524. *Radiation Chemistry of Carbohydrates. Part IX.*[1] *The Action of γ-Radiation on Deœrated* D-*Mannose Solutions.*

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γ-Irradiation of D-mannose solutions *in vacuo* gives D-mannonic acid, D-glucosone, 2-oxogluconic acid, D-arabinose, D-erythrose, two- and three-carbon aldehydic fragments, oxalic acid, and polymeric material. By use of radioactive tracer and paper-chromatographic methods, the products were estimated at several doses. By reference to the yield–dose curves the following primary processes were identified: (*a*) oxidation at C-1 to give mannonic acid, (*b*) attack at C-2 to give glucosone, (*c*) scission between C-3 and C-4 to give three-carbon aldehydic fragments, and (*d*) scission between C-2 and C-3 to give erythrose and a two-carbon aldehydic fragment with simultaneous, direct conversion of the hexose molecule into three two-carbon aldehydic fragments. D-Arabinose, 2-oxogluconic acid, and smaller fragments are formed by secondary processes.

Initial processes account for an initial $-G$(D-mannose) 2·4; the experimentally determined value is 3·5. It is suggested that the primary processes unaccounted for relate to the dimerisation of primary radicals formed as a preliminary step in the formation of polymer. The results indicate that, although initial radicals formed from D-mannose in oxygen and *in vacuo* are similar, their subsequent fate is different in the absence and in the presence of oxygen, thereby giving rise to different final products.

PREVIOUS papers in this series have compared the chemical changes resulting from the irradiation of D-sorbitol solutions in oxygen and *in vacuo*. Although the products formed under the two sets of conditions are somewhat different, a common primary abstraction process $R{\cdot}CH_2{\cdot}OH \longrightarrow R{\cdot}CH(OH){\cdot}$ is indicated by the results. Secondary reactions of the first-formed radical, which follow different courses in absence and in presence of oxygen, appear to be responsible for the overall chemical changes. In order that the influence of oxygen on the chemical changes may be further elucidated, we compare, in the present paper, the effect of ^{60}Co-γ-radiation on deærated D-mannose solutions with the changes observed in oxygen.[2] As with D-sorbitol it appears that the primary processes are not markedly different, although secondary processes may lead to substantially different products.

[1] Part VIII, *J.*, 1961, 3763.
[2] Phillips and Criddle, *J.*, 1960, 3404.

Experimental and Results

The irradiation and analytical techniques have been previously described in the Series. The dose rate employed was $0{\cdot}9 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ in volumes which varied from 30 to 50 ml. Before irradiation the solutions were de-gassed by pumping directly on the solutions. This method proved more convenient than the freeze–pump technique and yielded identical results.

Chromatographic Analysis of Irradiated Solutions.—An evacuated solution (40 ml.) of D-mannose (2·42 millimoles) was irradiated to a total energy input of $7{\cdot}8 \times 10^{22}$ ev and chromatographed on paper in (*a*) butan-1-ol–acetic acid–water (4 : 1 : 5) and (*b*) butan-1-ol–pyridine–water (10 : 3 : 3). With irrigant (*a*) the following spots were detected with *p*-anisidine: immobile material, polymer; pink, R_F 0·12, 2-oxogluconic acid; brown, R_F 0·18, glucosone; brown, R_F 0·21, mannose; yellow, R_F 0·45, erythrose. Spraying with alkaline silver nitrate revealed a further spot, R_F 0·31, corresponding to mannono-γ-lactone and increased the intensity of the spot of R_F 0·12, indicating the presence of an additional non-reducing component, possibly mannono-δ-lactone. A further characteristic is the pronounced streaking in the vicinity of the starting lines on the chromatograms. Treatment of the irradiated solution with Amberlite IRA-400 (OH^-) and Deacidite J (HCO_3^-) before chromatography removed the components with R_F 0·12 and 0·31 and reduced markedly the amount of immobile material,

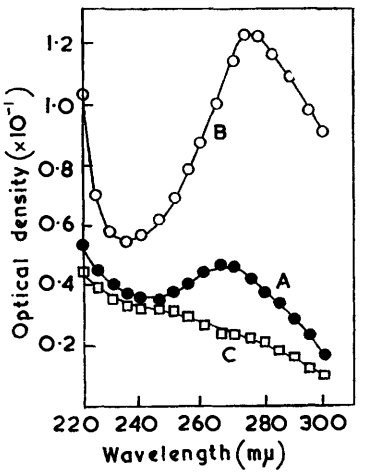


Fig. 1. Ultraviolet absorption spectra of D-mannose solutions (100 ml.) irradiated *in vacuo*.
(A) Energy input $3{\cdot}9 \times 10^{22}$ ev.
(B) With added $KHCO_3$.
(C) With added HCl.

indicating the acidic nature of these constituents. Gentle warming of the irradiated solution (30 min. at 60°) with activated charcoal (Norite) removed the polymer, although not affecting significantly the remaining products.

Irrigant (*b*) revealed no new components. In keeping with its previously adduced acidic origin, the pink spot of R_F 0·12 in irrigant (*a*) ran with R_F 0·03 in irrigant (*b*), which corresponds to 2-oxogluconic acid and mannono-δ-lactone.

Acid Formation.—The rate of acid formation is shown in Table 1, indicating an initial G 0·5, the acid produced being assumed to be monobasic. The rate of formation with dose is initially linear but increases slightly at higher doses.

Table 1.

Acid formation during the irradiation of evacuated D-mannose solutions.

Acid (10^{17} molecules/ml.) ...	0·25	0·60	0·75	0·95	1·30	1·60	1·90	2·20
Dose (10^{19} ev/ml.)	0·60	1·20	1·65	2·20	2·75	3·30	3·80	4·40

Absorption Spectra of Irradiated Solutions.—The absorption spectrum of evacuated D-mannose solutions after irradiation show the characteristic maximum at 265 mμ (Fig. 1), which is enhanced upon the addition of alkali (potassium hydrogen carbonate) and reduced on addition of mineral acid.

The increase of optical density at 265 mμ with dose is linear (Table 2).

TABLE 2.

Increase of absorption maximum (265 mμ) with dose.

Optical density (265 mμ) ...	0·195	0·36	0·55	0·65	0·84	1·02	1·50	1·20
Dose (10^{19} ev/ml.)	0·60	1·20	1·65	2·20	2·75	3·30	3·80	4·40

Hydrogen Peroxide Formation.—The rate of formation of hydrogen peroxide, measured by the colour produced with titanium sulphate solution, is slow, with an initial G value 0·3 (Table 3).

TABLE 3.

Hydrogen peroxide formation in evacuated D-mannose solutions during irradiation.

Hydrogen peroxide (10^{17} molecules/ml.)	0·15	0·32	0·50	0·65	0·80	0·90	1·00	1·15
Dose (10^{19} ev/ml.)	0·60	1·20	1·65	2·20	2·75	3·30	3·80	4·40

Estimation of Products by Isotope Dilution.—An evacuated solution (40 ml.) of D-mannose (2·42 millimoles) containing sufficient [^{14}C]-D-mannose to give a specific activity of 4·0 μc/millimole was irradiated to a total energy input of $3·9 \times 10^{22}$ ev. The following constituents were estimated in the irradiated solution:

D-*Mannose.* (*a*) As phenylosazone. The irradiated solution (5 ml.) was treated with carrier D-mannose (1·0 millimole), acetic acid (1 ml.), and phenylhydrazine (2·5 ml.), and the mixture was refluxed at 100° for 1 hr. The solid which separated on cooling was recrystallised eight times, to give pure D-glucosazone, m. p. 199°, with constant specific activity 0·57 μc/millimole.

(*b*) As phenylhydrazone. The method used has been previously described.[2]

D-*Lyxose.* The irradiated solution (5 ml.), after freeze-drying, was treated with carrier D-lyxose (1·0 millimole) and then a solution of benzylphenylhydrazine (2 ml.) in ethanol (20 ml.), and was refluxed for 1 hr. The solid which remained after removal of the solvent was recrystallised seven times from ethanol, to give pure D-lyxose benzylphenylhydrazone, m. p. 128°, with specific activity 0·0005 μc/millimole.

D-*Mannonic acid.* The irradiated solution (5 ml.) was treated with carrier D-mannonic acid (0·80 millimole) (prepared according to Isbell and her co-workers [3]). After addition of brucine (0·3 g.), the mixture was heated for 1—2 hr. at 100°, cooled, extracted three times with benzene to remove the excess of brucine, and evaporated to dryness *in vacuo*. The gum which formed solidified on treatment with hot ethanol, and nine recrystallisations from aqueous ethanol gave the pure brucine salt of mannonic acid with m. p. 210° and constant specific activity 0·14 μc/millimole.

D-Arabinose, three-carbon and two-carbon aldehydic fragments, oxalic acid, and formaldehyde were estimated as previously described.[2] The results are shown in Table 4.

TABLE 4.

Products when aqueous D-mannose is irradiated with γ-radiation *in vacuo*.

Initial D-mannose, 2·42 millimoles.
Energy input, $3·9 \times 10^{22}$ ev (vol. 40 ml.).

Product	D-Mannose		D-Arabinose	D-Mannonic acid	Three-carbon fragments
	a	*b*			
Carrier (millimoles)	1·0	1·0	1·20	0·80	1·30
Spec. activity (μc/millimole) ...	0·50	0·57	0·007	0·14	0·04
Yield (millimoles)	1·14	1·34	0·02	0·23	0·21

Product	Two-carbon fragment	Oxalic acid	Formaldehyde	D-Lyxose
Carrier (millimoles)	2·0	2·0	1·0	1·0
Spec. activity (μc/millimole) ...	0·04	0·016	0·007	0·0005
Yield (millimoles)	0·49	0·19	0·085	0·001

Oxogluconic acid estimated from paper chromatography, 0·15 millimole.
Erythrose estimated from paper chromatography, 0·10 millimole.
a, As phenylhydrazone. *b*, As phenylosazone.

[3] Isbell and Frush, *J. Res. Nat. Bur. Stand.*, 1931, **6**, 1145; Isbell, Frush, and Bates, *ibid.*, 1932, **8**, 571.

Rate of Formation of Products.—To calculate accurate initial G values for the main products, yield–dose curves were obtained by application of the isotope dilution method at varying doses. Individual samples of D-mannose (spec. activity 4·0 μc/millimole) were irradiated in 30-ml. amounts at a dose rate of $0{\cdot}9 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ to doses of 0·65—$5{\cdot}8 \times 10^{20}$ ev ml.$^{-1}$, and D-mannose, D-arabinose, D-mannonic acid, D-glucosone, and two- and three-carbon aldehydic fragments were estimated at each dose. Table 5 shows the rate of consumption of D-mannose, and the rates of formation of products are shown in Fig. 2.

TABLE 5.

Rate of degradation of D-mannose during irradiation in evacuated solution.

D-Mannose (10^{19} molecules/ml.) ...	3·70	3·48	3·20	2·70	2·30	1·8
Dose (10^{20} ev/ml.)	0	0·60	1·75	3·20	5·80	10·0

From the yield–dose curves, $-G$(D-mannose) is 3·5, and initial G (mannonic acid) 0·45, glucosone 0·5, two-carbon aldehydic fragments 0·95, three-carbon aldehydic fragments 0·5.

Yield–dose curves were also obtained for comparison from paper chromatography. Accurately known amounts (0·05 ml.) of the irradiated solutions used for isotope dilution

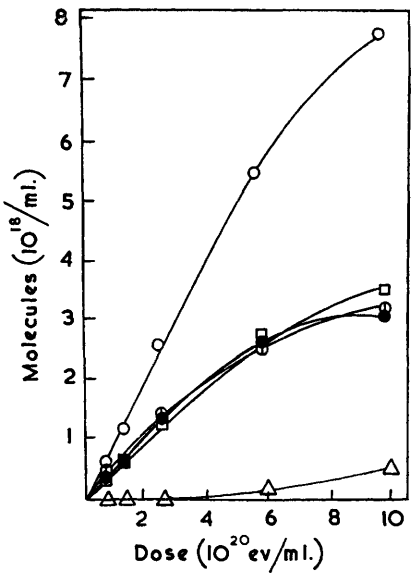


FIG. 2. Rate of formation of products during irradiation of D-mannose solutions *in vacuo*, determined by isotope dilution.

○ Two-carbon aldehydic fragments.
□ D-Mannonic acid. ⓘ D-Glucosone.
● Three-carbon aldehydic fragments.
△ D-Arabinose.

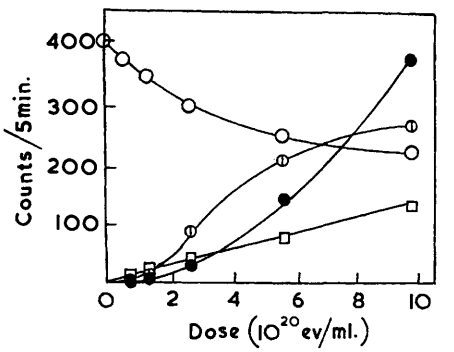


FIG. 3. Rate of formation of products during irradiation of D-mannose solutions *in vacuo*, determined by paper chromatography.

○ D-Mannose. ⓘ 2-Oxogluconic acid.
□ D-Erythrose. ● Polymer.

measurements described above were chromatographed on paper in butan-1-ol–acetic acid–water (4 : 1 : 5), and the radioactivities of the spots were measured. For standardisation the rate of consumption of D-mannose was measured and, as no carrier materials were available for estimation of 2-oxogluconic acid and D-erythrose by isotope dilution, these products were also measured by paper chromatography. The rate of polymer formation was estimated by measuring the radioactivity remaining on the starting line at increasing doses. The results are shown in Fig. 3, and indicate that D-erythrose, the only primary product measured in this way, is formed with initial G 0·25.

Discussion

The constituents present in evacuated D-mannose solutions after irradiation to a total energy input of $3{\cdot}9 \times 10^{22}$ ev are shown in Table 4, and these account for more than 95% of the initial mannose present. As in oxygen, greater attention was devoted to the products formed at low doses in order that initial degradation processes may be elucidated. Primary and secondary product formation may be distinguished from the yield–dose curves of the main products. For such processes the terms primary and secondary are used. However, it must be remembered that they are subsequent to primary and secondary radical formation, the truly primary and secondary effects of radiation action.

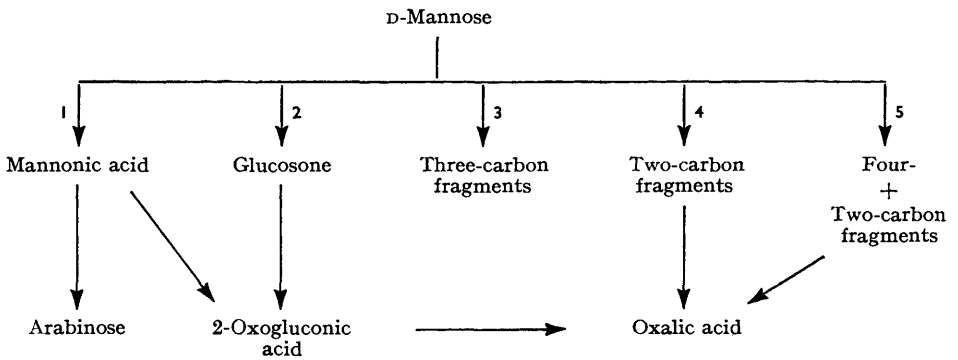
Acid formation (Table 1) is a primary process with initial G 0·5, which is in reasonable agreement with initial G for the production of mannonic acid (0·45). It is concluded, therefore, that the acid formed initially is mannonic acid, but, since the rate of total acid formation increases at high doses, it is clear that acids are further produced by secondary processes. From the yield–dose curve (Fig. 3) it is probable that 2-oxogluconic acid is a secondary product. Analytical difficulties make it impossible to detect a slow initial rate of formation of this acid. However, secondary formation is clearly dominant as indicated by the marked increase in its rate of formation at high doses. Attack at C-2 in D-mannonic acid is probably one route to the formation of 2-oxogluconic acid; another may involve oxidation of D-glucosone, formed as a primary product with initial G 0·5 (Fig. 2). In view of the formation of mannuronic acid in oxygenated systems, it is pertinent to note that, *in vacuo*, it was not possible to detect mannuronic acid by paper chromatography: no derivative suitable for the extensive purification necessary for isotope dilution analysis could be prepared.

D-Arabinose does not appear to be formed by a primary process (Fig. 2), and D-lyxose is present in negligible amounts. Previously, in oxygen, it was observed that some pentose formation occurred as a result of secondary decarboxylation reactions of the D-mannonic and D-mannuronic acid, which were present as primary products. The results reported here support this view. The primary scission between C-1 and C-2 to give arabinose and formaldehyde, observed in oxygenated solution, does not occur *in vacuo*.

Ring scission, similar to that occurring in oxygen, is indicated by the yield–dose curves for two- and three-carbon aldehydic fragments, although the latter fragments are formed in higher yields than those previously found in oxygen. The primary scission between C-2 and C-3, and between C-4 and C-5, to give three two-carbon fragments occurs with initial G 0·95. Comparison with initial G for erythrose formation (0·25) indicates that scission to give a two- and a four-carbon fragment takes place simultaneously with the formation of three two-carbon fragments, with the latter process dominant.

Another primary process with initial G 0·5 is the symmetrical scission to give two three-carbon fragments. In oxygen this was not in evidence as a primary process.

The proposed degradation of D-mannose in evacuated solution may therefore be represented as follows:

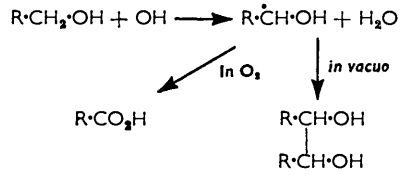


D-Glucosone, 2-oxogluconic acid, and the three-carbon aldehydic fragments will contribute to the overall ultraviolet absorption spectrum of the irradiated solution (Fig. 1). The shift in position (to 290 mμ) and intensity of the absorption maximum on the addition of alkali occurred with solutions irradiated in oxygen and was attributed to enolisation. Reductone, for example, absorbs in this region in alkali.[4]

A distinctive feature of D-mannose solutions irradiated *in vacuo*, and not encountered in oxygen, is the formation of a polymer. The yield–dose curve establishes that polymer formation is a secondary process, with the rate of formation increasing markedly only at high doses.

The primary processes discussed (reactions 1—5) account for an initial G(D-mannose) 2·4, which may be compared with the observed value, 3·5. The primary processes unaccounted for probably relate to the dimerisation of primary radicals formed as a preliminary step in the production of polymer. Such primary dimerisation processes, with initial G *ca.* 1, are observed when D-sorbitol solutions are irradiated *in vacuo*. The observation that mannuronic acid is formed only in oxygen supports the existence of a comparable process for D-mannose irradiations *in vacuo*. In the absence of oxygen, sugar radicals, formed as a result of primary hydrogen abstraction at C-6 by hydroxyl radicals, would likewise be expected to dimerise.

Evidence in support of this postulate has been provided by Bailey, Barker, Brimacombe, Pooley, and Spencer[5] as a result of an electron-spin resonance spectral study of γ-irradiated carbohydrates. The peak separation and anisotropic broadening observed with polycrystalline D-glucose is consistent with the radical R·ĊH·OH formed by H-ejection at C-6. This conclusion may be applied with some justification to irradiation of aqueous solutions, since it was previously demonstrated that there is a close correlation between the radicals formed on irradiation of glycollic acid in the solid state[6] and in solution.[7]

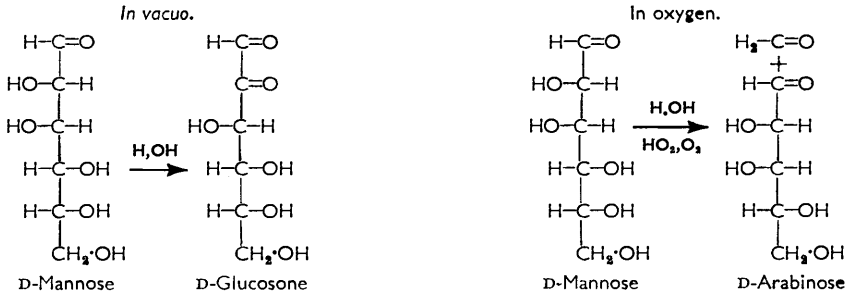


However, from the nature of the products, it is clear that attack by free radicals formed during the primary radiolysis of water is not confined to C-6. Attack at C-1 leads to mannonic acid: at the 3,4-bond to give three-carbon fragments; at the 2,3- and the 4,5-bond to give three two-carbon fragments. Thus, the primary sugar radicals which lead to these products, and secondary radicals arising subsequently from the products, may add to the growing polymer chain, giving a complex polymer with a carbon–carbon skeleton composed of widely differing monomer units. Polymer formation would therefore be an insufficient coupling process rather than a chain reaction.

Primary abstraction processes involving hydroxyl radicals would lead to identical primary sugar radicals in oxygen and *in vacuo*. Subsequently, the secondary effect of oxygen and hydroperoxy-radicals may lead to differing products in oxygen. However, some correlation should be possible between the two systems, and our results indicate that this is so. A striking feature is the identical rate of disappearance of D-mannose under both conditions ($-G$ 3·5), pointing to comparable primary abstraction processes which are independent of oxygen. Under both conditions D-mannonic acid is formed, and the products arising from ring scission are also similar. The products arising from attack at

[4] von Euler, Hasselquist, and Haushoff, *Arkiv Kemi*, 1953, **6**, 471.
[5] Bailey, Barker, Brimacombe, Pooley, and Spencer, *Nature*, 1961, **190**, 259.
[6] Grant, Ward, and Whiffen, *J.*, 1958, 4635.
[7] Grant and Ward, *J.*, 1959, 2659.

C-2 are different in oxygen and *in vacuo*. In oxygen, arabinose and formaldehyde are formed, but *in vacuo* ring scission does not occur and glucosone is formed. This may be represented as annexed.



Clearly, the same primary radical could lead to all the products. This behaviour is analogous to that of aqueous glycollic acid. The carbon–carbon scission which occurs in oxygen is diminished under a vacuum.[7] Similarly dimerisation is only observed in the absence of oxygen.

The G value 3·5 is significant and indicates that hydroxyl radicals are not the only species which may initiate reaction following abstractions of the type: $RH + OH \longrightarrow R\cdot + H_2O$. If all the hydroxyl radicals were scavenged by this process, G for the disappearance of aldohexose could not rise above $G_p(OH)$, the primary yield of hydroxyl radicals, which at the pH range of the experiments is less than 3. The situation is similar to that encountered with alcohols when irradiated at high concentrations.[8] One possibility is that hydrogen atoms may participate in the initiating process; an alternative is that hydroperoxy-radicals may initiate reactions, as proposed for irradiations of L-ascorbic acid.[9] A third possibility, which appears to us more probable, is that sub-excited electrons may contribute to the initial step of the reaction, and this possibility is now being investigated further. It is clear, however, that, on irradiation in solution, aldohexoses exhibit changes similar in character to those observed under comparable conditions in related compounds, particularly hydroxy-acids and alcohols.[10]

The authors thank Dr. W. Wild for valuable discussions, Professor A. G. Evans for his interest, and the United Kingdom Atomic Energy Authority (Research Group) for financial support. A maintenance grant (to W. J. C.) from U.K.A.E.A. (Research Group) is also gratefully acknowledged.

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[8] Jayson, Scholes, and Weiss, *J.*, 1957, 1358; Allen, Hayon, and Weiss, *J.*, 1959, 3913.
[9] Barr and King, *J. Amer. Chem. Soc.*, 1956, **78**, 303.
[10] Phillips, *Adv. Carbohydrate Chem.*, 1961, **16**, 13.