575. *Effects of γ-Radiation. Part IV.* The Degradation of* D-*Glucose in Aqueous Solution by γ-Irradiation* in Vacuo.

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The effect of ^{60}Co γ-radiation on aqueous solutions of glucose *in vacuo* has been studied. Gluconic acid and 2-oxo-D-*arabino*-aldohexose have been isolated as products; the latter has been determined by a new method utilising its ready reaction with *o*-phenylenediamine. Evidence for other products is presented and a degradation mechanism discussed.

Increasing attention is being paid to the effect of radiation on solutions of sugars, and recently irradiation of oxygenated glucose solutions has been studied.[1] A number of other investigations have been made (see, *e.g.*, refs. 2, 3), but no definite degradation processes appear to have been proposed and substantiated for the evacuated system.

* Part III, *J.*, 1959, 2659.

[1] Phillips, Moody, and Mattock, *J.*, 1958, 3522.
[2] Wolfrom, Brinkley, McCabe, Shen-Han, and Michelakis, *Radiation Res.*, 1959, **10**, 37.
[3] Bothner-by and Balazs, *ibid.*, 1957, **6**, 302.

The present investigation arose from observations that the reducing power of irradiated maltose solutions decreased with increasing dose, although the formation of glucose could be readily detected by chromatography. This was not in agreement with findings, then incomplete, on the irradiation of glucose in oxygen,[4] which led one to expect the formation of uronic acids. Irradiation of evacuated maltose solutions was also shown to produce relatively large yields of acids and lactones, and some evidence for a structure of 2-oxo-D-*arabino*-aldohexose type was obtained (see below).

These studies indicated that the preliminary mechanisms advanced for oxygenated solutions were not valid for evacuated solutions. Consequently glucose solutions which had been irradiated with γ-rays *in vacuo* were freeze-dried and examined by paper chromatography and paper ionophoresis. Gluconic acid, 2-oxo-D-*arabino*-hexonic acid, arabinose, arabonic acid (trace), and glucosaccharic acid (trace) were among the products detected. Gluconic acid was present as the major product. When the irradiated solution was chromatographed and the " glucose " band cut out and eluted, the eluate, on chromatography in phenol–water, showed the presence also of 2-oxo-D-*arabino*-aldohexose. Glucuronic acid and 2-oxo-D-*arabino*-hexonic acid can only with difficulty be distinguished by chromatography. Additional evidence was obtained by oxidising the irradiated solution with alkaline hypoiodite before ionophoresis. Under these conditions uronic acids are oxidised to saccharic acids. This treatment did not increase the minute amount of saccharic present, indicating effective absence of glucuronic acid. This simple oxidation technique was later used to verify the presence of glucuronic acid in oxygenated systems.[1] It also shows that polyols are absent, in agreement with the complete absorption of the irradiated solute on excess of Amberlite IRA-400 resin (OH^- form).[5] 2-Oxo-D-*arabino*-aldohexose was identified by the preparation of glucose 2 : 4-dinitrophenylosazone at a low temperature under conditions which did not afford the derivative from glucose.

Gluconic acid was identified as its calcium salt, after partial purification on 2% cross-linked " micro-bead " Deacidite FF resin in the carbonate form.[6] The mixture of acids was eluted with N-ammonium carbonate. Although this mild resin-treatment caused some degradation of 2-oxo-D-*arabino*-hexonic acid and 2-oxo-D-*arabino*-aldohexose, it was shown that the degradation did not lead to gluconic acid. Further, the amounts of these labile compounds were relatively small, compared with that of gluconic acid.

Acid production (essentially gluconic acid in the early stages of the degradation) was measured over a range of doses by potentiometric titration. The large differences between the values obtained at pH 7 and pH 9 indicate lactone formation (Tables 1 and 2).

TABLE 1. *Radiation degradation of* 0·1% *glucose solution* in vacuo.

Total dose (10^{18} ev ml.$^{-1}$)	Gram-equiv. of carboxylic acid pH 7	pH 9·3	pH 10	2-Oxo-D-*arabino*-aldohexose yield (mg./g.)	log I_0/I (267 mμ)
0	~0	~0	~0	0	0·0013
1·14	0·003	0·005	0·006	3·2	0·0158
2·28	0·006	0·012	0·014	6·2	0·0548
4·56	0·013	0·026	0·033	12·8	0·0904
6·84	0·018	0·034	0·041	17·2	0·129
11·4	0·031	0·052	0·061	30·6	0·228
18·2	0·041	0·074	0·087	42·5	0·334

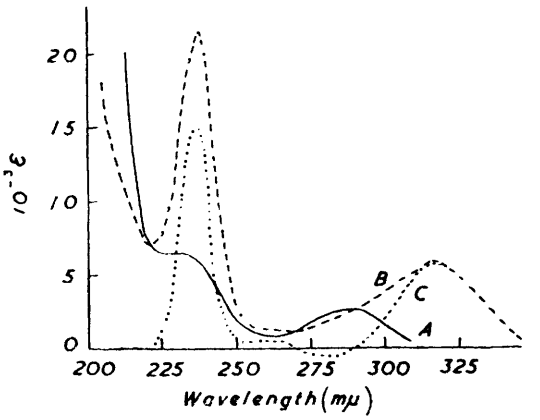
No satisfactory method was available for determination of 2-oxo-D-*arabino*-aldohexose.[7] It was found that *o*-phenylenediamine and 2-oxo-D-*arabino*-aldohexose gave 2-(*arabino*-tetrahydroxybutyl)quinoxaline quantitatively in oxygen-free water at 60°. This product differed markedly in ultraviolet absorption at 237 and 318 mμ from unchanged *o*-phenylenediamine and 2-oxo-D-*arabino*-aldohexose (see Figure). The 320 mμ region was chosen for

[4] Phillips, *Nature*, 1954, **173**, 1044.
[5] Barker, Bourne, Salt, and Stacey, *J.*, 1958, 2736.
[6] Machell, *J.*, 1957, 3389.
[7] Bayne and Fewster, *Adv. Carbohydrate Chem.*, 1956, **11**, 43.

TABLE 2. *Radiation degradation of* 0·1% *maltose solution* in vacuo.

Total dose (10^{18} ev ml.$^{-1}$)	Gram-equiv. of carboxylic acid pH 7	pH 8	pH 9	Reducing power (%)	log I_0/I (262 mμ)
0	0	0	0	100	—
19·7	0·053	0·054	0·09	89	0·32
53·5	0·15	0·19	0·23	81	0·70
74·0	—	—	—	79	0·85
150	0·34	0·40	0·45	67	1·22
380	0·50	0·59	0·66	59	1·61
760	0·55	0·69	0·77	44	2·17

the estimation of the 2-oxo-D-*arabino*-aldohexose, since it was free from interference caused by possible slight oxidation of the diamine. With *o*-phenylenediamine as control (Table 1) the amount of 2-oxo-D-*arabino*-aldohexose was calculated from the difference in molar extinction coefficient ($\Delta\varepsilon_{320} = 5600$). The absorption curve obtained under these conditions closely approximated to the theoretical difference curve (curve *C* in the Figure)



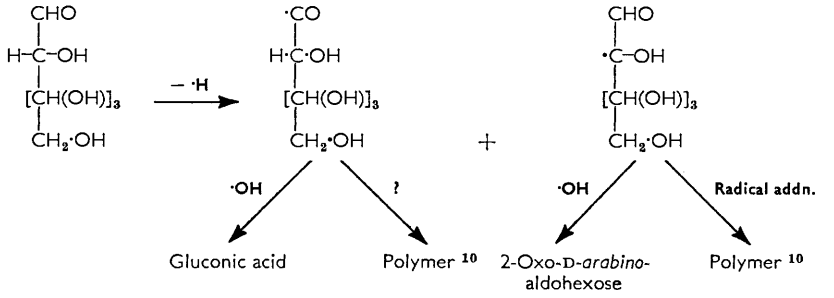
Spectrophotometric determination of 2-(arabino-*tetrahydroxybutyl*)*quinoxaline.*
A, o-*Phenylenediamine.*
B, 2-(arabino-*Tetrahydroxybutyl*)*quinoxaline.*
C, *Differential molar extinction coefficient from B–A* (Δε).

A similar curve was obtained when irradiated maltose solutions were heated with *o*-phenylenediamine in the same way, which indicates formation of maltosone.

Considerable interest has been shown in the ultraviolet absorption spectra of irradiated carbohydrate solutions.[1,3] The maxima occur at 262 mμ (maltose) and 267 mμ (glucose). 2-Oxo-D-*arabino*-aldohexose itself behaves similarly, but the absorption of the irradiated solution exceeded that calculated from the yield of this component. Glyceraldehyde in neutral or acidic solution shows negligible absorption, but 1 : 3-dihydroxypropan-2-one solutions slowly produce the observed absorption pattern, and this production has been advanced to explain the spectrum of glucose solutions irradiated in oxygen.[1] However, 1 : 3-dihydroxypropan-2-one has a molar extinction coefficient $\varepsilon_{270} \not> 30$ in neutral solution or at pH values between 3 and 7.[8] Hence to account for the spectrum this compound would have to be formed at a rate of 100 mg. l.$^{-1}$ hr.$^{-1}$ and would equal the initial weight of glucose within 10 hr. (Table 1). Even in 0·1N-sodium hydroxide the compound has $\lambda_{max.}$ ~290 mμ (ε 1000).[8] However, the required absorption maximum is given by enediol structures such as L-ascorbic acid ($\lambda_{max.}$ 263 mμ; ε 8500) in neutral or slightly acid solution. D-Glucoascorbic acid has been claimed as a product from the irradiation of gluconolactone in aqueous solution [9] and it has been found that irradiated glucose solutions readily decolorise dichlorophenolindophenol. However, these properties would be exhibited by any enediol structure which was not precluded by lactone-ring formation. Further, similar absorption is given by the polymeric materials produced from maltose, glucose, and gluconolactone on prolonged irradiation *in vacuo* (Part I [10]).

[8] Petuely and Meixner, *Monatsh.*, 1953, **84**, 1061.
[9] Coleby, *Chem. and Ind.*, 1957, 111.
[10] Barker, Grant, Stacey, and Ward, *J.*, 1959, 2648.

The degradation mechanism postulated is closely analogous to the radiation-induced degradation of glycollic acid (Parts II and III).[11] Primarily, a hydrogen atom is abstracted from the solute by solvent radicals, and the annexed sequence follows.



Irradiation of crystalline glycollic acid gives the carboxyhydroxymethyl radical,[12] and degradation of glycollic acid in dilute aqueous solution *in vacuo* is consistent with the initial formation of this radical, the product being modified by the presence of oxygen due to peroxy-radical formation.[11] The effect of radiation on crystalline glucose may be expected to be similar. The paramagnetic resonance spectra of a number of *X*-irradiated carbohydrates indicate [13] that the radical system is closely associated with a C_2 fragment. The two radicals postulated above would be in agreement with this. Further, auto-radiolysis of C^{14}-labelled glucose [14] gives a mixture of degradation products similar to that obtained in dilute aqueous solution *in vacuo*.

It should be emphasised that early carbon–carbon scission is not precluded, but it appears to occur only to a small extent. Later in the degradation sequence carbon–carbon scission occurs easily, *e.g.*, in the oxidative decarboxylation of gluconic acid to arabinose.[2,15]

Degradation of glucose solutions by irradiation in the presence of oxygen might be expected to yield the same radicals, which would rapidly be converted into the corresponding peroxy-radicals, preventing radical addition but yielding essentially the same oxidised compounds. However, observations on the degradation of glucose solutions in oxygen [1] suggest that neither 2-oxo-D-*arabino*-aldohexose nor 2-oxo-D-*arabino*-hexonic acid is formed, but instead glucuronic acid is formed and extensive carbon–carbon scission occurs. This may be analogous to the carbon–carbon scission which occurs on irradiation of aqueous glycollic acid in oxygen and is reduced on irradiation of aqueous glycollic acid *in vacuo*.[11]

Experimental

Determination of 2-*Oxo*-D-arabino-*aldohexose*.—(i) *Preparation of* o-*phenylenediamine*, 2-*oxo*-D-arabino-*aldohexose and* 2-(arabino-*tetrahydroxybutyl*)*quinoxaline*. *o*-Phenylenediamine was recrystallised (charcoal) twice from oxygen-free hot water under nitrogen and dried *in vacuo*. The colourless material had m. p. 102°. The ultraviolet spectrum and molar extinction coefficients were $\lambda_{max.}$ 231 (ε 5400) and 288 mμ (ε 2750) (Figure).

2-Oxo-D-*arabino*-aldohexose [16] was purified by elution from a column of powdered cellulose with the organic phase of butan-1-ol–ethanol–water (4 : 1 : 5 v/v) and obtained as a pale yellow, amorphous, slightly hygroscopic solid. The material showed only one component on chromatography in the systems described below.

2-(*arabino*-Tetrahydroxybutyl)quinoxaline was prepared by warming 2-oxo-D-*arabino*-aldohexose (12 mg.) with *o*-phenylenediamine (11 mg.) in water (1 ml.) at 60° for 10 min. The

[11] Grant and Ward, *J.*, 1959, 2654, 2659.
[12] Grant, Ward, and Whiffen, *J.*, 1958, 4635.
[13] Williams, Geusic, Wolfrom, and McCabe, *Proc. Nat. Acad. Sci. U.S.A.*, 1958, **44**, 1128.
[14] Bourne, Hudson, and Weigel, unpublished work.
[15] Barker, Grant, Stacey, and Ward, *Nature*, 1959, **183**, 376.
[16] Fischer, *Ber.*, 1889, **22**, 87.

crystals deposited on cooling were filtered off, and after recrystallisation from water had m. p. 190°, $\lambda_{max.}$ 237·5 (ε 21,200) and 318 mμ (ε 5800) (Figure).

(ii) *Quantitative formation of* 2-(arabino-*tetrahydroxybutyl*)*quinoxaline from* 2-*oxo*-D-arabino-*aldohexose.* *o*-Phenylenediamine had unchanged ultraviolet absorption after being heated in oxygen-free water for 3 hr. under nitrogen at temperatures up to 100°.

2-Oxo-D-*arabino*-aldohexose (10·4 mg.) was heated in an oxygen-free solution of *o*-phenylenediamine (12 mg.) in water (6 ml.) at 60° under nitrogen and aliquot parts (1 ml.) were removed after 10, 30, 60, 90, and 120 min. Each aliquot part was diluted to 100 ml. and the absorption at 320 mμ determined against a control solution containing *o*-phenylenediamine (0·02 mg./ml.). By using the Δε curve of the Figure, $\Delta\varepsilon_{320} = 5600$ was obtained, enabling the apparent percentage conversion of 2-oxo-D-*arabino*-aldohexose into 2-(*arabino*-tetrahydroxybutyl)quinoxaline to be calculated. The values after the above periods of heating were 74, 90, 97, 96, and 97% respectively. It was concluded that the 2-oxo-D-*arabino*-aldohexose sample was 97% pure.

(iii) *Interference of glucose, gluconic acid and* 2-*oxo*-D-arabino*hexonic acid.* Glucose (1 g.), gluconic acid (45 mg.), and 2-oxo-D-*arabino*-hexonic acid (10 mg.) were separately heated in the absence of oxygen with 0·2% *o*-phenylenediamine solution (6 ml.) at 60° for 90 min. under nitrogen. The solutions were diluted a hundred-fold and the absorption at 320 mμ was measured against 0·002% *o*-phenylenediamine solution as described above. The three determinations gave absorption values of 0·006, −0·005, and −0·006 respectively, which were interpreted as effective errors of <2% in the determination of 2-oxo-D-*arabino*-aldohexose.

(iv) *Trial analysis of a weighed sample and a standard solution.* 2-Oxo-D-*arabino*-aldohexose (10·0 mg.) was heated with oxygen-free 0·2% *o*-phenylenediamine solution (6 ml.) for 90 min. at 60° under nitrogen. An aliquot part (1 ml.) was diluted to 100 ml., and the absorption at 320 mμ measured against 0·002% *o*-phenylenediamine solution. The absorption corresponded to 9·7 mg. of 2-oxo-D-*arabino*-aldohexose (97%).

A mixture containing glucose (0·507 g.), 2-oxo-D-*arabino*-aldohexose (8·0 mg.), 1 : 4-gluconolactone (18·3 mg.), and 2-oxo-D-*arabino*-hexonic acid (8·0 mg.) was estimated (as above) to contain 7·7 mg. (96·5%) of 2-oxo-D-*arabino*-aldohexose.

Irradiation of Solutions.—The sugar solution (0·1%) was degassed and irradiated in an evacuated sealed vessel by means of a 200 c ^{60}Co source at a dose-rate of $3{\cdot}8 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$, as described earlier.[10,11,15] The solution was freeze-dried.

Examination of the Irradiated Solutions.—Irradiated solutions (0·1%) of maltose and glucose were prepared. In general only the operations effected on the glucose solutions are described below. The results obtained from the less detailed treatment of the other solutions are discussed in the theoretical section.

(i) *Chromatographic and ionophoretic analysis.* Chromatography was in butan-1-ol–ethanol–water (4 : 1 : 5 v/v), butan-1-ol–propionic acid–water (6 : 3 : 4 v/v), butan-1-ol–water–90% formic acid (4 : 3 : 1 v/v), ethyl acetate–90% formic acid–acetic acid–water (18 : 1 : 3 : 4 v/v), and pentanol–water–90% formic acid (4 : 3 : 1 v/v). Ionophoresis was in 0·2M-acetate buffer (pH 5), 0·2M-borate buffer (pH 10), and 0·75M-formate buffer (pH 2). The components were developed with aniline hydrogen phthalate,[17] alkaline silver nitrate,[18] naphtharesorcinol,[19] and Chlorophenol Red.[20]

Glucose solution (0·1%), irradiated for 14 hr. (total dose $3{\cdot}2 \times 10^{19}$ ev ml.$^{-1}$), was examined by chromatography and ionophoresis. Comparison of a number of papers enabled some conclusions to be drawn in spite of rather extensive streaking. Gluconic acid, 2-oxo-D-*arabino*-hexonic acid, arabinose, arabonic acid (trace), and glucosaccharic acid (trace) appeared to be present among several other components. The first three appeared to be the major products. No glucuronic acid was found (see below). 2-Oxo-D-*arabino*-aldohexose was not separated from glucose by the solvents used. Consequently the "glucose" band was cut from a number of chromatograms on Whatman No. 3 paper which had been irrigated with the butan-1-ol–ethanol–water system. The bands were eluted and reapplied to a chromatogram which was eluted with phenol–water (3 : 1 w/w). On development, two spots were present, behaving identically with

[17] Partridge, *Nature*, 1949, **164**, 443.
[18] Trevelyan, Procter, and Harrison, *ibid.*, 1950, **166**, 444.
[19] Forsyth, *ibid.*, 1948, **161**, 239.
[20] Block, Durrum, and Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press, New York, 1955, p. 169.

glucose and 2-oxo-D-*arabino*-aldohexose. On longer irradiation (>24 hr.) no interpretation could be put on the severely streaked chromatograms.

(ii) *Potentiometric titration.* The freeze-dried solids (0·2—1·6 g.) from solutions of glucose (0·1%) irradiated for various periods were dissolved in water (10 ml.) and titrated potentiometrically, under nitrogen, with carbonate-free 0·0217N-sodium hydroxide. Between pH 7·5 and pH 9·2, the immediate pH change on addition of alkali was followed by a slow drift towards neutrality. This is similar to the behaviour of a typical lactone such as 1 : 4-gluconolactone.

Control titrations on equal weights of glucose were made and subtracted from those for the irradiated materials. The results (Tables 1 and 2) are expressed in terms of acidimetric equivalent weights per mole of original sugar at various pH values.

(iii) *Ultraviolet absorption spectra.* The freeze-dried solid from irradiated solutions was dissolved in water (5 ml.) and its ultraviolet absorption spectrum measured. In all cases a maximum occurred in the range 260—270 mμ (Tables 1 and 2).

(iv) *Infrared absorption spectra.* These were measured for the freeze-dried solutes.

(v) *Reducing power.* Irradiated maltose solution (2 ml.), after various doses, was treated by Shaffer and Hartmann's method [21] to determine the reducing power. The determinations were unaltered by prior adjustment of the irradiated solution to pH 7 with alkali and addition of catalase to destroy any hydrogen peroxide (Table 2).

(vi) *Glucuronic acid.* Irradiated glucose solutions (100 ml.) were freeze-dried and treated with iodine solution [4 ml., containing iodine (8·47 g./l.) and potassium iodide (16·7 g./l.)] and N-sodium hydroxide (0·4 ml.). The solution was left at room temperature for 15 min., then neutralised with excess of solid carbon dioxide. The solution was then subjected to paper ionophoresis in 0·2M-acetate buffer (pH 5) and developed with alkaline silver nitrate.[18] Glucosaccharic acid was not formed, and no immobile components (*e.g.*, polyols) remained; components were detectable only in the hexonic acid region.

(vii) *Polyols.*[5] Amberlite IRA-400 resin (5 ml.) was washed with N-sodium hydroxide saturated with barium hydroxide (25 ml.) and then with carbon dioxide-free water (250 ml.). The freeze-dried solute from irradiated glucose (27 mg.) was dissolved in water (10 ml.) and applied to the column which was eluted with water (50 ml.). The eluate was freeze-dried to yield a trace of residue which showed no trace of polyols on chromatography and development with alkaline silver nitrate.

Characterisation of Products.—(i) 2-*Oxo*-D-arabino-*aldohexose.* The freeze-dried solute from irradiated glucose solution (1 g.) was dissolved in water (250 ml.), and the solution filtered and cooled to 15°. A filtered solution of 2 : 4-dinitrophenylhydrazine [250 ml., containing 5 g. in 2N-hydrochloric acid (300 ml.)] was warmed to 50° and added with rapid stirring. The solution was kept at 35° and filtered after 15 min. The product (58 mg.), recrystallised twice from aqueous ethanol, had m. p. 251° unaltered on admixture with authentic glucose 2 : 4-dinitrophenylosazone. 2-Oxo-D-*arabino*-aldohexose yielded an identical product on similar treatment, but glucose gave no precipitate at 35° in 2 hr., although it rapidly gave the same product at 100°.

(ii) *Gluconic acid.* The freeze-dried solute from irradiated solution of glucose (2 g.) was stirred for 2 hr. with Deacidite FF microbead resin (200—400 mesh; 2% nominal cross-linking; the carbonate form) (40 ml.).[6] The resin was then packed into a column and eluted with water (250 ml.) and N-ammonium carbonate (250 ml.). The ammonium carbonate eluate was treated with excess of Zeo-Karb 225 (H^+ form) and passed immediately into a stirred suspension of calcium carbonate. The mixture was stirred for 72 hr., boiled, and filtered (charcoal). The solution was concentrated, and the crude calcium salt precipitated by ethanol was recrystallised from water at 0° (yield 0·121 g.).[22] The product had $[\alpha]_D^{20}$ 8·5° (*c* 1·9 in water) and showed only one component corresponding to gluconic acid on chromatography and ionophoresis.

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[21] Shaffer and Hartmann, *J. Biol. Chem.*, 1921, **45**, 349, 365.
[22] Hudson and Isbell, *J. Amer. Chem. Soc.*, 1929, **51**, 2225.