311. *Effects of γ-Radiation. Part VIII.*[1] *Irradiation of D-Glucal in Aqueous Solution.*

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The effect of ^{60}Co γ-radiation on dilute aqueous solutions of D-glucal has been studied. Hydroxylation of the unsaturated linkage to give D-glucose and D-mannose occurred on irradiation both *in vacuo* and in oxygen. Scission of the double bond to give D-arabinose, however, occurred to any appreciable extent only in the presence of oxygen. Evidence for these and other products is presented and a degradative mechanism discussed.

Evidence obtained during previous studies on the irradiation products of cyclohexene [1] and 2,3-dihydro-4*H*-pyran [1,2] indicated that hydroxylation and scission of the unsaturated linkage would be the predominant reactions on irradiation of D-glucal. The stereospecificity of the hydroxylation and the ratio of hydroxylation to scission could readily be determined in this case as reference compounds are available.

D-Glucal was irradiated in dilute aqueous solution in the presence of barium carbonate to prevent hydrolysis to 2-deoxy-D-glucose under the acidic conditions caused by the irradiation. After irradiation either *in vacuo* or in the presence of oxygen the solutions showed identical absorption peaks at 265 mμ.

Table 1.

Concentration ratios of ^{14}C-irradiation products and *G* values from paper chromatography.

	Concentration ratio by D-[^{14}C]glucose		*G* Value by paper chromatography	
	Oxygen	*In vacuo*	Oxygen	*In vacuo*
D-Glucose	1·0	1·0	1·1	0·5
D-Mannose	0·72	0·85	0·8	0·45
D-Arabinose	2·6	0·5	3·2	0·1
1-Deoxy-D-glucose	0·105	0·84	—	—
2-Deoxy-D-glucose	0·48	1·5	—	—
Arabonolactone	0·31	—	—	—

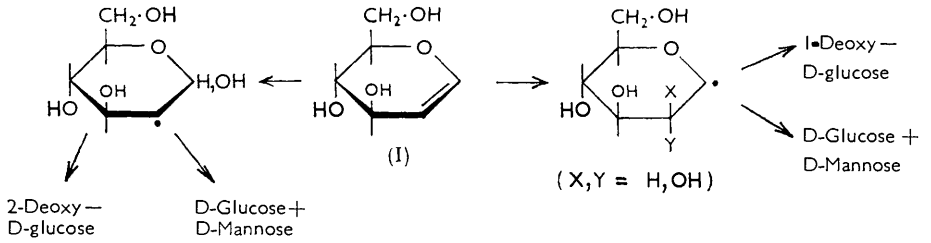
Most of the components of the irradiated solution were identified by paper chromatography and reaction with specific sprays. The predominant effect in irradiation of the D-glucal in oxygen was scission of the double bond to give D-arabinose. Very little scission occurred *in vacuo* but hydroxylation to D-glucose and D-mannose took place under both conditions. These three aldoses were determined quantitatively by paper chromatography (see Table 1). The concentration ratios of these and other components were also determined (see Table 1) by paper chromatography of the products from D-[^{14}C]glucal and assay in a liquid scintillator, the results being consistent with those of the above method. Less value was placed on the *G* values than on the ratios calculated by the latter technique, owing to the inadequacies of the method.

Electron spin resonance spectra of the irradiated crystalline D-glucal have previously indicated [3] that radicals associated with C-1 or C-2 are formed. In aqueous solution similar radicals could arise on hydrogen abstraction by the radicals formed directly from the solvent. Subsequent reaction of these reactive sites (C-1 and C-2) with hydrogen atoms would result in the formation of 1- and 2-deoxy-D-glucose, respectively. Alternatively reaction with the hydroxyl radicals could give D-glucose and D-mannose.

[1] Part VII, preceding paper.
[2] Bailey, Barker, Moore, and Stacey, *J.*, 1961, 4086.
[3] Bailey, Barker, Brimacombe, Pooley, and Spence, *Nature*, 1961, **190**, 259.

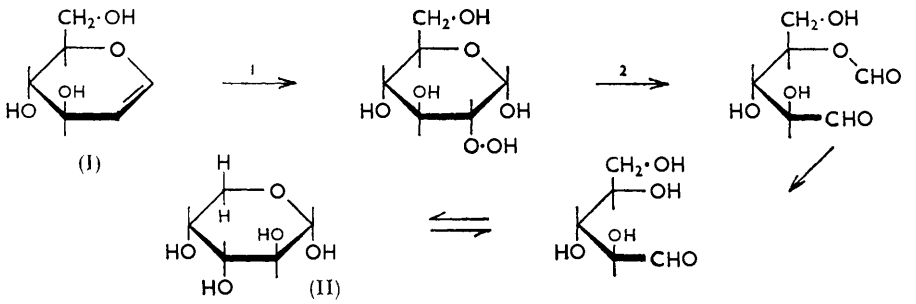
In the presence of oxygen the hydrogen atoms are removed by the reaction $H\cdot + O_2 \longrightarrow HO_2\cdot$, thus reducing the yield of 1- and 2-deoxy-D-glucose.

The virtual non-formation of D-arabinose (II) on irradiation *in vacuo* suggests that bond



scission of the D-glucal (I) occurs by formation of a hydroxy-hydroperoxide,[4] this mechanism being favoured by the production of the $HO_2\cdot$ radical.

The high *G* value for D-arabinose cannot be explained, but suggests that a mechanism additional to that proposed may also operate, possibly involving hydrogen peroxide.



1, (*a*) HO·, (*b*) $HO_2\cdot$–O_2. 2, $-H_2O$.

Oxidation of the primary irradiation product D-arabinose to D-arabonolactone probably proceeds by abstraction of the hydrogen atom from C-1 and fission of the ether linkage, as postulated previously[2] to account for the formation of δ-valerolactone and tetrahydropyran-2-ol from tetrahydropyran.

Experimental

Synthesis of D-*Glucal and* D-[^{14}C]*Glucal.*—D-Glucose (50 g.) was converted[5] into 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide, m. p. 84—86° (76 g., 92%), and the latter was dehydrobrominated[6] with zinc dust (150 g.) and 50% acetic acid (750 ml.) to yield 3,4,6-tri-*O*-acetyl-D-glucal, m. p. 50—52° (31 g., 44%). Deacetylation of part (15 g.) of the triacetate by the Zemplén reaction[7] with sodium (50 mg.) in methanol (300 ml.) afforded crude D-glucal (7·5 g.). After removal of a trace of glucose by fractionation on a cellulose column (40 × 8 cm.) and elution with butan-1-ol–ethanol–water (4 : 1 : 5), the syrupy D-glucal obtained crystallized (m. p. 55—58°). Generally labelled D-[^{14}C] glucose (6 mg.; 0·2 mc) was diluted with inactive D-glucose (20 g.) and converted by the three-stage synthesis above into generally labelled D-[^{14}C]glucal (4·9 g.).

Irradiation of D-*Glucal and* D-[^{14}C]*Glucal.*—D-Glucal (~4 g.) was dissolved in deionized distilled water (4 l.), which had been degassed and then saturated with oxygen, and was irradiated by a 200-c ^{60}Co source for 24 hr. (0·77 Mrad) in the presence of washed barium carbonate (5 g.) and a stream of oxygen. After freeze-drying of the filtered solution, the products were extracted with aqueous ethanol and evaporated under reduced pressure to a brown syrup (3·751 g. from 4·160 g.; 90%). A similar irradiation (dose 0·77 Mrad) was carried out in the absence of oxygen, by degassing the 0·1% aqueous solution of D-glucal under a high vacuum.

[4] Daniels, Scholes, and Weiss, *J.*, 1956, 832.
[5] Barczai-Martos and Korosy, *Nature*, 1950, **165**, 369.
[6] Fischer, *Ber.*, 1914, **47**, 196.
[7] Zemplén, *Adv. Carbohydrate Chem.*, 1957, **12**, 172.

The products were recovered as a light brown oil (3·684 g. from 3·878 g.; 95%). D-[^{14}C]Glucal was irradiated under similar conditions; 1·836 g. yielded 1·543 g. (84%) *in vacuo*, and 1·745 g. yielded 1·2734 g. (73%) in oxygen (doses as before).

Chromatography of Irradiation Products.—Most of the components detectable after separation on paper chromatograms irrigated with butan-1-ol–ethanol–water (4 : 1 : 5) and sprayed with alkaline silver nitrate [8] were identified by means of reference compounds (see Table 2). Confirmation was by use of selective sprays and the solvent system methyl ethyl ketone–acetic acid–saturated aqueous boric acid (9 : 1 : 1) which afforded a good separation of D-glucose, D-mannose, and D-arabinose. However, in this solvent the arabonolactone tended to overlap with the D-glucal. The same products were obtained from ^{14}C-labelled and inactive D-glucal.

Glucono-1,4-lactone could not be detected. Glucosone was also absent as shown by (1) chromatography [13] of the glucose fraction in phenol–water (3 : 1 w/w) and (2) analysis of the products with butanol as solvent after removal of the glucose with glucose-oxidase.

Quantitative Determination of D-*Glucose,* D-*Mannose, and* D-*Arabinose.*—A known amount of the inactive irradiation mixture (60 mg. from the irradiation in oxygen; 230 mg. from that *in vacuo*) was dissolved in distilled water (5 ml.). 15 μl. of each solution were applied to a chromatogram by an Agla syringe. After separation in the solvent described in the preceding paragraph, for 18 hr., the chromatogram was dipped in aniline hydrogen phthalate solution and dried at 105° for 15 min. Components corresponding to D-glucose, D-mannose, and D-arabinose were cut out, and the papers were placed in test-tubes containing (4 ml.) 0·7N-hydrochloric acid and 80% v/v ethanol and shaken intermittently for an hour, then filtered into 1 cm. cells

TABLE 2.

Identification of irradiation products.

Component	$R_{glucose}$	Oxygen	*in vacuo*	Reagent *
—	0·1	+	+	1
—	0·4	+	+	1
D-Glucose	1·0	++	++	1—3
D-Mannose	1·3	+	+	1—3
D-Arabinose	1·4	+++	+	1—3
1-Deoxy-D-glucose	1·7	—	+	1
2-Deoxy-D-glucose	2·1	+	++	1, 4
D-Glucuronolactone	2·3	trace	—	1, 5
D-Arabonolactone	2·6	+	—	1, 5
D-Glucal	3·6	+++	+++	1, 3

* (1) Alkaline silver nitrate.[8] (2) Aniline hydrogen phthalate.[9] (3) Periodate–benzidine.[10] (4) Periodate–nitroprusside.[11] (5) Hydroxylamine–ferric chloride.[12]

TABLE 3.

Quantitative determination of irradiation products.

Sugar analysed	Amount of sugar on chromatogram (γ) on irradn. in O_2	Amount of sugar on chromatogram (γ) on irradn. *in vacuo*	Wt. (g.) of sugar after irradn. in O_2	Wt. (g.) of sugar after irradn. *in vacuo*
D-Glucose	32·3	72·0	0·60	0·28
D-Mannose	23·5	64·2	0·44	0·25
D-Arabinose	94·1	14·1	1·7	0·05

of a Beckman spectrophotometer; the colours were determined at 390 mμ. The amount of sugar was determined by reference to a calibration curve of known amounts of each reference sugar after separation on the chromatogram.[14] From the concentrations of the sugars on the chromatogram (Table 3), the total weight of the irradiation products, and the dose, the *G* values were calculated (Table 1).

[8] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.
[9] Partridge, *Nature*, 1949, **164**, 443.
[10] Cifonelli and Smith, *Analyt. Chem.*, 1952, **26**, 1132.
[11] Edwards and Waldron, *J.*, 1952, 3631.
[12] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1957, **73**, 5859.
[13] Grant and Ward, *J.*, 1959, 2871.
[14] Wilson, *Analyt. Chem.*, 1959, **31**, 1199.

Concentration Ratios of Radioactive Products.—After separation of the irradiated mixture with ethyl methyl ketone as solvent, as above, appropriate circles, each 3 cm. in diameter, were cut out of the paper, the components having been located by development with alkaline silver nitrate of a strip run alongside. Development of the strip before counting tended to reduce the radioactivity. The activity of each component was determined in an Ekco type scintillation counter with a 3% solution of 2,5-diphenyloxazole in toluene as scintillator liquid and a check was made, by spraying, that the component had been correctly located. At least 10,000 counts were made in each case to ensure a statistical error of $<1\%$, and the background count was determined under the same conditions. The counting rates of the irradiation products gave the concentration ratios of the various components (Table 1). Attempts were made to determine the G values by placing a known amount of the active irradiation product on the chromatogram. The results, however, were not completely reproducible and more reliance was placed on the ratios of the counts for each component on a single chromatogram, and these are seen to be in good agreement with the results of paper chromatography.

The authors are grateful to Dr. J. Bevington for providing scintillator facilities, and to the U.K.A.E.A. Harwell for finance and for a maintenance grant to A. J. B.

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[*Received, March 9th*, 1962.]