481. *Radiation Chemistry of Carbohydrates. Part XV.** *Mechanism of Self-decomposition of* [^{14}C]D-*Glucose*

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Radioisotopic methods have been used to measure the rate of self-decomposition of [^{14}C]D-glucose under standardised conditions of geometry, so that the fraction of the emitted energy absorbed by the solid can be calculated. From the results initial $-G$ for the self-decomposition is 26—29. Moisture increased the decomposition rate. The high $-G$ values are similar to those encountered during γ-irradiation of polycrystalline anhydrous α-D-glucose. Under normal freeze-dried conditions, direct action effects are considered to be responsible for the major portions of the self-decomposition.

Previous studies indicated that the self-decomposition of ^{14}C-labelled sugars can be attributed, in the main, to reactive species produced by radiolysis of non-bonded water retained by the samples after freeze-drying.[1,2] However, it has been pointed out that a serious objection to this conclusion arises from the fact that high destructive coefficients ($-G$ values), in the range 40—100, have been reported for self-decomposition, while only *ca.* 4 radical pairs per 100 ev are formed during the radiolysis of water.[2] However, there is uncertainty in all the reported values of $-G$ for self-decomposition about the fraction

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* Part XIV, G. O. Phillips and K. W. Davies, *J.*, 1964, 3981.

[1] Bayly and Weigel, *Nature*, 1960, **188**, 384.

[2] Phillips, Criddle, and Moody, *J.*, 1962, 4216.

of the energy emitted during storage which is actually absorbed by the sample. For this reason there has always been considerable doubt about the dose actually received by the sugar.[2,3] The uncertainty arises from the irreproducible geometry of the stored film or globule of labelled material which results on freeze-drying. A prime consideration in the present study was, therefore, to standardise the geometry of the [^{14}C]D-glucose undergoing self-decomposition, so that more accurate $-G$ values may be obtained. Previously[4,5] it was shown that the state of aggregation profoundly influences the extent of radiation decomposition of α-D-glucose. Such effects have been related to the facility of energy transport in the various aggregates. Comparison with established direct action processes might then yield more information about the mechanism of self-decomposition.

EXPERIMENTAL

Materials and Methods.—[^{14}C]D-Glucose was supplied by the Radiochemical Centre, Amersham. Analytical methods for estimating the extent of self-decomposition have been described.[2]

Self-decomposition of Freeze-dried Aggregate.—[^{14}C]D-glucose (500 μc) of specific activity 5·4 mc/millimole as supplied in the tube from Amersham was stored at room temperature for 48 weeks and amount of decomposition estimated.

Isotope Dilution.—After the sample was dissolved in water (250 ml.), 1·0 ml. was withdrawn, treated with water (9 ml.) and carrier D-glucose (0·555 millimole). Combustion and conversion into barium carbonate established the activity at this stage to be 3·595 μc/millimole. An aliquot portion (5 ml.) was treated with carrier D-glucose (1 millimole), freeze-dried, and rigorously dried *in vacuo* over phosphoric oxide. Anhydrous sodium acetate (0·2 g.) and freshly distilled acetic anhydride (1 ml.) were added, the mixture refluxed for 1½ hr. and the mixture poured on crushed ice. The solid which separated was recrystallised eight times from aqueous ethanol to give pure penta-*O*-acetyl-β-D-glucose with m. p. 131° and constant specific activity 0·7491 μc/millimole, which is equivalent to 96·0% of the original activity being present in the form of D-glucose.

Paper Chromatography.—The remainder of the initial radioactive solution (4·5 ml.) was concentrated by freeze-drying and applied to four two-dimensional chromatographic papers (Whatman No. 54). The papers were irrigated in (i) butan-1-ol–pyridine–water (10 : 3 : 3) and in a second direction with (ii) butan-1-ol–acetic acid–water (4 : 1 : 5). The location of 19 products and unchanged [^{14}C]D-glucose was established by autoradiography and the proportion of activity in each product estimated by direct end-window counting. The results for the four papers are given in Table 1. From their behaviour on the paper chromatogram components 1—10 behave similarly to neutral fragments and components 11—19 are acidic. The

TABLE 1

Organic constituents separated by paper chromatography from the self-decomposition of [^{14}C]D-glucose after 47 weeks

Component	1	2	3	4	5	6	7	8	9
R_G * irrigant (i) ...	streak	0·48	0·71	1·35	1·75	2·20	2·45	2·70	3·00
R_G irrigant (ii) ...	0	0·32	0·57	1·35	2·00	2·30	2·60	2·90	3·30
Counts/5 min. ...	36,109	7278	2979	17,689	4441	7334	1087	95	175

Component	10	11	12	13	14	15	16	17	18
R_G irrigant (i) ...	3·20	0·67	0·91	1·23	1·40	1·60	1·95	2·35	2·50
R_G irrigant (ii) ...	3·70	0·19	0·32	0·57	0·65	0·83	1·10	1·22	1·65
Counts/5 min. ...	7423	11,970	8794	3454	2521	4947	185	140	75

Component	19	D-Glucose
R_G irrigant (i) ...	2·80	1·00
R_G irrigant (ii) ...	1·90	1·00
Counts/5 min. ...	75	3,010,283

* R_G indicates movement of component relative to D-glucose.

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[3] Rochlin, Picatinny Arsenal Technical Report 3078.

[4] Phillips and Baugh, to be published.

[5] Phillips and Baugh, *Nature*, 1963, **198**, 282; *Discuss. Faraday Soc.*, 1963, No. 36, 281.

results indicate a 96·3% purity of the original D-glucose. The pure [^{14}C]D-glucose was eluted from the paper chromatogram with triply distilled water (6 ml.).

Storage of [^{14}C]D-*Glucose under Standardised Conditions.*—The solution (5 ml.) was concentrated to *ca.* 1 ml. by freeze-drying. Glass microscope slides were cut to a size which would fit into a 6 in. × 1 in. ampoule and carefully cleaned by repeated boiling in chromic acid, washed with triply distilled water, and dried. Using an " Agla " microburette we applied the solution containing [^{14}C]D-glucose in volumes having the ratio 3 : 1 : 1 to three sets of slides with 5 slides in each set. In each application 0·01 ml. was deposited on the slide which was cooled over liquid air to ensure initial localisation of the deposit. Water was removed after each application by the passage of a current of warm air over the surface of the slide. Repeated attempts were necessary before deposits with the necessary localised weight and thickness (estimated radiochemically) could be prepared. Finally the deposits were confined to a circular area of diameter *ca.* 0·4 cm. The slides were placed in the glass ampoules, evacuated and dried by repeated exposure to fresh P_2O_5 without the introduction of air. Two sets of 5 slides containing one and three thicknesses of [^{14}C]D-glucose deposit were sealed, while dry, and the remaining group sealed after deliberate exposure to moist air. All the slides were stored at −15°.

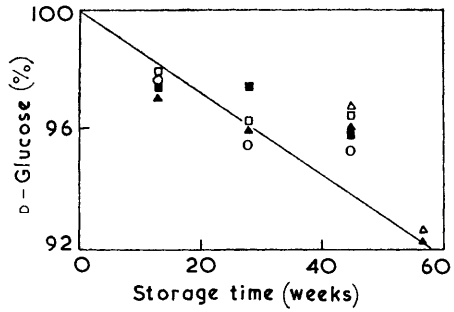

FIGURE 1. Self-decomposition of [^{14}C]D-glucose deposited as three thicknesses on slide and stored dry *in vacuo* at −15°

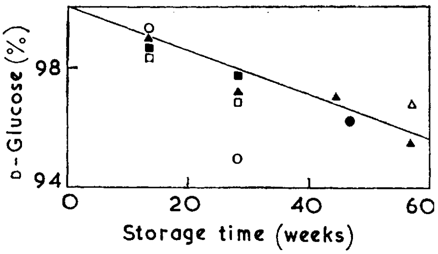

FIGURE 2. Self-decomposition of [^{14}C]D-glucose deposited as one thickness on slide and stored dry *in vacuo* at −15°

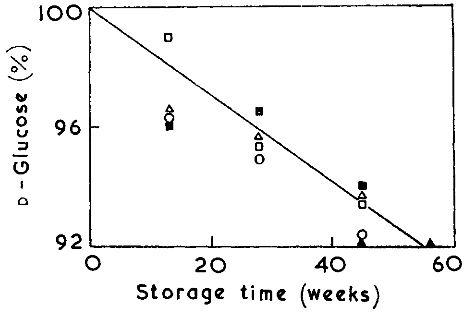

FIGURE 3. Self-decomposition of [^{14}C]D-glucose deposited as one thickness on slide and stored moist *in vacuo* at −15°

Symbols for all Figures as follows:
○ One-dimensional chromatogram; irrigant butan-1-ol–pyridine–water (10 : 3 : 3)
■ ,, ,, ,, butan-1-ol–acetic acid–water (4 : 1 : 5)
□ ,, ,, ,, butan-1-ol–ethanol–water (4 : 1 : 2)
△ Two-dimensional chromatogram; irrigants (*a*) butan-1-ol–pyridine–water (10 : 3 : 3)
(*b*) butan-1-ol–acetic acid–water (4 : 1 : 5)
▲ Isotope dilution analysis
● Self-decomposition in original tube, estimated by isotope dilution analysis

Analysis of Stored Samples.—After 13, 28, 45, and 58 weeks storage samples were analysed for [^{14}C]D-glucose by isotope dilution and paper chromatographic analysis after irrigation in several solvent mixtures. A typical analysis is as follows. The radioactive deposit was dissolved in water (4 ml.) and an aliquot portion (1 ml.) removed, and the proportion of D-glucose present estimated by isotope dilution analysis as described above. The results are given in

Figures 1—3. The remainder (3 ml.) was concentrated by freeze-drying and aliquot parts (0·05 ml.) applied to Whatman No. 1 filter paper strips (2·75 cm. × 50 cm.) and irrigated in (*a*) butan-1-ol–acetic acid–water (4 : 1 : 5); (*b*) butan-1-ol–pyridine–water (10 : 3 : 3); and (*c*) butan-1-ol–ethanol–water (4 : 1 : 1). After drying the distribution of radioactivity along the filter paper strips was determined using the automatic radiochromatogram scanner, and from the resulting histograms the proportion of [^{14}C]D-glucose to total activity determined.

The samples which had been stored for 45 and 57 weeks contained more products than could be separated cleanly by one-dimensional chromatography. The % decomposition was, therefore, estimated by preparing autoradiographs and directly counting the spots using an end-window Geiger counter as described above. Results are summarised in Figures 1—3.

DISCUSSION

Self-decomposition of [^{14}C]D-glucose during 48 weeks' storage led to 4% decomposition and gave 19 products. This greatly exceeds the number formed during the γ-irradiation of D-glucose in aqueous solution for an equivalent amount of D-glucose degraded.[6] A closer similarity is shown with the products from the γ-irradiation of solid α-D-glucose,[7] and points to extensive participation of direct action effects during self-decomposition. The rate of decomposition does not vary significantly for individual sugars on γ-irradiation in aqueous solution.[6] Self-decomposition rates, however, show wide variations. For example, the results given here for [^{14}C]D-glucose stored in the glass ampoule, as supplied from Amersham, correspond to an initial decomposition rate of 0·79%/year/mc/millimole, which may be compared with [^{14}C]D-mannose 0·43% and [^{14}C]D-ribose 1·17%/year/mc/millimole when stored under identical conditions. No conclusion, however, may be drawn about the relative susceptibilities of these sugars to radiation damage since variations in the geometry of the stored samples might lead to different proportions of the emitted energy being absorbed. To standardise the geometry of the self-decomposing [^{14}C]D-glucose in this investigation, the method has been described whereby the samples are deposited on to glass slides, which had been stringently cleaned to prevent decomposition by chemical effects. The thickness of deposit was varied three-fold to examine the effect of changing the proportion of energy absorbed on amount of decomposition. The initial rates of dedecomposition, calculated from measurements after 13, 28, 45, and 58 weeks (Figures 1—3) are in Table 2. Reasonable agreement was obtained for individual analyses, having regard to the small percentage decompositions estimated.

Moisture accelerated the decomposition. When three thicknesses of [^{14}C]D-glucose (each 0·069 mg.) had been applied, the decomposition was appreciably greater than for a single deposit, which clearly demonstrates the effect of absorbing a greater proportion of emitted β-radiation.

TABLE 2

Self-decomposition of [^{14}C]D-glucose stored on glass slides at −15°

Sample	Wt. (mg.)	Radioactivity (μc)	Decompn. (%)	Decomposition (% yr.$^{-1}$ mc^{-1} millimole^{-1})
Slide (×3)	0·2069	6·166	6·7	1·28
Slide (×1)	0·0690	2·055	3·8	0·72
Slide (×1 Wet)	0·0690	2·055	7·6	1·46
Original tube	16·8	500	4·2	0·79

The decomposition due to the disintegration of the compound's unstable nuclei or primary (internal) effect[1] is negligible and does not exceed 0·001%. This value was calculated from the number of emissions during storage in relation to the number of molecules initially present.

For a pure β-emitter, the fraction of the liberated energy absorbed by the sample (F) is given by the expression

$$F = \frac{pl}{2r}\left(1{\cdot}5 + \ln\frac{r}{pl}\right)$$

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[6] Phillips, *Adv. Carbohydrate Chem.*, 1961, **16**, 13.

[7] Phillips, Baugh, and Lofroth, unpublished results.

where l is the even thickness of deposit (cm.), p the density (mg. cm.$^{-3}$) and r the mean range of the particles.[1,3] It has been assumed in calculating this expression that $r \nless pl$ and that all particles have the energy corresponding to their mean energy. From the total activity recovered during isotope dilution analysis the weight of deposit was estimated and, by assuming that the weight was deposited in the form of a cylinder of radius *ca.* 0·2 cm., which was measured for each deposit, the thickness may be calculated. On this basis $F = 0{\cdot}55$ when three thicknesses are deposited and 0·28 when one thickness is deposited on the slide.* The dose absorbed during storage may therefore be calculated and $-G$ values estimated. For the thicker deposit $-G$ is 26 and for the thinner layer 29. When moisture is present $-G$ values cannot be estimated because of the uncertainty about the value of F. When dry polycrystalline α-D-glucose is exposed to γ-radiation [5] G(acid) is 13—15 and recently initial $-G$ values for this process of *ca.* 25 have been found.[7] As energy transport becomes less efficient in the textured solid produced by freeze-drying initial $-G$ (α-D-glucose) falls to *ca.* 7. The high $-G$ values reported here for self-decomposition are, therefore, compatible with degradation resulting in the main by way of direct action processes. There is clearly a closer similarity between self-decomposition and γ-irradiation of the solid state than with the behaviour of aqueous solutions on irradiation. Under normal freeze-dried conditions it is therefore not necessary to invoke contributions from reactive species formed by radiolysis of non-bonded water to account for the high $-G$ values as has been previously suggested.[1,2] Furthermore, despite the amorphous nature of the deposits studied, the high $-G$ values indicate an efficiency in localisation of energy which is approached during external irradiations only by the crystalline state.

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* For ^{14}C β-radiation the mean energy 0·045 Mev and $r = 3{\cdot}26$ mg. cm.$^{-2}$ have been used in the calculations.[3, 8]

[8] Jenks and Sweeton, *Phys. Rev.*, 1952, **86**, 803; Radiological Health Handbook, Rev. edn., U.S. Public Health Service PB 121784R, p. 53 (1960).