The Photochemistry of Phosphorus Compounds. Part VII.[1] The Far-ultraviolet Spectroscopy and Photochemistry of Glycerol 1-Phosphate and of Glycerol 2-Phosphate in Aqueous Solutions

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The u.v. absorption of the disodium salts of both glycerol 1- and of glycerol 2-phosphate in aqueous solutions exhibits a weak absorptivity from 300 to 220 nm, which is almost unaffected by changes in temperature or by replacement of water with D_2O as a solvent. Below 220 nm, the absorptivity increases sharply. This steep absorption edge is red-shifted with increasing temperature; in D_2O solution it is shifted towards shorter wavelengths. On the basis of these environmental effects, the weak absorption in the 300—220 nm region is assigned to an internal electronic transition, while the intense absorption band below 220 nm is assigned to a ' charge-transfer-to-solvent transition '.

The photochemistry of the glycerophosphates was studied by irradiation of aqueous solutions at 253·7 nm. In a nitrogen atmosphere, the quantum yield for release of orthophosphate both from glycerol 1- and from glycerol 2-phosphate was $\phi = 0{\cdot}01$. In an oxygen atmosphere, the quantum yield increased to $\phi = 0{\cdot}023$ and 0·030 for glycerol 1- and glycerol 2-phosphate, respectively. Photolysis of glycerol 1-phosphate in $H_2{}^{18}O$ in evacuated ampoules resulted in 98% C—O bond fission. Both in nitrogen and in oxygen atmospheres, an acid- and alkali-labile phosphate ester was formed as an initial product, which disappeared upon further photolysis, and which was identified as dihydroxyacetone phosphate. The yield of hydrogen produced by photolysis of glycerol 1-phosphate in evacuated tubes was only 10% of that equivalent to the orthophosphate released. The main non-volatile products in the photolysis in evacuated tubes of ^{14}C-labelled glycerol 1-phosphate were glycidol (40% of the orthophosphate released), glyceraldehyde or dihydroxyacetone (10%), and glycollic acid (9%). Glycollic acid and carbon dioxide were released only in traces by photolysis of both glycerol 1- and glycerol 2-phosphate in a nitrogen atmosphere, but evolved readily in an oxygen atmosphere. Glycidol itself in aqueous solutions was found to undergo photolysis to glycollic acid and carbon dioxide, very slowly under nitrogen and in evacuated tubes, but immediately in oxygenated solutions.

THE photochemical decomposition of glycerol 1-phosphate, an important intermediate of lipid metabolism, as well as of glycerol 2-phosphate, has been briefly described; it has been found to occur more readily than that from unsubstituted alkyl phosphates.[2] In contrast to hydrolysis,[3] the photolytic release of orthophosphate was lowest at pH 4, occurring more readily both at higher and lower pH values. In previous studies of inorganic phosphorus oxyanions,[4,5] ethyl dihydrogen phosphate,[6] α-D-glucose 1-phosphate, and glucose 6-phosphate,[1] photochemical effects in the 300—200 nm region were shown to be due to an internal electronic transition, while the effects in the 200—180 nm region were found to be due to a ' charge-transfer-to-solvent ' transition, with formation of the hydrated electron. The purpose of the present work is a study of the photochemistry of glycerol 1- and of glycerol 2-phosphate, by examining their far-u.v. absorption spectra, and by identifying and determining the photolysis products.

EXPERIMENTAL

Materials.—DL-α-Glycerophosphate, disodium salt, hexahydrate (Sigma, Grade X) was recrystallized (to remove traces of glycerol) by dissolution in a minimal amount of hot water followed by addition of ethanol until the appearance of turbidity; the solution was then kept for 24 h at −6° when crystals forming on the walls of the flask were found to be free of glycerol.

β-Glycerol phosphate, disodium salt, pentahydrate (Sigma, Grade I), α-glycero-UL-C-14-phosphate (International Chemical and Nuclear Corp.), deuterium oxide (Norsk Hydro-Elektrisk), and glycidol (Fluka AG) were used without further purification. ^{18}O-Enriched water (from the separation plant of this Institute) was redistilled in a vacuum system.

Absorption Spectra.—These were recorded as previously described,[1] with a Zeiss PMQII spectrophotometer specially fitted for the far-u.v. region. For experiments below 200 nm, the whole optical path was flushed with nitrogen gas. Fluorescence spectra were taken with a Heathkit spectrophotometer fitted with two monochromators.†

Photochemical Light Sources.—(a) A high-pressure mercury immersion lamp (Hanau Q-81) was used, in a previously described multi-jacketted irradiation assembly built of concentric quartz tubes,[6] in which a 0·5-cm layer of distilled water (thermostatted at 30 ± 1°) circulated continuously between the light source (the central tube) and the irradiated solution (the outermost tube). The water layer served both for cooling and to filter out the far-u.v. emission of the lamp. Thus, most of the light reaching the reacting solution is at 254 nm. The irradiated solution layer is *ca.* 1 cm thick, and its volume is 100—150 ml. An inlet tube fitted with a sintered glass disk at the bottom of the solution enabled bubbling of a gas (usually nitrogen or oxygen) through the irradiation region.

(b) A low-pressure mercury lamp (Thermal Syndicate, model T/M5/544) was used mainly for the actinometry

† We are indebted to Dr. Meir Shinitsky for kindly measuring the fluorescence spectra.

[1] Part VI, M. Trachtman and M. Halmann, *Carbohydrate Research*, 1971, **19**, 245.

[2] E. Bamann, K. Gubitz, and H. Trapmann, *Arch. Pharm.*, 1961, **294**, 240.

[3] L. Kugel and M. Halmann, *J. Amer. Chem. Soc.*, 1966, **88**, 3566.

[4] M. Halmann and I. Platzner, (*a*) *Proc. Chem. Soc.*, 1964, **261**; (*b*) *J. Chem. Soc.*, 1965, 1440; (*c*) *J. Phys. Chem.*, 1966, **70**, 2281.

[5] Ch. Benderli and M. Halmann, *J. Phys. Chem.*, 1967, **71**, 1053.

[6] M. Halmann and I. Platzner, *J. Chem. Soc.*, 1965, 5380.

experiments, and for the irradiation of sealed quartz tubes.

Actinometry.—The quantum yield of the reaction was determined at 253·7 nm, using the low-pressure mercury lamp. The flat end of the lamp touched one side of the flat polished windows of a cylindrical quartz absorption cell of 5-cm optical path, which was filled with water to filter out the emission of the lamp at 184·9 nm. The light coming out of this filter cell passed through a similar 5·0 cm long quartz cell containing the reaction mixture. For determination of the light flux, the reaction cell was filled with 0·1M-chloroacetic acid. The quantum yield for release of chloride ion in this reaction at 253·7 nm at 25° was taken as $\phi = 0{\cdot}33$.[7] The amount of chloride ion released was determined by titration with mercuric nitrate (0·01M) using diphenylcarbazone as an indicator (the titration mixture was acidified with nitric acid and contained 10 vol. of ethanol per vol. of aqueous solution). The average of two measurements gave $4{\cdot}0 \times 10^{-4}$ einstein l^{-1} min^{-1} for the light flux in the irradiation cell. Attempts were also made to use potassium ferric oxalate as an actinometer. However, this actinometer was found to be too sensitive in our irradiation set-up (which fits the low quantum yields of glycerophosphate photolysis) and was completely decomposed within *ca.* 2 min.

Quantum Yields.—For determination of the quantum yield in our reaction, the same reaction cell was filled with a 0·05M-solution of either glycerol 1- or glycerol 2-phosphate, but in this case either nitrogen or oxygen gas was bubbled slowly from a thin glass capillary through the photolysis solution. The irradiation time was chosen so as to keep the extent of decomposition of the glycerophosphates to less than 1%. The quantum yield ϕ was derived from the equation $\phi = n/[It(1 - 10^{-\varepsilon A e})]$ where n = number of mol of orthophosphate released, I = light flux of the lamp, $4{\cdot}0 \times 10^{-4}$ einstein l^{-1} min^{-1}, t = irradiation time in min, ε = absorptivity of the solution at the wavelength of irradiation (254 nm); for glycerol 1- and 2-phosphate, $\varepsilon = 0{\cdot}27$ and $0{\cdot}21$ M^{-1} cm^{-1}, respectively, $A = 0{\cdot}05M$, concentration of the glycerophosphate solutions, and e = 5·0 cm, the optical path in the irradiated cell.

Analysis of Photolysis Products.—Orthophosphate. This was determined by the Molybdenum Blue colorimetric method of Fiske and Subbarow at 660 nm.[8a]

Acid- or alkali-labile phosphate esters. These were determined by the Lowry and Lopez modification (at 700 nm) of the above method.[8b]

Acid-catalysed hydrolysis (in 0·5M sulphuric acid at 100°) and alkaline hydrolysis (in 1M sodium hydroxide at room temperature) were both carried out for 30 min.[9]

TABLE 1

Paper-chromatography and electrophoresis of photolysis products of glycerol 1- and of glycerol 2-phosphate

Compound	Solvent (I) R_F	Solvent (II) R_F	Solvent (III) R_F	Solvent * (IV) R_P	Solvent * (V) cm Movement	Solvent * (VI) cm Movement	Electrophoresis pH 3·5 R_P
Glycerol 1-phosphate	0·33	0·39	0	0·50			0·86
Glycerol 2-phosphate	0·33	0·39		0·50			0·80
Glycerol	(0·45)	(0·73)	(0·28)				(0·14)
Glyceraldehyde / Dihydroxyacetone	0·58	0·73	0·4		16—21	25	0·14
Glycidol	0·63	0·73	0·80				0·14
Glycollic acid	0·61	0·52	0·20	3·6—3·76			0·54—0·60
Glyceric acid	(0·48)			(2·0)			(0·60)
Glyoxylic acid		(0·4)			(5·5)	(9)	
Tartaric acid	(0·38)						(0·78)
Lactic acid	(0·72)	(0·54)	(0·27)				(0·47)
Formaldehyde					36	29	
Orthophosphate	0·42			1·00			1·00
Ethylene glycol	(0·63)	(0·74)					

Solvent I: n-butanol–acetic acid–water (4 : 1 : 1).
Solvent II: n-propanol–ammonia–water (6 : 3 : 1)
Solvent III: chloroform–ethanol (8 : 2).
Solvent IV: n-pentyl alcohol–5N-formic acid (1 : 1)
Solvent V: light petroleum–ether (95 : 5)
Solvent VI: ethanol–light petroleum (4 : 1).

Electrophoresis (pH 3·5) pyridine–acetic acid–water (6·6 : 66 : 30000).

* 2,4-Dinitrophenylhydrazone derivatives.

In the iodine test for glyceraldehyde 3-phosphate, a photolysed glycerol 1-phosphate solution (1 ml) was treated with a solution of iodine (0·5 ml; 0·01M, in aqueous potassium iodide, containing sodium carbonate, 0·1M) for 15 min at room temperature. The mixture was then saponified with an excess of 1M sodium hydroxide, as above.[9,10]

Paper-chromatography and electrophoresis. Whatman No. 1 paper was used. Paper chromatography was done in descending mode. Sodium salts in the samples were converted before spotting with Dowex-50W-H^+ into the free acids. Paper electrophoresis was performed at 2·5 kvolt (40 volt cm^{-1}) for 1·5 h in a Savant electrophoresis tank with paper sheets wetted in a pH 3·5 buffer. Values of R_F and R_P (movement relative to orthophosphate) are presented in Table 1. Data for compounds which were *not* detected in the photolysis of the glycerophosphates are shown in parentheses.

Phosphorus-containing compounds were detected by the ammonium molybdate–perchloric acid spray, followed by drying at 80 °C for a few minutes and exposure to sunlight.[11] Carboxylic acids were detected by spraying with a Bromocresol solution (1% in ethanol).

[7] J. G. Calvert and J. N. Pitts, jun., 'Photochemistry,' Wiley, New York, 1966, p. 787.

[8] L. F. Leloir and C. E. Cardini in 'Methods in Enzymology' S. P. Colowick and N. O. Kaplan, eds., Academic Press, New York, 1957, vol. III, (*a*) p. 843; (*b*) p. 845.

[9] G. Scholes, W. Taylor, and J. Weiss, *J. Chem. Soc.*, 1957, 235.

[10] O. Meyerhof and K. Lohmann, *Biochem. Z.*, 1934, **271**, 89; W. Kiessling, *Chem. Ber.*, 1934, **67**, 869.

[11] I. M. Hais and K. Macek, 'Paper Chromatography,' Publishing House of the Czechoslovak Academy of Sciences, Prague, 1963, p. 819.

Glycidol, glyceraldehyde, dihydroxyacetone, glycerol, and the glycerophosphates were detected on the paper chromatogram by their reaction with silver nitrate. For detection of glycidol, we found it necessary to hang the paper sheet after chromatography (in solvent III, see Table 1) for *ca.* 10 h in a tank saturated with ammonia vapour, in order to hydrolyse glycidol to glycerol. The chromatogram was then dried at room temperature and dipped into a silver nitrate solution (10 g in 5 ml water and 900 ml acetone). After the wet sheet had been allowed to hang for 5—10 min, the chromatogram was then dipped in a ' developer ' (20 g sodium hydroxide in 200 ml water, 600 ml ethanol, and 400 ml isopropyl alcohol). After *ca.* 10 min, brownish-grey spots developed. The sheet was then dipped into a solution of Kodak *X*-Ray fixer and finally washed with water and dried. Throughout the ' development ', exposure to intense light must be avoided.*

Colour test for glycollic acid and glycidol with 2,7-dihydroxynaphthalene. Glycollic acid was also detected as described [12] by heating with a solution of 2,7-dihydroxynaphthalene in conc. sulphuric acid. A violet-red colour is formed, for which we found $\lambda_{max.} = 550$ nm. This colour test was clearly positive after u.v.-irradiation of solutions of both glycerol 1- and of glycerol 2-phosphate in an oxygen atmosphere. However, after irradiation in a nitrogen atmosphere, a brownish colour appeared, with a pronounced green fluorescence; this does not occur in the colour test with pure glycollic acid. An explanation was eventually found in the identification of glycidol as a photolysis product. The fluorescence spectrum obtained by 2,7-dihydroxynaphthalene with the irradiated solutions of glycerophosphates was compared with the fluorescence spectrum obtained with authentic glycidol. The excitation was measured by exciting the sample at different wavelengths (400—540 nm was used) and measuring the total emission (at a constant wavelength 510 nm), while the fluorescence spectrum was obtained by exciting the sample with monochromatic light (475 nm was used) and measuring the spectral distribution of the fluorescent light. Both for authentic glycidol and for the glycerol 1- and glycerol 2-phosphate solutions photolysed in a nitrogen atmosphere, the hydroxynaphthalene derivative had an absorption maximum at 475 nm, and a fluorescence spectrum with a maximum at 500 nm.

Carbon dioxide. For analysis of carbon dioxide produced during the photolysis (with the high-pressure mercury lamp), either nitrogen or oxygen gas was bubbled through the glycerophosphate solution. The out-comimg gas was then passed through a tube packed with glass-wool (to hold back droplets) and then bubbled through two flasks with saturated solutions of barium hydroxide. During photolysis in an oxygen gas stream, the formation of carbon dioxide (observed by precipitation of barium carbonate) was immediate and copious. In a nitrogen atmosphere, the formation of carbon dioxide was very much slower. Quantative determination of the carbon dioxide evolved was made by titration of the barium hydroxide with hydrochloric acid, using Phenolphthalein as an indicator.

Hydrogen.—Analysis of the molecular hydrogen formed during photolysis was carried out by gas chromatography on a Molecular Sieve 5A (Linde; 30—40 mesh) column (1·5-m long; inner dia., 3·0 mm) at room temperature in an argon carrier gas stream (30 ml min^{-1}) and using a Gow–Mac thermal conductivity detector.

The photolysis was carried out in a quartz reaction tube (I.D. 1·0 cm, height, 18·0 cm), which was fitted with a gas inlet tube (to sweep only the gas phase above the reaction solution), and with a gas outlet tube. This reaction tube was connected (using two three-way T-shaped pressure stopcocks) to a bypass to the inlet system of the gas chromatograph, between the argon gas tank and the column entrance.

Samples of glycerol 1-phosphate (1 ml; 0·5M) were repeatedly frozen in a solid CO_2 bath, degassed by connecting to a high vacuum, purged with argon, and again frozen and degassed. The tube was then brought to room temperature and was photolysed at 254 nm by irradiation with the low-pressure mercury lamp through a 0·7 cm layer of flowing distilled water at 25°. For analysis, the tube was frozen in solid CO_2, and argon was made to sweep the gaseous products into the column of the gas chromatograph. This procedure was found to determine all the hydrogen produced. (By thawing the reaction mixture, refreezing and again sweeping argon through the tube, no additional hydrogen was detected). The yield of hydrogen produced by photolysis of the glycerophosphate solution (2·21 mmol H_2 after 10 h) was corrected for the amount of hydrogen released by photolysis of pure water under the same conditions (1·05 mmol).

^{18}O-Tracer Experiments.—Solutions of glycerol 1-phosphate (0·5 and 1·0M) in ^{18}O-enriched water (*ca.* 15 atom % ^{18}O) in evacuated sealed quartz ampoules or in a quartz tube with bubbling through of nitrogen, were irradiated with the low-pressure mercury lamp. The orthophosphate released was precipitated with barium chloride at pH 9, centrifuged down, washed with acetone, and dried. The precipitate was dissolved in 2N-hydrochloric acid, and reprecipitated by addition of 2N-sodium hydroxide to pH 9. Three such precipitations were necessary to free the barium phosphate from traces of glycerophosphate, as shown by paper electrophoresis.

^{18}O-Analysis. This was carried out by heating a mixture of barium orthophosphate with guanidine hydrochloride (40 mg) in an evacuated sealed glass ampoule (fitted with a breakseal) to 300° for 3 h.[13] The resulting carbon dioxide was analysed by mass spectrometry for its ^{18}O content.

Analysis of Photolysis Products using ^{14}C-Labelled Glycerol 1-Phosphate.—Irradiations were carried out in sealed quartz ampoules containing degassed and evacuated solutions of glycerol 1-phosphate (1 ml; 0·05 or 0·10M of total glycerophosphate and *ca.* 1 μCi of uniformly ^{14}C-labelled glycerol 1-phosphate) and using a low-pressure mercury lamp. Analysis of products was made by paper chromatography and electrophoresis of the solution before and after irradiation. After drying of the paper sheet, strips of 1 × 3 cm^2 were cut and were counted for ^{14}C in a liquid scintillation spectrometer. Results after irradiation were corrected for traces of impurity (particularly glycerol) in the labelled glycerophosphate.

Potentiometric titration of the glycerophosphate solution before and after photolysis was carried out in order to determine the total amount of acids liberated by irradiation. Titrations were made with standard 0·1M-sodium hydroxide

* We are indebted to Mr. Y. Korn for advice on this modified silver nitrate method.

[12] F. Feigl, ' Spot Tests, in Organic Analysis,' Elsevier, Amsterdam, 1956, 5th edn., p. 346.

[13] P. D. Boyer, D. J. Graves, C. H. Suelter, and M. E. Dempsey, *Analyt. Chem.*, 1961, **33**, 1906.

or hydrochloric acid, with a TTTI Radiometer pH meter fitted with a scale expander.

RESULTS

Ultraviolet Spectroscopy

Concentration Dependence.—The u.v. absorption spectra of aqueous solutions of glycerol 1-phosphate and of glycerol

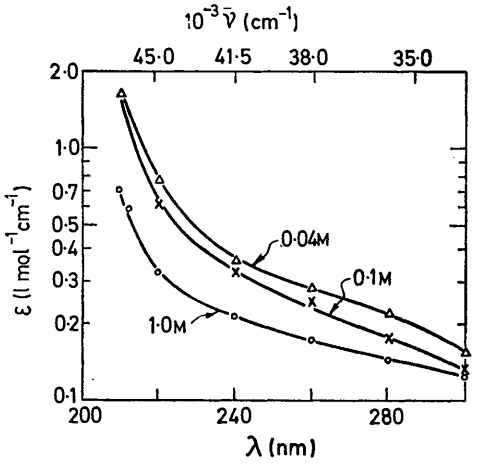


FIGURE 1 Absorption spectrum of aqueous glycerol 1-phosphate at various concentrations

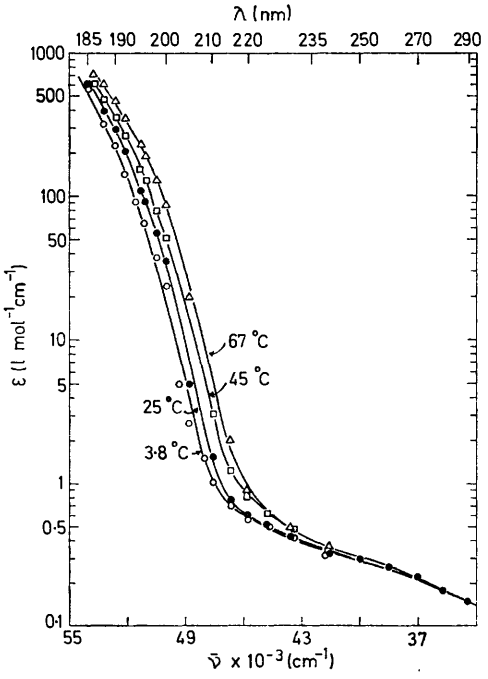


FIGURE 2 Temperature dependence of the absorption spectrum of glycerol 1-phosphate

2-phosphate do not observe the Beer–Lambert Law. Increasing dilution causes a considerable rise in absorptivity. The failure of the Beer–Lambert Law is most severe at high concentrations, from 1·0 to 0·1M solutions. Further dilution, from 0·1 to 0·01M concentrations, has a much smaller effect (see Figure 1 for glycerol 1-phosphate).

The absorptivity of both glycerol 1- and of glycerol 2-phosphates increases very slowly with decreasing wavelength in the 300—220 nm region, and rises sharply below 220 nm. The absorption maximum is below 185 nm (the limit of the spectrophotometer used).

Temperature Dependence.—The variation in absorptivity with temperature in the 3·8—67° range is presented in Figure 2 for aqueous solutions of glycerol 1-phosphate. Similar results were obtained for glycerol 2-phosphate. For both phosphate esters, there exists a clear distinction between two spectral regions. Above 220 nm, there is essentially no temperature effect. Below 220 nm, there is a very marked increase in absorptivity with rising temperature. As shown in the Figure, the spectral bands at

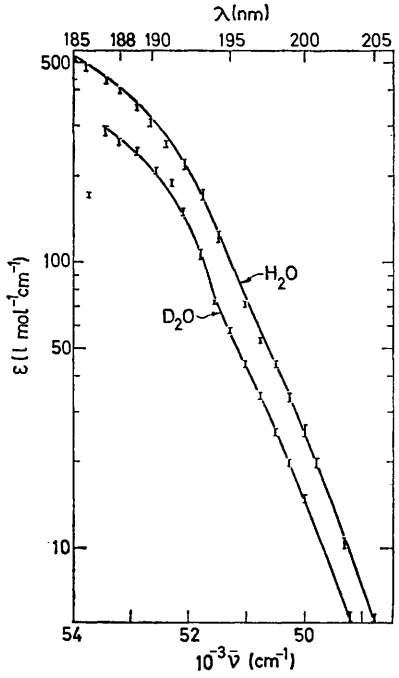


FIGURE 3 Absorption spectra of glycerol 2-phosphate in water and in deuterium oxide solutions

higher temperatures are red-shifted towards longer wavelengths.

Deuterium Solvent Isotope Effect.—In the far-u.v. region, below 205 nm, replacement of water by deuterium oxide as the solvent causes a considerable shift in the spectral curve of both glycerophosphates towards shorter wavelengths (see Figure 3 for glycerol 2-phosphate).

Photochemistry

Quantum Yields for Orthophosphate.—Values for the quantum yields of orthophosphate release from the glycerophosphates by irradiation at 254 nm are as follows:

Atmosphere	Nitrogen	Oxygen
Irradiation time (h)	12	4
	Quantum yield $10^2\,\phi$	
Glycerol 1-phosphate	0·96 ± 0·06	2·3 ± 0·1
Glycerol 2-phosphate	1·16 ± 0·02	3·07 ± 0·01

The quantum yields are seen to be very low, only *ca.* 0·01 in a nitrogen atmosphere, and increasing to 0·02—0·03 in an oxygen atmosphere. The low values are similar to what had been previously reported for the photolysis of ethyl dihydrogen phosphate.[6]

Glycollic Acid and Carbon Dioxide.—The photolysis of

aqueous glycerophosphate resulted in an increase of acidity in the solutions, as observed by a decrease in pH, and which was measured by the amount of standard base required to bring the solution by potentiometric titration back to its original acidity, pH 8·9. As shown in Table 2, the amount of the acidic products is much larger in an oxygen atmosphere than in a nitrogen atmosphere. In both nitrogen and oxygen atmospheres, the amount of acid production is slightly larger for glycerol 1-phosphate than for glycerol 2-phosphate. Part of this acid was shown to be carbon dioxide, observed by bubbling the effluent gas from the photolysis tube into aqueous barium hydroxide (see Experimental section). Another part was glycollic acid, detected by paper chromatography and determined by colorimetry. Both acids were formed only in minor amounts during irradiations in a nitrogen atmosphere, but were produced in major and easily detectable amounts in oxygenated solutions. The yields of orthophosphate and of glycollic acid release as a function of time by irradiation of glycerol 1-phosphate in an oxygen atmosphere are shown in Figure 4.

TABLE 2

Yields of products after photolysis of aqueous glycerophosphates (initially 0·05M) at 254 nm

Glycerol	Atmosphere	Photolysis time (h)	pH drop	HPO_4^{2-} (%)	Titratable acid (%)	CO_2 (%)	Glycollic acid (%)
1-Phosphate	N_2	17·0	8·9 to 7·6	44·4	8·8	<1	Traces
2-Phosphate	N_2	17·2	8·9 to 7·7	45·2	7·4	<1	Traces
1-Phosphate	O_2	3·12	8·9 to 6·9	20·9	20	11·2	10·8
2-Phosphate	O_2	4·33	8·9 to 7·0	25·3	18·2	7·8	12

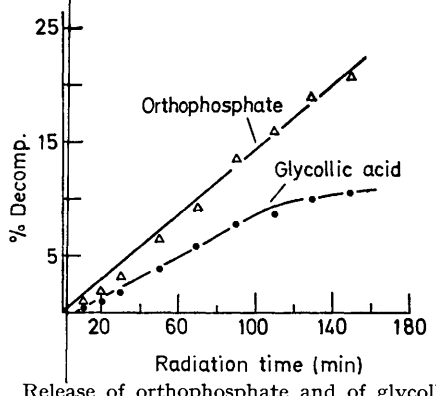


FIGURE 4 Release of orthophosphate and of glycollic acid as a function of time in the photolysis of an oxygenated solution of glycerol 1-phosphate

Part or all of the glycollic acid and the carbon dioxide formed must be secondary, by photochemical oxidation of glycidol (see below). Photolysis of aqueous solutions of pure glycidol under similar conditions in a nitrogen atmosphere gave rise to a slow release of glycollic acid and carbon dioxide. In oxygenated solutions, this release was immediate and copious.

Hydrogen.—The yield of hydrogen released by irradiation of evacuated aqueous solutions of glycerol 1-phosphate during 7·5 h was only 10% of the yield of orthophosphate produced during the same period.

Glycerol.—In a previous study, the photolysis of the glycerophosphates was considered to be a ' photochemical hydrolysis ',[2] thus possibly implying that the organic product may be glycerol. We tested for the formation of glycerol during the photolysis of glycerol 1-phosphate (paper chromatography in solvent III, Table 1). The result was negative; glycerol, if formed, was produced in less than 2% yield, relative to the orthophosphate released. (For this experiment, freshly recrystallized glycerol 1-phosphate had to be used, freed from glycerol impurities; see Experimental section). Also, no ethylene glycol could be detected.

Glycidol, Glyceraldehyde, Diydroxyacetone, and Glycollic Acid.—Glyceraldehyde and dihydroxyacetone were expected as major products in the photolysis of glycerol 1- and 2-phosphate, respectively, by analogy with the photolysis of ethyl dihydrogen phosphate, which resulted in equimolar amounts of acetaldehyde, hydrogen, and orthophosphate.[5] Our methods could not differentiate between glyceraldehyde and dihydroxyacetone, which have closely similar properties. Glyceraldehyde and/or dihydroxyacetone were indeed produced in the photolysis of glycerol 1- and glycerol 2-phosphate, and were detected by isolating the crystalline 2,4-dinitrophenylhydrazone derivatives, (m.p. 163—165°, from both glycerophosphates photolysed under nitrogen), and by paper chromatography and electrophoresis (see Table 1). Using ^{14}C-labelled glycerol 1-phosphate, and counting of the paper chromatograms, the yields of the non-volatile organic products could be determined, relative to the orthophosphate released. In such an irradiation of an evacuated ampoule containing glycerol 1-phosphate (0·1M), the yields obtained were glycidol, 40%, glyceraldehyde and/or dihydroxyacetone, 10%, and glycollic acid, 9%.

As mentioned in the Experimental section, glycollic acid and glycidol were further identified by their colour test with 2,7-dihydroxynaphthalene—which in the case of glycidol was found to give a beautiful green fluorescence.

Acid- and Alkali-labile Phosphate Esters.—In the radiolysis of aqueous glycerophosphates by *X*-rays, it had been reported that a very considerable fraction of the substance was converted into a labile phosphate ester, possibly glyceraldehyde 3-phosphate or dihydroxyacetone phosphate.[9] We found that also in the photolysis of the glycerophosphates, some labile phosphate was formed, which was converted into orthophosphate by heating for 30 min with either 0·5M-sulphuric acid at 100° or 1M-sodium hydroxide at room temperature, but which was stable at room temperature and neutral pH for three days. As shown in Table 3, the contents of labile phosphate ester after short irradiation times (1—3 h) amounted to only *ca.* 15% of the glycerophosphate which had decomposed. This labile phosphate ester disappeared after more prolonged photolysis. Thus the photolysis of the glycerophosphates differs from the radiolysis by ionizing radiations in that only a small fraction of the material is converted into a labile phosphate ester.

This labile phosphate ester was shown not to be glyceraldehyde 3-phosphate by treating the reaction mixture (glycerol 1-phosphate irradiated in a nitrogen atmosphere)

TABLE 3

Acid- and alkali-labile phosphate esters formed by photolysis of aqueous glycerophosphates as a function of irradiation time

			Orthophosphate (mmol) after photolysis		
	Atmosphere	Time (h)	No hydrol.	Acid hydrol.	Base hydrol.
Glycerol 1-phosphate	N_2	1·25	1·6	1·75	2·0
		4	3·8	4·20	4·4
		6	5·8	5·9	5·9
Glycerol 1-phosphate	O_2	3	8·5	9·8	10·0
		5	15·0	16·0	16·2
		7	20·0	20·6	20·8
Glycerol 2-phosphate	O_2	1	3·4	4·0	3·8
		2	6·5	7·0	6·9
		3	8·0	8·3	8·0

with iodine in mild alkali. This reagent is known to oxidize glyceraldehyde 3-phosphate to β-phosphoglyceric acid, which is alkali-stable, while dihydroxyacetone phosphate is not attacked by iodine under these conditions.[9,10] As shown in Table 4, iodine treatment in mild

TABLE 4

Photolysis of glycerol 1-phosphate (0·1M) in a nitrogen atmosphere. Test for glyceraldehyde 3-phosphate

Photolysis time (min)	% Orthophosphate released, photolysis only	% Orthophosphate, photolysis and alkaline hydrolysis	% Orthophosphate, photolysis, I_2, alkaline hydrolysis	% Labile phosphate *
15	0·495	0·555	0·555	11
30	0·94	1·07	1·07	12

* Relative to the total phosphate undergoing photolysis.

alkali, followed by saponification in strong alkali (1M-NaOH as above) caused the same release of orthophosphate as saponification alone (11—12%). Thus, there was no loss of alkali-labile phosphate by the iodine treatment, and hence the labile photolysis product must be essentially only dihydroxyacetone phosphate. This result for the photolysis is similar to that obtained in the radiolysis of glycerol 1-phosphate by *X*-rays.[9]

Determination of the Point of Bond Breakage.—In order to determine to what extent the photolytic release of orthophosphate from glycerol 1-phosphate occurs with P–O or C–O breakage, the photolysis was carried out in ^{18}O-enriched water, and the resulting orthophosphate was analysed for its ^{18}O-content. As shown below, both in a nitrogen atmosphere and *in vacuo*, 98—99% of the photolysis involves C–O bond breakage.

	Atom % excess ^{18}O		% P–O Bond breakage
Atmosphere	Initial water A	Orthophosphate B	$4 \times 100 \times B/A$
Nitrogen	14·6	0·087	2·4
Vacuum	13·8	0·039	1·1

DISCUSSION

The ultraviolet absorption spectra of aqueous solutions of both glycerophosphates indicate two regions of electronic excitation: a weak and wide band ranging from 300 to 200 nm, and a steep rise below 220 nm, with the maximum beyond 185 nm. In the main features, the ultraviolet absorption spectra of the two glycerophosphates are rather similar to each other, and also to the absorption spectrum of the orthophosphate dianion, HPO_4^{2-}, and of other phosphorus oxyanions.[1,4-6] Environmental effects on the spectra, changes in temperature and solvent, are also closely similar to those previously observed in various phosphorus oxyanions. For the weak absorption around 250 nm, the electronic transition may possibly be of the type $n \rightarrow \pi^*$, by excitation of an electron from a non-bonding orbital on one of the oxygen atoms to an antibonding orbital of the P–O bond. The low intensity may reflect the small amount of overlap between the n orbital localized on one atom and the π^* orbital which is delocalized over the whole phosphate group of the molecule.

The very intense absorption below 220 nm indicates, as with the other phosphorus oxyanions, the onset of a ' charge-transfer-to-solvent ' (c.t.t.s.) type of transition. Both the temperature effect, red-shift with rise of temperature, and the solvent-isotope effect, blue-shift by replacing water with deuterium oxide as a solvent, are typical of c.t.t.s. transitions. For spherical uni- and di-valent anions, the theory of c.t.t.s. transitions [14] permits calculation of the crystalline ionic radius and the vertical ionization potential of the ion. On attempting to apply this theory to our observed environmental effects in the absorption of glycerol 1- and glycerol 2-phosphates, we obtained for the crystalline radii of these dianions values of 2·71 and 2·57 Å, respectively. These values seem much too small to describe the radii of the whole glycerophosphate ion. They are only slightly larger than those reported for the orthophosphate dianion, HPO_4^{2-}, for which measurements similar to ours, and using the theory of c.t.t.s. spectra, result in a value of 2·36 Å, in agreement with that derived from thermochemical measurements, 2·38 Å.[4b]

These values for the crystalline radii, as well as the similarity between the absorption spectra of the glycerophosphates and of the orthophosphate ions, could possibly be explained by considering the absorption site with the glycerophosphates to be localized mainly in the phosphate portion of the molecule. According to the model of Stein and Treinin for c.t.t.s. transitions,[14b] the central anion is surrounded with a thin layer of solvent molecules. During the very rapid electronic transition, *ca.* 10^{-15} s, the solvent layer remains stationary—as postulated in the Franck–Condon principle. The glycerophosphate ion, and other phosphate ester anions, may be considered as a phosphate ion surrounded by a solvent layer, consisting partly of water molecules and partly of the organic group, which plays the role of some of the solvent molecules in the solvation shell nearest to the phosphate ion. The same results had been obtained in the far-u.v. absorption spectra of aqueous solutions of the dianions of α-D-glucose 1-phosphate and of glucose 6-phosphate,[1] in which the temperature and

[14] (*a*) M. Smith and M. C. R. Symons, *Discuss. Faraday Soc.*, 1957, **24**, 206; (*b*) G. Stein and A. Treinin, *Trans. Faraday Soc.*, 1959, **55**, 1086; (*c*) A. Treinin, *J. Phys. Chem.*, 1964, **68**, 893; (*d*) M. J. Blandamer and M. F. Fox, *Chem. Rev.*, 1970, **70**, 59; (*e*) M. F. Fox, *Quart Rev.*, 1970, **24**, 565 giving further references.

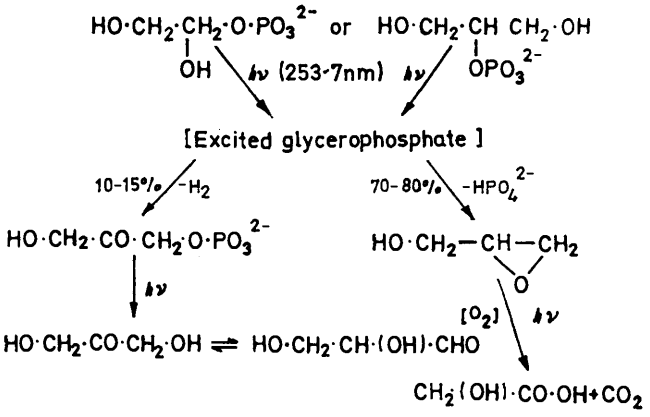
deuterium isotope effects are similar to the present data for the glycerophosphates.

The photochemical experiments were all carried out at 254 nm, in which the above spectroscopic data had shown that the light absorption process is probably due to an internal electronic transition in the glycerophosphate molecules. The predominant reaction in this photolysis in aqueous solutions, as with other alkyl dihydrogen phosphates,[2,15] is the release of orthophosphate. The tracer experiments using ^{18}O-enriched water showed that the phosphate release occurs essentially only with C–O bond fission. This result is in contrast with that in the hydrolytic decomposition of the glycerol 2-phosphate dianion, which occurs with P–O bond fission only.[3] The photolytic reaction is thus completely different from hydrolysis.

An acid- and alkali-labile phosphate ester, probably dihydroxyacetone phosphate, is produced in the early stages of photolysis, but it accounts only for about 15% of the total phosphate released. The photolysis reaction thus differs from the radiolysis of the glycerophosphates by *X*-rays, in which dihydroxyacetone phosphate is the predominant product.[9]

The different pathways in radiolysis and photolysis may be accounted for by considering that radiolysis acts primarily on the solvent water, producing hydrated electrons, hydroxyl radicals, and other very reactive species, which are responsible for the radiolytic effects on the glycerophosphates. In our photolysis experiments at 254 nm, most of the excitation is directly on the glycerophosphate ion.

The marked overall similarity in the spectroscopic and photochemical results for both glycerol 1- and glycerol 2-phosphate leads us to assume that the primary photochemical step is formation of a similar short-lived intermediate. This intermediate then breaks up mainly by phosphate release to form glycidol (leading to glycollic acid and carbon dioxide) and partly by hydrogen release to form dihydroxyacetone phosphate (leading to dihydroxyacetone and/or glyceraldehyde). The nature of this excited intermediate cannot be deduced



from the present data, but may possibly be elucidated from flash photolysis measurements. Such measurements for the orthophosphate dianion, HPO_4^{2-}, indicated formation of the $HPO_4^{\cdot -}$ radical-ion, detected by its transient absorption spectrum at 500 nm.[16] In view of the similarity of the absorption spectra of the glycerophosphates with that of the orthophosphate ion, analogous glycerophosphate radical-ions may be formed, possibly by an $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transition on the phosphate group of the molecule.

The photolytic dehydrogenation of glycerol 1-phosphate to dihydroxyacetone phosphate may be considered a chemical model of the oxidation of glycerol 1-phosphate in living cells, which uses NAD and α-glycerophosphate dehydrogenase as coenzyme and enzyme, respectively, and which provides a link between carbohydrate and lipid metabolism.

[1/2222 *Received*, 23*rd November*, 1971]

[15] M. Halmann, 'Topics in Phosphorus Chemistry,' M. Grayson and E. J. Griffiths, eds., Interscience, 1967, vol. 4, p. 49.
[16] J. R. Huber and E. Hayon, *J. Phys. Chem.*, 1968, **72**, 3820.