525. *Radiation Chemistry of Carbohydrates. Part X.*[1] *Action of γ-Radiation of Aqueous* D-*Ribose Solutions in Oxygen.*

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D-Ribose is degraded on γ-irradiation in dilute aqueous solution with initial $-G$ 3·2—3·3. Primary degradation processes, identified by reference to yield–dose curves, lead to the formation of D-ribonic acid, three- and two-carbon aldehydic fragments, and a further unidentified acid. D-Erythrose arises, in the main, from secondary processes. Thus, the main effects of radiation which have been identified are end-group oxidation and chain scission to give two- and three-carbon aldehydic fragments.

Continuing our previous studies on the chemical effects of ionising radiations on aldohexoses [2] and ketohexoses,[3] the present paper describes the changes which are induced during irradiation of D-ribose in dilute oxygenated solution. Interest in the genetic effects of ionising radiations led to extensive investigations of the changes following irradiation of nucleic acids.[4] The precise chemical changes remain uncertain.[4,5] Attention has been mainly concentrated on the physical changes which are observed during and after irradiation, particularly changes in viscosity.[4,6] Model experiments have been carried out with purine and pyrimidine nucleotides [7] and solutions of simple phosphates.[8] Considerable attention has been given to the post-irradiation release of inorganic phosphate from these compounds, and it has been suggested that changes in the sugar component may be responsible for this behaviour. However, no investigation into the chemical changes accompanying the irradiation of D-ribose solutions has previously been reported.

Results and Experimental

The ^{60}Co source used for irradiations, irradiation cells, and dosimetric and analytical methods have been described in previous Papers in this series. The dose rates employed in the investigation were $1{\cdot}45 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ in the large cell (100 ml.) and $0{\cdot}90 \times 10^{17}$ ev ml.$^{-1}$ min.$^{-1}$ in the small cell (40 ml.). In all irradiations the solutions were continuously oxygenated.

Chromatographic Analysis of Irradiated Solutions.—A solution of D-ribose (3·33 millimoles) in water (100 ml.) was irradiated to a total energy input of $7{\cdot}1 \times 10^{22}$ ev, and chromatographed on paper with butan-1-ol–acetic acid–water (4 : 1 : 5). Using *p*-anisidine as spray revealed the following spots: pale yellow, R_F 0·10; green, R_F 0·18; pink, R_F 0·35, ribose; yellow, R_F 0·44, erythrose. Using alkaline silver nitrate showed up a further spot, R_F 0·38, corresponding to ribono-γ-lactone. Treatment of the irradiated solution with Amberlite IRA-400 (OH^-) resin before paper chromatography removed the constituents with R_F 0·10, 0·18, and 0·38, indicating their acidic nature.

Acid Formation.—The rate of acid formation with increasing dose is shown in Table 1. The rate is initially linear and corresponds to an initial G value for acid formation of 1·65, the

Table 1.

Acid formation during irradiation of D-ribose solutions.

Dose (10^{19} ev/ml.)	1·80	3·90	5·20	7·10	8·50	10·8	12·4	14·0	15·7	17·5
Acid (10^{18} molecules/ml.)	0·30	0·65	0·95	1·20	1·65	1·80	2·25	2·65	2·95	3·50

[1] Part IX, preceding paper.
[2] Phillips, Moody, and Mattok, *J.*, 1958, 3522; Phillips and Criddle, *J.*, 1960, 3404.
[3] Phillips and Moody, *J.*, 1960, 754.
[4] Butler, *Rad. Res., Suppl. No.* 1, 1959, 403.
[5] Baron, Johnson, and Corbure, *Rad. Res.*, 1954, **1**, 410; Scholes and Weiss, *Exp. Cell. Res., Suppl.*, 1952, **2**, 219; Scholes and Weiss, *Biochem. J.*, 1953, **53**, 567.
[6] Taylor, Greenstein, and Hollaender, *Arch. Biochem.*, 1948, **16**, 19.
[7] Daniels, Scholes, and Weiss, *J.*, 1956, 3771.
[8] Scholes, Taylor, and Weiss, *J.*, 1957, 235; Wilkinson and Williams, *J. Chim. phys.*, 1955, **52**, 600.

acid being assumed to be monobasic. At higher doses, the rate increases, indicating a contribution to the total acid formation by secondary processes.

Absorption Spectra of Irradiated Solutions.—Fig. 1 shows a typical absorption spectrum of the irradiated D-ribose solution in comparison with an unirradiated solution. The characteristic absorption maximum at 265 mμ increases in intensity on addition of alkali (sodium hydrogen carbonate) and is reduced on addition of mineral acid.

Estimation of Products by Isotope Dilution Analysis.—In a typical irradiation, a solution (100 ml.) of D-ribose (3·33 millimoles) containing sufficient D-[^{14}C]-ribose to give a specific activity for the irradiated pentose solution of 5·0 μc/millimole was irradiated to a total energy input of $1{\cdot}06 \times 10^{23}$ ev in oxygen.

A sample of the irradiated solution was chromatographed on paper in butan-1-ol–acetic acid–water (4 : 1 : 5) and the resulting autoradiograph scanned with a Hilger photoelectric densitometer. Fig. 2 shows the relative concentration of ^{14}C along the chromatogram. The peaks correspond to the constituents detected with spray reagents and confirm the view that no further non-reducing product remains undetected.

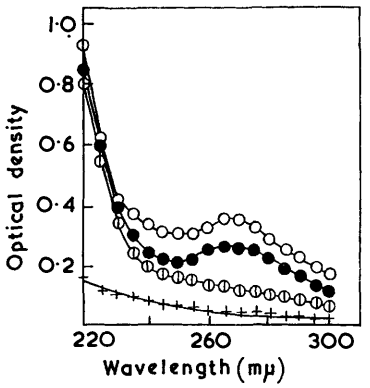


FIG. 1. Ultraviolet absorption spectra of irradiated D-ribose solutions (100 ml.) in oxygen. Energy input $2{\cdot}0 \times 10^{22}$ ev.

○ With added $KHCO_3$. ⓘ With added HCl. ● Irradiated solution. + Unirradiated solution.

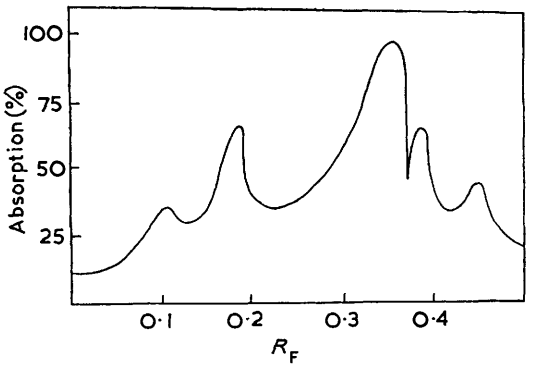


FIG. 2. Density of spots on autoradiograph, giving a measure of ^{14}C concentration along chromatogram.

The main products were estimated by application of isotope dilution analysis directly to the irradiated solution as follows:

D-*Ribose.* The irradiated solution (5·0 ml.) was treated with carrier D-ribose (1·02 millimoles) and freeze-dried. The solid was heated in ethanol (25 ml.) with benzylphenylhydrazine (0·5 ml.) at 100° for 1 hr., then the solvent was removed. Seven recrystallisations of the resulting solid from ethanol–benzene gave pure D-ribose benzylphenylhydrazone, m. p. 127°, constant specific activity 0·036 μc/millimole.

D-*Ribonic acid.* The irradiated solution (5·0 ml.) was treated with carrier D-ribono-γ-lactone (0·95 millimole) and, after equilibration for 24 hr., freeze-dried. The solid was heated in ethanol (25 ml.) with 95% hydrazine hydrate (0·5 ml.) at 100° for 1 hr., giving a solid which after seven recrystallisations from ethanol gave pure D-ribonohydrazide with m. p. 150° and constant specific activity 0·079 μc/millimole.

D-*Erythrose.* Carrier D-erythrose was freshly prepared by the method of Frush and Isbell.[9] The irradiated solution (5·0 ml.) was treated with carrier D-erythrose (0·85 millimole), glacial acetic acid (1 ml.), and phenylhydrazine (1·5 ml.), and refluxed for 20 min. The solid formed was further refluxed with benzene (20 ml.), and the solution chromatographed on a column of calcined alumina (4 parts) and chromatographic alumina (Brockman activity 1; 1 part). The band corresponding to erythrosazone was eluted with benzene; evaporation and four

[9] Frush and Isbell, *J. Res. Nat. Bur. Stand.*, 1953, **51**, 307.

recrystallisations from benzene gave the pure compound with m. p. 159° and constant specific activity 0·10 μc/millimole.

D-*Erythronic acid.* Pure carrier material was first prepared by the procedure of Hardegger, Kreis, and El Khadem.[10] Subsequently, the irradiated solution (5·0 ml.) was treated with potassium D-erythronate (1·0 millimole) and the solution made slightly alkaline with potassium hydroxide. After 24 hr., the mixture was freeze-dried and the resulting solid required seven recrystallisations from water to give pure potassium D-erythronate with constant specific activity 0·009 μc/millimole.

Glycollic acid. The irradiated solution (5·0 ml.) was treated with carrier glycollic acid (2·50 millimoles) and an excess of calcium carbonate. After 24 hr. the solution was boiled for

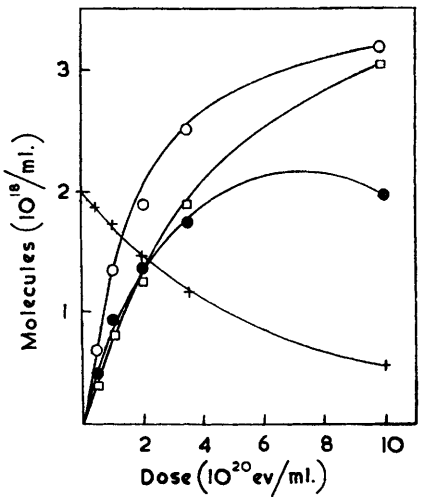


FIG. 3. Rate of formation of products during irradiation of D-ribose solutions in oxygen, determined by isotope dilution.

○ Two-carbon aldehydic fragments. □ Three-carbon aldehydic fragments. ● D-Ribonic acid. + D-Ribose (× 10^{-1}).

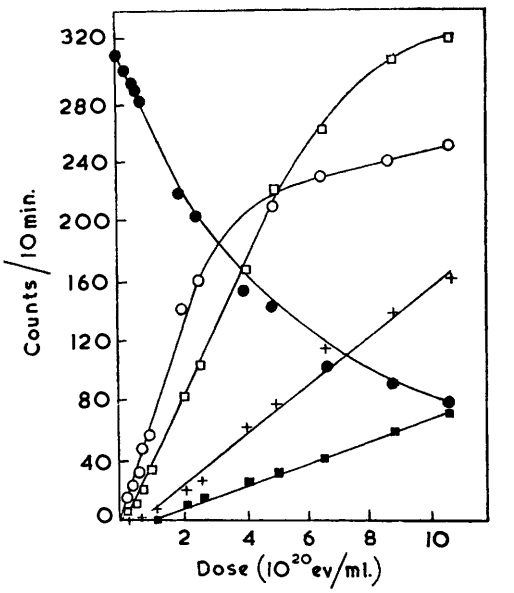


FIG. 4. Rate of formation of products during irradiation of D-ribose solutions in oxygen, determined by paper chromatography.

● D-Ribose. □ Product R_F 0·18. ○ D-Ribono-γ-lactone. ▪ D-Erythrose.

10 min. and the excess of calcium carbonate removed while hot. Crystals of calcium glycollate separated on cooling, and required seven recrystallisations to achieve the constant specific activity of 0·0024 μc/millimole.

Glycollaldehyde. The irradiated solution (5·0 ml.) was treated with carrier glycollaldehyde (2·40 millimoles) and a saturated solution of 2,4-dinitrophenylhydrazine in 2% sulphuric acid (25 ml.). The orange solid which separated required seven recrystallisations from ethyl acetate to give pure glycollaldehyde 2,4-dinitrophenylhydrazone with m. p. 155° and constant specific activity 0·014 μc/millimole.

Oxalic acid and two- and three-carbon aldehydic fragments were estimated as described previously. The results of this estimation are shown in Table 2.

Rate of Formation of Products.—By applying the isotope dilution method to estimate the products at four doses in the range 0·5—3·5 × 10^{20} ev/ml., yield–dose curves were obtained for D-ribonic acid and two- and three-aldehydic fragments. The initial rate of degradation of D-ribose was also measured, and the results are shown in Fig. 3, giving the following initial G values: $-G$(D-ribose) 3·2; initial G(ribonic acid) 0·9; initial G(two-carbon fragments) 1·3; G(three-carbon fragments) 0·8.

Paper chromatography at increasing doses was used to determine yield–dose curves for the

[10] Hardegger, Kreis, and El Khadem, *Helv. Chim. Acta*, 1951, **34**, 2343.

TABLE 2.

Products when aqueous D-ribose is irradiated with γ-radiation in oxygen.

Initial D-ribose, 3·33 millimoles. Energy input $1{\cdot}06 \times 10^{23}$ ev.

Product	D-Ribose	D-Ribonic acid	D-Erythrose	Three-carbon fragments	Two-carbon fragments
Carrier (millimoles)	1·02	0·95	0·85	1·40	2·00
Spec. activity (μc/millimole) ...	0·19	0·079	0·10	0·05	0·026
Yield (millimoles)	0·80	0·31	0·42	0·50	0·52

Product	Glycollaldehyde	Glycollic acid	Oxalic acid	D-Erythronic acid
Carrier (millimoles)	2·40	2·50	2·00	1·01
Spec. activity (μc/millimole) ...	0·014	0·002	0·012	0·009
Yield (millimoles)	0·32	0·05	0·24	0·04

Unidentified product of R_F 0·10 estimated from paper chromatography 0·08 millimole.
Unidentified product of R_F 0·18 estimated from paper chromatography 0·33 millimole.

products not conveniently estimated by isotope dilution analysis (Fig. 4). For comparison of the two methods, the rate of degradation of D-ribose was also measured by paper chromatography. Details of the method have been described previously. The results indicate the following initial G values: $-G$(D-ribose) 3·3; initial G(ribono-γ-lactone) 0·7; initial G(acid R_F 0·18) 0·4. D-Erythrose and the unidentified product of R_F 0·10 appear to be formed by secondary processes.

DISCUSSION

Paper chromatography of irradiated D-ribose solution showed that several constituents are formed during irradiation. The presence of D-erythrose, revealed in this way, was confirmed by isotope dilution analysis. Two further constituents present in the irradiated solution, with R_F 0·10 and 0·18, respectively, in butan-ol–acetic acid–water, have not been identified. Their behaviour towards the ion-exchange resin Amberlite IRA-400 (OH^-) indicates that they are acidic. It is evident from Fig. 2, which shows the distribution of ^{14}C along the paper chromatogram, that there are no further major products formed which were not revealed by spray reagents. The considerable background along the paper, however, confirms the pronounced streaking observed on spraying and indicates the presence of lower fragments. A comprehensive estimation of products by isotope dilution analysis after 0·5% D-ribose solution has been irradiated to a total energy input of $1{\cdot}06 \times 20^{23}$ ev is shown in Table 2. The total products account for *ca.* 80% by weight of initial D-ribose.

Yield–dose curves from isotope dilution analysis (Fig. 3) and paper chromatography (Fig. 4) allow primary and secondary degradation processes to be identified. Acid formation (Table 1) contributes extensively to the overall degradation. When it is assumed that the acid formed is monobasic, initial G(acid) is 1·6, which represents half the initial degradation of D-ribose (initial $-G$ 3·2).

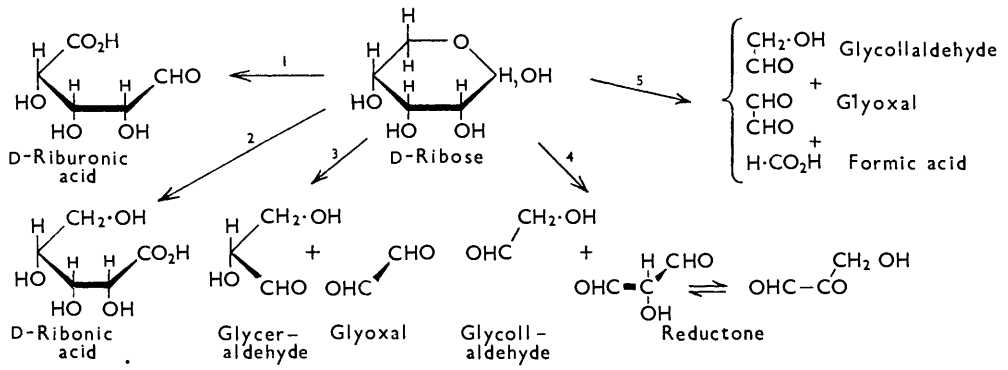
This $-G$ value for D-ribose is slightly lower than the previously reported initial degradation rates for 1% solutions of sugars irradiated in oxygen [2] and *in vacuo*,[3] where the initial is $-G$ 3·5. D-Ribonic acid is formed by a primary process (Figs. 3 and 4). By isotope dilution analysis initial G(ribonic acid) is 0·9 and from paper chromatography 0·7; the latter value represents the formation of ribono-γ-lactone only. The unknown reducing acid, R_F 0·18, is formed with initial G 0·4 when estimated by paper chromatography (if it is a five-carbon acid). In view of the well-established susceptibility of primary alcohol groups in hexoses and hexitols, and our evidence that the acid of R_F 0·18 is a primary product, reducing in character, it is probable that the acid is formed by attack at C-5. So far as we are aware, however, the existence of D-riburonic acid has not been previously reported. We are continuing our investigation into the nature of this unidentified primary acid.

In addition to oxidation at the extremities of the molecule, chain scission appears to be

a dominating process. Two- and three-carbon aldehydic fragments are formed by primary processes. The former occurs with initial G 1·3 and the latter 0·8. These different values indicate that more than one type of chain scission occurs, a view supported by the difference between the amount of glycollaldehyde formed and total two-carbon aldehydic fragments, which is a measure of glyoxal and glycollaldehyde formation (since they form the same osazone). The primary end-group oxidations and ring scission described may be represented as annexed.

Thus, on the basis of our isotope dilution data, initial G(three-carbon fragments) 0·8 represents the sum of reactions 3 and 4, since both reductone and glyceraldehyde would yield glycerosazone. For reactions 3 and 4, an equivalent formation of two-carbon fragments would be expected, equal therefore to an initial G 0·8. The difference between this value and the overall initial G for two-carbon fragments (1·3) may thus be attributed to the amount formed by reactions of type 5. The accompanying formation of a further acid, possibly formic acid, may therefore account for the difference observed between initial G(total acid) 1·6 and the sum of the acid due to ribonic acid (initial G 0·9) and unknown acid of R_F 0·18 (initial G 0·4).

The slow initial rate of D-erythrose formation is consistent with a secondary production, although it is difficult to differentiate between a primary and a secondary product when the initial portion of the yield–dose curve cannot be accurately measured. The marked increase in rate of formation at higher doses, however, indicates that the secondary formation is the main process responsible for D-erythrose formation, although slow primary reaction giving erythrose cannot be precluded.



The unidentified acid with R_F 0·10 appears to be a secondary product, possibly a keto-acid formed from D-ribonic acid.

Thus, when D-ribose is irradiated in oxygenated solution end-group oxidation and chain scission, to yield lower aldehydic fragments, are the main effects.

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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[*Received, February 13th,* 1962.]