NOTES

Studies of the Recoil Tritium-labeling Reaction

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In the reaction of the energetic tritium atom produced in the $Li^6(n, \alpha)T$ reaction with organic compounds, radiation-damage products with specific activities higher than the labeled parent molecule have been detected[1-3]. In order to investigate the specificity of the chemical reactions of recoil tritium, studies have been made of the reaction with crystalline glucose, lactose and α-hydroximinopropionic acid.

A finely powdered mixture of 20 g. of each sample and 2 g. of lithium carbonate was irradiated in the JRR-1 reactor for 21 hr. at a flux of $\sim 2.5 \times 10^{11}$ n/cm^2/sec. The irradiated materials were somewhat brownish as a result of radiation decomposition. Sugars were separated from lithium carbonate by hot water extraction and adsorption on Amberlite IR-120 resin. Since Rowland et al.[4] reported that macro amounts of organic acids were separated during the purification of the irradiated galactose, attempts were made to separate the neutral and acidic constituents by the use of ion-exchange resin.

The solution of sugars was passed through a column of Amberlite IR-45, which was washed with water to produce a neutral fraction. Acids were removed from the column with a 2 N ammonia solution, and free acids were liberated by passage of the solution through a column of Amberlite IR-120.

The constituents in both the neutral and the acidic fraction were separated chromatographically on Toyo paper No. 50. The acidic sugars

1) M. A. El-Sayed, P. J. Estrup and R. Wolfgang, *J. Phys. Chem.*, **62**, 1356 (1958).
2) R. M. White and F. S. Rowland, *J. Am. Chem. Soc.*, **82**, 5345 (1960).
3) J. K. Lee, B. Musgrave and F. S. Rowland, ibid., **82**, 3545 (1960).
4) F. S. Rowland, C. N. Turton and R. Wolfgang, ibid., **78**, 2354 (1956).

were developed with *n*-butanol-acetic acid-water (4 : 1 : 5) and detected with bromophenol blue or benzidine hydrochloride. The neutral sugars were irrigated with *n*-butanol-pyridine-water (6 : 4 : 3) and detected with benzidine hydrochloride or ammoniacal silver nitrate.

Paper chromatography of the neutral fraction of lactose showed five spots, but that of glucose showed only one spot. The R_f values of the three spots derived from lactose were identical with those of lactose (0.12), galactose (0.19) and glucose (0.23). The chromatogram of the acidic fraction of lactose revealed three spots which were identified as mucic acid (0.01), gluconic acid (0.12) and saccharic acid (0.20).

The irradiated oxime was extracted with ether, and the residue was chromatographed with *n*-butanol-acetic acid-water (4 : 1 : 1). The two spots produced by spraying ninhydrine corresponded to DL-alanine and glycine. The results obtained with the undegraded samples are summarized in Table I.

Table I. Incorporation of recoil tritium into organic compounds

Sample	Total tritium d.p.m./mg.	Nonlabile tritium incorporated d.p.m./mg.	Entry of tritium %	Recovery %
Glucose	2.2×10^5	5.3×10^4	24	89.0
Lactose	2.2×10^5	2.2×10^4	10	47.4
Oxime	2.2×10^5	2.0×10^4	9	55.0

The total tritium content, which was calculated from the bombardment condition, represents the number of tritons produced by the nuclear reaction. The low recovery of lactose as compared with that of glucose may be attributed to the fact that the glucoside linkage is ready to undergo radiolysis. The amount and the specific activity of the radioactive mucic acid produced by the irradiation of lactose was determined by the double dilution method. For a comparison of the specific activity, the isolated lactose was oxidized with nitric acid to mucic acid. As a result, the radiation-damage mucic acid had a specific activity about ten times higher than the chemically degraded mucic acid, as is shown in Table II.

Table II. Comparison of the specific activity of mucic acid produced by radiation damage and chemical degradation

Sample	Specific activity μc./mM	Yield %
Radiation-damage mucic acid	12.0	0.09
Chemically-degraded mucic acid	1.26	—
Lactose	3.4	—

Table III. The chemical state of tritium produced by the neutron irradiation of α-hydroximinopropionic acid

Sample	Specific activity μc./mM	Yield %
DL-Alanine	169.0	0.34
Glycine	75.0	0.17
Oxime	0.93	55.0

The production of mucic acid of high specific activity from lactose suggests that the initial substitution of T for H on the -CH- carbon leaves an excited molecule which eventually decomposes to the labeled mucic acid.

Table III shows the specific activities and the yields of tritium-labeled products formed by the reaction of recoil tritium with α-hydroximinopropionic acid.

The great difference in the specific activity between DL-alanine and oxime can be explained on the basis of the preferential utilization of the tritium atom for the addition of the double bond. White and Rowland[2] have demonstrated that the addition of recoil tritium to the double bond occurs in fumaric acid and maleic acid.

The radioactive glycine is presumably produced by the substitution of T for CH_3, Hoff and Rowland[5] have already shown the production of labeled acetaldehyde in the reaction of recoil tritium with acetone.

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5) W. J. Hoff, Jr. and F. S. Rowland, ibid., 79, 4867 (1957).