**652.** *Photo-degradation of Carbohydrates. Part III.[1] Sensitized Photo-oxidation of D-Sorbitol.*

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When oxygenated aqueous solutions of D-sorbitol containing, as sensitizers, sodium anthraquinone-2-sulphonate or -2,6-disulphonate are irradiated with 3650 Å radiation, the main reaction is hexose formation. Hexonic acids are produced at a slower rate. The sensitized photo-oxidation is first-order in D-sorbitol, and the initial rate is proportional to the amount of light absorbed in the sensitizer until absorption is complete. The sensitizer is regenerated by a cyclic mechanism involving oxygen, but particularly at low D-sorbitol concentrations, is destroyed by a side-reaction which leads to coloured products. The proposed mechanism is in agreement with that previously suggested for the photo-sensitized oxidation of alcohols. Deactivation of the photo-excited sensitizer (Q^*) is due to competition between

$$RCH_2 \cdot OH + Q^* \longrightarrow \cdot QH + R\dot{C}H \cdot OH$$

and

$$Q^* \longrightarrow Q$$
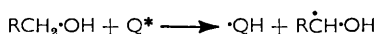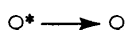
Thus, quantum yields for hexose production are dependent on hexitol concentration. Values are the same for D-sorbitol and D-mannitol.

RADIATION in the far ultraviolet initiates direct photolysis of carbohydrates.[1,2] In the presence of certain dyes and substances such as zinc oxide and titanium oxide, photo-degradation may occur with light in the near-ultraviolet or visible parts of the spectrum, and such photo-oxidations have been studied mainly for cellulose and cellulosic materials.[2,3] In contrast to the direct photolysis it has been shown conclusively that, for the photo-sensitized deterioration of cellulose, the presence of oxygen is necessary and dyes generally act as initiators rather than inhibitors.[4] For carbohydrates little attention has been given to the course of such reactions, although the autoxidation of alcohols and related compounds in aqueous solutions has been studied.[5-7] In this Paper, as a model for more complicated carbohydrates, the sensitized photo-oxidation of D-sorbitol in aqueous solution is described. Sodium anthraquinone-2-sulphonate and -2,6-disulphonate were employed since the wide difference in reactivity shown by these sensitizers allows photo-oxidation to be studied over a wide range of conditions.

EXPERIMENTAL AND RESULTS

The irradiation procedures using a Hanovia 220 w medium-pressure mercury lamp have been described,[1] and, to remove all radiation which might initiate direct photolysis, a Pyrex filter was used. Generally, the incident wavelengths were confined to the 2940—4000 Å region by use of a Chance OX1 filter which had maximum transmission (67%) at 3650 Å. Sodium anthraquinone-2-sulphonate and 2,6-disulphonate were recrystallised from alcohol. Oxygen was passed continuously through the solution during irradiation. Highly purified water[8] was used in the preparation of the solutions. Reducing products were estimated using the Somogyi reagent,[9] and the energy incident on the irradiation vessel was determined using a potassium ferrioxalate actinometer.[10] When a layer of x cm. of filter solution is interposed between the

[1] Part II, Phillips and Barber, *J.*, 1963, 3990.
[2] Phillips, *Adv. Carbohydrate Chem.*, 1963, **18**, 9.
[3] See symposium, *J. Soc. Dyers and Colourists*, 1949, **65**, 585.
[4] Egerton, *J. Soc. Dyers and Colourists*, 1949, **65**, 764; *ibid.*, 1947, **63**, 161; *Textile Res. J.*, 1948, **18**, 659; *J. Textile Inst.*, 1948, **39**, T 293.
[5] Bolland and Cooper, *Proc. Roy. Soc.*, 1954, *A*, **225**, 405.
[6] Bridge and Porter, *Proc. Roy. Soc.*, 1958, *A*, **244**, 259, 276; Bridge and Reed, *Trans. Faraday Soc.* 1960, **56**, 1796.
[7] Wells, *Trans. Faraday Soc.*, 1961, **57**, 1703, 1719; *J.*, 1962, 3100.
[8] Amphlett, *Discuss. Faraday Soc.*, 1952, **12**, 145.
[9] Somogyi, *J. Biol. Chem.*, 1952, **22**, 195.
[10] Hachard and Parker, *Proc. Roy. Soc.*, 1954, *A*, **225**, 518.

lamp and the vessel, the expression $(d + x)$ cm. will be used, where d is the constant distance of the lamp from the quartz window (*ca.* 2 cm. of air).

The Absorbing Species.—Solutions (250 ml.) of D-sorbitol (0·275M) containing sodium anthraquinone-2-sulphonate (1×10^{-4}M) were irradiated at $(d + 1)$ cm. of (a) water, (b) 0·275M-D-sorbitol solution, and (c) 1×10^{-3}M-sodium anthraquinone-2-sulphonate. Fig. 1 shows that the rate of formation of reducing products is the same for filter solutions (a) and (b). No reaction occurs, however, after 120 min. irradiation using filter (c).

Effect of D-Sorbitol Concentration.—Solutions (250 ml.) of D-sorbitol (1×10^{-4}M in sodium anthraquinone-2-sulphonate) were irradiated at $(d + 0)$ cm. of water with unfiltered light, and the solute concentration was varied from 0·138 to 0·413M. The reaction rate and equilibrium position increased with D-sorbitol concentration (Fig. 2). Similar behaviour was observed over a wider concentration range (0·138—1·75M) when the sensitizer concentration was 5×10^{-5}M and irradiation was at $(d + 1)$ cm. of water through an OX1 filter. From the results, the initial rate of formation of reducing products can be shown to increase linearly with D-sorbitol
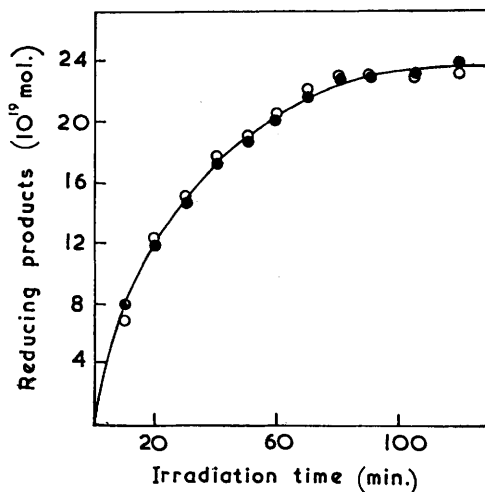

FIG. 1. Irradiation of aqueous D-sorbitol (0·275M) containing sodium anthraquinone-2-sulphonate (1×10^{-4}M) at $(d + 1)$ cm. Filter solutions:
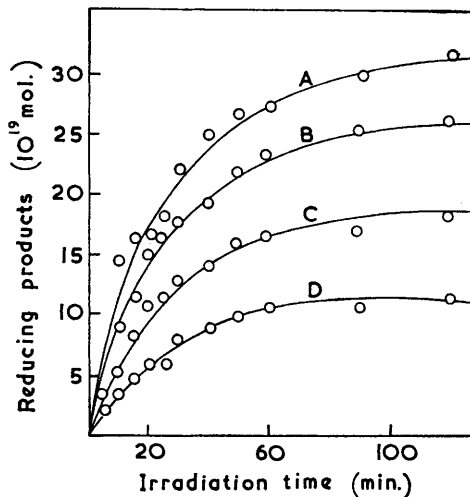
    ○ water.
    ● 0·27M-D-sorbitol

FIG. 2. Effect of D-sorbitol concentration on the photo-oxidation sensitized by sodium anthraquinone-2-sulphonate (1×10^{-4}M) using unfiltered light.

A, 0·413M.   C, 0·206M.
B, 0·344M.   D, 0·138M.

concentration (Table 1). A similar relationship was observed with sodium anthraquinone-2,6-disulphonate, but here an appreciably greater reactivity was found at equivalent sensitizer and D-sorbitol concentrations.

Solutions (50 ml.) of D-sorbitol (0·044—0·220M) (1×10^{-4}M in anthraquinone-2,6-disulphonate) were irradiated at $(d + 1)$ cm. of water through an OX1 filter. The rate of formation of reducing products was measured, and, from yield–dose curves similar to those already shown for the monosulphonate sensitizer, initial rates were measured (Table 1). A direct comparison of the reactivities of the two sensitizers under identical conditions is shown in Table 2.

TABLE 1.

Effect of D-sorbitol concentration on the rate of sensitized photo-oxidation.

(*a*) Sodium anthraquinone-2-sulphonate (5×10^{-5}M).

D-Sorbitol (M)	0·138	0·275	0·413	0·55	0·75	1·0	1·2	1·5	1·75
Initial rate (10^{16} mol. ml.$^{-1}$ min.$^{-1}$) ...	0·25	0·98	1·12	1·42	1·87	2·37	2·94	3·52	4·54

(*b*) Sodium anthraquinone-2,6-disulphonate (1×10^{-4}M).

D-Sorbitol (M)	0·043	0·087	0·131	0·176	0·211
Initial rate (10^{16} mol. ml.$^{-1}$ min.$^{-1}$) ...	3·1	4·5	7·6	8·8	10·3

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[1964] *Photo-degradation of Carbohydrates. Part III.* 3445

Effect of Sensitizer Concentration.—(a) *Sodium anthraquinone-2-sulphonate.* Using unfiltered light, 0·275M-D-sorbitol solutions (250 ml.) were irradiated at $(d + 0)$ cm. in the presence of 0·1—1·0 × 10^{-4}M-sensitizer. The rate of formation of reducing products increased with sensitizer concentration, and each yield–dose curve attained a point of equilibrium (Fig. 3).
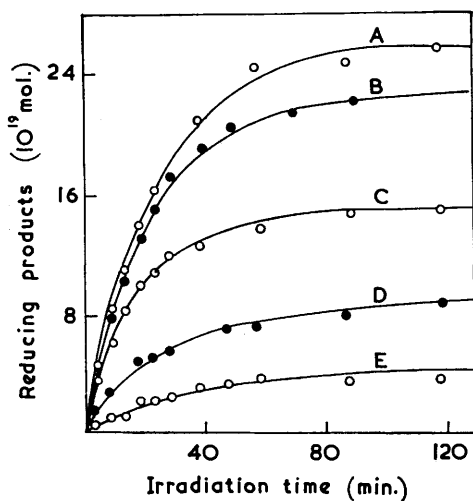

FIG. 3. Effect of sensitizer concentration on the photo-oxidation of D-sorbitol (0·275M) using unfiltered light.

A, 1 × 10^{-4}M.　　D, 0·25 × 10^{-4}M.
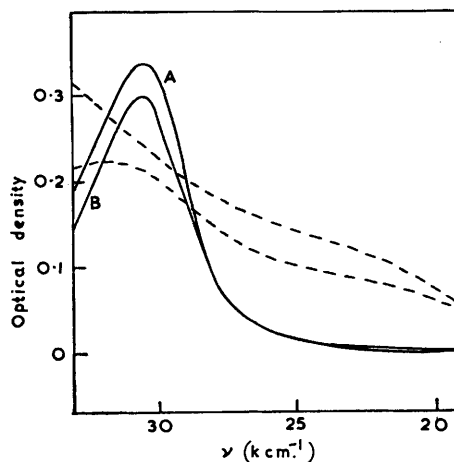B, 0·75 × 10^{-4}M.　E, 0·10 × 10^{-4}M.
C, 0·5 × 10^{-4}M.

FIG. 4. Absorption spectra of sensitizers used in the photo-oxidation of D-sorbitol (5·5 × 10^{-2}M).

A, sodium anthraquinone-2,6-disulphonate (0·6 × 10^{-4}M).
B, Sodium anthraquinone-2-sulphonate (0·5 × 10^{-4}M).
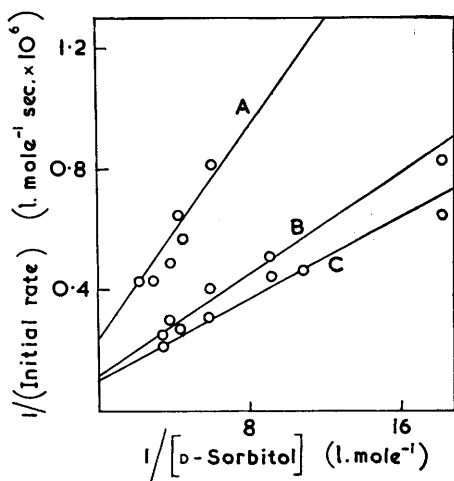
(The broken lines are the spectra after irradiation.)

FIG. 5. Plot of 1/(Initial rate) against 1/[D-Sorbitol] at various concentrations of sodium anthraquinone-2,6-disulphonate.

A, 0·5 × 10^{-4}M.　　C, 1·2 × 10^{-4}M.
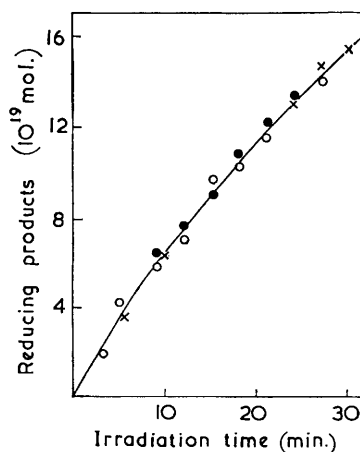B, 1·0 × 10^{-4}M.

FIG. 6. Effect of temperature and pH on the photo-oxidation sensitized by sodium anthraquinone-2-sulphonate (0·5 × 10^{-4}M).

○, pH 6·5 (23°).
●, pH 3·0 (23°); ×, pH 6·5 (7°).

TABLE 2.

A comparison of sensitizer (2×10^{-3}M) reactivity during the photo-oxidation of
D-sorbitol (0·055M) at ($d + 1$) cm. of water.

	Reducing molecules/ml. ($\times 10^{18}$)						
Sodium anthraquinone-2-sulphonate	0·43	0·81	0·90	1·26	1·53	1·80	1·89
Sodium anthraquinone-2,6-disulphonate	2·23	3·61	4·60	5·28	6·77	7·31	7·98
Time (min.)...	15	30	45	60	115	140	180

At this stage the ratio of the number of reducing molecules produced to the number of sensitizer molecules destroyed in each case was approximately 20 : 1. Using light passing through an OX1 filter, the effect of sensitizer concentration upon the initial rate of production of reducing molecules was examined at D-sorbitol concentrations of 0·275 and 1·75M. The results (Table 3a) show that the increase in initial rate depends linearly on sensitizer concentration at the two concentrations of D-sorbitol.

TABLE 3.

Effect of sensitizer concentration on the rate of photo-oxidation.

(a) Sodium anthraquinone 2-sulphonate.			(b) Sodium anthraquinone-2,6-disulphonate.		
Sorbitol (M)	Sensitizer (10^{-4}M)	Initial rate (10^{16} mol. ml.$^{-1}$ min.$^{-1}$)	D-Sorbitol (M)	Sensitizer (10^{-4}M)	Initial rate (10^{16} mol. ml.$^{-1}$ min.$^{-1}$)
0·275	0·25	0·49	0·055	0·1	0·2
,,	0·50	0·98	,,	0·5	1·2
,,	1·00	1·99	,,	0·8	2·6
1·75	0·10	0·96	,,	2	6·0
,,	0·25	2·56	,,	8	7·5
,,	0·50	4·94	,,	10	7·0
,,	0·75	7·44	,,	50	7·5

(b) *Sodium anthraquinone-2,6-disulphonate.* The rate of photo-oxidation showed a similar dependence on sensitizer concentration (Table 3b). No further increase in rate was observed at sensitizer concentrations above 4×10^{-4}M, when complete absorption of the light occurred.
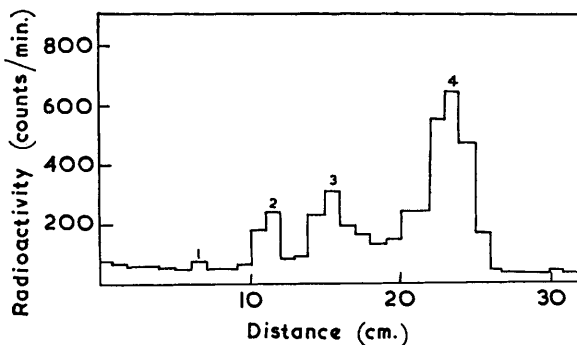

FIG. 7. Distribution of carbon-14 along paper chromatogram of irradiated D-[^{14}C]sorbitol solutions containing sodium anthraquinone-2,6-disulphonate (4×10^{-3}M).

1, Acids; 2 and 3, hexoses.
4, D-sorbitol.

The molar extinction of the 2,6-disulphonate at the absorption maximum (3310 Å) is $5·9 \times 10^3$, and for the 2-sulphonate $5·7 \times 10^3$. Both sensitizers have similar absorption spectra (Fig. 4).

A plot of 1/(Initial rate) against 1/[D-Sorbitol] gives a straight line at sensitizer concentrations of 0·5—$1·2 \times 10^{-4}$M and D-sorbitol concentrations of 0·043—0·211M (Fig. 5). The equation [7] for the line is:

$$1/(\text{Initial rate}) = \text{Intercept} + \text{Slope}/[\text{D-Sorbitol}].$$

Values of the intercept and slope are shown in Table 4.

Effect of Temperature.—D-Sorbitol solution (250 ml.; 1·0M) containing sodium anthraquinone-2-sulphonate ($0·5 \times 10^{-4}$M) was irradiated with the surrounding bath at 23·3 and 7°.

TABLE 4.

Effect of variation in sensitizer and D-sorbitol concentration on photo-oxidation rates.

Sensitizer (M)	Intercept (l. mole⁻¹ sec. × 10⁻⁶)	Slope (sec. × 10⁻⁶)	(Slope)/(Intercept)
0.5×10^{-4}	0.24	0.068	0.28
1.0×10^{-4}	0.11	0.034	0.31
1.2×10^{-4}	0.10	0.027	0.27

The pH was also varied from 7 to 3 with sulphuric acid. Fig. 6 shows that the reaction rates are independent of temperature and pH in this range.

Reaction Products.—Nature of the reducing products. For the product studies, sodium anthraquinone-2,6-disulphonate was used as sensitizer because the greater reactivity allows a higher conversion of D-sorbitol into products before destruction of the sensitizer by side-reactions. Paper-chromatographic and radioisotopic techniques previously used to examine the γ-irradiation of D-sorbitol solutions [11] were applied. In a typical experiment D-sorbitol solution (0·055M;

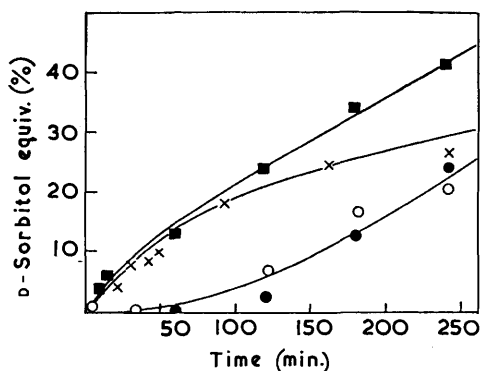

FIG. 8. Product formation and D-sorbitol disappearance during photo-oxidation of D-sorbitol sensitized by sodium anthraquinone-2,6-disulphonate.

■, D-Sorbitol disappearance from radiochromatogram.
×, reducing products from direct measurement.
○, D-sorbitol disappearance not accounted for by reducing products.
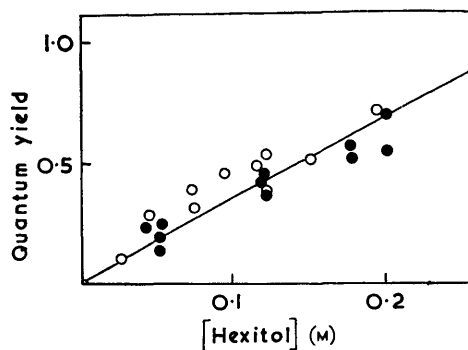●, acid production from radiochromatogram.

FIG. 9. Variation in quantum yields of hexose production with hexitol concentration.

○, D-Mannitol.
●, D-Sorbitol.

50 ml.) containing D-[¹⁴C]sorbitol (50 μc) and sodium anthraquinone-2,6-disulphonate (4×10^{-3}M) was irradiated at $(d + 1)$ cm. of water for 240 min. Samples (*ca.* 0·3 ml.) were removed at successively increasing doses, and aliquots (0·04 ml.) were applied to filter-paper (Whatman No. 1) strips 50 cm. long and 2·75 cm. wide. After irrigation in methyl ethyl ketone–saturated aqueous boric acid–acetic acid (9 : 1 : 1) the papers were dried and scanned using an automatic radiochromatogram scanner. A typical histogram is shown in Fig. 7, which reveals unchanged D-sorbitol and the main products, *viz.*, D-glucose, L-gulose, and the corresponding hexonic acids. From histograms prepared after varying doses, the extent of decomposition of D-sorbitol was estimated, and the % decomposition with dose is shown in Fig. 8, which gives also the rate of hexose formation when estimated from reducing-power measurements. Hexose production initially parallels the amount of D-sorbitol degraded, but, thereafter, only represents a proportion of it. The extent to which these yield–dose curves diverge parallels the hexonic acid formation.

Side-reaction of the sensitizer. When mixtures of D-sorbitol and sodium anthraquinone-2-sulphonate were irradiated, a yellow colour was produced, and was particularly marked at low D-sorbitol concentration. This colour is formed also when sodium anthraquinone-2-sulphonate

[11] Phillips and Criddle, *J.*, 1961, 3756, 3763; Phillips and Davies, *J.*, 1964, 205.

(0.5×10^{-3}M) was irradiated at ($d + 0$) cm. of water for 3 hr. in the absence of D-sorbitol. Concentration of this solution and addition of 2N-sodium hydroxide (0.5 ml.) led to an intense wine-red solution.   Four coloured bands were produced when this solution was separated on an alumina column using aqueous sodium hydroxide (2N) as eluent.[12]   The irradiated solution containing D-sorbitol behaved similarly, but the separated bands have not been identified.   In an experiment using D-[^{14}C]sorbitol, no radioactivity was associated with the coloured fractions. The absorption spectra of the sensitizers before and after irradiation at two D-sorbitol concentrations are shown in Fig. 4.   The increase in absorption at 4000 Å due to the side-reaction is significantly greater at a D-sorbitol concentration of 0.055 than at 1.75M (Table 5).

Quantum yields of products.   Fig. 9 shows the increase in quantum yields of reducing products during the irradiation of D-sorbitol and D-mannitol solutions, when the sensitizer was sodium anthraquinone-2,6-disulphonate.   All measurements were carried out using an OX1 filter.   The amount of light incident on the vessel was measured with the potassium ferrioxalate actino-

TABLE 5.

Increase in optical density at 4000 Å during the irradiation of D-sorbitol solutions
0.25×10^{-4}M in sodium anthraquinone-2-sulphonate.

D-Sorbitol (M)	Optical density					
0.055	0.010	0.045	0.050	0.060	0.080	0.082
1.75	0.010	—	—	0.013	—	0.020
Time (min.)	0	5	10	15	25	35

TABLE 6.

A comparison of quantum yields of reducing products and hydrogen peroxide;
sensitizer, sodium anthraquinone-2-sulphonate.

D Sorbitol (M)	0.138	0.275	0.413	0.55	0.75	1.0	1.2	1.5	1.75
Reducing products	0.02	0.07	0.08	0.10	0.14	0.17	0.22	0.26	0.33
Hydrogen peroxide	—	—	—	—	—	0.16	0.19	0.18	—

meter [10] and the proportion absorbed estimated on the basis that the active wavelength was the 3650 Å mercury line.

In the same way, quantum yields for reducing products and hydrogen peroxide were estimated, and Table 6 shows a comparison of the results for a range of D-sorbitol concentrations.

DISCUSSION

The direct photolysis of D-sorbitol is initiated by the absorption of radiation in the region 2300—2400 Å.[1,2]   The sensitized photo-oxidation reported here, however, is due to light-absorption by the sensitizer, as indicated by the absence of reaction on irradiating through a filter of ($d + 1$) cm. of sodium anthraquinone-2-sulphonate solution (10^{-3}M) and the absence of any inhibiting influence of a filter of D-sorbitol solution (0.275M) (Fig. 1).   The photo-oxidation yields reducing products and hydrogen peroxide, but the rate decreases as irradiation is continued (Fig. 2) owing to the destruction of the sensitizer with the formation of a yellow colour.   A similar behaviour accompanied the photo-sensitized oxidation of ethanol, when a coloured product showing spectroscopic characteristics identical with those observed by us was identified as sodium 9,10-dihydroxyanthracene-2,6-disulphonate.[5,6]   Disproportionation of the intermediate semiquinone radicals was considered to lead to this product.[5,6]   Mooney and Stonehill,[12] however, whilst observing similar coloured products formed during irradiation of sodium anthraquinone-2-sulphonate with light of wavelengths higher than 4000 Å, claim that the products arise by hydroxylation of the sensitizer by hydroxyl radicals formed during photodecomposition of water.   Our observations favour the former explanation since the side-reaction is minimised by increasing D-sorbitol concentration (Table 4), and the stationary concentration of reducing products formed on termination of the reaction is in direct proportion to initial D-sorbitol concentration (Fig. 2).   Disproportionation as distinct from the fast reaction of OH

[12] Mooney and Stonehill, *Chem. and Ind.*, 1961, 1309.

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[1964] *Photo-degradation of Carbohydrates. Part III.* 3449

radicals appears, therefore, to be a competing reaction of the excited sensitizer with D-sorbitol.

The main initial reaction, however, is oxidation of D-sorbitol to hexose; D-glucose and L-gulose would arise from each extremity of the molecules. Hexose formation initially corresponds to the amount of D-sorbitol degraded (Fig. 7). Acid production from the yield–dose curves shows the characteristics of a secondary process, but the possibility that there is a small but significant initial rate of acid formation cannot be eliminated.

The sensitizer clearly operates by a cyclic mechanism, since, on termination of the reactions, at least 20 hexose molecules are produced for each sensitizer molecule destroyed, a ratio which is independent of sensitizer concentration.

The rate of photo-oxidation is first-order in D-sorbitol and in excited sensitizer (Q^*), a limiting factor here being the point at which all the light is absorbed (Table 3b). Thus, as for the corresponding alcohol studies, two competing processes may be envisaged: [7]

$$RCH_2 \cdot OH + Q^* \xrightarrow{k_1} \cdot QH + R\dot{C}H \cdot OH \qquad (1)$$

and

$$Q^* \xrightarrow{k_0} Q \qquad (2)$$
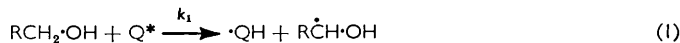
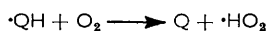giving effective kinetics represented by

$$-1\{d(RCH_2 \cdot OH)/dt\} = 1/I + (1/I)(1/[RCH_2 \cdot OH])(k_0/k_1)$$

where I = rate of activation, proportional to intensity of light. This relationship is obeyed, as shown by the linear relationship between 1/(Initial rate) and 1/[Sorbitol] (Fig. 5). As observed by Wells,[7] the process $Q^* \longrightarrow Q$ is not affected by variation in D-sorbitol or sensitizer concentration as indicated by the constant values of (Slope)/(Intercept). Thus, the physical modes of deactivation by collision with solvent molecules, or water, or sensitizer are not significant. Furthermore, no fluorescence was observed by us in any of our studies. The absence of any temperature effect in the range 7—23·3° indicates that thermal activation energy is not required for the primary processes responsible for the deactivation of the sensitizer (reactions 1 and 2). It is probable also that any significant contribution of ionic processes to the photo-oxidation would be reflected by an influence on the rate with change of pH. The fact that none was found further supports the free-radical mechanism.
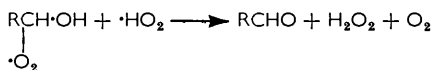
When light-absorption is complete, the initial rate is independent of sensitizer concentration (Table 3), but, as required by the competition between reactions (1) and (2), the quantum yield for hexose formation increases with hexitol concentration (Fig. 9). Since oxygen is continuously bubbled through the system during photodecomposition, a source of error is introduced into the quantum-yield measurements. However, the values demonstrate that no chain process involving D-sorbitol is present. Wells[7] was able to attain quantum yields approaching unity at propan-2-ol concentrations of 4M. At this high concentration, which cannot be achieved with the solid hexitols, all the photo-excited sensitizer is deactivated by reaction (1).

No significant steric effects were observed between D-sorbitol and D-mannitol such as Wells[7] observed with ring-sugars. However, no such stringent steric requirements for confirmation, present in hexose derivatives, would in any event be expected for the hexitols, particularly since the predominant reaction is at the primary alcohol extremities of the molecule.

The mechanism of formation of hexoses from the intermediate free radical $R\dot{C}H \cdot OH$ in oxygen requires the formation of equimolar amounts of hexose and hydrogen peroxide according to:

$$\cdot QH + O_2 \longrightarrow Q + \cdot HO_2$$

and

$$\underset{\underset{\cdot O_2}{|}}{RCH \cdot OH} + \cdot HO_2 \longrightarrow RCHO + H_2O_2 + O_2$$

Such an equivalent is approached (Table 5). The yellow colour produced by the side-reaction, particularly at low D-sorbitol concentration, interferes with hydrogen peroxide estimations, which are therefore not as accurate as the hexose estimations.

From our results, therefore, the features of the sensitized photo-oxidation of D-sorbitol reported here fully support the mechanisms advanced by Wells [7] for alcohols.

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