*Carbohydrate Research*, 100 (1982) 131–141
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

# PHOTOLYSIS OF D-FRUCTOSE IN AQUEOUS SOLUTION

CHRISTIAN TRIANTAPHYLIDES,
*Ecole Supérieure de Chimie, Université de Droit, d'Economie et des Sciences, Marseille (France)*

HEINZ-PETER SCHUCHMANN, AND CLEMENS VON SONNTAG
*Institut für Strahlenchemie im Max-Planck-Institut für Kohlenforschung, Stiftstr. 34–36, D-4330 Mülheim a.d. Ruhr (West Germany)*

(Received March 20th, 1981; accepted for publication, July 10th, 1981)

## ABSTRACT

In the 254-nm photolysis of aqueous solutions of D-fructose, only the open-chain form, which is present to an extent of ~0.8% in equilibrium with the cyclic forms, absorbs the light. A study of the products and their quantum yields reveals that the main, primary process is C–C bond cleavage $\alpha$ to the carbonyl group. In the absence of oxygen, the subsequent reactions of the resulting radicals are (*a*) loss of CO from the hydroxyalkylacyl radicals (estimated rate constant $k \sim 3 \times 10^6$ $s^{-1}$); (*b*) consecutive elimination of two molecules of water from the tetritol radicals; and (*c*) disproportionation and combination reactions. A peculiar product is *trans*-4-hydroxy-2-butenal, whose precursor is formed from the tetritol radical through elimination of two molecules of water. This compound is a good radical-scavenger and during photolysis quickly attains a low steady-state concentration. One of the products derived from it is a 2,3-dideoxy-2,3-di-*C*-hydroxymethyltetrose. In the presence of oxygen, the CO elimination process is partly, and the water elimination reactions are fully, suppressed by the fast addition of oxygen to the acylalkyl and hydroxyalkyl radicals. The peroxyl radicals react through unimolecular elimination of $HO_2^{\cdot}$ from $\alpha$-hydroxyalkylperoxyl radicals and bimolecular dismutation with loss of $O_2$, accompanied by loss of $CO_2$ when hydroxyalkylacylperoxyl radicals are involved.

## INTRODUCTION

Carbohydrates are degraded if their aqueous solutions are exposed to u.v. light. At the turn of this century, it had been observed that carbon monoxide is evolved thereby (*e.g.*, refs. 1–3). Despite more-recent efforts[4,5], the nature of the carbohydrate products remained largely unrevealed up to the advent of g.l.c. methods in carbohydrate analysis, which were applied in a study of the photolysis of D-fructose 6-phosphate[6].

In aqueous solution, such carbohydrates as D-fructose undergo mutarotation. The pyranoid and furanoid forms of D-fructose do not absorb in the near u.v., but

the open-chain form, which carries a carbonyl group, exhibits a weak absorption with the maximum at 278 nm. The absorption is very weak because it is due to a (forbidden) $n \rightarrow \pi^*$ transition [$\varepsilon$(278 nm) $\sim 54 M^{-1}.cm^{-1}$, ref. 7], and also because the relative concentration of the open-chain form in the equilibrium is very low (0.7–0.8%, refs. 7 and 8). Excited ketones are well known to undergo bond cleavage $\alpha$ to the carbonyl group. In D-fructose, this would lead to hydroxyalkyl and acyl radicals (*cf.* also glyceraldehyde[9] and trioses[10,11]). Some of the chemistry of such radicals is already known[12] from radiation-chemical work on carbohydrates and related compounds. Further data are now reported.

## RESULTS AND DISCUSSION

Deoxygenated as well as oxygenated aqueous solutions of D-fructose (0.1M) were photolysed with the 254-nm line from a Hg low-pressure arc, the 185-nm light emitted by this lamp being filtered off by a 2-mm Vycor quartz plate. Fig. 1 shows the absorption spectrum of a M aqueous solution of D-fructose (1-cm path-length). It can be seen that, at 254 nm, only the C=O chromophore of the carbonyl group of the open-chain form is excited. The $n \rightarrow$Rydberg transitions of the oxygen atoms in the OH groups, as well as in the ether groups of the cyclic components of the mutarotational mixture, occur at lower wavelengths.

The carbohydrate products were analysed by g.l.c.–m.s. as their trimethylsilyl derivatives with or without prior reduction by $NaBD_4$. Fig. 2 shows a typical gas chromatogram obtained from $NaBD_4$-reduced products after prolonged irradiation of a deoxygenated solution. The identification of the products was based on their

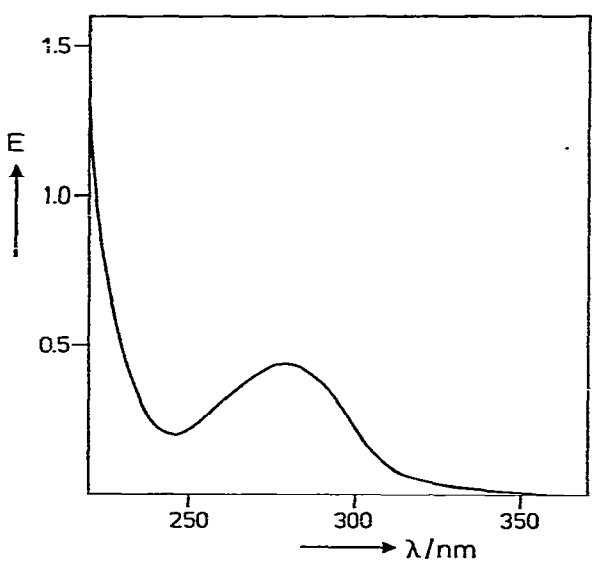



Fig. 1. Extinction (220–350 nm) of a M aqueous solution of D-fructose.

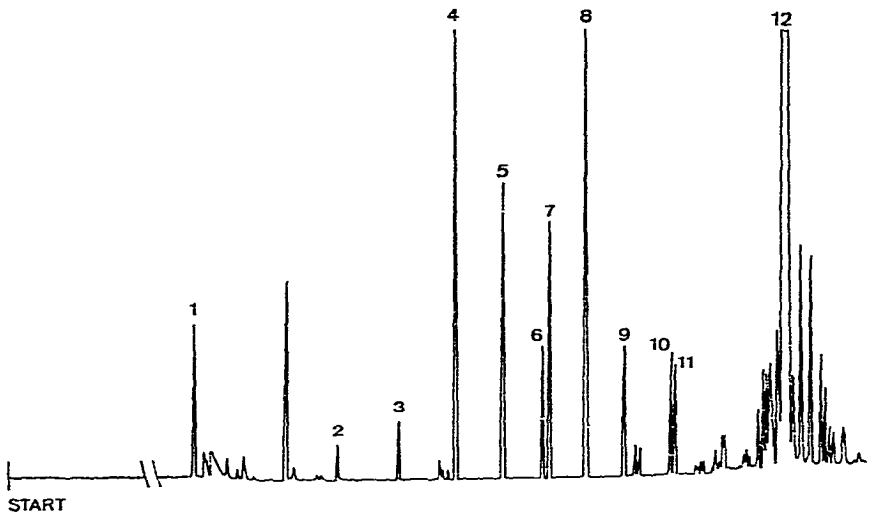



Fig. 2. Gas chromatogram of 254-nm photolysis products of D-fructose: glass capillary, OV-101 coated; temperature-programmed; carrier gas, $H_2$. Key: 1, ethylene glycol; 2, 2-butene-1,4-diol; 3, glycerol; 4, 2-deoxytetritol; 5, 2,3-dideoxy-2-*C*-hydroxymethyltetritol; 6, threitol; 7, erythritol; 8, 2-deoxy-2-*C*-hydroxymethyltetritol; 9, 2,3-dideoxy-2,3-di-*C*-hydroxymethyltetritol; 10, arabinitol; 11, ribitol; 12, mannitol + glucitol.

well-known mass-spectral fragmentation patterns[13]. In some cases, reference material was synthesised, or material isolated by preparative g.l.c. for identification by n.m.r. spectroscopy (see Experimental), in order to aid in the interpretation of the mass spectra. In deoxygenated solution, there was considerable formation of material of a molecular weight similar to, as well as higher than, that of the starting material. This range of products is only partially shown in Fig. 2, and no attempt has been made to identify them. However, it will be shown below that suggestions can be made as to the type of these products.

At the D-fructose concentration used in this work, the absorbance was 0.027 $cm^{-1}$ at 254 nm. Because of this, the conversion of D-fructose had to be kept very low (<0.5%) in order not to build up too high a concentration of products which would themselves be photolysed. The apparent quantum yield of some products (*e.g.*, some carbonyl compounds) tends to fall with increasing time of irradiation, whereas that of their photolysis products [*e.g.*, carbon monoxide and (in oxygenated solutions) carbon dioxide] rises. The quantum yields given in Table I were obtained by extrapolating a plot of quantum yields *vs.* irradiation time to zero time. Even with such a correction, the quantum yields given in Table I can only be considered as approximate.

(*a*) *Deoxygenated solutions.*

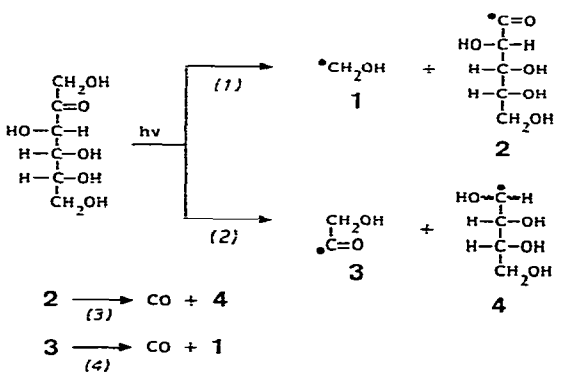
The primary reaction of many electronically excited, aliphatic ketones is C–C bond cleavage $\alpha$ to the C=O chromophore (Norrish Type I). This reaction is especially facilitated if the neighbouring carbons carry a hydroxyl group (*e.g.*, dihydroxy-

TABLE I

APPROXIMATE QUANTUM YIELDS IN THE 254-NM PHOTOLYSIS OF D-FRUCTOSE IN DEOXYGENATED AND OXYGENATED AQUEOUS SOLUTIONS

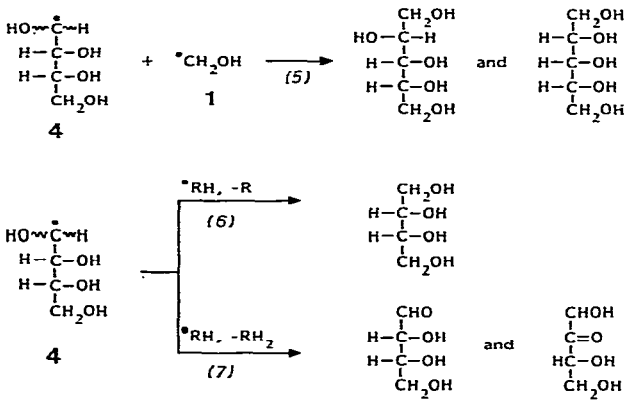
| *Product* | *Deoxygenated solutions* | *Oxygenated solutions* |
|---|---|---|
| Carbon monoxide | 0.2 | 0.02 |
| Carbon dioxide | absent | 0.07 |
| Formic acid | absent | 0.08 |
| Formaldehyde | 0.05 | 0.06 |
| Methanol | <0.02 | absent |
| Ethylene glycol | 0.03 | absent |
| Glycolic acid | traces | 0.06 |
| Glyceraldehyde | 0.015 | 0.04 |
| *trans*-4-Hydroxy-2-butenal | present | [a] |
| 2-Deoxytetrose | 0.035 | absent |
| Erythritol | 0.01 | absent |
| D-Erythrose | 0.03 | 0.08 |
| D-*glycero*-Tetrulose + D-threose | 0.01 | <0.01 |
| D-Erythronic acid | absent | 0.08 |
| D-Arabinitol | 0.02 | absent |
| Ribitol | 0.02 | absent |
| D-Arabinose | absent | absent |
| D-Arabinonic acid | absent | present |
| 2-Deoxy-2-*C*-hydroxymethyltetrose | 0.02 | absent |
| 2,3-Dideoxy-3-*C*-hydroxymethyltetrose | 0.015 | absent |
| 2,3-Dideoxy-2,3-di-*C*-hydroxymethyltetrose | 0.004 | absent |
| Hydrogen peroxide | absent | 0.06 |

[a]Not observed, most likely absent.

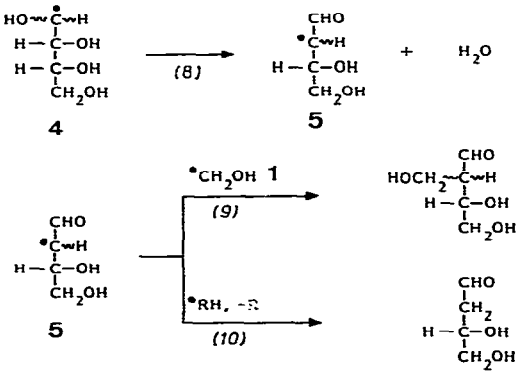



acetone[10,11]). Thus, it is not surprising that the open-chain form of D-fructose yields the primary radicals **1**–**4** (reactions *1* and *2*). Two of these are hydroxyalkylacyl radicals (**2** and **3**) which are known[14] to lose carbon monoxide readily (reactions *3* and *4*). The self-termination of the hydroxymethyl radical **1** gives largely the combination product ethylene glycol and only very little of the disproportionation products

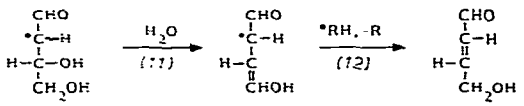
formaldehyde and methanol[15]. Radical **4** is only slightly pyramidal and rapidly inverts at the radical center. Combination with the hydroxymethyl radical **1** gives D-arabinitol and ribitol, respectively (reaction *5*). The two pentitols are formed in about equal yields, indicating that there is no significant preference with respect to either conformation of radical **4**. The combination of two radicals **4** would yield octitols, but these have not been measured. The disproportionation products (reactions *6* and *7*) D-erythrose and erythritol are also observed. It is to be expected (*cf.* ref. 16) that a considerable part of the disproportionation reactions leads to the enol (reaction *7*), which reverts to D-erythrose, D-threose, and D-*glycero*-tetrulose. The last two products are the precursors of the threitol formed on reduction.



It is well known[12] that 1,2-dihydroxyalkyl radicals such as **4** (ref. 17) readily eliminate water (reaction *8*). From **4**, another acylalkyl radical **5** is formed which exists in two conformations. Two stereoisomeric 2-deoxy-2-*C*-hydroxymethyltetroses arise on addition of the hydroxylmethyl radical **1** to **5** (reaction *9*). Acylalkyl radicals are strongly oxidising radicals[18]. Therefore, their reduction products, but never their oxidation products, have been observed[12]. Here, the reduction product of **5** is 2-deoxytetrose (reaction *10*, *cf.* ref. 17).

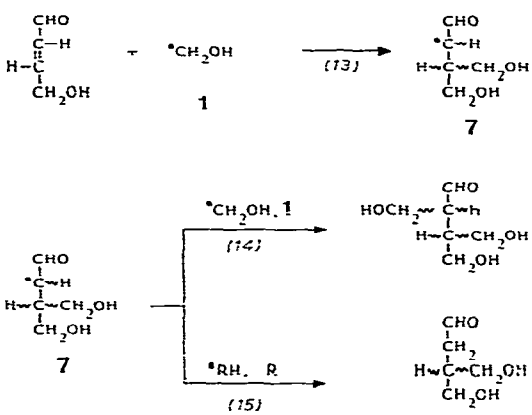


Up to this point, the free-radical chemistry is well established, especially by the numerous studies of the radiation chemistry of carbohydrates[12]. However, there are two important products which cannot be explained by the interaction of the radicals **1**–**5**. These are 2,3-dideoxy-2,3-di-*C*-hydroxymethyltetrose and 2,3-dideoxy-3-*C*-hydroxymethyltetrose. We suggest that these products have *trans*-4-hydroxy-2-butenal as a common precursor, a compound which has been observed at very low concentration. It would then be necessary to assume that radical **5** further loses a molecule of water to give **6** (reaction *11*), which is reduced in a disproportionation reaction to *trans*-4-hydroxy-2-butenal (reaction *12*). An efficient reduction of **6** is expected, because it presumably retains some of the oxidising properties of its precursor **5**.



**5** **6**

Such olefinic compounds as *trans*-4-hydroxy-2-butenal are very good radical scavengers. In free-radical systems, they only build up to low steady-state concentrations, especially at such low initiation rates as prevail in the present study. The scavenging of a hydroxymethyl radical **1** will lead to **7** (reaction *13*). Radical **7** either reacts with **1** to give 2,3-dideoxy-2,3-di-*C*-hydroxymethyltetrose (reaction *14*), or is reduced to yield 2,3-dideoxy-3-*C*-hydroxymethyltetrose (reaction *15*). The oxidising property[18] of the acylalkyl radicals **5** and **7** is quite high, and not only such reducing radicals as **1** and **4** but also D-fructose might well be the hydrogen donor (*cf.* ref. 19).

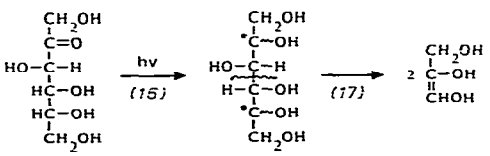


In Table I, quantum yields are given for the formation of 2,3-dideoxy-2,3-di-*C*-hydroxymethyltetrose and 2,3-dideoxy-3-*C*-hydroxymethyltetrose. In a strict sense, this is not correct because these are secondary products. However, the steady-state concentration of their precursor *trans*-4-hydroxy-2-butenal is very small, and hence the induction period is so short that it has not been possible to characterise these products as secondary on account of their build-up characteristics.

In support of the above mechanism, it should be mentioned that elimination of water, leading to an allyl-type radical, is not uncommon in carbohydrate free-radical chemistry, albeit observed with somewhat different kinds of radicals[12].

The present finding may lead to a better understanding of the high yields of polymeric material formed on γ-irradiation of carbohydrates in deoxygenated, aqueous solutions[20–22], where the intermediates of low molecular weight analogous to *trans*-4-hydroxy-2-butenal have so far not been detected. In the present system, material of higher molecular weight was also seen, but the intervention of a small radical (**1**) enables us to observe the first representatives of the series of products from the polymerisation process. In the radiolysis of aqueous solutions of carbohydrates, no such small radicals are formed and a reaction sequence similar to that described above has escaped detection.

Besides the Norrish type I reaction, ketones often undergo the Norrish type II reaction, a process by which a H atom is abstracted intramolecularly by the excited carbonyl (reaction *16*). In the D-fructose system, the resulting biradical **8** would break down into two molecules of the enol form of glyceraldehyde and dihydroxyacetone (reaction *17*). As only little of this material has been found, this process appears to be of minor importance in the present system.

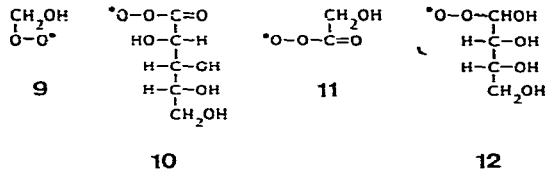



**8**

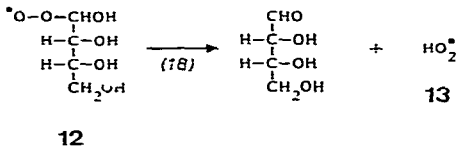
(*b*) *Oxygenated solutions*

In oxygenated solutions, the quantum yield for formation of carbon monoxide is considerably diminished, possibly because of a physical quenching of the excited ketone by the molecular oxygen, or because the acylalkyl radicals are scavenged by oxygen which prevents their decomposition (reactions *3* and *4*). The formation of carbon dioxide as a major product points to the latter reaction (see below), but, in the absence of an acceptable material balance, the former cannot be excluded.

Scavenging of the primary radicals **1–4** by oxygen leads to the corresponding peroxyl radicals **9–12**. Two of these (**9** and **12**) are α-hydroxyalkylperoxyl radicals which are known[12,23] to eliminate $HO_2^-$, forming the corresponding carbonyl compound (*e.g.* reaction *18*). The $HO_2^-$ elimination rate from **12** is quite fast[24], whereas that from **9** is comparatively slow[23] at natural pH and proceeds rapidly only at high pH. Thus, in the present case, an important reaction of **12** will be the unimolecular decay according to reaction *18*, but there will also be a contribution from bimolecular-decay processes. Radical **9** is considered to participate in the bimolecular-decay processes, as do **10** and **11**. It has been shown[25] that two hydroxymethylperoxyl radicals **9** predominantly react according to reaction *20*, but only to a small extent

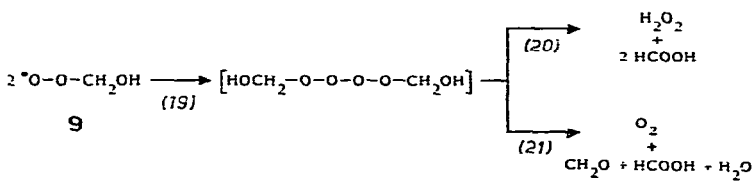


9 10 11 12

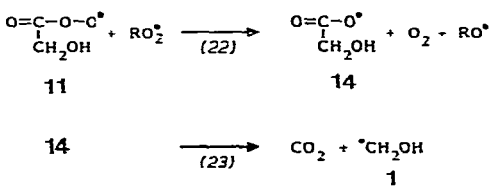


12 13

*via* the Russell mechanism (reaction *21*). A tetraoxide is believed to be an intermediate in these reactions (reaction *19*). Apparently, little is known about the decay of such hydroxyalkylacylperoxyl radicals as **10** and **11**, and their interaction with hydroxymethylperoxyl radicals **9** and **12** and hydroperoxyl **13** or superoxide radicals ($O_2^{\cdot-}$) [(pK($HO_2^{\cdot}$) = 4.8, *cf.* ref. 26], which must also be present in this system as they are generated by reaction **18**. The intermediate tetraoxides not only undergo molecular fragmentation processes [for example, the Russell mechanism and reactions equivalent to reaction *20* (possible products: glycolic acid and arabinonic acid)], but also a breakdown into oxyl radicals and molecular oxygen, *e.g.*, reaction *22*. The resulting oxyl radical **14** may fragment (reaction *23*, *cf.* ref. 27). Such a reaction would explain the formation of carbon dioxide.



9



11 14

14 1

acid $HO_2^{\cdot}$ (reaction *24*). The rate constant of its self-termination is negligible[26]. In their interaction with other peroxyl radicals, an ·OH radical may be generated (reaction *25*), leading to further oxidative degradation of the substrate[28].

$$HO_2^{\cdot} + O_2^{\cdot-} \xrightarrow[(24)]{H^+} H_2O_2 + O_2$$

$$RO{-}O^{\cdot} + HO_2^{\cdot} \xrightarrow[(25)]{} RO^{\cdot} + O_2 + {}^{\cdot}OH$$

Since the addition of oxygen to the primary radicals **1**–**4** is likely to be nearly diffusion-controlled[29], only very fast reactions can compete with the oxygen fixation at the oxygen concentrations used in these experiments. Water elimination (reaction *8*) is too slow to take place to a significant extent under such conditions. This has been shown with the aqueous D-glucose system, using $\gamma$-irradiation to generate the radicals[30]. Much faster than the water elimination reaction is the decarbonylation of the hydroxyalkylacyl radicals **2** and **3**. The $CH_3$-$\dot{C}O$ radical decarbonylates rather slowly[31] ($k = 1\ s^{-1}$), but the hydroxymethylacyl radical **3** decomposes very much faster, a rate constant near to $10^8\ s^{-1}$ having been suggested[11]. This value appears somewhat high. At such a value, carbon monoxide formation in our system would not have been suppressed and carbon dioxide would not have been formed. Assuming a diffusion-controlled rate ($k \sim 10^{10}\ M^{-1}.s^{-1}$) of oxygen addition to the hydroxyalkylacyl radicals **2** and **3** which prevents carbon monoxide formation and induces carbon dioxide formation, one calculates, from the oxygen concentration ($\sim 10^{-3}$M) and the 1:3 ratio of carbon monoxide to carbon dioxide, a decarbonylation rate constant of $\sim 3 \times 10^6\ s^{-1}$ for the hydroxyalkylacyl radicals **2** and **3**. This value, although lower than the earlier estimate, might still be on the high side considering the value measured[32] for the decarbonylation of the pivaloyl radical ($1.6 \times 10^5\ s^{-1}$), but it shows quite clearly the strong effect of the hydroxyl substituent on the unimolecular decay of the acyl radicals.

## EXPERIMENTAL

Before irradiation, 0.1M aqueous solutions of D-fructose in a suprasil QS cell (thickness, 1 cm; cross section, 4 $cm^2$; and capacity, 4 mL), fitted with a device[33] for bubbling with the desired gas, were saturated with oxygen, or purged with argon, for 20 min. Light from a low-pressure Hg lamp (Graentzel, Karlsruhe) was passed through a Vycor quartz plate that removed the 185-nm component; the incident intensity at 254 nm was $1.85 \times 10^{18}$ quanta/min/4 mL, of which $\sim 6\%$ are absorbed in a 0.1M solution. During irradiation, the cell was thermostated at $15 \pm 1°$, and its contents were stirred with a Teflon-coated magnetic bar. Irradiation times ranged up to 3 h.

Carbohydrate products were analysed by g.l.c.–m.s. after trimethylsilylation, which in some instances was preceded by reduction with sodium borodeuteride. Hydrogen peroxide was determined photometrically by the method of Allan *et al.*[34],

and formaldehyde by the method of Nash[35]. The gaseous products CO and $CO_2$ were transferred into a sampling bulb by bubbling with argon, and an aliquot was subjected to g.l.c. on 1m + 2m Poropak N at 45°. For protection of the catalyst column (see below), a backflush technique was used (*cf.* ref. 36). Downstream from the separating columns, hydrogen was added to the carrier gas, and CO and $CO_2$ were reduced to $CH_4$ on a Nickel catalyst [50% of $Ni(NO_3)_2$ on Chromosorb P activated by hydrogen within the column (12 cm, 2 mm i.d. at 350°)]. The catalyst column was mounted in the manifold of a Perkin–Elmer 900 gas chromatograph, and the manifold was heated to 350°. Methane was measured by flame ionisation.

Authentic 2,3-dideoxy-2,3-di-*C*-hydroxymethyltetritol was prepared[37] by reduction with $LiAlH_4$ of tetraethyl ethanetetracarboxylate, which was obtained from diethyl malonate by oxidation with bromine. *trans*-1,4-Dihydroxybutene is a minor component of the commercially available (Roth) *cis*-isomer. G.l.c. retention times and mass spectra of these two isomers, as well as of their $Me_3Si$ derivatives, are similar but not identical.

The $Me_3Si$ ether of 2,3-dideoxy-2-*C*-hydroxymethyltetritol was isolated by preparative g.l.c. from a $NaBH_4$-reduced sample after derivatisation. P.m.r. data ($CDCl_3$): $\delta$ 0.13(s), 0.15(s), 1.47(m), 1.69(m), 3.43(d), and 3.53(t). The mass spectrum showed characteristic fragment ions at *m/z* 73 (100%), 103 (30), 116 (20), 143 (20), 147 (50), 156 (60), and 321 (M − 15; 2%).

## REFERENCES


1 H. Bierry, V. Henri, and A. Ranc, *C.R. Acad. Sci.*, 151 (1910) 316–318.
2 D. Berthelot and H. Gaudechon, *C.R. Acad. Sci.*, 156 (1913) 707–710.
3 A. Ranc and R. Wurmser, *L'Ind. Chim.*, 7 (1920) 429–431.
4 G. O. Phillips, *Adv. Carbohydr. Chem.*, 18 (1963) 9–59.
5 G. O. Phillips and T. Rickards, *J. Chem. Soc., B*, (1969) 455–461.
6 C. Triantaphylides and R. Gerster, *J. Chem. Soc., Perkin Trans. 2*, (1977) 1719–1724.
7 W. Funcke, C. von Sonntag, and C. Triantaphylides, *Carbohydr. Res.*, 75 (1979) 305–309.
8 L. D. Hayward and S. J. Angyal, *Carbohydr. Res.*, 53 (1977) 13–20.
9 K.-G. Seifert and J. Bargon, *Angew. Chem.*, 85 (1973) 768–769.
10 K.-G. Seifert, *Tetrahedron Lett.*, (1974) 4513–4516.
11 S. Steenken, W. Jaenicke-Zauner, and D. Schulte-Frohlinde, *Photochem. Photobiol.*, 21 (1975) 21–26.
12 C. von Sonntag, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 7–77.
13 M. Dizdaroglu, D. Henneberg, and C. von Sonntag, *Org. Mass Spectrom.*, 8 (1974) 335–345.
14 S. Steenken and D. Schulte-Frohlinde, *Tetrahedron Lett.*, (1973) 653–654.
15 H. Seki, R. Nagai, and M. Imamura, *Bull. Chem. Soc. Jpn.*, 41 (1968) 2877–2881.
16 B. Blank, A. Henne, G. P. Laroff, and H. Fischer, *Pure Appl. Chem.*, 41 (1975) 475–494.
17 M. Dizdaroglu, H. Scherz, and C. von Sonntag, *Z. Naturforsch., Teil B*, 27 (1972) 29–41.
18 S. Steenken, *J. Phys. Chem.*, 83 (1979) 595–599.
19 C. von Sonntag and E. Thoms, *Z. Naturforsch., Teil B*, 25 (1970) 1405–1407.
20 S. A. Barker, P. M. Grant, M. Stacey, and R. B. Ward, *J. Chem. Soc.*, (1959) 2648–2654.
21 A. J. Bailey, S. A. Barker, and M. Stacey, *Radiat. Res.*, 15 (1961) 538–545.
22 J. B. Snell, *J. Polym. Sci., Part A*, 3 (1965) 2591–2607.
23 E. Bothe, M. N. Schuchmann, D. Schulte-Frohlinde, and C. von Sonntag, *Photochem. Photobiol.*, 28 (1978) 639–644.
24 E. Bothe, D. Schulte-Frohlinde, and C. von Sonntag, *J. Chem. Soc., Perkin Trans 2*, (1978) 416–420.

25 E. BOTHE AND D. SCHULTE-FROHLINDE, *Z. Naturforsch., Teil B*, 33 (1978) 786–788.
26 B. H. J. BIELSKI, *Photochem. Photobiol.*, 28 (1978) 645–649.
27 J. A. HOWARD, in J. K. KOCHI (Ed.), *Free Radicals*, Vol. II, Wiley, New York, 1973, pp. 3–62.
28 M. N. SCHUCHMANN AND C. VON SONNTAG, *J. Phys. Chem.*, 83 (1979) 780–784.
29 G. E. ADAMS AND R. L. WILLSON, *Trans. Faraday Soc.*, 65 (1969) 2981–2987.
30 M. N. SCHUCHMANN AND C. VON SONNTAG, *J. Chem. Soc., Perkin Trans. 2*, (1977) 1958–1963.
31 J. A. KERR AND J. G. CALVERT, *J. Phys. Chem.*, 69 (1965) 1022–1029.
32 H. SCHUH, E. J. HAMILTON, JR., H. PAUL, AND H. FISCHER, *Helv. Chim. Acta*, 57 (1974) 2011–2024.
33 F. WEEKE, E. BASTIAN, AND G. SCHOMBURG, *Chromatographia*, 7 (1974) 163–170.
34 A. O. ALLEN, C. J. HOCHANADEL, J. A. GHORMLEY, AND T. W. DAVIS, *J. Phys. Chem.*, 56 (1952) 575–586.
35 T. NASH, *Biochem. J.*, 55 (1953) 416–421.
36 D. R. DEANS, *Chromatographia*, 1 (1968) 18–22.
37 A. F. NORRIS, Thesis, University of Salford, 1977, p. 80.