Photoreactions of Fructose 6-Phosphate in Oxygenated and Deoxygenated Aqueous Solutions [1]

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The photoreactions of fructose 6-phosphate in water, irradiated at 254 nm in the presence and in the absence of oxygen (ϕ 0.60 and 0.50, respectively), were studied. The most abundant photoproducts were carbon monoxide and orthophosphate; the other main products were identified by g.l.c.–mass spectrometry of the trimethylsilyl derivatives as 2-deoxyerythrose 4-phosphate, 2,4-dihydroxycyclobutyl phosphate, and glycolaldehyde phosphate, with glyceraldehyde 3-phosphate in the presence of oxygen. These results can be explained by assuming the free carbonyl function of the sugar phosphate to be excited. Carbon monoxide is obtained in the primary process *via* a Norrish type I reaction. The resulting phosphorylated radical undergoes elimination of water leading to 2-deoxyerythrose 4-phosphate, which gives further important Norrish type II reactions. Orthophosphate is the result of secondary degradation processes, and also probably arises from hydrolysis of labile organic phosphates. This photochemical behaviour is in contrast with the hydrogen abstraction occurring generally with carbohydrates, in particular glucose 6-phosphate (ϕ 0.8 × 10^{-2}).

FRUCTOSE-6-PHOSPHATE is an important intermediate in the glycolytic pathway of sugar metabolism. In aqueous solution this compound shows a weak absorption at 260—280 nm which was first assigned to a 20% proportion of the free keto form,[2] and then to impurities as only 2.5% of this form was found by i.r. spectroscopy.[3] On the other hand it was shown by ^{13}C n.m.r. spectroscopy that the compound exists principally as the two pyranose anomers with an α : β ratio of 20 : 80,[4] and more recently by using ^{13}C-enriched fructose 6-phosphate that the proportion of the free keto form is 4.1%.[5] Thus it is not clear which of the open-chain or cyclic species is absorbing in the 270 nm range, nor whether the open-chain keto form is to some extent responsible for this absorption.

The photosensitized hydrolysis of glucose 6-phosphate

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has been reviewed; [6] orthophosphate release was shown to be due to secondary degradation processes.[7] The weak absorption of this compound at 220—240 nm was attributed to an internal transition,[8] and the open aldehydic form was not detected; [3] indeed the photolysis of glucose 6-phosphate is in many respects similar to that of glucose,[9] with anomeric hydrogen abstraction leading to further degradation at C-1.[7] With fructose 6-phosphate, the hemiacetal position has no attached hydrogen atom, and the existence of the open-chain carbonyl form is symptomatic of basic differences in structure between these two compounds, so that other photoreactions could be expected. We have studied the direct effect of light (254 nm) on fructose 6-phosphate in aqueous solution, in the absence and in the presence of oxygen.

EXPERIMENTAL

Materials and Irradiations.—Disodium D-fructose 6-phosphate (Sigma) was used without further purification; [2-^{18}O]fructose 6-phosphate was prepared as described by Model *et al.* for fructose 1,6-diphosphate.[10] Water was twice distilled.

Irradiations of the sugar phosphate (usually 0.01M) were carried out as previously described.[7] A high-pressure mercury lamp (Hanau TQ 150) fitted into a double-jacketted quartz reactor (volume of solution *ca.* 150 ml) was generally employed. A low-pressure mercury lamp (Thermal Syndicate T/MS/544; volume of solution 2—5 ml) was employed for tracer and actinometry experiments. The irradiated solution was stirred continuously by a gas stream (helium or helium + 10% oxygen at 6 l h^{-1}).

Quantum yields (ϕ) were determined at 254 nm using chloride release from chloroacetic acid as reference (ϕ 0.33).[11] Irradiations were carried out using identical volumes and optical densities.

Analysis of Photoproducts.—Paper chromatography. Chromatography of solutions obtained after photolysis and of the sugars released after enzymic hydrolysis by alkaline phosphatase was carried out on Whatman no. 1 paper (see Table 1). Compounds (B) and (C) were separated by preparative chromatography (Whatman no. 3) and eluted with water for structure determination.

Periodate oxidation of compound (C). Oxidation by periodate was performed as described by Khym.[12a] Formic acid [12b] and malonaldehyde [13] were determined by the 2-thiobarbituric acid method; periodate and iodate were removed on Dowex 1 resin (100—200 mesh; acetate form), and formaldehyde was detected in the eluate by the chromotropic acid reaction.[14a]

G.l.c.–Mass Spectrometry.—A fraction of lyophilized material was directly trimethylsilylated for g.l.c.–mass spectrometric analysis (LKB Producer 9000 S) as previously described.[7] Another fraction was reduced by $NaBH_4$ or $NaBD_4$ prior to trimethylsilylation and g.l.c.–mass spectrometric analysis.[15] The structures of compounds (B) and (C) were determined in the same way.

TABLE 1

Paper chromatography after 3 h irradiation; R_F values

	Solvent A *	Solvent B †
(a) Products of photolysis ($h\nu$–He and $h\nu$–O_2)		
Compound I	0.104	0.20
Compound II	0.076	0.155
Compound III (fructose 6-phosphate)	0.024	0.115
(b) After alkaline phosphatase hydrolysis (Tris buffer)		
Compound A ($h\nu$–O_2 only)	0.75	
Compound B (2-deoxyerythrose)	0.57	0.58
Compound C (cyclobutane-1,2,3-triol)	0.40	0.52
Compound D (Tris)	0.25	0.37
Compound F (fructose)	0.15, 0.18	0.23, 0.28, 0.30
(c) Reference compounds		
2-Deoxyerythrose	0.57	0.56
Erythrose	0.42	0.44
2-Deoxyribose	0.42	0.50
Ribonolactone	0.34	0.52
Ribose	0.27	0.36
Xylose	0.21	0.30
Arabinose	0.18	0.28
Fructose	0.15, 0.18	0.20, 0.27, 0.30
Fructose 6-phosphate	0.022	0.115
Tris	0.25	0.37

* Butan-1-ol–acetic acid–water (4 : 1 : 1) (migration time 16—20 h). † Ethyl acetate–acetic acid–water (9 : 2 : 2) (migration time 9—10 h).

Kinetics and tracer experiments. Orthophosphate was analysed by the colorimetric method of Fiske and Subba Row; [14b] acids were determined by titration with sodium hydroxide (0.01M; phenolphthalein) and fructose 6-phosphate was determined enzymically by the reduction of NADP in the presence of phosphoglucose isomerase and glucose 6-phosphate dehydrogenase.[16]

Effluent gases were analysed by mass spectrometry (Atlas CH 4; a part of the effluent was allowed to flow through a capillary tube into the ion source). Concentrations of CO and CO_2 were determined by measuring the intensities of the peaks at *m/e* 28 and 44. Standardization was achieved with known mixtures of helium with 5% CO (v/v) and with 0.65% CO_2 (v/v).

In tracer studies, the ^{18}O content of the evolved CO and CO_2 was determined similarly. [2-^{18}O]Fructose 6-phosphate isotopic analysis was performed by g.l.c.–mass spectrometry of the trimethylsilyl derivative.

RESULTS

The main photoproducts were orthophosphate and carbon monoxide (see Figure 1). Both in the presence and in the absence of oxygen the rate of orthophosphate release was

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[12] (*a*) J. X. Khym, *Methods Carbohydrate Chem.*, 1972, **6**, 87; (*b*) J. F. Kennedy, *ibid.*, p. 93.
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low as compared with that of fructose 6-phosphate disappearance, which indicates an important transformation of the substrate into other organic phosphates.

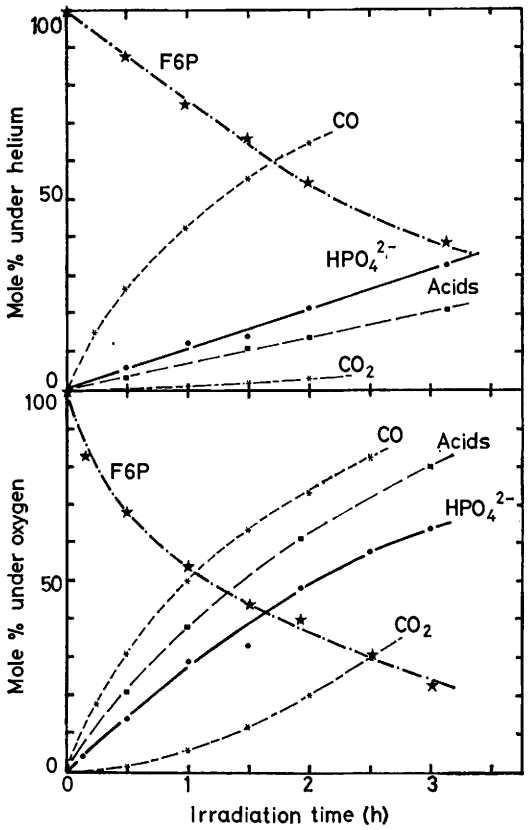

FIGURE 1 Photolysis of disodium fructose 6-phosphate (0.01M) in the absence and in the presence of oxygen

In the absence of oxygen, irradiation of [2-^{18}O]fructose 6-phosphate with a low-pressure mercury lamp resulted in evolution of carbon monoxide of ^{18}O abundance identical with that of the starting material. As shown in Table 3, the decrease of ^{18}O label in the evolved CO, and its increase when the experiment was done with fructose 6-phosphate in $H_2{}^{18}O$, were the same. This indicates that isotopic exchange occurs between water and the substrate, which explains the decrease in ^{18}O abundance of the carbon monoxide. Therefore CO formation could be a primary process of the photolysis. The quantum yield (ϕ) determined on the basis of the CO evolved during isotopic and blank experiments was found to be 0.50. Measurements based on fructose 6-phosphate disappearance gave ϕ 0.23 (see Table 2) which seems inconsistent with the previous result; however in this case one at least of the photoproducts was interfering with NADPH during the enzymic analysis so that the substrate concentration was overestimated. The same difference was also found in photoreactions carried out with a high-pressure mercury lamp. Production of CO showed first-order kinetics and a rate of about twice that of the measured fructose 6-phosphate disappearance at the beginning of the irradiation (see Figure 1).

In the presence of oxygen only 77% of the ^{18}O present in the [2-^{18}O]fructose 6-phosphate was found in the evolved CO, showing that there is another source of carbon monoxide. The quantum yield (ϕ) determined on the basis of fructose 6-phosphate disappearance was 0.60 (see Table 2). The previous remarks are also valid for this analysis; however as shown in Figure 1, the amount of organic phosphate is less important in the presence of oxygen, and this leads to more accurate results.

TABLE 2

Quantum yields

	Glucose 1-phosphate [a]	Glucose 6-phosphate	Fructose 6-phosphate
ε 254/mol^{-1} l cm^{-1}	0.5	0.5	2.5
ϕ under He	1.02×10^{-2}	$(0.8 \pm 0.2) \times 10^{-2}$ [b]	0.23 ± 0.01 [c]
			0.50 ± 0.05 [d]
ϕ in the presence of O_2	3.56×10^{-2}		0.60 ± 0.05 [c]

[a] Values from ref. 24. [b] Average value of three experiments (10^{-2}M; irradiation times 2, 4, and 6 h) determined from orthophosphate release. [c] Average value of five experiments (10^{-2}M; irradiation time 10—60 min) determined enzymically from fructose 6-phosphate disappearance. [d] Average value of three experiments, determined on the basis of carbon monoxide formation.

TABLE 3

^{18}O Abundance in the CO evolved during photolysis under He from [2-^{18}O]fructose 6-phosphate (F6P) in H_2O and from F6P in $H_2{}^{18}O$

Time [a] (min)	[2-^{18}O]F6P–H_2O [b] τ/τ_i (%)	F6P–$H_2{}^{18}O$ [c] $1 - \tau/\tau_\infty$ (%)
14	92	
15	92.5	
28		92.1
35	90.5	89
45	87.6	
50	87	86.5

[a] Time after F6P dissolution in water: irradiation begun after 10 min of He bubbling in order to eliminate dissolved oxygen. [b] ^{18}O Abundance of the CO (τ) referred to that of starting [2-^{18}O]F6P (τ_i 85%). [c] ^{18}O Abundance of the CO (τ) referred to that of $H_2{}^{18}O$ (τ_∞ 10%); for comparison with the preceding results, the complement to 100% is given.

As expected, the rate of formation of CO_2 and acids was low, and was increased in the presence of oxygen. In both cases malonaldehyde was formed but with a lower yield in oxygenated solution (0.5 and 0.1%, respectively, after 3 h irradiation).

Identification of Products.—Trimethylsilyl derivatives of sugar phosphates are suitable for g.l.c.–mass spectrometric analysis.[17,18] However identification of products in mixtures can be difficult because of the various cyclic isomers of each compound giving rise to several chromatographic peaks. A convenient means of simplifying the analysis consists of reductions of the carbonyl function with borohydride and with borodeuteride, which permits the location of this function.[15] This reaction has been tested with various sugar phosphates and has been shown to be quantitative. A typical chromatogram of the trimethylsilyl derivatives obtained before and after reduction is shown in Figure 2. Compounds were also identified by hydrolysis with alkaline phosphatase,[19] followed by paper chromatography (Table 1) and g.l.c.–mass spectrometry of the trimethylsilyl derivatives.

When disodium fructose 6-phosphate in aqueous solution

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was irradiated in the absence of oxygen, three major products were observed by g.l.c.–mass spectrometry: orthophosphate, 2,4-dihydroxycyclobutyl phosphate, and

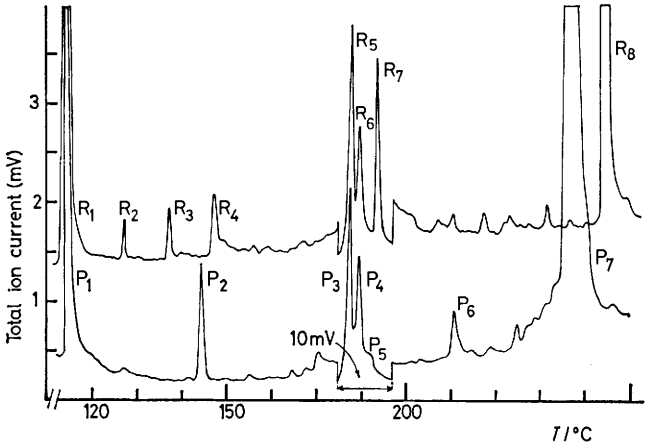

FIGURE 2 G.l.c. of photolysis products under helium (time of irradiation 3.25 h); lower chromatogram direct silylation; P_1 orthophosphate, P_2 not identified, P_3, P_4, and P_5 2,4-dihydroxycyclobutyl phosphate, P_6 not identified, P_7 fructose 6-phosphate; upper chromatogram (1 mV shifted) silylation after borohydride reduction; R_1 orthophosphate, R_2 reduced 2-deoxyerytrose, R_3 not identified, R_4 reduced glycolaldehyde phosphate, R_5 and R_6 2,4-dihydroxycyclobutyl phosphate, R_7 reduced 2-deoxyerythrose 4-phosphate, R_8 reduced fructose 6-phosphate

2-deoxyerythrose 4-phosphate. Of three minor compounds produced, two were identified as 2-deoxyerythrose and glycolaldehyde phosphate.

In the presence of oxygen, the same products were observed in smaller quantities, and another minor compound, glyceraldehyde 3-phosphate.

2-Deoxyerythrose 4-phosphate. After hydrolysis with alkaline phosphatase 2-deoxyerythrose [compound (B); Table 1] was identified by co-chromatography with an authentic sample obtained by Ruff degradation of arabinose.[20] The compound was also separated by preparative paper chromatography and after reduction was subjected to g.l.c.–mass spectrometry of the trimethylsilyl ether; the characteristic mass spectrum described by Dizdaroglu *et al.* was obtained.[21] The phosphate ester was also analysed by g.l.c.–mass spectrometry after borohydride reduction and derivatization (Figure 2; peak R_7). The mass spectrum obtained was attributed to the tetrakis-*O*-trimethylsilyl derivative of 2-deoxytetritol 4-phosphate (see Figure 3c); with borodeuteride reduction, the following ions were shifted by incorporation of one deuterium atom *m/e* 459 ⟶ 460; *m/e* 219 ⟶ 220; *m/e* 103 ⟶ 104, indicating that the reduced compound was 2-deoxyerythrose 4-phosphate.

2,4-Dihydroxycyclobutyl phosphate. Compound (C) (Table 1) was extracted by paper chromatography after orthophosphate release; when treated with borohydride it was not reduced. G.l.c.–mass spectrometry of the trimethylsilyl ethers showed three chromatographic peaks with the same mass spectra; from the fragmentation pathways (Figure 3a) (C) was tentatively identified as a cyclobutanetriol. In order to confirm this result, compound (C) was oxidized with periodic acid. Tests for formic acid and malonaldehyde were positive. However, the stoicheiometry could not be ascertained because of the presence of material extracted from the paper, which after oxidation gave positive formaldehyde and formic acid tests but negative reaction for malonaldehyde; however the proposed cyclobutane-1,2,3-triol structure was confirmed.

Before and after treatment with borohydride, g.l.c.–mass spectrometry of the trimethylsilyl derivatives of the phosphate mixture showed three unchanged chromatographic peaks (P_3, P_4, and P_5; Figure 2) with the same mass spectra (Figure 3b). The main fragmentation pathway is shown in Figure 3b; furthermore, ions at *m/e* 315 (21%), 299 (91), 227 (15), and 211 (16) indicated the presence of the phosphate end group; the absence of $(CH_2OSiMe_3)^+$ (*m/e* 103) and an intense peak at *m/e* 356 (23%) showed a cyclic structure; ions at *m/e* 341 (19%) and 328 (62%) are

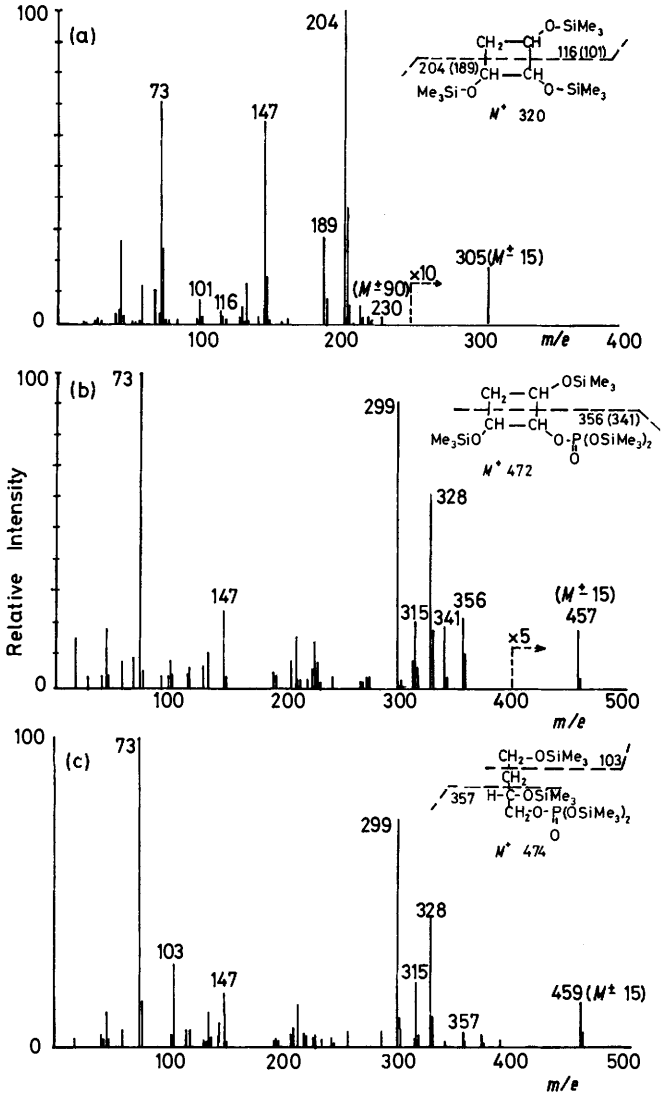

FIGURE 3 Mass spectra of trimethylsilyl derivatives of (a) cyclobutane-1,2,3-triol, (b) 2,4-dihydroxycyclobutyl phosphate, (c) reduced 2-deoxyerythrose 4-phosphate

probably obtained from this last ion (*m/e* 356) by methyl loss, internal transposition, and loss of CO; with *m/e* 204 $(Me_3SiO{\cdot}CH{:}CH{\cdot}SiMe_3)^+$ absent from the spectrum these

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phosphate esters were identified as isomers of tetrakis-trimethylsilyl derivatives of 2,4-dihydroxycyclobutyl phosphate.

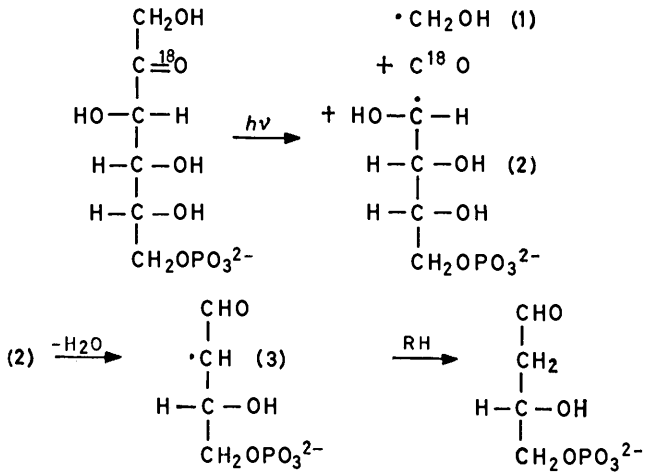
Glycolaldehyde phosphate. Reduction, derivatization, and g.l.c.–mass spectrometric analysis showed the presence of the tristrimethylsilyl derivative of ethylene glycol phosphate (peak R_4, Figure 2): M^+, *m/e* 358 not observed; $M^+ - CH_3$, *m/e* 343 (13%); phosphate *m/e* 315 (10%), 299 (57), 227 (8), and 211 (14); $(CH_2{\cdot}CH_2OSiMe_3)^+$ *m/e* 117 (13%); $(CH_2OSiMe_3)^+$ *m/e* 103 (7%); $(Me_3Si)^+$ *m/e* 73 (100%). Reduction by borodeuteride resulted in incorporation of one atom of deuterium and shifted the ions *m/e* 343 ⟶ 344, 117 ⟶ 118, and 103 ⟶ 104. This allowed R_4 to be identified as reduced glycolaldehyde phosphate.

Glyceraldehyde 3-phosphate. In the presence of oxygen and after reduction with borohydride, derivatization and subjection to the same chromatographic conditions (see Figure 2) gave another peak at 173 °C, identified as the tetrakistrimethylsilyl derivative of glycerol 1-phosphate by its mass spectrum (comparison with that of an authentic sample). Deuterium was incorporated in position 1 on reduction with borodeuteride [*m/e* 445 ⟶ 446 ($M^+ - 15$) and 103 ⟶ 104]. This shows that glyceraldehyde 3-phosphate was produced.

DISCUSSION

When aqueous solutions of fructose 6-phosphate were irradiated in the presence and in the absence of oxygen, carbon monoxide was immediately evolved at a high rate (see Figure 1). Tracer experiments with [2-^{18}O]-fructose 6-phosphate showed that this carbon monoxide originated from the carbonyl function. These results can be explained by assuming that the free keto form of the sugar phosphate is excited during the primary process, leading to a Norrish type I reaction [22] (see Scheme 1). This assumption is supported by the formation of 2-deoxyerythrose 4-phosphate: after CO loss, the phosphorylated radical (2) obtained undergoes rapid elimination of water to give the radical (3), which leads to 2-deoxyerythrose 4-phosphate; this elimination is well known and has been observed with various radicals from glycols,[23] monosaccharides,[24] and polyhydroxyorganic phosphates.[25]

A Norrish type I reaction has not been observed previously in the photochemistry of sugars and sugar phosphates. Indeed in aqueous solutions such compounds are usually in the cyclic pyranose or furanose forms and undergo hydrogen abstraction, so that photolysis is in many respects similar to radiolysis.[9,24] Glucose 6-phosphate and glucose 1-phosphate give this type of reaction;[7,26] the quantum yields for the photoreactions of these sugar phosphates in aqueous solution are low (0.8×10^{-2} and 1.02×10^{-2}, respectively) in the absence of oxygen (see Table 2) and a value of the same magnitude would be expected for hydrogen abstraction from fructose 6-phosphate. In fact the quantum yield obtained for this compound is much greater (ϕ 0.50; see Table 2), in good agreement with carbonyl excitation; as the u.v. absorption can be only partially attributed to the free keto form of the sugar phosphate, this value has to be taken as a minimum. Equilibration amongst the various anomeric and open-chain forms of sugar phosphates is rapid,[5,27] and such a photoreaction of fructose



SCHEME 1

6-phosphate would be possible even if the concentration of the free keto form in aqueous solution were low. A Norrish type I reaction can be the consequence of carbonyl excitation; stable products from a type II reaction [22,28] have not been identified. The same type of photochemical behaviour was observed with hydroxyacetone and 1,3-dihydroxyacetone: [29] with a neighbouring hydroxy-group the excited carbonyl is unable to abstract a hydrogen atom from H donors and exclusively undergoes C–C fragmentations.

2-Deoxyerythrose 4-phosphate from the primary reaction cannot give a stable acetal cyclic form in aqueous solutions, and undergoes further photoreactions of the carbonyl function: glycolaldehyde phosphate and 2,4-dihydroxycyclobutyl phosphate are obtained by way of a Norrish type II reaction as shown in Scheme 2. Cyclization can lead to three possible isomers, which were all observed by g.l.c.–mass spectrometry.

As noticed for glucose 6-phosphate [7] and 1-phosphate,[26] the presence of oxygen accelerates the photoreaction (ϕ 0.60), probably by causing chain reactions. The same photoproducts as in deoxygenated solutions are produced, but in lower yields; this shows that the

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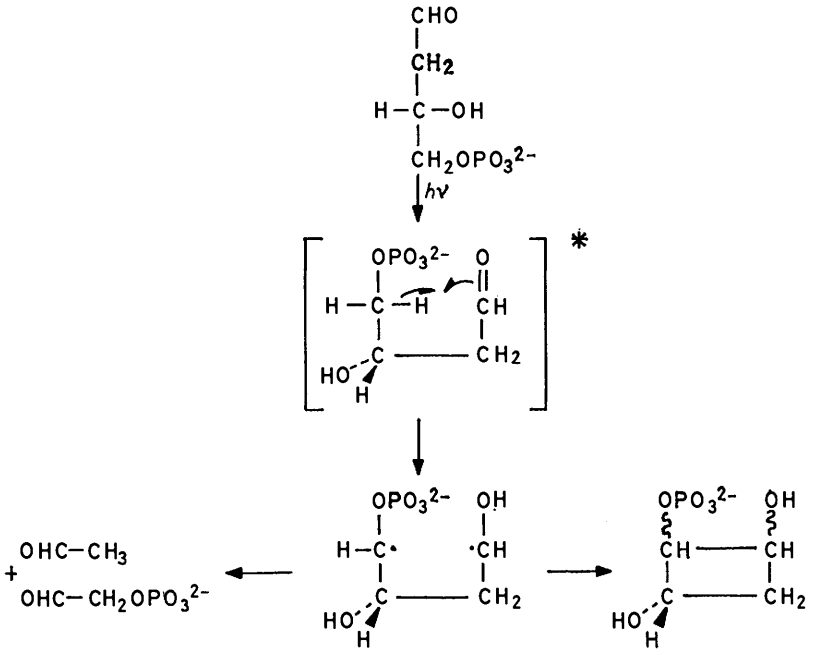
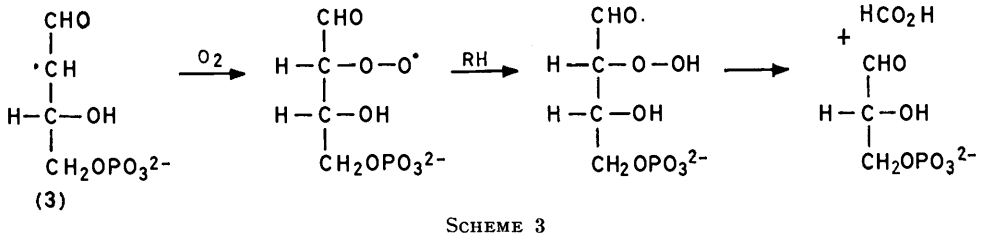
various radicals obtained after the primary reaction may react with oxygen:[30] radical (1) ($^{\bullet}CH_2OH$) is well known to give the peroxyl radical, which leads to formaldehyde and formic acid;[31] radical (2) has a too short a lifetime [23a,24] and oxidation of radical (3) is more likely, giving the observed glyceraldehyde 3-phosphate (Scheme 3).

Conclusions.—The photochemical behaviour of fructose 6-phosphate is in contrast with that of glucose 6-phosphate and 1-phosphate. This compound is rapidly decomposed by u.v. irradiation, giving a typical photoreaction of the carbonyl function. Thus the weak absorption observed in the 270 nm region can be, at least partially, attributed to the free keto form.



SCHEME 2



SCHEME 3

Orthophosphate formation seems to be due to secondary photodegradation processes and to hydrolysis of labile organic phosphates; 2-deoxyerythrose is probably obtained in this way from 2-deoxyerythrose 4-phosphate, which could be as labile as is erythrose 4-phosphate.[32] In the presence of oxygen, orthophosphate release is enhanced; significant amounts of organic acids are also formed, most probably with short carbon chains since in g.l.c.–mass spectrometry no long-chain carboxylated compounds were identified.

As noticed previously, malonaldehyde formation seems to be a general result of photolysis [26] or radiolysis [33] of carbohydrates and related compounds.

2-Deoxyerythrose 4-phosphate, an intermediate in the photodegradation, undergoes the same type of photoreaction. It appears that the photoreactions of sugar phosphates in aqueous solution depend markedly on the structural form of the solvated compound, a small amount of the free carbonyl form being sufficient to give the classical photoreactions of this function.

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