon) or oxygen-saturated aqueous solutions. The release of orthophosphate was found to be the primary and predominant reaction in both argon and oxygen, with quantum yields of $(1.04 \pm 0.02) \times 10^{-2}$ and $(3.56 \pm 0.01) \times 10^{-2}$, respectively. Glucose was not observed. Under argon, the most important organic products identified were deoxy-sugars and malondialdehyde. In oxygenated solutions, carbon dioxide and monoxide, gluconate, arabinose, glyoxal, and glycolate were the major products. A tentative mechanism for the primary photochemical step may be the release of orthophosphate from the glucose 1-phosphate dianion, producing a glycosyl radical, which then undergoes further degradation.

α-D-GLUCOSE 1-PHOSPHATE, a crucial intermediate in carbohydrate metabolism, was shown to have a weak absorption band in the 220—260 nm region in aqueous solution, which was assigned to an internal electronic transition, and a steeply rising absorption below 220 nm, assigned to a ' charge-transfer-to-solvent-transition '.[2] The degradation of sugar phosphates by ionizing radiations has been the subject of several studies;[3,4] however, only limited data have been reported on the photolysis of these compounds by u.v. light.[1,5] It has been shown in the radiolytic studies that the phosphate ester link at the glycosidic C-1 of glucose is more labile to radiation than that at the primary 6-position of glucose.[3a] In the photolysis of glucose 6-phosphate we found that the reaction is not merely a simple release of orthophosphate, and that glucose 6-phosphate undergoes degradation to other sugar phosphates of shorter chain length.[1] In the present work we irradiated α-D-glucose 1-phosphate at 254 nm, a region in which the photolysis of water is negligible, and in which the effect of light is therefore directly on the sugar phosphate. Argon was chosen as an inert gas to study the photolysis in the absence of oxygen, and to compare with the results in solutions saturated in oxygen gas.

EXPERIMENTAL

Materials.—Dipotassium α-D-glucose 1-phosphate (Sigma) was used without further purification; water used as

[1] Part 10, C. Triantaphylides and M. Halmann, *J.C.S. Perkin II*, 1975, 34.

[2] M. Trachtman and M. Halmann, *Carbohydrate Res.*, 1971, **19**, 245.

[3] N. K. Kotchetkov, L. I. Kudryashov, M. A. Chlenov, and L. P. Grineva (*a*) *Zhur. obschei Khim.*, 1971, **41**, 2071; (*b*) *Doklady Chem.*, 1972, **202**, 115; (*c*) *Carbohydrate Res.*, 1974, **35**, 235.

[4] L. Stelter, C. von Sonntag, and D. Schulte-Frohlinde, *Internat. J. Radiation Biol.*, 1974, **25**, 515; *Z. Naturforsch.*, 1975, **30***b*, 609; 656; C. L. Greenstock and E. Shierman, *Internat. J. Radiation Biol.*, 1975, **28**, 1.

[5] H. Trapman and M. Devani, *Naturwiss.*, 1965, **52**, 208.

solvent in the photolysis was redistilled; malondialdehyde was prepared by heating a weighed amount (*ca.* 0.2 g) of 1,1,3,3-tetraethoxypropane (Fluka) with concentrated hydrochloric acid (3 ml) for 1—3 min at 45—50°, releasing the malondialdehyde quantitatively.[6] α-D-[*U*-^{14}C]Glucose 1-phosphate was obtained from the Radiochemical Centre, Amersham.

Absorption Spectra.—These were measured using a Zeiss PMQII spectrophotometer.

Irradiations.—The procedures were similar to those described previously.[1,7] A medium pressure mercury lamp (Hanau Q-81) was fitted into a double-jacketted quartz photochemical reactor. Distilled water maintained at 30 ± 1° was circulated between the lamp and the reaction solution, in a jacket of *ca.* 1 cm width, to assure a constant temperature and also to filter out the 185 nm emission of the lamp. The irradiated sugar phosphate solution (usually 0.01M) had an optical path of *ca.* 1 cm and a volume of *ca.* 150 ml. It was stirred by a gas stream, either argon [bubbled through solutions of (a) sodium dithionate (15% w/v) and (b) of indigo carmine (1.5% w/v) in 10% KOH in order to remove traces of oxygen and carbon dioxide] or oxygen (bubbled through aqueous saturated barium hydroxide to remove traces of carbon dioxide).[8] The gases entered at the bottom of the reaction vessel through a sintered glass disc. Samples were removed without interrupting the irradiated solutions, using a syringe pierced through a rubber septum fitted in a side port of the reactor.

For the photolysis of solutions of [^{14}C]glucose 1-phosphate, aqueous solution (2 ml; 0.01M in glucose 1-phosphate; 20 μCi mmol^{-1}) was placed in a quartz tube surrounded by a quartz jacket through which distilled water was circulated at 30 ± 1°. A small glass inlet tube permitted the passage of a gas (argon or oxygen) through the irradiated solution. The apparatus was illuminated from the outside with a low pressure mercury lamp (Ultraviolet Products, model PCQX-1).

Quantum Yields for Orthophosphate Formation.—These were measured at 253.7 nm using a low pressure mercury lamp (Thermal Syndicate, model T/MS/544) with its flat end pressed against the polished window of a cylindrical quartz cell of 5 cm optical path, which was filled with water to filter out the 185 nm emission of the lamp. Subsequently, the light passed through a similar 5 cm long quartz cell containing the reaction mixture.[9,10]

For the determination of the light flux the reaction cell was filled with 0.1M-chloroacetic acid. The photolytic release of chloride ion was determined by the titration of chloride ions with mercuric nitrate (0.01M), using diphenylcarbazone as an indicator. The quantum yield for the release of chloride ion in this reaction at 253.7 nm and 25° was taken as $\phi = 0.33$.[10] The average of two measurements gave $I = 2.12 \times 10^{-4}$ einstein l^{-1} min^{-1} for the light flux in the irradiation vessel.

For the determination of quantum yields, the same reaction vessel was filled with a 0.05M solution of glucose 1-phosphate, while a slow stream of either argon or oxygen was passing through the solution from a thin glass capillary. The irradiation time was chosen so as to keep the extent of decomposition to < 1%. The quantum yield was derived from the equation $\phi = n/[It(1-10^{-\epsilon Ad})]$, where n = number of moles of orthophosphate released, $I = 2.12 \times 10^{-4}$ einstein l^{-1} min^{-1} (the light flux), t = irradiation time in min, $\varepsilon = 0.50$ l mol^{-1} cm^{-1} (the molar absorptivity of aqueous glucose 1-phosphate at 254 nm), $A = 0.05$M (concentration of glucose 1-phosphate), and $d = 5.0$ cm (optical path).

Analysis of Products.—Orthophosphate was determined by the method of Fiske and Subbarow.[11]

Carbon dioxide. The gas issuing from the reactor was passed *via* a three-way stopcock through two barium hydroxide absorption flasks (100 ml; 0.02M) in series. After a measured interval the stopcock was turned to lead the gas stream into an alternative train of barium hydroxide absorption flasks, while portions of the first two flasks were titrated with 0.1N-hydrochloric acid. The procedure was repeated periodically.

Acid formation. The acid produced during the photolysis was determined by the difference in the titration result of 0.01N-sodium hydroxide (phenolphthalein) before and after photolysis.

Malondialdehyde and glyoxal. These were qualitatively detected and quantitatively determined by the 2-methylindole reaction.[6] Malondialdehyde in neutral solutions produced a deep red colour with $\lambda_{max.}$ 555 nm, whereas glyoxal produced a brown-red colour with $\lambda_{max.}$ 505 nm. Both compounds were assayed in the photolytic solutions by comparison with standard curves each time from fresh authentic materials. Glyoxal was also determined as a partially volatile component, b.p. 50.4°, in the barium hydroxide traps used to measure the production of carbon dioxide during the photolysis under oxygen. In the presence of barium hydroxide, the brown-red colour upon addition of 2-methylindole required *ca.* 16 h to develop, whereas in the absence of barium hydroxide, about 10 min were sufficient. In the barium hydroxide containing solutions, the absorption maximum for the photolysis product with 2-methylindole was shifted to 482 nm. The same effect of ' blue-shift ' and delay in colour development was found in the reaction with authentic glyoxal.

Glycolic acid. The photolysed sample was treated with a solution of 2,7-dihydroxynaphthalene (0.01%) in concentrated sulphuric acid and heated at 100° for 20 min.[12a] A violet-red colour was formed, $\lambda_{max.}$ 530 nm.

The spectrum of the samples photolysed under oxygen indicated the initial absence of glycolic acid (within 2 h), its appearance as a ' shoulder ' after 6 h, and its becoming the dominant feature after 15.5 and 21 h. The measurements also indicated the absence of glycolic acid after the photolysis under argon.

Carbon monoxide. Its presence was detected by the passage of the effluent gas through a wash-bottle containing equal volumes of palladium chloride–phosphomolybdic acid reagent and acetone heated at 60°. The yellow colour of the reagent changed to blue-green.[12b] For quantitative determination the carbon monoxide was converted to carbon dioxide using iodine pentaoxide.[8,12c]

[6] H. Scherz, G. Stehlik, E. Bancher, and K. Kaindl, *Mikrochim. Acta*, 1967, 916.
[7] H. P. Benschop and M. Halmann, *J.C.S. Perkin II*, 1974, 1175.
[8] A. I. Vogel, ' Quantitative Inorganic Analysis,' Longmans, London, 1961, 3rd edn., p. 1080.
[9] J. Greenwald and M. Halmann, *J.C.S. Perkin II*, 1972, 1095.
[10] J. G. Calvert and J. N. Pitts, ' Photochemistry,' Wiley, New York, 1966, p. 787.
[11] L. F. Leloir and C. E. Cardini, in ' Methods in Enzymology,' eds. S. P. Colowick and N. O. Kaplan, Academic Press, New York, 1957, vol. 3, p. 543.
[12] F. D. Snell and C. T. Snell, ' Colorimetric Methods of Analysis,' Van Nostrand, New York, 1957, 3rd ed., (*a*) vol. 3, p. 328; (*b*) vol. 2, p. 838; (*c*) vol. 2, p. 829.

Paper chromatography was done in the descending mode, using Whatman No. 3 chromatography paper. Detection of phosphorus compounds was made with molybdate spray,[13a] reducing sugars were detected with aniline hydrogen phthalate spray [13b] or with silver nitrate dip,[13c] while

TABLE 1

Movements of various compounds relative to glucose 1-phosphate in chromatography solvent systems I—III

System	I	II	III
Orthophosphate	4.4		
Glucose *	6.1—7.3	4.7	11.1
Deoxyglucose	10.2—12.1		16.5
Fructose *	6.0	6.1—8.2	
Xylose	7.1—9.6	8.2—11.6	
Arabinose	6.5—8.7	6.9—9.5	12.3
Ribose *	10.8		
Gluconate	4.7—5.5	4.4—5.0	8.6
Glucuronate *	4.3—4.8	3.1—3.6	8.0
Deoxyribose	12.9—14.5		24.0

* These compounds were not detected as photolysis products.

gluconic acid was detected with sodium periodate [13d] and bromophenol [13e] sprays. Quantitative yields of arabinose, gluconic acid, deoxyribose, and deoxyglucose were measured by radiochromatography, after photolysis of ^{14}C-labelled

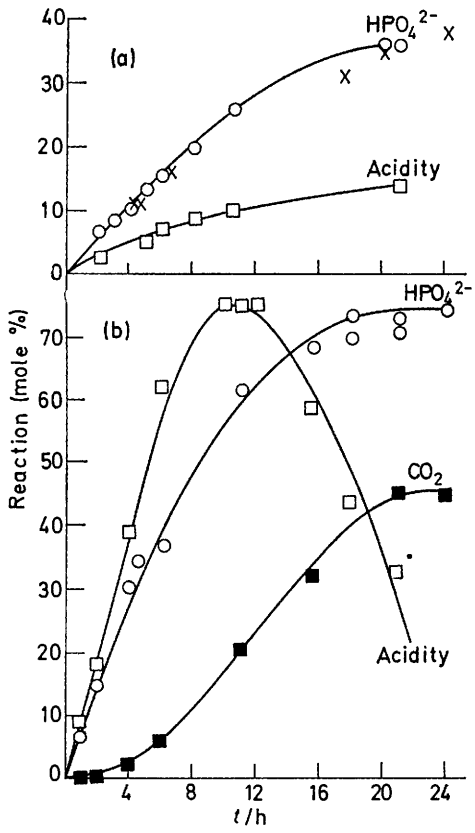


FIGURE 1 Photolysis of potassium α-D-glucose 1-phosphate: yields of orthophosphate, acidity, and carbon dioxide as a function of time (a) under argon, (b) under oxygen

glucose 1-phosphate. The dried paper was cut into strips (1 × 3 cm) which were counted for ^{14}C by liquid scintillation. Chromatography solvent systems used were: I, n-butanol–acetic acid–water (4 : 1 : 1); II, ethyl acetate–acetic acid–water (9 : 2 : 2); III, pyridine–ethyl acetate–water (11 : 40 : 6). Movements of the various compounds

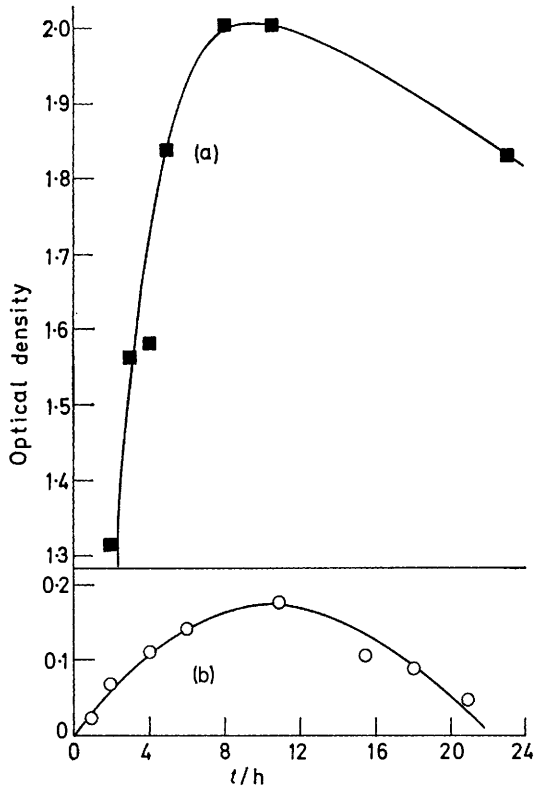


FIGURE 2 Optical density at 270 nm as a function of time during the photolysis of glucose 1-phosphate (0.01M): (a) under argon; (b) under oxygen

tested relative to glucose 1-phosphate are given by Table 1.

T.l.c. was carried out on silica gel plates (Riedel-De Haen, Hannover), with the solvent systems: A, ethyl acetate–acetone–water (4 : 5 : 1) [14a] for detection of gluconolactone and 2-deoxygluconolactone (R_F 0.5 and 0.6, respectively), using successive sprays of hydroxylamine and ferric chloride; [14b] B, pyridine–ethyl acetate–water (11 : 40 : 6) for detection of glucose, ribose, arabinose, 2-deoxyglucose, and 2-deoxyribose (R_F 0.14, 0.32, 0.20, 0.28, and 0.37 respectively), using a diphenylamine–aniline spray; [14c] C, ethyl acetate–methanol (2 : 3) for detection of 2-deoxyglucose and 2-deoxyribose (R_F 0.74 and 0.69 respectively), using successive dips in periodic acid and thiobarbituric acid.[14d]

RESULTS

(a) *Photolysis in an Argon Atmosphere.*—The primary degradation process of glucose 1-phosphate under u.v. irradiation appears to be the release of orthophosphate (Figure 1a). Very little carbon dioxide (only *ca.* 3% within

[13] I. M. Hais and K. Macek, 'Paper Chromatography,' Publishing House of the Czechoslovak Academy of Science, Prague, 1963 (*a*) p. 819; (*b*) p. 793; (*c*) p. 782, (*d*) p. 791; (*e*) p. 783.

[14] (*a*) M. Dizdaroglu and C. v. Sonntag, *Z. Naturforsch.*, 1975, **28***b*, 635; (*b*) M. Abdel-Akher and F. Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859; (*c*) S. A. Hansen, *J. Chromatog.*, 1975, **107**, 224; (*d*) P. J. Anderson, *ibid.*, 1966, **21**, 163.

21 h) is evolved concurrently with the release of orthophosphate. The yield of carbon monoxide is even lower. The photolysis in an argon atmosphere produces acid at a much slower rate than that produced in an oxygen atmosphere (Figure 1b). The acid production in the argon atmosphere is delayed, but becomes significant after prolonged irradiation, reaching 14% within 21 h. This is in agreement with the results observed with glucose 6-phosphate, in which in the first few hours of photolysis under nitrogen only trace quantities of acid were released, while photolysis under oxygen caused immediate and major production of acids.[1]

diate production of orthophosphate and of acid (Figure 1b). The release of orthophosphate is more immediate in the photolysis of glucose 1-phosphate than that obtained under similar conditions for glucose 6-phosphate.

The release of orthophosphate levels off after 18 h whereas the formation of acid attains a maximum after 11 h and then decreases rapidly.

There is an induction period of *ca.* 2 h before the production of carbon dioxide becomes significant, indicating that dephosphorylation may be the primary step in the photolysis. The rate of acid formation is more rapid than that of carbon dioxide, indicating that after dephosphorylation an

TABLE 2

Yields (mole %) of products of photolysis of glucose 1-phosphate (0.01M) by a medium pressure Hg lamp

	Photolysis under Ar					Photolysis under O_2					
t/h	HPO_4^{2-}	CO_2	Acids	CO	MDA [a]	HPO_4^{2-}	CO_2	Acids	CO	Glyoxal	Glycolate
1						6.7	<0.03				
2	6.3	<0.03	2.5			14.8	<0.03	18.0		2.9	
4	10.3	<0.03				30.3	2.4		0.6	9.3	
4.25					0.41						
6	15.3	<0.03		<0.03		36.9	6.1		1.1		
6.5					0.54						
8	19.7	<1.0		Trace							
10.5	26.1	1.0	10.0								
11						61.5	20.8	75.0			
15.5						68.5	32.5			18.0	2.5
17.5					0.48						
21	36.1	2.7	14	1.2		73.1	45.8	33.0	7.0	6.2	3.2
24	37.7	2.8		2.2	0.43	74.6	45.4		7.6		

[a] MDA = Malondialdehyde.

TABLE 3

Yields of products (mole %) after photolysis of ^{14}C-labelled glucose 1-phosphate (0.01M) at 254 nm

	Photolysis under Ar			Photolysis under O_2		
t/h	HPO_4^{2-}	Deoxyglucose	Deoxyribose	HPO_4^{2-}	Arabinose	Gluconate
3.5					0.38	
4.5					0.70	
10				4.0	1.29	0.18
21		0.22			1.03	0.20
24					1.23	0.35
26		0.31		8.2	1.55	0.39
47		0.33	0.49			
51		0.34				
70	7.8	0.48	0.73			
90	10.6	0.48	0.66			

The photolysis of aqueous solutions of glucose 1-phosphate resulted in the formation of a product absorbing in the 265—270 nm region (Figure 2), similar to that obtained during the photolysis of glucose 6-phosphate,[1] as well as the γ-radiolysis [15] and photolysis [16] of aqueous solutions of carbohydrates. This compound was identified as malondialdehyde, using the specific colour reaction with 2-methylindole.[6]

Paper chromatography of the photolysed solution showed the presence of traces of deoxy-sugars, in yields reaching about 1% after *ca.* 90 h. By t.l.c. it was proven that the photolysis products do not include 2-deoxygluconolactone or gluconolactone (<0.3% lactone formed after 70 h illumination).

It should be noted that while u.v.-photolysis of glucose 1-phosphate does produce extensive dephosphorylation, the bulk of the carbon products have not been identified; about one-third of these are acid products.

(b) *Photolysis in an Oxygen Atmosphere.*—Illumination of aqueous glucose 1-phosphate at 254 nm caused the immediate acid is formed, followed by decarboxylation. The formation of products absorbing in the 265—270 nm region after illumination under oxygen was very weak (Figure 2).

After 5—10% release of orthophosphate had occurred, gluconate, arabinose, carbon dioxide, and glyoxal were the main primary products detected. Secondary decomposition of these initial products then became significant, as noted by the formation of glycolate after 15.5 h of photolysis, whereas initial measurements indicated the absence of this compound. Carbon monoxide, which increased linearly (see Table 2) may also be a secondary product.

(c) *Quantum Yields for Orthophosphate.*—The values for the quantum yield of orthophosphate release from glucose 1-phosphate irradiated at 254 nm are in Table 4. The quantum yields are seen to be very low in the argon atmosphere, but to be almost four times higher in oxygenated

[15] (*a*) J. Morre, *Compt. rend.*, 1967, **462**, 2650; (*b*) H. Scherz, *Experientia*, 1968, **24**, 420; (*c*) P. Seidler and C. von Sonntag, *Z. Naturforsch.*, 1969, **24***b*, 780.

[16] H. Scherz, *Carbohydrate Res.*, 1970, **14**, 417.

solutions. The above values are similar to those previously reported for the photolysis of ethyl dihydrogen phosphate,[17] trimethyl phosphate,[7] and glycerol 1- and 2-phosphate.[9]

TABLE 4

Quantum yields for release of orthophosphate from glucose 1-phosphate

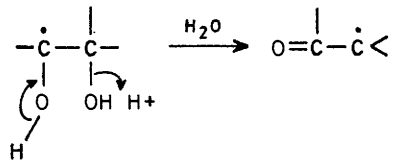
Atmosphere	Argon	Oxygen
Irradiation time (h)	12	4
Quantum yield ($10^2\phi$) [a]	1.04 ± 0.02	3.56 ± 0.01

[a] Average value of four experiments.

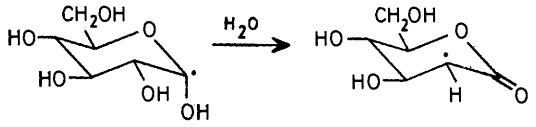
DISCUSSION

Photolysis in an Argon Atmosphere.—In the u.v. photolysis of aqueous glucose 1-phosphate, a product with an absorption maximum at 265 nm was found, which on the basis of its specific colour reaction with 2-methylindole was identified as malondialdehyde, $CH_2(CHO)_2$. Previous work on the u.v. irradiation of aqueous solutions of D-glucose and of other sugars in the absence of oxygen showed similar absorption characteristics.[18] In the present study we found that the photolysis of glucose 1-phosphate under argon also yielded deoxysugars, very tentatively identified as deoxy-ribose and -glucose. It is possible that deoxy-compounds may be formed after dephosphorylation in a manner similar to that described for the β- and γ-ray irradiation of aqueous solutions of polyhydroxy-compounds, in which it was shown that the formation of free malondialdehyde is strongly pH dependent, and that in an acid medium no free malondialdehyde is obtained.[15,16,18] The formation of deoxy-compounds and free malondialdehyde seems to be a general reaction occurring upon irradiation of open chain or cyclic carbohydrate molecules. It was proposed that OH radicals are the main precursors responsible for the formation of malondialdehyde and deoxysugars during radiolysis,[18] the formation of these compounds being particularly favoured by the CH_2OH–$CHOH$–$CHOH$– configuration, involving the non-aldehyde end of the molecule.

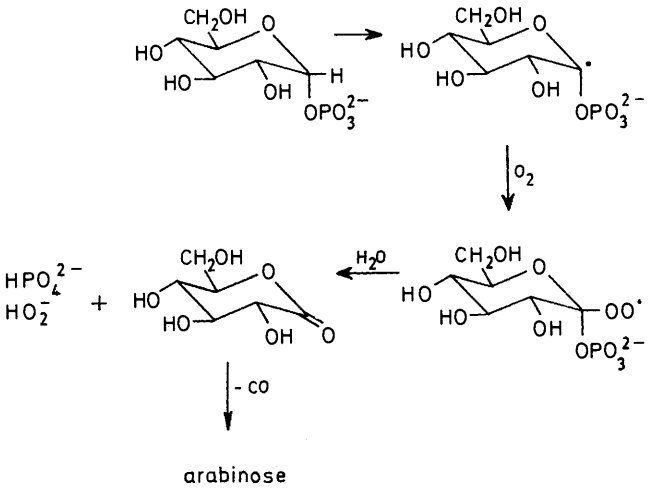
In the radiolysis of hexoses in aqueous solutions, hexose radicals were reported to be primary products.[19] Under deoxygenated conditions, a major proportion of the radicals were dehydrated to deoxyhexosuloses.[20,21] Evidence on the fate of the primary radicals formed in the radiolysis of polyhydroxy-compounds was obtained by e.s.r. spectroscopy.[22] Thus, in the radiomimetic oxidation of monosaccharides (by $^{\bullet}OH$ radicals derived from Ti^{III}–H_2O_2), the primary radicals, produced by hydrogen abstraction, underwent rapid acid-catalysed elimination of water to produce carbonyl-conjugated radicals, which were stable enough to be detected by e.s.r.[22a]



In the case of D-glucose, the preferred point of the initial hydrogen abstraction was at the hemiacetal C-1; water elimination then produced the 2-deoxygluconolactone radical, observed by e.s.r. However, radical



formation occurred also at the other carbon atoms of glucose, as indicated by the nature of the final reaction products.[20,21] In the photolysis of glucose 1-phosphate, a similar glucosyl radical may be formed by phosphate elimination, and may lead to deoxysugars. An analogous phosphate elimination has been detected by e.s.r. in the radiolysis of glycerol 2-phosphate, leading from an initial glycerol 2-phosphate radical to the observed 2-deoxy-radical $CH(O)$–CH–CH_2OH.[22b] However, the photolysis of glucose 1-phosphate did not yield 2-deoxy-gluconolactone as a major product, which would have



been expected on the basis of such a mechanism, by analogy with the results obtained in the radiolysis of D-glucose.[20,21]

Photolysis in an Oxygen Atmosphere.—While orthophosphate elimination was the initial observed process in the photolysis of glucose 1-phosphate, D-glucose was not formed as a product. Nevertheless, the photolysis of glucose 1-phosphate shows certain similarities with

[17] M. Halmann and I. Platzner, *J. Chem. Soc.*, 1965, 5380.
[18] H. Scherz, *Radiation Res.*, 1970, **43**, 12.
[19] G. O. Phillips, W. Griffiths, and J. V. Davies, *J. Chem. Soc.* (*B*), 1966, 194.
[20] (*a*) V. Hartmann, C. von Sonntag, and D. Schulte-Frohlinde, *Z. Naturforsch.*, 1970, **25b**, 1394; (*b*) M. Dizdaroglu, D. Henneberg, G. Schomburg, and C. von Sonntag, *ibid.*, 1975, **30b**, 416.
[21] S. Kawakishi and M. Namiki, *Carbohydrate Res.*, 1973, **26**, 252; S. Kawakishi, Y. Kito, and M. Namiki, *ibid.*, 1973, **30**, 220; 1975, **39**, 263.
[22] R. O. C. Norman and R. J. Pritchett, *J. Chem. Soc.* (*B*), 1967, 1329; A. Samuni and P. Neta, *J. Phys. Chem.*, 1973, **77**, 2425.

the photolysis of D-glucose, in which the main products after 10—20% decomposition were gluconic acid, arabinose, and a tetrose.[23]

The more rapid photolytic degradation of glucose 1-phosphate, by comparison with glucose 6-phosphate,[1] may possibly be attributed to the more labile nature of the glycosidic ester bond relative to the primary ester bond of glucose 6-phosphate. Similar results had been reported in the radiolysis of sugar phosphates.[3]

A tentative mechanism of the oxidative photolysis is the initial formation of a glucosyl phosphate radical, which traps an oxygen molecule to form a peroxyl radical, which then is hydrolysed to gluconolactone, which more slowly undergoes decarbonylation to arabinose. The photolysis and radiolysis [3] of glucose 1-phosphate also show similarity in that the main degradation process seems to be the direct rupture of the phosphate ester link. By contrast, in the radiolysis [3] and photolysis [1] of glucose 6-phosphate, there are other major primary reactions, resulting in dehydrogenation as well as carbon–carbon bond breakage, but preserving the phosphate ester bond.

[5/1585 *Received*, 11*th August*, 1975]

[23] G. O. Phillips and T. Rickards, *J. Chem. Soc.* (*B*), 1969, 455.