Studies on Tritium Labeled Compounds. II.[1] *Preparation of Tritium Labeled Stearic Acid*

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In an attempt to prepare ^{3}H-labeled stearic acid as a biochemical tracer, we have carried out three different methods; chemical method, electric discharge method and recoil tritium labeling method. Wolfgang, Pratt and Rowland[2] investigated the labeling of organic compounds by a electric discharge in tritium in 0.05 mmHg region on the assumption that accelerated tritium ions will be active transients required for labeling. Dorfman and Wilzbach[3] have recently conducted investigations on the labeling method by means of electric discharge in a 10 mmHg region. We have also studied the labeling method by electric discharge in tritium-hydrogen in 100 mmHg region[1].

Wolfgang and Rowland[4] have developed a ^{3}H-labeling method of organic compounds. According to their reports, the compound to be labeled is mixed with lithium salt and the mixture is irradiated by slow neutrons in a reactor. A considerable fraction of the energetic tritium produced in the ^{6}Li(n, α)T reaction will replace bound hydrogen in the molecules of stopping medium to form a radioactive form of the original hydrogenous molecule.

We have made investigations on the recoil tritium labeling method using a mixture of stearic acid and lithium carbonate in a reactor of the Japan Atomic Research Laboratory (JRR-1).

Experimental and Results

Chemical Method.—*Reduction of Ethyl Linoleate*[5]. —154 mg. of ethyl linoleate, 5 mg. of platinum oxide catalyst and 2 ml. of ethyl acetate are mixed together in a reaction vessel connected with a vacuum apparatus. Then in the apparatus, H^3-labeled ethyl stearate was prepared by catalytic hydrogenation of ethyl linoleate with ^{3}H-H gas liberated by electrolysis of ^{3}HHO. After about 20 min., 23 ml. (theoretical: 22.4 ml.) of ^{3}H-H was absorbed and the uptake of ^{3}H-H has ceased. The catalyst was removed by filtration and after the solvent was evaporated, ^{3}H-labeled ethyl stearate was hydrolyzed by heating under reflux for three hours with potassium hydroxide-methanol solution. The solution was acidified with 10 N-hydrochloric acid, cooled and filtered. Then, deposited ^{3}H-stearic acid was extracted with ether. The yield of ^{3}H-stearic acid was 90% on the basis of ethyl linoleate, with a specific activity of 0.9 μc/mg.

Exchange Method in the Presence of Catalyst. — In an attempt to prepare ^{3}H-stearic acid, exchange method for deuterium labeling of fatty acid developed by Heyninger, Rittenberg and Schoenheimer[6] was employed. In a glass tube, 20 mg. of PtO$_2$ suspended in 1 ml. of ethyl alcohol was reduced in a hydrogen stream. After alcohol was distilled, 200 mg. of stearic acid, 5 mg. of potassium hydroxide and 0.4 ml. of ^{3}HHO (50 mc./ml.) were added. The mixture was frozen, the tube was sealed and then heated at 135°C for a week. After the period of reaction, the tube was opened and connected with a vacuum apparatus, and the liquid was distilled in vacuo. The dried residue was dissolved in potassium hydroxide-ethyl alcohol solution and filtered, and the filtrate was acidified with hydrochloric acid. The deposited ^{3}H-stearic acid was recrystallized from ether. The yield of ^{3}H-stearic acid was 90% on the basis of stearic acid, with a specific activity of 6.2 μc/mg.

Electric Discharge Method. — Discharge was carried out in a sample cell of approximately 20 ml., with tungsten electrodes (15 mm. apart). A sample to be labeled was placed on the bottom of the glass cell. Discharge was normally run from 20 sec. to 180 sec. in ^{3}H-H mixture gas under a constant pressure of 100 mm Hg. The following is the procedure: A quantity of approximately 20 mg. was placed on the bottom of a glass cell and the cell was degassed. After the degassing was completed, ^{3}H-H gas liberated by electrolysis of tritiated water (30 mc./ml.) was introduced into the cell and the discharge was made to run. In running of the discharge, the electrodes were over-heated. The electrodes were cooled by stopping the discharge. The cell was again degassed and air was introduced into the cell.

The stearic acid was then assayed for tritium in a gas-flow counter. The following table summarize the results.

Table I shows activity levels obtained for stearic acid subjected to the discharge technique. The

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[1] Y. Sato, T. Meshi, T. Takahashi and N. Sugimoto, *Japanese J. Pharm. and Chem.* (*Yakugaku Kenkyu*), **32**, (5) 317 (1960).

[2] R. Wolfgang, T. Pratt and F. S. Rowland, *J. Am. Chem. Soc.*, **78**, 5132 (1956).

[3] L. M. Dorfman and K. E. Wilzbach, *J. Phys. Chem.*, **63**, 799 (1959).

[4] F. S. Rowland, C. N. Turton and R. Wolfgang, *J. Am. Chem. Soc.*, **78**, 2354 (1956).

[5] R. F. Glascock and L. R. Reinius, *Biochem. J.*, **62**, 529 (1956).

[6] W. E. Van Heyningen, D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, **125**, 495 (1938).

Chart I

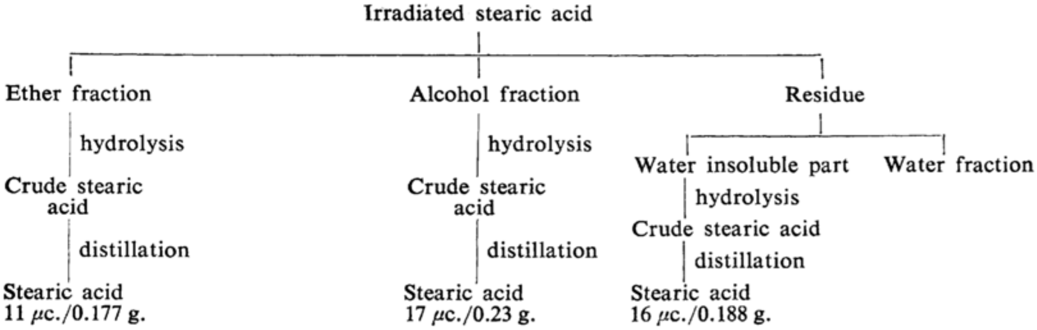

TABLE I. LABELING BY ELECTRIC DISCHARGE IN ^{3}H-H GAS

^{3}H-H Pressure, mm Hg	Discharge Time, sec.	Activity, μc./g.
100	20	1.9
100	40	5.7
100	60	8.6
100	120	23.6
100	180	91.6

tritium content of the product increases approximately with the discharge time.

Recoil Tritium Labeling Method. — A mixture of 2 g. stearic acid and 1 g. lithium carbonate was irradiated with thermal neutrons for 17 hr. at a flux 2.5×10^{11} n/cm^2 sec. The irradiated product was first extracted with anhydrous ether, and then with anhydrous ethyl alcohol. Infrared spectra of the ether fraction, alcohol fraction and the residue are shown in Fig. 1. It was recognized, that ester of fatty acid was produced by irradiation. Hydrolysis of each fraction was carried out by alcoholic potassium hydroxide. The hydrolyzed compounds were identified as stearic acid by infrared spectra, and they all distilled in vacuo (10 mm Hg) at 206~208°C.

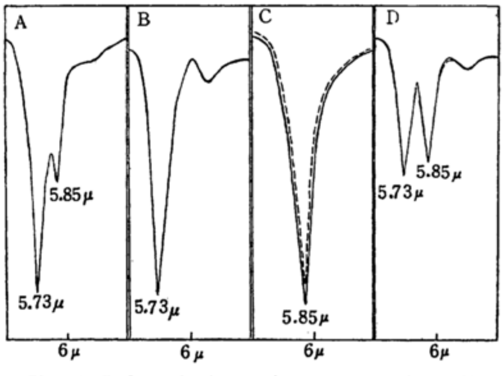

Fig. 1. Infrared absorption spectra of recoil tritiated stearic acid.
A : Residue, B : Alcohol fraction, C : —— Authentic stearic acid, ---- Hydrolyzed specimens of A, B and D, D : Ether fraction.

The stearic acid was assayed for tritium by means of a gas-flow counter. Chart I represents the experimental treatments described above.

The total yield of radio active stearic acid was about 0.6 g. (about 30%). Activity (A) of recoil tritium atoms produced by irradiation was calculated on the basis of the following equation.

$$A=\sigma Nf(1-e^{-0.693/t})$$

σ : Cross section 945 barn, N: Number of Li atom, f: Neutron flux, T: Half-life, t: Irradiation time.

When a mixture of stearic acid and lithium carbonate was irradiated by a 2.5×10^{11} neutron flux, value of A was 832 μc. The tritium content of the obtained stearic acid was therefore about 5% of the theoretical value.

Radioactivity Measurement of Tritium Labeled Stearic Acid. — Tritium activity was counted according to the Jackson and Lampe's method.[7] A windowless gas flow counter was used for radioassay of ^{3}H-stearic acid. The self-absorption characteristics of tritium labeled stearic acid samples are given in Fig. 2.

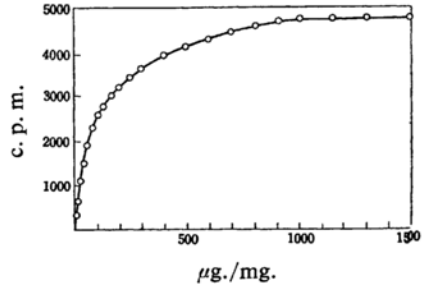

Fig. 2. Self-absorption curve of ^{3}H-stearic acid.

These curves indicate that self-absorption is negligible up to sample weights of about 20 μg. per sq. cm.

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[7] F. L. Jackson and H. W. Lampe, *Anal. Chem.*, **28**, 1735 (1956).

Discussion

By using tritium gas with higher specific activity than in above chemical process, it is easy to get a much higher specific activity. A higher specific activity can also be obtained using the discharge method when the discharge time is made prolonged and the tritium gas is of a higher specific activity. Tritium labeling in a reactor, on the other hand, is less usable, since this method gives rise to high destruction and lower specific activity. From the infrared absorption spectra of each fraction of irradiated products, it was recognized that the irradiated products contain fatty acid ester, because a 5.73 μ band of these products was identical with the carboxyl band of ethyl stearate.

Summary

Chemical reduction method, exchange method, electric discharge method and recoil tritium labeling method were applied for preparing tritium labeled stearic acid. As the result of the recoil tritium labeling, the irradiated stearic acid was found to contain high destruction products which are mainly composed of esters of fatty acid.

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