Carbohydrate Research, 39 (1975) 263–269

γ-RADIOLYSIS OF D-GLUCOSE IN AERATED, AQUEOUS SOLUTION

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(Received April 16th, 1974; accepted for publication in revised form, August 29th, 1974)

ABSTRACT

γ-Radiolysis of D-glucose in aerated, aqueous solution gives mainly D-glucono-1,5-lactone, D-*arabino*-hexosulose, and D-*ribo*-hexos-3-ulose, together with D-*xylo*-hexos-4-ulose, D-*xylo*-hexos-5-ulose, and other pentose, tetrose, and triose derivatives as minor products, which were estimated by mass spectrometry of their alditol-*d* acetates. These hexose derivatives appear to be produced by the decomposition of D-glucose peroxy-radicals which are formed by the reaction of the primary radicals of D-glucose with oxygen. Bond scission of the peroxy-radicals yields triose, tetrose, and pentose. A radiolysis mechanism for the degradation of D-glucose in aerated, aqueous solution is proposed, based on the reaction of several kinds of D-glucose radical with oxygen.

INTRODUCTION

Irradiated sugar solutions are known to have bactericidal and bacteriostatic effects on several micro-organisms[1,2], and also to enhance the browning reaction of sugars with amino acids[3]. These properties are considerably influenced by the nature of the sugar and the irradiation conditions, especially by the presence or absence of oxygen. Radiolysis of D-glucose and D-fructose in oxygen-free conditions gives deoxyhexosuloses and deoxyhexodiuloses, respectively[4,5], and a small proportion of lower aldoses resulting from bond scission of the hexose molecule[5]. Radiolysis of D-glucose under oxygenated conditions has been reported[6] to yield glyoxal, D-glucuronic acid, D-arabinose, D-gluconic acid, and dihydroxyacetone as major products, and D-erythrose, D-xylose, saccharinic acid, and formaldehyde as minor products. However, except for formaldehyde, these products did not exhibit bactericidal and bacteriostatic effects[1] or enhance the browning reaction[3].

In order to clarify the differences in the radiolysis mechanism of sugars in oxygenated and deoxygenated aqueous solution, we have reinvestigated the radiolytic products from D-glucose under aerated conditions and now report on the formation of hexosuloses by radiation-induced oxidation of D-glucose.

EXPERIMENTAL

Materials and irradiation. — D-Glucose and other reagents used were all Guaranteed Grade reagents. D-Glucose solutions (10–20mM) were prepared with triply-distilled water and irradiated with a ^{60}Co γ-ray source (4 kCi) to a dose of 2.0 Mrad at a dose rate of 8.7×10^4 rad per hour under aeration.

Preparation of alditol acetates from radiolytic products. — The irradiated solution was treated with excess sodium borohydride below 50° until reduction was complete. Amberlite IR-120(H^+) resin was slowly added to the mixture until the evolution of hydrogen gas ceased. The filtered solution was concentrated to dryness under reduced pressure, and boric acid was removed from the residue by repeated distillation of methanol therefrom. The residue was acetylated by treatment with acetic anhydride containing sulphuric acid (2.5%) at 60° for 10 min. In the preparation of deuterium-substituted alditol acetates, the freeze-dried matter from the irradiated D-glucose solution was dissolved in D_2O, treated with $NaBD_4$, and then acetylated as described above.

G.l.c. of the alditol acetates. — G.l.c. was performed with a Hitachi Gas Chromatograph model K 53 with a stainless steel column (0.3×200 cm) and 8% silicone DC QF-1 on Chromosorb W (AW). Column temp., 200°; injection temp., 280°; N_2 gas, 20 ml/min. Preparative g.l.c. was carried out with a column (0.4×200 cm) at 230° and an injection temp. of 300°. Each alditol acetate collected from preparative g.l.c. was subjected to mass spectrometry, using an Hitachi RMS-4 spectrometer with direct insertion.

RESULTS

G.l.c. (Fig. 1) of the alditol acetates derived from the radiolytic products indicated the formation of many products in the irradiated D-glucose solution. Nine alditol acetates (P1, P3, P6, and P8 ~ P13) were identified by comparison of their relative retention times with those of the authentic samples. The identifications were supported by mass-spectral data; the mass spectra of alditol acetates, generally, do not contain molecular ion peaks, but fragmentation ions can be used to identify the structure[7].

Both Peak 3 (P3) and Peak 4 (P4) exhibited the mass spectrum of a tetritol tetra-acetate, but the relative retention time of P3 agreed with that of erythritol tetra-acetate and so P4 was inductively identified as threitol tetra-acetate. Peaks P9–P13 showed mass-spectral fragmentation characteristic of hexitol hexa-acetates (*A*, Scheme 1), and their retention times on g.l.c. indicated that they were the hexa-acetates of allitol, mannitol, galactitol, glucitol, and iditol, respectively. Peaks P5 and P8 were methyl pentonate and methyl hexonate, respectively, since their mass spectra contained fragment peaks (*m/e* 317, 275, 203 in P5, and *m/e* 389, 347, 275, 203 in P8) characteristic of methyl aldonate acetate. However, these methyl esters were artifacts arising during the methanol treatment (see Experimental). P5 was not identified, but

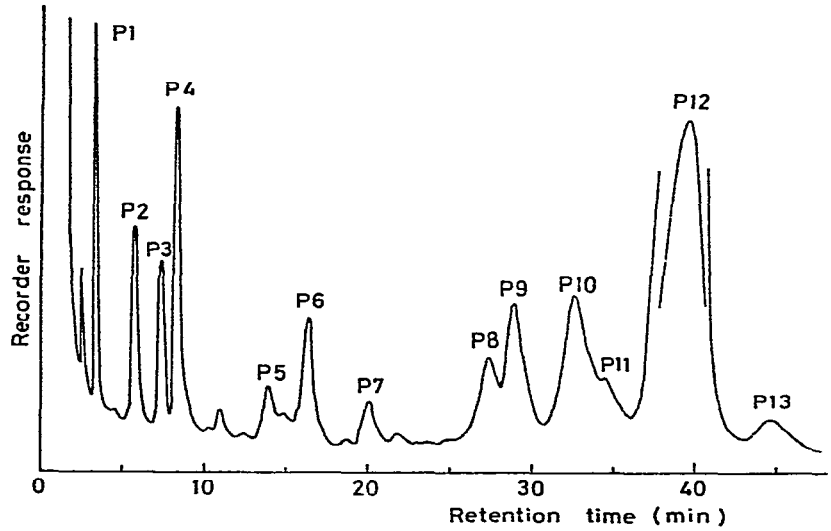
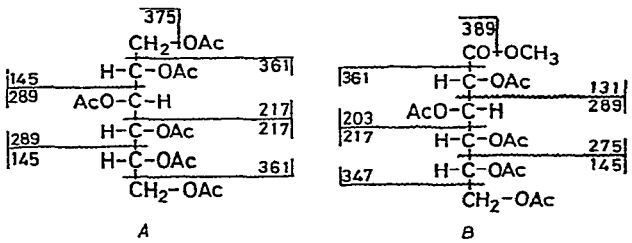


Fig. 1. G.l.c. of the acetylated alditols prepared from 20mM D-glucose irradiated under aerated conditions: 8% DC QF-1 on Chromosorb W, stainless steel column (0.3 × 200 cm) at 200°, injection temp. 280° N_2 carrier gas (20 ml/min.), flame-ionization detector. Assignment of peaks: P1 glycerol (relative retention time, 0.09), P2 unidentified (0.15), P3 erythritol (0.19), P4 threitol (0.21), P5 methyl pentonate (0.35), P6 arabinitol (0.42), P7 unidentified (0.51), P8 methyl gluconate (0.70), P9 allitol (0.74), P10 mannitol (0.84), P11 galactitol (0.87), P12 glucitol (1.00), P13 iditol (1.14).

P8 was identified as methyl D-gluconate penta-acetate by comparison with an authentic sample; the fragmentation pattern (*B*) of P8 is shown in Scheme 1.



Scheme 1.

The alditols listed above would be derived from aldoses and their ulose derivatives produced by the γ-radiolysis of D-glucose, and the hexitols, except glucitol, were not derived from the corresponding hexose, but from hexosuloses. To clarify the structure of these products, the freeze-dried residue of the irradiated solution was reduced with $NaBD_4$ in D_2O, and then acetylated and fractionated by preparative g.l.c. The structure of each alditol-*d* hexa-acetate could be deduced from the fragmentation pattern by comparison with that of the undeuterated compound.

For glycerol-*d* triacetate (P1), the ratio of the peak strengths of *m/e* 145

$(CH_2OAc \cdot CHOAc)^{+\cdot}$, 146 $(CHDOAc \cdot CHOAc)^{+\cdot}$, and 147 $(CHDOAc \cdot CDOAc)^{+\cdot}$ suggested it to be a mixture of glycerol-*1-d*, *-2-d*, and *-1,2-d_2*, and this conclusion is supported by the relative abundances of *m/e* 103, 104, and 105 which are formed by the elimination of ketene from the fragments *m/e* 145, 146, and 147, respectively. These glycerol-*d* compounds are considered to be derived from D-glyceraldehyde, dihydroxyacetone, and triosulose, respectively.

Erythritol-*d* tetra-acetate (P3) was not a single substance because of the groups of fragment ions at *m/e* 145, 146, 147, and 217 $(CH_2OAc \cdot CHOAc \cdot CHOAc)^{+\cdot}$, 218 $(CHDOAc \cdot CHOAc \cdot CHOAc)^{+\cdot}$, 219 $(CHDOAc \cdot CDOAc \cdot CHOAc)^{+\cdot}$, which suggest the presence, at least, of the tetra-acetates of erythritol-*1-d* and *-1,2-d_2* derived from D-erythrose and D-*glycero*-tetrosulose, respectively. P4 was assumed to be threitol-*1,4-d_2* from the peaks *m/e* 146 (base peak) and 218, and the compound must therefore be derived from L-*threo*-tetrodialdose.

The mass spectrum of P6 was complex; the triplicity of the fragment ions, *m/e* 145 (146, 147), 187 (188, 189), 217 (218, 219), and 289 (290, 291) indicated the presence of arabinitol-*1,2-d_2* in addition to arabinitol-*1-d*. The former compound was derived from D-*erythro*-pentosulose and the latter from D-arabinose.

P8, the peak of methyl gluconate penta-acetate was not homogeneous since there were peaks at *m/e* 390, 362, and 348 in addition to those for methyl gluconate penta-acetate (*m/e* 389, 361, and 347), indicative of a deuterated compound the structure of which was not determined.

P9 was shown to be allitol-*d_2* hexa-acetate from the peaks at *m/e* 377 $(M^+ - OAc)$, 363 $(M^+ - CH_2OAc)$, and 362 $(M^+ - CHDOAc)$. From the fragment-ion pairs *m/e* 145/146, 217/219, and 290/291, P9 was identified as allitol-*1,3-d_2*. G.l.c. indicated P10 to be mannitol hexa-acetate. The fragment ions, *m/e* 377, 363, and 362 indicated the presence of two deuterium atoms, and the fragment-ion pairs *m/e* 145/147, 217/219, and 289/291 were consistent with the structure mannitol-*1,2-d_2*. Allitol-*d_2* and mannitol-*d_2* would be derived from D-*ribo*-hexos-3-ulose and D-*arabino*-hexosulose, respectively.

G.l.c. showed P13 to be iditol hexa-acetate, but the position of the deuterium could not be determined because of the small yield of material after preparative g.l.c. However, the presence of iditol after reduction suggests that D-*xylo*-hexos-5-ulose must be formed in the irradiated D-glucose solution, as under deaerated conditions[5].

Since P2 and P7 did not correspond with any authentic acetylated alditol, they remain to be identified. P5 was assumed to be methyl pentonate tetra-acetate from its mass spectrum because it gave a characteristic peak at *m/e* 275 but not at 347. G.l.c. indicated P11 to be galactitol hexa-acetate.

DISCUSSION

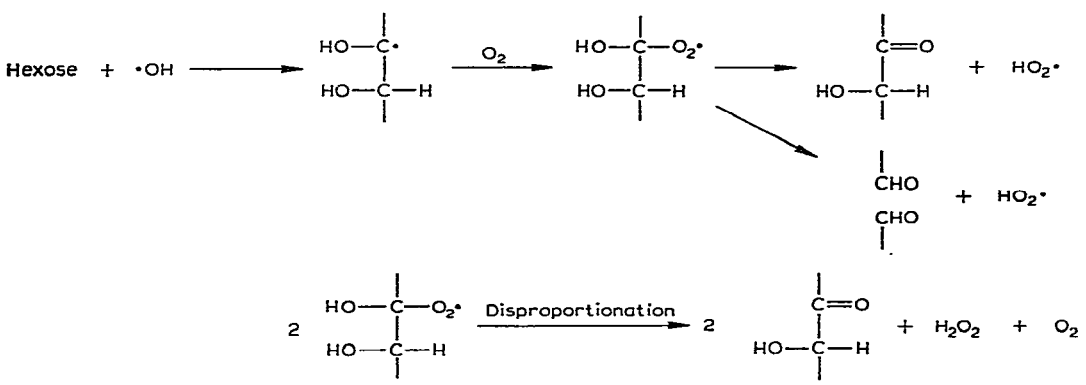
γ-Radiolysis of D-glucose under aerated conditions produces mainly hexose derivatives, and also minor proportions of bond-scission products such as triose, tetrose, and pentose derivatives, as for radiolysis under deoxygenated conditions[5].

However, the amount of the bond-scission products is increased by aeration, which strongly suggests an oxygen effect on the radiation-induced degradation of D-glucose.

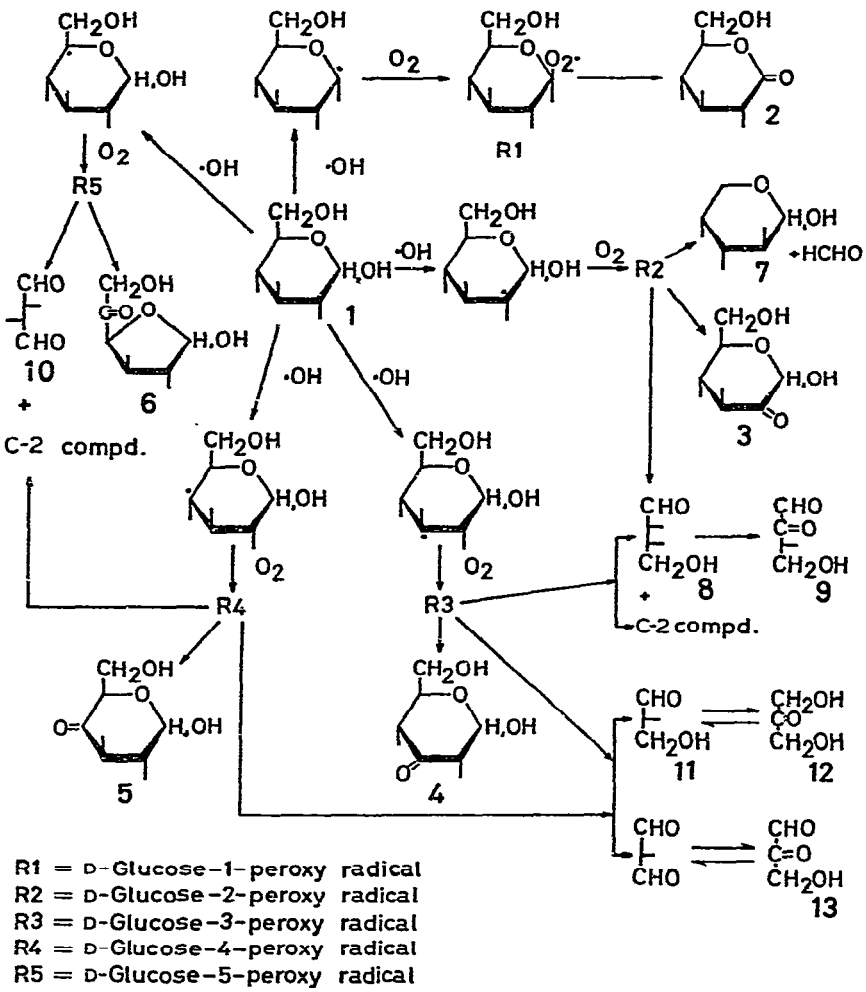
Among the scission products of D-glucose, D-glyceraldehyde (**11**), dihydroxyacetone (**12**), triosulose (**13**), D-erythrose (**8**), and L-*threo*-tetrodialdose (**10**) were identified. The trioses arise by scission of the C-3–C-4 bond in D-glucose, and **8** and **10** by scission of the C-2–C-3 and C-4–C-5 bonds. All of the hexose derivatives in the radiolytic products from D-glucose were oxidation products, that is, D-*arabino*-hexosulose (**3**), D-*ribo*-hexos-3-ulose (**4**), and D-glucono-1,5-lactone (**2**) as main products, and D-*xylo*-hexos-4-ulose (**5**) and D-*xylo*-hexos-5-ulose (**6**) as minor products. The product **3** is a radiolysis product of D-glucose[6], D-fructose[8], and D-mannose[9]. The products **4**[10] and **6**[11] are known compounds, which have been prepared by chemical and enzymic methods. The hexosuloses, **4**, **5**, and **6** have not hitherto been reported as products of the γ-radiolysis of aldohexoses. The main products, 2-deoxy-D-*arabino*-hexono-1,4-lactone[4], and 2-deoxy- and 3-deoxy-hexose derivatives[5], formed on radiolysis of D-glucose under deoxygenated conditions are not formed in the presence of oxygen. These differences arise because, under oxygenated conditions, the primary radical of D-glucose may react with oxygen at a greater rate than that with other radical species or other substances.

Generally, in the radiolysis of a hexose in aqueous solution, non-hydroxyl hydrogen atoms in the hexose molecule are abstracted by hydroxyl radicals, a primary radiolysis product of water, to give hexose radicals[12]. The formation of hexosulose under deoxygenated conditions probably proceeds by the disproportionation of a hexose radical, but a major proportion of the radicals are dehydrated to give deoxyhexosuloses[5,13,14].

Under aerated conditions, hexose radicals rapidly react with oxygen to give peroxy radicals, which decompose to hexosuloses by elimination of the hydroperoxy radical and to lower aldoses by cleavage of the carbon chain. Moreover, the possibility that disproportionation of the peroxy radicals occurs cannot be excluded, as shown by the radiolysis of *myo*-inositol[15]. The overall reaction may therefore be summarized as follows:



Thus, the C-1 radical from D-glucose afforded D-glucono-1,5-lactone (**2**) and D-arabinose (**7**), with participation of oxygen. The C-2 radical gave D-*arabino*-hexosulose (**3**) by decomposition of its peroxy radical, and D-arabinose (**7**) and D-erythrose (**8**) by scission of the C-1–C-2 and C-2–C-3 bonds, respectively. Similarly, D-*ribo*-hexos-3-ulose (**4**) was derived from the C-3 radical, which was also decomposed to D-erythrose (**8**) by scission of the C-2–C-3 bond, and D-glyceraldehyde (**11**)



Scheme 2. The proposed mechanism of radiolysis of D-glucose in the presence of oxygen.

and hydroxymalonaldehyde by scission of the C-3–C-4 bond. These trioses undergo intramolecular rearrangements to give dihydroxyacetone **12**) from **11**, and triosulose (**13**) from hydroxymalonaldehyde. The minor products, D-*xylo*-hexos-4-ulose (**5**) and -5-ulose (**6**) were derived from the C-4 and C-5 radicals, respectively, of D-glucose,

and these radicals were also decomposed to L-*threo*-tetrodialdose (**10**). The proposed mechanisms of radiolysis are represented in Scheme 2.

The C-6 radical from D-glucose decomposed to D-*gluco*-hexodialdose, but the glucitol-*1,6-d*$_2$ hexa-acetate derived therefrom was substantially contaminated with glucitol-*1-d* hexa-acetate derived from unreacted D-glucose, and could not be determined by mass spectrometry.

The amounts of **3** and **4** produced were greater than those of **5** and **6**, as deduced from the peak areas in g.l.c., which suggests that hydrogen abstraction from D-glucose by the OH radical occurred more readily at H-2 and H-3 than at H-4 and H-5. D-Glucono-1,5-lactone (**2**) produced by the abstraction of H-1 in D-glucose gave two products in the work-up procedure, namely, glucitol by reduction and methyl D-gluconate by esterification of the free acid. It appeared that H-1 in D-glucose is as easily abstracted as H-2 and H-3. A parallel is provided with deoxygenated conditions, abstraction of H-1 in D-glucose gave 2-deoxy-D-*arabino*-hexono-1,4-lactone as the main product[4].

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