34. *Radiation Chemistry of Carbohydrates. Part XIII.* Action of γ-Radiation on Oxygenated Solutions of Maltose.*

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When maltose is irradiated with ^{60}Co γ-radiation in oxygenated solution, the predominating initial process is a hydrolytic type of scission to glucose. There is accompanying oxidative scission, to yield gluconic acid. Maltobionic acid, and an additional disaccharide acid, probably formed by oxidation of a 6-CH_2·OH group, are also initial products. Radioactive-tracer and paper-chromatographic methods were used to obtain yield–dose curves for the products and to measure the rate of degradation of maltose (initial $-G$ 4·0), which is of the first order in maltose. Secondary degradative processes lead to additional products at doses higher than 2×10^{20} ev ml.$^{-1}$. The effect of maltose concentration on the radiative decomposition is examined, and possible mechanisms are discussed.

A post-irradiation process leads to the formation of acid, reduction in hydrogen peroxide, and decrease in intensity of the ultraviolet absorption at 267 mμ. This after-effect is shown to be a thermal reaction due to hydrogen peroxide.

When maltose and cellobiose are irradiated with fast electrons in air-equilibrated 50% aqueous solutions, the predominating reaction appears to be hydrolysis.[1] For doses of 50—400 Mrep, at dose rates of 5 Mrep/min., it was demonstrated paper chromatographically that irradiated maltose solutions contained glucose, while irradiated cellobiose solutions showed only unchanged cellobiose on paper chromatograms. It has been concluded from these observations that the α-glycosidic linkage in maltose is more labile than the β-link in cellobiose, which is in accord with the relative ease of acid hydrolysis of these disaccharides. The exact path of the γ-induced hydrolysis is not known and the nature of the products was not investigated in detail. A number of samples of aqueous maltose solutions (20%) were irradiated with fast electrons to doses of 20—100 Mrep. The apparent hydrolysis, as determined by the reducing power, increased linearly with dose, and at a dose of 100 Mrep there was 15% of apparent hydrolysis. The results were interpreted in terms of scission of the glycosidic bond only.

The reducing power of maltose solution (0·1%) irradiated *in vacuo* decreases with energy input.[2] The presence of glucose was readily detectable by paper-chromatographic methods. Initial G for acid production *in vacuo* is 1·5—1·8. Some evidence for the formation of compounds of the 2-oxo-D-*arabino*-aldohexose type was obtained. The ultraviolet absorption spectrum of solutions irradiated *in vacuo* showed a maximum absorption at 262 mμ, which increased in intensity at increasing doses. Prolonged irradiation of 0·1% aqueous evacuated maltose solutions gave rise to an acidic polymer,[3] which showed similarities to polymers formed during irradiation of glucose, 1,4-gluconolactone, lactic acid, and glycollic acid.

In the present study, the products formed when aqueous maltose solutions are irradiated with ^{60}Co γ-radiation, in oxygen, are examined. After previous investigations on monosaccharides,[4] maltose now provides a model system for the study of polysaccharides containing predominantly α-1,4-glycosidic linkages.

Results and Experimental

Details of the ^{60}Co source of γ-radiation, irradiation vessels, dosimetry, and analytical techniques have been described previously. The dose rates employed were: $1{\cdot}18 \times 10^{17}$ ev ml.$^{-1}$

* Part XII, *J.*, 1963, 297.

[1] Wolfrom, Binkley, McCabe, Shen Han, and Michelakis, *Radiation Res.*, 1959, **10**, 37.
[2] Grant and Ward, *J.*, 1959, 2871.
[3] Barker, Grant, Stacey, and Ward, *J.*, 1959, 2648.
[4] Phillips and Criddle, *J.*, 1960, 3404, 3756; 1962, 2733; Phillips and Moody, *J.*, 1958, 3522.

in the large annular cell (300 ml.); $1{\cdot}38 \times 10^{17}$ ev ml.$^{-1}$ in the small annular cell (140 ml.); and $1{\cdot}25 \times 10^{17}$ ev ml.$^{-1}$ min.$^{-1}$ in the small cells (45 ml.); cells were irradiated in a turntable revolving at 3 rev./min. around the source.

Chromatographic Analysis of Irradiated Maltose Solutions.—Paper-chromatographic and radioactive-tracer techniques were combined in a study of irradiated oxygenated maltose solutions ($2{\cdot}78 \times 10^{-2}$M). Initially, a qualitative paper-chromatographic study was undertaken to identify the products. In a typical experiment, an aqueous solution (140 ml.) of maltose was irradiated in oxygen to a dose of 2×10^{20} ev ml.$^{-1}$. Without further treatment, an aliquot part (0·05 ml.) of the irradiated solution was subjected to paper chromatography with the following irrigants: (i) pyridine–butan-1-ol–water (3 : 10 : 3); (ii) acetic acid–butan-1-ol–water (1 : 4 : 5); and (iii) ethanol–butan-1-ol–water (1 : 4 : 2). Four products were revealed when the paper chromatograms were sprayed with alkaline silver nitrate (Table 1). Substance I was unidentified, but shows the characteristics of a disaccharide acid. Substance II ran identically with control maltobionic acid. Substance III corresponds to gluconic acid, IV to maltose, and V to glucose. Treatment of the irradiated solution with Deacidite J (HCO_3^- form) removed substances I, II, and III, which further indicates their acidic nature.

TABLE 1.

Constituents in irradiated maltose solutions after a dose of 2×10^{20} ev ml.$^{-1}$.

Constituent	I	I	III	IV	V
R_G, irrigant (i)	Immobile	0·12	0·30	0·40	1·0
R_G, irrigant (ii)	0·1	0·45	0·76	0·56	1·0
R_G, irrigant (iii)	Immobile	0·30		0·56	1·0

R_G indicates movement relative to glucose.

At higher doses, two-dimensional paper chromatography with inactive maltose and [^{14}C]-maltose was used to examine the irradiated solutions. Autoradiographs were prepared from the radiochromatograms as previously described.[5] The following is a typical experiment. An aqueous solution (140 ml.) of maltose, containing sufficient [^{14}C]maltose to give a specific activity of 8 μc per millimole, was irradiated in oxygen to doses varying from 2·3 to $8{\cdot}0 \times 10^{20}$ ev ml.$^{-1}$. The irradiated solution was treated by paper chromatography two-dimensionally with irrigants (i) and (ii). The results are summarised in Table 2. The constituents present are numbered in the order in which they were detected at increasing doses. Up to a dose of 2×10^{20} ev ml.$^{-1}$, only constituents I—V were detectable (Table 1). The number of secondary products increased

TABLE 2.

Constituents detected in irradiated maltose solutions by paper chromatography.

Dose (10^{20} ev ml.$^{-1}$)	2·3		5·0		6·0		8·0		
Irrigant	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	Constituent
R_G	0·1	0·32	0·06	0·18	0·09	0·35	0·08	0·35	I
	0·12	0·42	0·14	0·42	0·12	0·45	0·15	0·45	II
	0·11	0·52			0·16	0·52	0·21	0·54	VI
							0·22	0·64	
	0·20	0·70	0·24	0·70	0·22	0·65	0·24	0·70	III
	0·45	0·56	0·41	0·54	0·43	0·60	0·37	0·57	IV
							0·45	0·85	
							0·55	0·65	
							0·60	0·65	
			0·70	0·75	0·71	0·73	0·65	0·75	VII
	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	V
				1·45	1·25	1·30	1·25	1·30	VIII

at higher doses. Substance VI corresponds to glucuronic acid and was first observed after a dose of $2{\cdot}3 \times 10^{20}$ ev ml.$^{-1}$. Substance VIII corresponds to arabinose and VII is a neutral product first observed at 5×10^{20} ev ml.$^{-1}$. After 8×10^{20} ev ml.$^{-1}$ the pattern is complex and eleven products, in addition to unchanged maltose, were observable.

Rate of Formation of Products.—Yield–dose curves for maltose degradation and the formation of primary products were obtained from a quantitative study of one-dimensional chromatograms

[5] Phillips and Criddle, Proc. Intern. Conf. on the Uses of Radioisotopes, Copenhagen, 1962, p. 326.

at low irradiation doses. In a typical experiment an aqueous solution (45 ml.) of maltose ($2{\cdot}78 \times 10^{-2}$M) containing [^{14}C]maltose to give a specific activity of 8 μc/millimole was irradiated in oxygen to doses of $0—2{\cdot}0 \times 10^{20}$ ev ml.$^{-1}$. The maximum dose, therefore, corresponds to the product study summarised in Table 1. Aliquot parts (0·05 ml.), after increasing doses, were applied to filter paper (Whatman No. 54) strips 40—45 cm. long and 2·5 cm. wide and were irrigated in solvents (i) and (ii). The papers were dried and the distribution of radioactivity along the strip was estimated by using an automatic radiochromatogram scanner.[6] Values were corrected for variations in total radioactivity due to inaccuracies in applying the aliquot sample to the paper. Yield–dose curves expressed as percentages of the initial maltose present

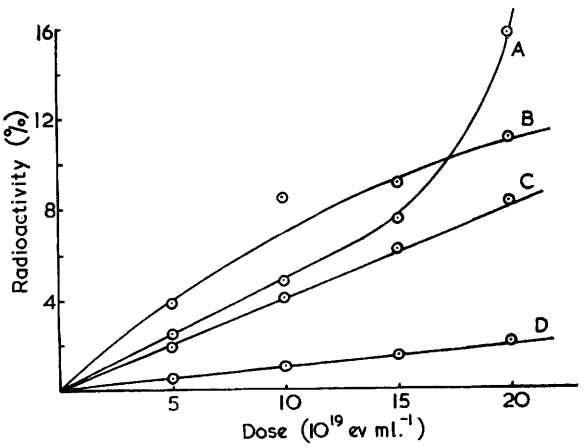


FIG. 1. Rate of formation of products during irradiation of maltose solutions in oxygen, determined by paper chromatography.

(A) Maltobionic acid. (B) Glucose. (C) Disaccharide acid. (D) Gluconic acid.

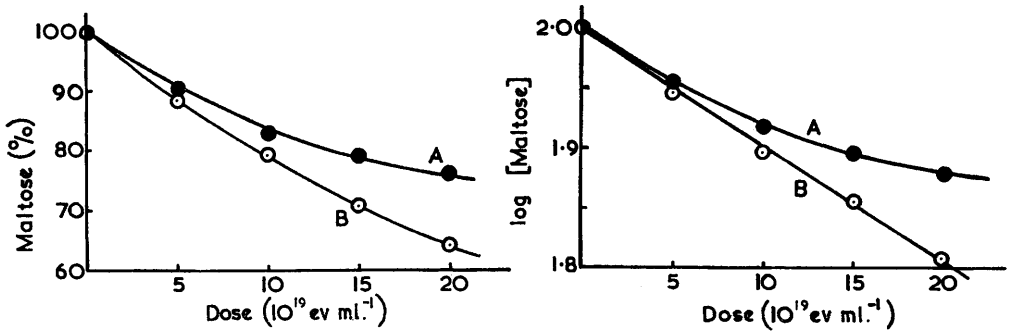


FIG. 2. Degradation of maltose during γ-irradiation in oxygenated solution.

(A) Irrigant (i), pyridine–butan-1-ol–water (3 : 10 : 3). (B) Irrigant (ii), acetic acid–butan-1-ol–water (1 : 4 : 5).

are shown in Figs. 1 and 2. The [^{14}C]maltose used initially contained 6·8% of impurities due to self-decomposition. After this observation, all [^{14}C]maltose was purified by paper chromatography before use. Table 3 shows the initial G values obtained. The rate of degradation of maltose ($-G$) is lower in irrigant (i) than in (ii) because products run so near to maltose that it

TABLE 3.

Initial G values of maltose and initial products of irradiation.

	Maltose (IV)	Glucose (V)	Maltobionic acid (II)	Gluconic acid (III)	Disaccharide acid (I)
Irrigant (i)	−3·3	+2·4			+0·7
Irrigant (ii)	−4·0	+2·4	+0·7	+0·3	+0·7

[6] Phillips, unpublished work.

is impossible to distinguish them. Such overlapping may be seen from autoradiographs prepared from two-dimensional radiochromatograms at a dose of $2{\cdot}3 \times 10^{20}$ ev ml.$^{-1}$ (Table 2).

Estimation of the Rate of Degradation of Maltose by Isotope Dilution Analysis.—An aqueous solution of maltose (45 ml.; $2{\cdot}78 \times 10^{-2}$M) containing sufficient [^{14}C]maltose to give a specific activity of 7·696 or 4·686 μc/millimole was irradiated to doses of 4·2, 8·4, and $12{\cdot}6 \times 10^{19}$ ev ml.$^{-1}$. The irradiated solution (5 ml.), after treatment with carrier maltose (*ca.* 1 millimole), was left to equilibrate for 24 hr. and then freeze-dried. After rigorous drying, freshly prepared fused sodium acetate (0·4 g.) and acetic anhydride (2 ml.) were added, and the mixture was heated at 100° for 2—3 hr. The solid which separated when the mixture was poured on ice was recrystallised ten times from aqueous ethanol, to give pure octa-acetyl-β-D-maltose, m. p. 158°, with constant specific activity. Table 4 shows the results.

TABLE 4.

Degradation of maltose ($2{\cdot}78 \times 10^{-2}$M) during γ-irradiation in oxygenated aqueous solution (45 ml.).

Dose (10^{19} ev ml.$^{-1}$)	0	4·2	8·4	12·6
Initial spec. activity (μc/millimole)		7·696	4·686	4·686
Carrier (millimoles)		1·014	1·00	1·00 (3 ml.)
Final spec. activity (μc/millimole)		0·8519	0·4881	0·2858
Maltose (10^{19} molecules/ml.)	1·668	1·520	1·398	1·303

Estimation of D-*Gluconic Acid by Isotope Dilution Analysis.*—The low initial rate of formation of D-gluconic acid makes it difficult to conclude whether it is a primary or secondary product. The presence of D-gluconic acid as impurity formed during self-decomposition of the [^{14}C]-maltose used for paper-chromatographic experiments further complicates the interpretation of the results. Therefore, an independent isotope dilution study was undertaken to establish the form of the yield–dose curve of this acid. A solution (45 ml.) of [^{14}C]maltose ($2{\cdot}78 \times 10^{-2}$M) of specific activity 7·185 μc/millimole was irradiated to doses ranging from 0 to $2{\cdot}0 \times 10^{20}$ ev ml.$^{-1}$. To the irradiated solution (5 ml.) carrier D-gluconolactone was added and left to equilibrate for 48 hr. Phenylhydrazine (1·5 ml.) and acetic acid (3 ml.) were added and solution was heated at 100° for 1 hr. The solid which separated was recrystallised 8 times from hot water, to give pure D-gluconophenylhydrazide with m. p. 197° and constant specific activity. The results are given in Table 5. In Tables 4 and 5 the yield recorded at zero dose corresponds to the amount of carrier added.

Products Estimated by Isotope Dilution Analysis after a Dose of 8×10^{20} ev ml.$^{-1}$.—A solution (140 ml.) of [^{14}C]maltose ($2{\cdot}78 \times 10^{-2}$M) with a specific activity of 10·19 μc/millimole was irradiated. The products which were indicated by paper chromatography were estimated as follows.

TABLE 5.

Formation of D-gluconic and during γ-irradiation of aqueous maltose solutions ($2{\cdot}78 \times 10^{-2}$M) in oxygen.

Dose (10^{19} ev ml.$^{-1}$)	0	5	10	15	20
Carrier (millimoles)	1·00	0·96	0·98	1·15	0·96
Final spec. activity ($\times 10^2$) (μc/millimole)	2·097	2·759	3·096	3·304	4·762
Gluconic acid (10^{17} molecules/ml.)	7·00	8·802	10·10	12·06	15·30

Initial G (gluconic acid) = 0·3.

D-*Glucose.* Carrier D-glucose (1·313 millimoles) was added to the irradiated solution (5 ml.) and allowed to equilibrate for 24 hr. Phenylhydrazine (1 ml.) and acetic acid (0·5 ml.) were added. The precipitate, after 7 recrystallisations from ethanol, gave pure glucosazone with m. p. 201° and constant specific activity 0·2268 μc/millimole.

D-*Gluconic acid.* After addition of carrier gluconic acid (0·9118 millimole), D-gluconophenylhydrazide was prepared as described above with constant specific activity 0·0628 μc/millimole.

D-*Arabinose.* The irradiated solution (5 ml.) was mixed with carrier D-arabinose (1 millimole) and treated with *NN*-diphenylhydrazine (0·5 ml.) at 100° for 1 hr. The solid which separated on cooling was recrystallised 6 times from ethanol, to give pure D-arabinose diphenylhydrazone with m. p. 196° and constant specific activity 0·01877 μc/millimole.

Maltobionic acid. Carrier maltobionic acid was prepared as described by Glattfield and

Hanke.[7] Attempts to estimate this product, however, proved unsuccessful because it was impossible to prepare a crystalline derivative sufficiently pure. Minute traces of impurity invalidate the method.

Three-carbon aldehydic fragments. The irradiated solution (5 ml.) was treated with carrier 1,3-dihydroxyacetone (1·75 millimoles), acetic acid (1 ml.), and phenylhydrazine (1·5 ml.). The mixture was heated at 100° for 15 min. The solid which separated on cooling was recrystallised 8 times from benzene, to give pure glycrosazone with m. p. 127° and constant specific activity 0·0628 μc/millimole.

Two-carbon aldehydic fragments. The irradiated solution (5 ml.) was treated with carrier glyoxal monohydrate (1·628 millimoles), glacial acetic acid (0·5 ml.), and phenylhydrazine (1·5 ml.). Pure glyoxal bisphenylhydrazone was obtained, after 8 recrystallisations from benzene, with m. p. 170° and constant specific activity 0·0351 μc/millimole. The results are given in Table 6.

TABLE 6.

Estimation of products formed during γ-irradiation of aqueous maltose solution (140 ml.; $2·78 \times 10^{-2}$M) in oxygen.

Product	D-Glucose	D-Gluconic acid	D-Arabinose	Three-carbon aldehydic fragments	Two-carbon aldehydic fragments
Carrier (millimoles)	1·313	0·9118	1·00	1·75	1·628
Spec. activity (μc/millimole)	0·2268	0·0677	0·0188	0·0628	0·0351
Yield (millimoles)	1·711	0·3435	0·1243	1·239	0·8468

Carbon dioxide. Only small amounts of carbon dioxide are formed at doses below 2×10^{20} ev ml.$^{-1}$. When the gas stream from the irradiation of maltose ($2·78 \times 10^{-2}$M) solution (45 ml.) was passed into baryta, 20·5 mg. of barium carbonate were formed.

Hydrogen peroxide. Aqueous maltose solutions (45 ml.) were irradiated at a dose rate of $1·25 \times 10^{17}$ ev ml.$^{-1}$ min.$^{-1}$ to doses ranging from 0 to $5·40 \times 10^{19}$ ev ml.$^{-1}$. Hydrogen peroxide was estimated, as described previously,[4] at various maltose concentrations in the range $2·78 \times 10^{-3}$M to 1·39M. The results are shown in Fig. 3 and initial G values in Table 7.

TABLE 7.

Hydrogen peroxide and acid formation during irradiation of aqueous maltose solutions in oxygen.

Maltose (M)	Initial $G(H_2O_2)$	Initial G(acid)	Maltose (M)	Initial $G(H_2O_2)$	Initial G(acid)
$2·78 \times 10^{-3}$	2·6	1·1	$8·34 \times 10^{-1}$	1·2	3·5
$2·78 \times 10^{-2}$	2·0	1·8	1·12	0·9	—
$2·78 \times 10^{-1}$	1·4	2·7	1·39	1·2	4·6
$5·56 \times 10^{-1}$	—	3·2			

Effect of Maltose Concentration on Acid Formation.—Aqueous maltose solutions (45 ml.) were irradiated to doses of 0—$6·0 \times 10^{19}$ ev ml.$^{-1}$ at a dose rate of $1·25 \times 10^{17}$ ev ml.$^{-1}$ min.$^{-1}$. The concentration of maltose was varied from $2·78 \times 10^{-3}$M to 1·39M, and the acid formation was estimated potentiometrically. The results, summarised in Fig. 4, show that initial G(acid) increases with increasing maltose concentration (Table 7).

Absorption Spectra of Irradiated Solutions.—The absorption spectrum of irradiated maltose solutions is typical of irradiated carbohydrates, having a maximum at 265 mμ. As irradiation progresses, the optical density at 265 mμ increases linearly with dose. The rate of increase is dependent on maltose concentration (Table 8).

TABLE 8.

Change in optical density at 265 mμ during the γ-irradiation of maltose solutions in oxygen.

Maltose (M)	Dose (10^{19} ev ml.$^{-1}$)	0·75	1·50	2·25	3·0	3·75	4·50	11·25
$2·78 \times 10^{-3}$	Optical density	0·015	0·025	0·03	0·05		0·06	0·35
$2·78 \times 10^{-2}$	,, ,,	—	0·02	0·03	0·05	0·06	0·06	0·33
$2·78 \times 10^{-1}$	,, ,,	—	0·04	0·09	0·09	0·10	0·11	0·94
$5·56 \times 10^{-1}$	,, ,,	—	0·04	0·09	0·09	0·10	0·12	0·94

[7] Glattfield and Hanke, *J. Amer. Chem. Soc.*, 1918, **40**, 973.

Post-irradiation Processes.—When irradiation of aqueous maltose solutions was terminated, it was observed that (*a*) the amount of acid increased, (*b*) the amount of hydrogen peroxide decreased, (*c*) the absorption at 265 mμ decreased, and (*d*) the infrared spectrum of the product changed.

(*a*) *Acid formation.* Aqueous maltose solutions (45 ml.; $2{\cdot}78 \times 10^{-2}$M) were irradiated to a dose of $15{\cdot}75 \times 10^{19}$ ev ml.$^{-1}$. Aliquot parts (10 ml.) were titrated with alkali potentiometrically

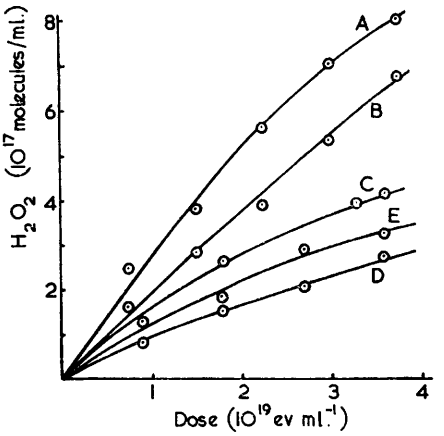


FIG. 3. Hydrogen peroxide formation in oxygenated maltose solutions during γ-irradiation.

[Maltose]: (A) $2{\cdot}78 \times 10^{-3}$, (B) $2{\cdot}78 \times 10^{-2}$, (C) $2{\cdot}78 \times 10^{-1}$, (D) $8{\cdot}34 \times 10^{-1}$, (E) 1·112, and (F) 1·39M.

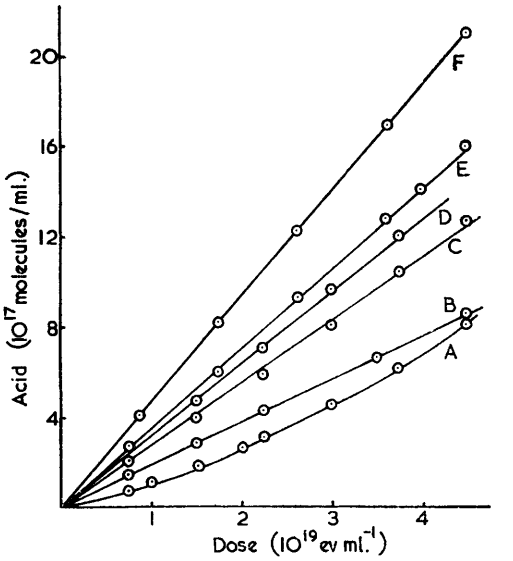


FIG. 4. Acid formation in oxygenated maltose solutions during irradiation.

[Maltose]: (A) $2{\cdot}78 \times 10^{-3}$, (B) $2{\cdot}78 \times 10^{-2}$, (C) $2{\cdot}78 \times 10^{-1}$, (D) $5{\cdot}56 \times 10^{-1}$, (E) $8{\cdot}34 \times 10^{-1}$, and (F) 1·39M.

0, 1, 4, 24, and 116 hr. after irradiation was terminated. One part was freeze-dried immediately after irradiation to remove hydrogen peroxide and volatile acid, redissolved in water (10 ml.) and left for 120 hr. before titration. This sample showed no post-irradiation production of acid. All estimations were conducted in an atmosphere of nitrogen, and pK_a values of the acids were calculated during each estimation. Each neutralisation point was obtained by a graphical analysis of the results. A summary of the findings is given in Table 9. In an independent estimation, pK_a for maltobionic acid was found to be 4·11. The form of the pH titration curves indicated extensive lactone formation.

TABLE 9.

Post-irradiation release of acid after irradiation of oxygenated maltose solutions ($2{\cdot}78 \times 10^{-2}$M) to a dose of $15{\cdot}75 \times 10^{19}$ ev ml.$^{-1}$.

Post-irradiation time (hr.)	0	1	2	24	116	120 *
Total acid (10^{18} molecules/ml.)	4·09	4·19	4·46	4·61	5·09	3·25
Volatile acid (10^{18} molecules/ml.)	0·81			0·80	0·80	
Post-irradiation release (10^{18} molecules/ml.)	—	0·10	0·35	0·52	1·00	—
pK_a	4·10	4·05	4·00	3·83	3·80	3·90

* Freeze-dried sample.

(*b*) *Hydrogen peroxide.* Hydrogen peroxide was estimated on the above irradiated maltose solution 0, 3, 6½, and 20 hr. after irradiation had ceased. The decrease in hydrogen peroxide is shown in Table 10.

TABLE 10.

Post-irradiation decrease in hydrogen peroxide after irradiation of oxygenated maltose solutions ($2{\cdot}78 \times 10^{-2}$M) to a dose of $15{\cdot}75 \times 10^{19}$ ev ml.$^{-1}$.

Post-irradiation time (hr.)	0	3	6·5	20
H_2O_2 (10^{18} molecules/ml.)	4·0	2·63	1·75	1·50
Decrease (10^{18} molecules/ml.)	0	1·37	2·25	2·5

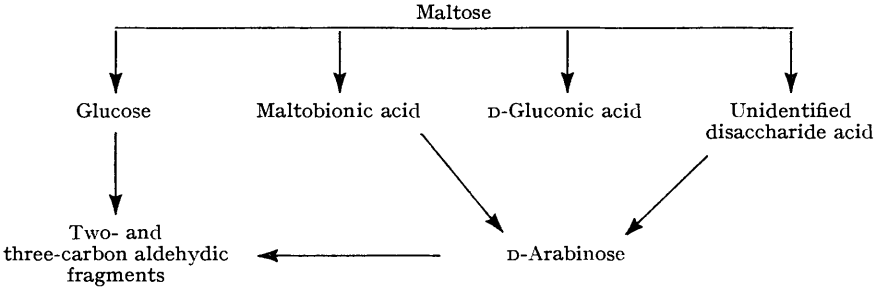
(*c*) *Absorption spectrum.* After a dose of $15{\cdot}75 \times 10^{19}$ ev ml.$^{-1}$ aqueous maltose solution ($2{\cdot}78 \times 10^{-2}$M) had an optical density of 0·46 at 265 mμ. After 26 hr. the density decreased to 0·22.

(*d*) *Infrared spectrum.* The maltose solution (10 ml.), after irradiation to a dose of $15{\cdot}75 \times 10^{19}$ ev ml.$^{-1}$, was freeze-dried and its infrared spectrum examined as a mull in Nujol, a Unicam S.P. 100 double-beam infrared spectrometer being used. The procedure was repeated for a solution which had been set aside for 144 hr. The spectra were identical apart from a peak at 800 cm.$^{-1}$ which disappeared during the post-irradiation process. Another significant observation is that the irradiated maltose showed two stable peaks, at 1640 and 1740 cm.$^{-1}$. γ-Lactones have characteristic peaks in this region.[8]

DISCUSSION

When aqueous maltose solutions had been irradiated in oxygen to a dose of 2×10^{20} ev ml.$^{-1}$, four products and unchanged maltose were revealed by paper chromatography. D-Glucose, D-gluconic acid, maltobionic acid, and an additional acid having the characteristics of a disaccharide acid were identified up to this dose. Autoradiography revealed seven additional products at a dose of 8×10^{20} ev ml.$^{-1}$. Examination at intermediate doses of 5·0 and $6{\cdot}0 \times 10^{20}$ ev ml.$^{-1}$ indicate pronounced secondary degradation of the initial products as irradiation progresses. To elucidate the initial pattern of degradation, therefore, detailed product studies were conducted at doses below 2×10^{20} ev ml.$^{-1}$.

Estimation of the rate of degradation of maltose by isotope dilution analysis gave an initial $-G$ 4·0, which is in accord with the value estimated by paper chromatography when the irrigant is butan-1-ol–acetic acid–water. In butan-1-ol–pyridine–water, $-G$(maltose) is 3·3, which indicates that in this irrigant there is incomplete separation of maltose from other products. It has been found that the plot of log (maltose concentration) against dose is linear (Fig. 2), indicating that maltose degradation is a first-order process in maltose.



Where incomplete separation of the products from maltose occurred, the first-order relationship with dose is not obeyed. The extent of deviation enabled a true degradation curve for maltose under these conditions to be calculated, and indicated that the acid which runs with maltose in the basic irrigant had initial G 0·7. The first-order relation during radiation degradation being thus established, it may usefully be applied to calculate the maltose concentration at any particular dose. The behaviour has been shown to be general for aqueous carbohydrate solutions on irradiation in oxygen or *in vacuo*.[9]

By reference to the yield–dose curves of the main products, it is possible to distinguish initial degradation processes. Isotope-dilution and paper-chromatographic estimations show good agreement. Initial G for D-glucose, the main product, is 2·4 and for maltobionic

[8] Barker, Bourne, Pinkard, and Whiffen, *Chem. and Ind.*, 1958, 658.
[9] Phillips, *Radiation Res.*, 1963, **18**, 446.

acid 0·7. A further unidentified disaccharide acid is formed with initial G 0·7 and may have arisen by oxidation of the 6-CH_2·OH group of one of the glucose molecules. Support for this conclusion is provided by the appearance of D-glucuronic acid on paper chromatograms at high doses. The initial production of D-gluconic acid (initial G 0·3) indicates that a small proportion of oxidative scission accompanies the main hydrolytic type of scission at the 1,4-glycosidic link. In this respect the degradative processes are similar to those operating during γ-irradiation of sucrose in aqueous solution. Here, the initial $-G$ is 4·0 and the main initial products are D-glucose and D-fructose. Accompanying oxidative scission leads to D-gluconic acid and D-glucosone. The formation of maltobionic acid (initial G 0·7) is analogous to the production of gluconic acid during irradiation of D-glucose solutions in oxygen (initial G 0·6).

The presence of products such as D-arabinose and two- and three-carbon aldehydic fragments at high doses indicates secondary degradation of D-glucose, which was shown to proceed along this route. Formation of the product which absorbs at 265 mμ is also secondary in character (Table 8) and is a process which also accompanies irradiation of aqueous D-glucose. The results indicate the annexed degradation processes. The processes which lead to acid formation account for initial G 1·7, which is in good agreement with acid estimated by direct titration for $2{\cdot}78 \times 10^{-2}$M maltose solutions (initial G 1·8). Approximately 25% of the maltose initially degraded is unaccounted for by the products in the middle row of the chart and direct conversion of maltose into fragments containing fewer carbon atoms may occur.

In interpreting the mechanism of the radiation decomposition of maltose, a difficulty is encountered which is common to all previously investigated aqueous sugar systems. The $-G$ for maltose (4·0) is greater than G(OH) which is 2·4 at the pH of the experiments. Degradation must, therefore, be initiated by processes other than the commonly encountered abstractions of OH radicals: $RH + OH \longrightarrow R\cdot + H_2O$. In oxygen, abstraction due to the hydrogen atom is considered negligible since relations $H + O_2 \longrightarrow HO_2$ or $e_{aq}^- + O_2 \longrightarrow O_2^-$ remove hydrogen atoms and their precursors. Furthermore, within the maltose concentration range studied ($2{\cdot}78 \times 10^{-2}$ to 1·39M), the yield of acid is never independent of solute concentration, as expected if the chemical change is caused by free radicals produced from water, that is, "indirect action." There is evidence from the decrease in yield of hydrogen peroxide as the maltose concentration is increased that hydrogen atoms or their precursors may be scavenged by maltose in competition with their reaction with oxygen.

Possible explanations of the high $-G$ (maltose) at $2{\cdot}78 \times 10^{-2}$M may be direct electronic excitation of maltose or additional scavenging of hydroxyl radicals from the back-reaction $H\cdot + \cdot OH \longrightarrow H_2O$ which takes place in the regions of intense ionisation or "spur." Investigation of the mechanisms is continuing.

Post-irradiation processes were observed which lead to an ~20% increase in the observed yield of acid, and a decrease in hydrogen peroxide concentration and in the intensity of the absorption at 265 mμ. The absence of post-irradiative release of acid when hydrogen peroxide was removed by freeze-drying immediately after irradiation indicates that the after-effect may be a thermal reaction between hydrogen peroxide and an oxidisable product of radiative action. This product is probably responsible for the absorption maximum at 265 mμ, which also decreases in intensity during the post-irradiation process. All carbohydrate solutions which have been examined show ultraviolet absorption maxima in this region, and the position and intensity are dependent on the pH of the solution. For this reason it was suggested that compounds of the reductone type may be responsible.[4]

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