816. *Radiation Chemistry of Carbohydrates. Part XI.* Self-decomposition of* D-[^{14}C]*Mannose,* D-[^{14}C]*Ribose, and* D-[^{14}C]*Fructose.*

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Paper-chromatographic and isotope-dilution analysis showed that considerable decomposition of D-[^{14}C]mannose, D-[^{14}C]ribose, and D-[^{14}C]-fructose occurred when these sugars were stored *in vacuo* in the freeze-dried state. The relative contributions to the decomposition of primary and secondary radiation effects are discussed. The results indicate that non-bonded water, retained by the sample after freeze-drying, may be responsible for most of the degradation, this being due to formation of hydrogen atoms and hydroxyl radicals by radiation. Storage of D-[^{14}C]mannose in frozen dilute aqueous solution reduces the decomposition significantly and indicates a possible method of storing ^{14}C-labelled carbohydrates of high specific activity for long periods.

Decomposition may occur in ^{14}C-labelled compounds on storage owing to the effects of their own radiation. ^{14}C-Labelled amino-acids, amino-alcohols, purines, calcium glycollate, cholesterol, tyrosine, succinic acid, methanol, and methyl iodide have all been shown to be degraded by their own radiation.[1] Although intensive studies have been undertaken into the chemical effects arising from the external action of ionising radiations on pure and aqueous solutions of organic compounds,[2] comparatively little attention has been devoted

* Part X, *J.*, 1962, 2740.

[1] Wagner and Quinn, *J. Amer. Chem. Soc.*, 1953, **75**, 4861; Tolbert, Adams, Bennett, Hughes, Kirk, Lemmon, Noller, Ostwald, and Calvin, *ibid.*, p. 1867; Lemmon, *Nucleonics*, 1953, **11**, 44; Skeaba, Burr, and Hess, *J. Chem. Phys.*, 1953, **21**, 1296.

[2] Swallow, "Radiation Chemistry of Organic Compounds," Pergamon Press, London, 1960.

to self-decomposition.[3] For comparison with our previous studies on the effect of ionising radiations on carbohydrates,[4-6] we now discuss the products of self-decomposition of D-[^{14}C]mannose, D-[^{14}C]ribose, and D-[^{14}C]fructose. It is important that the user of ^{14}C-labelled carbohydrates is aware of the nature of the products of self-decomposition in order to avoid erroneous interpretation in tracer experiments, and to devise suitable purification procedures. Previously a detailed investigation of the products has been undertaken only for D-[^{14}C]glucose.[7] Recently the principles controlling self-decomposition of compounds labelled with radioactive isotopes have been well described by Bayly and Weigel; [8] in the present work particular attention has been given to the nature of the degradation processes which operate in freeze-dried ^{14}C-labelled carbohydrates.

Experimental and Results

Materials.—The samples used in this investigation were supplied by the Radiochemical Centre, Amersham. D-[^{14}C]Fructose (generally labelled) was isolated from *Canna* leaves which had been prepared by photosynthesis in the presence of [^{14}C]carbon dioxide. Alkaline oxidation of this sugar gave D-[^{14}C]arabonic acid which was epimerised in the presence of pyridine to D-[^{14}C]ribonic acid. The ester of this acid was reduced to D-[^{14}C]ribose with sodium amalgam. D-[^{14}C]Mannose was prepared from the walls of *Chlorella* cells which had been grown on sodium hydrogen [^{14}C]carbonate as the sole source of carbon. The cell walls were hydrolysed with dilute sulphuric acid, and D-mannose was isolated by paper chromatography from the neutral hydrolysate. All the samples were initially free from impurities and were stored at room temperature *in vacuo* in the freeze-dried state.

Methods.—Paper chromatography and isotope dilution analysis were used to examine the samples of self-decomposed [^{14}C]sugars. For one-dimensional chromatography the irrigant was butan-1-ol–acetic acid–water (4 : 1 : 5) and for two-dimensional chromatography the additional irrigant was generally butan-1-ol–pyridine–water (10 : 3 : 3). Autoradiographs were prepared by leaving the dried chromatogram in contact with Ilford *X*-ray films for a suitable time. The relative concentration of ^{14}C along the chromatogram was estimated by using an automatic chromatogram-scanner utilising a thin window (1—7 mg./cm.^{2}) Geiger–Müller counter or by measuring the density of the spots on the autoradiograph with a Hilger photoelectric densitometer. Details of these methods, automatic chromatogram-scanner, and the techniques used in isotope analysis have been reported.[9]

Self-decomposition of D-[^{14}C]*Ribose.*—The sealed ampoule containing D-[^{14}C]ribose (1·3 mg.; 50 μc) which had been stored for 15 months was opened and the contents were dissolved in water (100 ml.). A known volume (5 ml.) was freeze-dried to *ca.* 0·1 ml., which was chromatographed in butan-1-ol–acetic acid–water. After location of the bands, the distribution of activity was accurately measured by direct counting (thin-window counter). The results from which the amount of decomposition of D-[^{14}C]ribose may be calculated are shown in Table 1. The amounts of material in the individual bands are too small for detection with spray reagents.

By two-dimensional paper chromatography the bands were further fractionated; a typical autoradiograph revealed 15 components, and immobile material on the starting line. The amount of decomposition was estimated by direct counting of the individual spots, and the results are shown in Table 2. Components 1—11 are neutral and components 12—15 are acidic. The results of one-dimensional chromatography indicate 5·4% of decomposition of D-[^{14}C]ribose (Table 1) and two-dimensional chromatography 6·6%.

Estimation of Products of Self-decomposition of D-[^{14}C]*Ribose.*—Products were estimated by

[3] Tolbert and Lemmon, *Radiation Res.*, 1955, **3**, 52; Dauben and Paycot, *J. Amer. Chem. Soc.*, 1956, **78**, 5637; Tolbert, *Nucleonics*, 1960, **18**, 74.

[4] Phillips and Criddle, *J.*, 1960, 3404; Phillips, Moody, and Mattok, *J.*, 1958, 3522; Phillips, Mattok, and Moody, Proc. Internat. Conference on Peaceful Uses of Atomic Energy, 1958, Vol. XXIX, p. 92; Phillips, *Adv. Carbohydrate Chem.*, 1961, **16**, 13.

[5] Phillips and Moody, *J.*, 1960, 754.

[6] Phillips and Criddle, *J.*, 1962, 2733.

[7] Bourne, Hutson, and Weigel, *J.*, 1960, 5153.

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

[9] Phillips and Criddle, Proc. Internat. Conference on Radioactive Isotopes (Copenhagen), 1960, p. 326.

TABLE 1.

One-dimensional separation of organic constituents present in a sample of self-decomposed D-[^{14}C]ribose after storage for 15 months.

Component	1	2	3	4	5	6	7	8	9	10	11
R_R *	0·05	0·16	0·24	0·36	0·45	0·72	1·00	1·32	1·48	1·60	1·85
Counts/5 min.	930	1301	992	1995	2630	2040	248, 130	1745	1025	302	903

* R_R indicates moment relative to ribose.

TABLE 2.

Two-dimensional separation of organic constituents present in a self-decomposed samples of D-[^{14}C]ribose (1·3 mg.; 50 μc) after storage for 15 months.

Component	R_R (acid irrigant)	R_R (basic irrigant)	Radioactivity (%)	Component	R_R (acid irrigant)	R_R (basic irrigant)	Radioactivity (%)
1	0·22	0·24	0·20	9	0·90	0·91	1·06
2	0·34	0·32	0·57	10	1·00	1·00	93·40
3	0·37	0·34	0·52	11	1·36	1·31	0·73
4	0·52	0·52	0·11	12	0·54	0·15	0·92
5	0·60	0·58	0·19	13	0·64	0·19	0·12
6	0·68	0·62	0·16	14	1·02	0·46	0·61
7	0·68	0·72	0·24	15	1·15	0·13	0·52
8	0·73	0·80	0·43	Starting line	—	—	0·22

applying isotope dilution analysis directly to the solution (100 ml.) obtained by dissolving the initial sample of D-[^{14}C]ribose in water.

D-*Ribose.* The active solution (5·0 ml.) was treated with carrier D-ribose (1·0 millimole), and the mixture was freeze-dried. The resulting solid was dissolved in ethanol and treated with benzylphenylhydrazine (0·5 ml.). After refluxing at 100° for 2 hr. the solution was concentrated *in vacuo*. Seven recrystallisations of the solid which separated were necessary to give pure D-ribose benzylphenylhydrazone, m. p. 127°, constant specific activity 2·53 μc/millimole.

D-*Ribonic acid.* The active solution (5 ml.) was treated with carrier D-ribono-γ-lactone and allowed to equilibrate for 24 hr. The solid obtained after freeze-drying of the solution was dissolved in ethanol (30 ml.) and treated with 96% hydrazine hydrate (0·5 ml.). After 1 hour's heating at 100° and then cooling, a solid separated, which, by six recrystallisations from ethanol, gave pure D-ribonohydrazide, m. p. 150°, constant specific activity 0·012 μc/millimole.

D-*Erythrose.* Carrier D-erythrose (0·85 millimole) was added to the active solution (5 ml.), and the mixture treated with glacial acetic acid (1 ml.) and phenylhydrazine (1·5 ml.). The mixture was heated at 100° for 30 min. and cooled. Water (10 ml.) was added and the solid which separated refluxed with benzene (30 ml.). The solution, after removal of solid by filtration, was chromatographed on a column packed with 4 : 1 calcined alumina–activated alumina (Brockmann activity 1). Elution with benzene separated the erythrosazone band, from which pure erythrosazone was recovered; after three recrystallisations from benzene, it had m. p. 160°, constant activity 0·016 μc/millimole.

D-*Erythronic acid.* The active solution (5 ml.) was treated with carrier sodium D-erythronate (1·0 millimole). 0·1N-Sodium carbonate (0·1 ml.) was added and the mixture left to equilibrate for 24 hr. After removal of the water by freeze-drying, addition of methanol (15 ml.), followed by storage for 5 hr. at 5°, yielded crystals of sodium D-erythronate. Eight recrystallisations from methanol–water were necessary to give material of constant specific activity 0·008 μc/millimole.

Three-carbon aldehydic fragments. Carrier 1,3-dihydroxyacetone (2 millimoles) was added to the active solution (5 ml.), and the mixture treated with glacial acetic acid (1 ml.) and phenylhydrazine (1·5 ml.), heated at 100° for 20 min., and then cooled. Water (10 ml.) was added. The resulting solid was recrystallised seven times, to give pure glycerosazone, m. p. 127°, constant specific activity 0·002 μc/millimole.

Glycollic acid. The active solution (5 ml.) was treated with water (10 ml.) and an excess of calcium carbonate. After 1 hr. at 70° the solid was removed and the solution evaporated under reduced pressure to 5 ml. The crystals which separated on cooling were recrystallised

six times from water, to give pure calcium glycollate with constant specific activity 0·005 μc/millimole.

Two-carbon aldehydic fragments. The active solution (5 ml.) was treated with carrier glyoxal (2·5 millimoles), glacial acetic acid (1 ml.), and phenylhydrazine (1·5 ml.). The solid which separated was recrystallised eight times from benzene, to give pure glyoxal bisphenylhydrazone, m. p. 170°, constant specific activity 0·0012 μc/millimole.

Oxalic acid. The active solution (5 ml.) was treated with carrier oxalic acid (2·5 millimoles) and after 12 hr. the solution was evaporated under reduced pressure until oxalic acid dihydrate crystallised. Six further recrystallisations from water gave the pure compound, m. p. 99°, constant specific activity 0·001 μc/millimole.

To determine the specific activity of the initial active sample, a 5-ml. portion was treated with carrier D-ribose (0·37 millimole), and sufficient of the solution was burnt to give 50 mg. of barium carbonate, which was counted in the form of a disc of thickness 20 mg. per cm.^{2}. The sample had a specific activity of 7·43 μc/millimole.

The combined results are shown in Table 3.

TABLE 3.

Analysis of D-[^{14}C]ribose (1·3 mg.; 50 μc) after storage for 15 months.

Product	Carrier (millimoles)	Specific activity (μc/millimole)	Yield (%)
D-Ribose	1·00	2·530	91·90
D-Ribonic acid	1·72	0·012	0·72
D-Erythrose	0·85	0·016	0·49
D-Erythronic acid	1·00	0·008	0·30
Three-carbon fragments	2·00	0·002	0·15
Glycollic acid	2·00	0·003	0·28
Two-carbon fragments	2·00	0·001	0·08
Oxalic acid	2·50	0·001	0·14

Total decomposition 8·1%.

Self-decomposition of D-[^{14}C]*Mannose.*—The analytical techniques were similar to those which were described for D-[^{14}C]ribose. For one-dimensional paper chromatography, the irrigant was butan-1-ol–ethanol–water (4 : 1 : 5). Two-dimensional paper chromatography, with, as additional irrigant, butan-1-ol–acetic acid–water, revealed 22 constituents. The proportions of the individual constituents are shown in Table 4. Components 1—10 are acidic and components 11—22 neutral. From the measurements recorded in Table 4, the total decomposition of D-mannose during storage is 15%.

TABLE 4.

Analysis of D-[^{14}C]mannose (0·9 mg.; 100 μc) after storage for 29 months.

Component	R_M * (acid irrigant)	R_M * (basic irrigant)	Radioactivity (%)	Component	R_M * (acid irrigant)	R_M * (basic irrigant)	Radioactivity (%)
1	0·45	0·15	0·53	13	1·15	1·15	2·60
2	0·50	0·20	0·24	14	1·38	1·38	0·30
3	0·70	0·25	0·65	15	1·30	1·50	0·96
4	0·84	0·26	0·70	16	1·50	1·63	0·83
5	0·86	0·13	0·18	17	1·62	1·75	0·35
6	1·10	0·14	1·42	18	1·65	1·78	0·40
7	1·16	0·28	0·24	19	1·71	1·82	0·19
8	1·44	0·20	0·04	20	1·75	1·85	0·08
9	1·52	0·20	0·54	21	1·80	1·85	0·04
10	1·61	0·21	0·04	22	1·77	1·76	0·04
11	0·80	0·80	3·50	Starting line	—	—	0·37
12	1·00	1·00	85·00				

* R_M indicates movement relative to D-mannose.

Estimation of Products of Self-decomposition of D-[^{14}C]*Mannose.*—D-[^{14}C]Mannose (0·9 mg.; 100 μc) was dissolved in water (100 ml.) after storage for 29 months, and the products of self-decomposition were estimated by applying isotope dilution analysis directly to the active solution as follows.

D-*Mannose.* The active solution was treated with carrier D-mannose (0·83 millimole), and the mixture treated with glacial acetic acid (1 ml.) and phenylhydrazine (1·5 ml.). After

8 hr. at 5°, the solid that had separated was recrystallised six times from aqueous ethanol, to give pure D-mannose phenylhydrazone, m. p. 185°, constant specific activity 2·70 μc/millimole.

D-*Arabinose*. Carrier D-arabinose (1·10 millimoles) was added to the active solution (5·0 ml.), and the mixture treated with ethanol (10 ml.) and diphenylhydrazine (1·0 ml.). After refluxing for 1 hr. at 100° the solution was left for 24 hr.; a solid separated, which after six recrystallisations from ethanol gave pure D-arabinose diphenylhydrazone, m. p. 196°, constant specific activity 0·017 μc/millimole.

D-*Lyxose*. The active solution was treated with carrier D-lyxose (1·40 millimoles) and after 12 hr. was freeze-dried. A solution of benzylphenylhydrazine (1 ml.) in ethanol (20 ml.) was added and the mixture refluxed for 1 hr. After removal of the solvent under reduced pressure a solid separated, which after seven recrystallisations from ethanol gave pure D-lyxose benzylphenylhydrazone, m. p. 128°, constant specific activity 0·009 μc/millimole.

D-*Arabonic acid*. The active solution (5 ml.) was treated with carrier D-arabono-γ-lactone (1·10 millimoles) and allowed to equilibrate for 24 hr. After freeze-drying of the solution, ethanol (20 ml.) was added and the mixture refluxed until dissolution was complete. 96% Hydrazine hydrate (0·5 ml.) was added and the mixture further refluxed for 1 hr. Then the excess of solvent was removed under reduced pressure and the resulting solid was recrystallised seven times from ethanol, to give pure D-arabonohydrazide with constant specific activity 0·009 μc/millimole.

D-Erythrose, D-erythronic acid, three- and two-carbon aldehydic fragments, glycollic acid, and oxalic acid were estimated as described above, in 5-ml. samples. The results are shown in Table 5. The specific activity of the initial active solution (5 ml.) was 13·41 μc/millimole after the addition of 0·28 millimole of inactive carrier.

TABLE 5.

Analysis of D-[^{14}C]mannose (100 μc; 0·9 mg.) after storage for 29 months.

Product	Carrier (millimoles)	Specific activity (μc/millimole)	Yield (%)
D-Mannose	0·83	3·685	81·40
D-Arabinose	1·10	0·017	0·50
D-Lyxose	1·40	0·009	0·33
D-Erythrose	1·10	0·006	0·18
D-Erythronic acid	1·09	0·004	0·13
D-Arabonic acid	1·10	0·009	0·29
Three-carbon aldehydic fragments	1·61	0·008	0·38
Two-carbon aldehydic fragments	2·20	0·005	0·32
Glycollic acid	5·20	0·004	0·41
Oxalic acid	1·15	0·002	0·09

Total decomposition 18·6%.

Rate of Decomposition of D-[^{14}C]*Mannose*.—D-[^{14}C]Mannose (~100 μc) was dissolved in water (100 ml.) and divided into twenty 5-ml. aliquot parts. Ten parts were freeze-dried and sealed in evacuated tubes, and the remainder frozen and stored at −5°. At approximately monthly intervals, the unchanged D-mannose was estimated in one frozen sample and in an evacuated freeze-dried sample. In this way the rate of degradation of D-[^{14}C]mannose under both conditions may be compared, and the results are shown in the Figure. For the frozen samples the rate of decomposition was *ca.* 1% per year and in the freeze-dried samples *ca.* 7%.

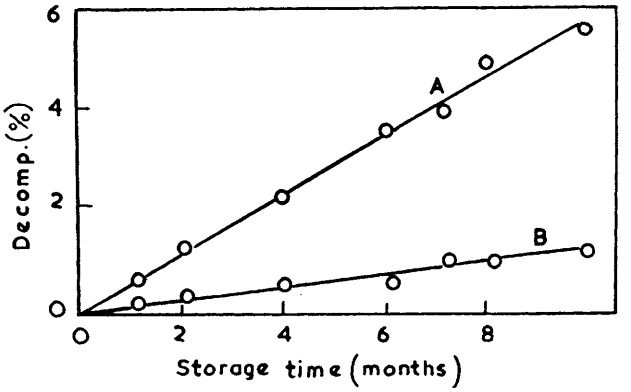
Self-decomposition of D-[^{14}C]*Fructose*.—Two-dimensional paper-chromatography of D-[^{14}C]-fructose (1·12 mg.; 50 μc) which had been stored in the freeze-drier *in vacuo* for 33 months revealed the presence of at least 16 constituents. By direct counting of the paper chromatogram the proportion of the individual constituents were estimated and the results are shown in Table 5. From the behaviour of the individual constituents on the paper chromatogram there are indications that components 1—5, 12, and 16 are acidic and that components 14 and 15 are neutral sugars. It is probable that the immobile constituent remaining on the starting line is a polymer formed during self-decomposition. From the measurements recorded in Table 6, decomposition of D-fructose was 7·3%.

Estimation of Products of Self-decomposition of D-[^{14}C]*Fructose*.—D-[^{14}C]Fructose (1·3 mg.; 50 μc) was dissolved in water (100 ml.) after being stored *in vacuo* for 33 months, and the products of self-decomposition were estimated as follows.

D-*Fructose*. The active solution (5 ml.) was treated with carrier D-fructose (0·67 millimole),

phenylhydrazine (2 ml.), and glacial acetic acid (1 ml.), and the mixture refluxed for 30 min. The solid which separated was washed with benzene (50 ml.) and recrystallised nine times from ethanol, to give pure glucosazone, m. p. 201°, constant specific activity 3·05 μc/millimole.

Glucosone. The active solution (5 ml.) was treated with carrier glucosone (0·55 millimole), and phenylhydrazine (2 ml.). After 2 hr. at 20° a solid had separated, which on eight recrystallisations from ethanol gave pure glucosazone, m. p. 199°, constant specific activity 0·012 μc/millimole. A control experiment confirmed that no glucosazone was produced when D-fructose was treated with phenylhydrazine under the conditions of the experiment.



Self-decomposition of D-[^{14}C]mannose: (A) freeze-dried; (B) frozen solution.

Glycollaldehyde. The active solution (5 ml.) was treated with carrier glycollaldehyde (3·13 millimoles) and a saturated solution of 2,4-dinitrophenylhydrazine in 2N-sulphuric acid (80 ml.). After 1 hr. at 20° the solid which had separated was filtered off, washed with water

TABLE 6.

Analysis of D-[^{14}C]fructose (50 μc; 1·12 mg.) after storage for 33 months.

Component	R_F * (acid irrigant)	R_F * (basic irrigant)	Radioactivity (%)	Component	R_F * (acid irrigant)	R_F * (basic irrigant)	Radioactivity (%)
1	0·54	0	0·20	10	0·69	0·67	0·70
2	1·01	0	0·06	11	0·75	0·75	0·48
3	0·40	0·09	0·33	12	0·82	0·38	0·44
4	0·51	0·12	0·15	13	1·00	1·00	92·70
5	0·70	0·16	0·59	14	1·27	1·37	0·72
6	0·33	0·20	0·07	15	1·52	1·60	1·10
7	0·40	0·24	0·37	16	2·26	0·93	0·34
8	0·47	0·31	0·59	Starting line	—	—	0·28
9	0·61	0·49	0·87				

* R_F indicates movement relative to D-fructose.

(200 ml.), and recrystallised eight times from ethyl acetate, to give pure glycollaldehyde 2,4-dinitrophenylhydrazone, m. p. 155°, constant specific activity 0·0007 μc/millimole.

Two- and three-carbon aldehydic fragments and glycollic acid and oxalic acid were estimated as described above, and the results are shown in Table 7. The specific activity of the initial

TABLE 7.

Analysis of D-[^{14}C]fructose (1·12 mg.; 50 μc) after storage for 33 months.

Product	Carrier (millimoles)	Specific activity (μc/millimoles)	Yield (%)
D-Fructose	0·67	3·05	91·30
Glucosone	0·55	0·012	0·29
Two-carbon aldehydic fragments	1·70	0·0016	0·12
Three-carbon aldehydic fragments	1·67	0·0045	0·33
Glycollaldehyde	3·13	0·0007	0·10
Glycollic acid	1·64	0·002	0·18
Oxalic acid	0·80	0·001	0·05

active solution (5 ml.) was 8·11 μc/millimole (carrier 0·277 millimole). Estimation of D-fructose as glucosazone gives a result which includes a contribution due to glucosone, which has been estimated independently. The true decomposition of D-fructose is therefore 9% if this contribution is included.

Discussion

Our results show that for each ^{14}C-carbohydrate studied, considerable self-decomposition occurred during storage of freeze-dried samples *in vacuo* for 18—33 months. The number of products formed, in each case, is large. A typical separation is shown in Table 4 for D-[^{14}C]mannose, which had been stored for 29 months; it reveals 22 components. Control samples of 2-oxogluconic acid, lyxose, and arabinose have similar R_M values to components 1, 14, and 13, respectively. Lactones of mannonic acid correspond to components 3 and 6. Two-, three-, and four-carbon aldehydic fragments and arabinose were identified unequivocally by isotope dilution analysis (Table 5). The radioactivity which remains on the starting line during paper chromatography is probably due to polymeric material. The nature of the products indicates certain similarities between self-decomposition of D-[^{14}C]mannose and the degradation resulting from γ-irradiation of mannose solutions *in vacuo*.[6] Here, oxidative processes lead initially to D-mannonic acid and glucosone, while two-, three-, and four-carbon aldehydic fragments are formed by primary ring scission. At high doses, initial products are extensively degraded to numerous secondary products, which include D-arabinose, D-lyxose, and 2-oxogluconic acid. An acidic polymer is formed on irradiation *in vacuo*.

However, in addition to the similarities shown between the products of self-decomposition and irradiation *in vacuo*, it is clear that there are a large number of additional products present in D-[^{14}C]mannose in amounts less than 1%, which are not present in the irradiated solution.

The self-decomposition of D-[^{14}C]ribose and D-[^{14}C]fructose follows similar paths. For the former, two-dimensional paper chromatography revealed 15 products in addition to immobile polymeric material; for the latter, 16 products and polymer. The products identified by isotope dilution analysis are shown in Tables 3 and 6 and, as for D-[^{14}C]-mannose, may be identified in some instances with the products formed during irradiation of aqueous solutions.[5,10]

Self-decomposition of labelled compounds may arise by one or more effects:[8] (*a*) primary (internal) radiation effects arising from the disintegration of one of the compounds unstable nuclei; (*b*) primary (external) radiation effect, which is decomposition of the compound by direct absorption of its own radiation; (*c*) secondary radiation effect arising from radiation of the labelled compound with reactive species formed by primary radiation action; (*d*) by chemical reactions which may not be connected with radiation action. For ^{14}C-labelled carbohydrates stored as thin freeze-dried films in scrupulously cleaned glass vessels, decomposition by chemical reactions is insignificant, and attention may be confined to effects (*a*)—(*c*).

Decomposition for our radioactive D-mannose, D-ribose, and D-fructose, measured by isotope dilution analysis, amounted to 18·6, 8·1, and 9·0%, respectively, and the corresponding values from paper chromatograms were 15, 6·6, and 7·3%. Self-absorption of the emitted β-rays and failure to isolate the initial ^{14}C-carbohydrate discretely from decomposition products may account for the fact that paper-chromatogram values were consistently lower than the more reliable values obtained by isotope dilution analysis.

The proportion of the degradation which may be attributed to the primary (internal) effect (*a*) is insignificant. The % contribution to the overall degradation was calculated from the number of emissions during storage in relation to the number of molecules of each sugar initially present, and is shown in Table 8.

Consideration must, therefore, be given to the relative importance of the direct

[10] Phillips and Criddle, Part X, *J.*, 1962, 2740.

TABLE 8.

Self-decomposition of ^{14}C-carbohydrates.

Sample	Wt. (mg.)	Radioactivity (μc)	Storage (months)	Decomp. (%)	Decomp. (% yr.$^{-1}$ mc^{-1} mmole^{-1})
D-[^{14}C]Mannose	0·9	100	29	18·6	0·43
D-[^{14}C]Ribose	1·3	50	15	8·1	1·17
D-[^{14}C]Fructose	1·12	50	33	9·0	0·42

Sample	No. of mol. destroyed/100 ev	Decomp. due to primary (internal) radn. (%)	Decomp. due to primary (external) radn. * (%)
D-[^{14}C]Mannose	4	0·009	1·9
D-[^{14}C]Ribose	10	0·002	0·59
D-[^{14}C]Fructose	4	0·004	0·9

* Calc. from the expression % decomposition $= [1 - \exp(-F\varepsilon a s_0 t \times 6{\cdot}14 \times 10^{-16})]100$ where F is the fraction of the liberated energy absorbed by the sample, ε is the mean energy of the radiation in ev, a is the number of molecules destroyed per 100 ev, s_0 the initial specific radioactivity of the compound in c/mole, and t the time in sec.

degradation of the ^{14}C-sugars due to bombardment by their own β-rays, and possible secondary radiation effects due to the action of hydrogen atoms and hydroxyl radicals formed by interaction of the β-rays with non-bonded water retained by the freeze-dried samples. From the infrared spectrum of a sample of D-glucose prepared in the same way as the radioactive samples, Bourne, Hutson, and Weigel [7] demonstrated that an appreciable quantity of water was present. We have confirmed in a similar manner that water is retained by freeze-dried mannose samples, unless subsequent rigorous drying procedures are adopted.

The natures of the products do not allow a clear choice to be made between the two possible mechanisms of degradation. Although obvious similarities may be seen between products of self-decomposition and irradiation *in vacuo*, it is possible that direct action may cause the same groups to split off in the primary process as are pulled off by hydrogen and hydroxyl radicals in solution. Electron spin resonance studies [11] in fact demonstrate that the identical radical R·CH(OH)· is formed by direct irradiation of solid D-glucose and by irradiation of glucose solutions *in vacuo*. Radicals formed in this manner have a considerable life at room temperature in the absence of water.[12] It is clear, therefore, that after reaction in water, radicals produced during irradiation of solids may lead to a similar skeletal breakdown as irradiation of aqueous solutions. Therefore, since the analyses of our samples were carried out in water, radicals formed by direct radiative action and by indirect action on non-bonded water might lead to similar final products.

Quantitative expression of the radiation chemical yields for irradiation of aqueous solutions is provided by the G value (the number of molecules undergoing change per 100 ev of energy input). On a similar basis, the number of molecules destroyed per 100 ev liberated by the ^{14}C-sugar during storage may be calculated. This value varies from 4 to 10 for the sugars studied (Table 8). Destruction coefficients ($-G$ values) for many pure organic compounds irradiated *in vacuo* vary from 5 to 10, and these values were used by Tolbert [3] to calculate the expected decomposition of ^{14}C-compounds during storage. To identify decomposition rates for irradiated systems with $G(-M)$ for the self-decomposition process, however, it must be established that all the emitted energy is absorbed by the sample. For the ^{14}C-carbohydrates studied, stored in amounts varying from 0·9 to 1·12 mg., this is extremely doubtful. For ^{14}C-sugars stored under conditions similar to ours, Bayly and Weigel [8] estimated the fraction of the energy absorbed by the sample. This fraction is critically dependent on the geometry of the sample, which was not recorded by us as the time of analysis. Comparison is possible, however, with the

[11] Barley, Barker, Brimacombe, Pooley, and Spencer, *Nature*, 1961, **190**, 259.

[12] Granath and Kinell, *Acta Chem. Scand.*, 1961, **15**, 141; Ehrenberg, Ehrenberg, and Lofroth, Proc. First Nordic Meeting on Food Preservation by Ionising Radiation, Riso Report 1960, No. 16, 25.

values calculated by Bayly and Weigel [8] for similar freeze-dried samples. For D-[^{14}C]-glucose (100 μC; 0·43 mg.) with a specific activity of 41·8 μC per millimole, the measured rate of decomposition was 0·51% per year per mC per millimole; the corresponding rate for our D-[^{14}C]mannose (100 μC; 0·9 mg.) with a specific activity of 20 mC per millimole was 0·43%. The amount of decomposition is in general proportional to specific activity, providing other factors are comparable. Therefore, as a working approximation in the calculation of $G(-M)$, we have assumed that the fraction of the energy absorbed by our samples is the same as the fraction estimated by Bayly and Weigel [8] for D-[^{14}C]glucose stored under comparable conditions (10%). On this basis $G(-M)$ for D-[^{14}C]mannose is 40, D-[^{14}C]fructose 40, and D-[^{14}C]ribose 100, and in relation to the influence of secondary radiation effects, the decomposition due to primary radiation effects is small. The results are summarised in Table 8. The proportion of the observed decomposition due to the primary (internal) effect is negligible, while 6—10% of the decomposition may be attributed to primary (external) radiation effects. The major proportion of the decomposition appears, therefore, to be due to reactive species formed by radiolysis of non-bonded water retained by the samples.

This conclusion is supported by the observed rate of decomposition for ^{14}C-sucrose ($-G$ 4·6).[8] Non-bonded water can be entirely removed from sucrose on freeze-drying. The observed decomposition may thus be attributed to direct action effects only, and the $-G$ value is comparable with the calculated contribution of direct action effects in the self-decomposition of D-[^{14}C]mannose ($-G$ 4). Further, for D-[^{14}C]glucose samples containing non-bonded water the rate of decomposition at −80° is reduced markedly below that at room temperature,[8] an observation which may be rationalised in terms of the reduction of mobility of hydrogen and hydroxyl radicals at low temperature.

An objection to the conclusion, however, arises from the fact that irradiation of water produces hydrogen and hydroxyl radical pairs at a rate of 4 per 100 ev. Therefore, to account for $G(-M)$ 40—100, it is necessary to invoke subsequent chain reactions. No such chain reactions, however, have been observed during irradiations of carbohydrates in aqueous solution when $G(-M)$ is 3·5—4·0.[4-6]

It should be emphasised that the value $F = 10\%$ is arbitrary, and was selected to obtain a direct comparison with the valuable analysis provided by Bayly and Weigel,[8] which is the first quantitative treatment applied to self-decomposition.

From a practical point of view, it is to be expected from our main conclusions that decomposition due to secondary radiation effects may be reduced by dispersion of the sample over an area or dilution with inactive material. The mobility of secondary hydrogen and hydroxyl radicals may also be reduced in frozen solution. We have compared the effect of storing the ^{14}C-carbohydrate in frozen solution and in the freeze-dried state, with a view to reducing the rate of decomposition without reducing the specific activity of the sample, such reduction being unavoidable if it is necessary to dilute the sample with inactive carrier. For the same quantity of radioactivity (5μC; 0·05 mg.), the rate of decomposition may be reduced from 0·43% in the freeze-dried state to 0·055% per year per mC per millimole in frozen solution. If it is necessary to store ^{14}C-carbohydrates of high specific activity for long periods, then storage in dilute frozen solution, followed by recovery of the sample by freeze-drying, is eminently suitable. This is a procedure we have followed satisfactorily with a variety of ^{14}C-carbohydrates.

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