

Degradation Products of γ -Irradiated Solid Carbohydrates Obtained after Dissolution in Water

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It is shown by paper chromatographic analysis that a number of substances are formed when γ -irradiated solid carbohydrates are dissolved in water. CO_2 is formed from α -D-glucose with a G-value of 2.5, while D-fructose and sucrose gave no CO_2 . The production of acidic groups of different carbohydrates showed a wide range of G-values, e.g. 1.0 for sucrose and 48 for lactose $\cdot \text{H}_2\text{O}$.

We have earlier studied radiation damage of solid carbohydrates by means of electron spin resonance^{1,2}, and the reactions occurring after the irradiated material is dissolved in water^{1,3,4}. Alongside with this work, efforts have been made to separate and identify the degradation products. Apart from the radiation chemistry viewpoint, it seemed to be of interest to isolate and identify these degradation products as some of them might have reactive properties causing indirect chemical and biological effects^{3,5-7}. The present communication will give preliminary results of this work, mainly on α -D-glucose.

The radiation damage of carbohydrates in aqueous solution has been extensively studied⁸⁻¹³. Only a few data have been reported on degradation products obtained after dissolution of irradiated solid carbohydrates. Wolf from *et al.*^{12,13}, using paper chromatography, found that sucrose gives glucose and fructose and that methyl- α -D-glucoside gives glucose. Collins¹⁴ also using paper chromatographic analysis states that only glucose is present in the endproduct after irradiation of glucose. Grant and Ward¹¹ refer to an unpublished work by Bourne, Hudson and Weigel, stating that auto-radiolysis of ¹⁴C-labelled glucose gives a mixture similar to that obtained in dilute aqueous solution *in vacuo*. According to the work of Grant and Ward these products are gluconic acid, 2-oxo-D-arabino-aldohexose, 2-oxo-D-arabino-hexonic acid, arabinose, arabonic acid and glucosaccharic acid.

EXPERIMENTAL

Irradiation. Solid carbohydrate samples were irradiated at room temperature and in air in small plastic boxes with ^{60}Co γ -rays. The dose rate was 4×10^5 rad/h in these samples.

Dissolution. The irradiated samples were dissolved in distilled water in equilibrium with air. The final concentration was 5–10 %.

Chromatography. The solutions were applied to Whatman No. 1 paper, the amount of solid substance was 3000–6000 μg per spot. The chromatograms were developed by the ascending technique, using several solvent systems known to separate carbohydrates and similar substances¹⁵. In order to obtain better separation from some spots, the chromatograms were mostly developed twice and they were dried between the developments. After spraying with different reagents, spots were detected in visible- and UV-light.

Potentiometric titrations. The solutions were titrated with carbonate-free 0.04 or 0.004 N NaOH and the pH was measured by a Radiometer pH meter model PHM 22.

CO₂-analysis. A weighed solid sample was dissolved in carbonate-free water in a vessel, which had been washed with CO₂-free nitrogen. The evolved CO₂ was taken up in 20 ml 0.005 N NaOH containing 5 % BaCl₂. The residual base was titrated with 0.01 N HCl in nitrogen atmosphere with a mixed indicator of thymol blue and cresol red. The errors were of the order of ± 20 %.

RESULTS

Carbon dioxide. 17 determinations in the dose interval 5–36 Mrad showed that CO₂-formation in α -D-glucose is nearly linearly dependent on the dose. The G-value was found to be 2.5 (40 eV/CO₂). Irradiation in closed vessels showed that no or negligible amounts of CO₂ were able to diffuse out from the crystals during a couple of days. It is noteworthy that neither D-fructose nor sucrose gave any CO₂ (dose 18 Mrad). These findings are in agreement with our IR-investigations of irradiated carbohydrates: α -D-glucose, D-fructose and sucrose give peaks at 2340 cm^{-1} with relative intensities of 50:3:4 (dose 45 Mrad).

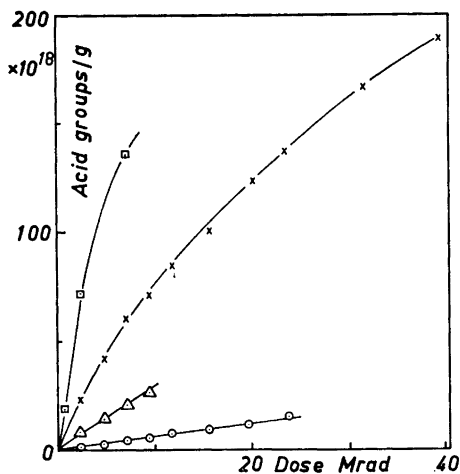


Fig. 1. Produced acid groups vs. dose for sucrose O, maltose-monohydrate Δ , α -D-glucose \times , and lactose-monohydrate \square .

Table 1. G-values for acid groups formed in some carbohydrates after irradiation with 2.37 Mrad in the polycrystalline state.

| Substance | G-value |
|---------------------|---------|
| Sucrose | 1.0 |
| D-Fructose | 1.0 |
| Maltose-monohydrate | 5.0 |
| α -D-Glucose | 15.2 |
| Lactose-monohydrate | 48 |

In addition, CO₂-analysis in α -D-glucose was made by means of the Warburg manometric technique, where the evolved CO₂ was taken up by KOH-solutions and the change of pressure was measured. These analyses gave figures in accordance with the above mentioned method. However, the measurements were disturbed by pressure changes due to changes of volume when dissolving unirradiated glucose, for which corrections had to be made. Using this technique we detected the formation from irradiated α -D-glucose of another gas (or gases), not absorbed by alkali. The amount of this gas was about equal to the volume of CO₂.

Acid groups. The yield of acidic groups in some carbohydrates against dose is given in Fig. 1 and G-values at 2.37 Mrad are given in Table 1. The amount of acidic groups is not changed when the solution of the irradiated α -D-glucose is kept in air for one or two days at room temperature. It is however known from UV-measurements that changes occur in the solution¹.

Chromatography. Paper chromatography on irradiated α -D-glucose has shown that at least seven different spots are obtained. A normally developed and sprayed chromatogram is shown in Fig. 2. We have found that the solvent butan-1-ol:acetic acid:water (4:1:5 v/v) gives the best separation. For two-

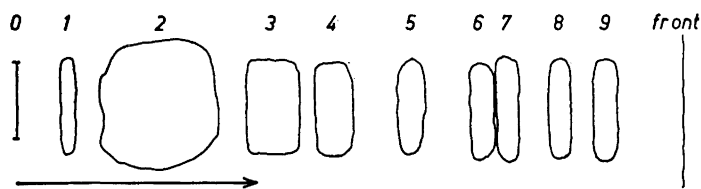


Fig. 2. Schematic picture of a chromatogram obtained from 45 Mrad irradiated α -D-glucose. 6 mg was applied at 0, developed twice with butan-1-ol:acetic acid:water (4:1:5 v/v) and sprayed with aniline-phthalic acid. In addition to the spots there is a streaking from the glucose spot to spot 7, appearing as a weak brown background. 1: Brown, weak; is also found in unirradiated glucose. 2: Brown; identical with glucose; might also contain degradation products. 3: Brownish yellow in UV-light. 4: Brownish red; red in UV-light; coincides with glucuronolacton ($\sim 10 \mu\text{g}$). 5: Blue in UV-light, very weak. 6: Pink in UV-light. 7: Blue in UV-light, weak. 8: Pink in UV-light, weak. 9: Brownish red; pink in UV-light.

dimensional chromatograms, however, other suitable solvents are used. The same spots, as shown in Fig. 2, are obtained from α -D-glucose, irradiated with 26 and 108 Mrad. Parallel experiments with D-fructose and sucrose have shown that separation of degradation products also in these cases is possible by paper chromatography. It is noteworthy that D-fructose in addition to five spots with $R_F > 0.5$, gives a strong spot at $R_F = 0.3$ (butan-1-ol:acetic acid:water, 4:1:5), which is brownish yellow after spraying with aniline-phthalic acid. After irradiation with 25 Mrad this spot is of about the same size and gives a colour reaction of about equal intensity as the intact D-fructose.

DISCUSSION

Our data show that many different substances can be detected after dissolution in water of irradiated solid carbohydrates. Except for carbon dioxide, none of the substances have so far been identified and quantitatively estimated. However, the results indicate that although the yields mostly are small, identification and quantitative estimation will be possible, e.g. with ^{14}C -labelled compounds.

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