# *Studies on Tritium Labeled Compounds. IV*. Preparation of Tritium Labeled Alanine by Means of Electric Discharge*

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On the assumption that accelerated-tritium ions are the active transients required for labeling, Wolfgang, Pratt and Rowland¹⁾ studied methods for preparing tritium labeled organic compounds using the electric discharge method. On the other hand, Dorfman and Wilzbach²⁾ investigated the labeling method under electric discharge in a 10 mmHg pressure region and reported that the labeling would be expected to occur as a result of the primary process of ionization, excitation and dissociation of tritium molecules in the gas phase. We recently reported³⁾ that the labeling would be produced in the same mechanism as that reported by Dorfman and Wilzbach. In this work, we carried out tritium labeling under electric discharge by employing α-hydroximinopropionic acid as the sample and obtained DL-alanine of a high specific activity.

## Experimental and Results

About 50 mg. of α-hydroximinopropionic acid was placed in the bottom of a glass cell which has two electrodes of tungsten as shown in Fig. 1. The cell was attached to a vacuum line and degassed. $^3$H-H gas, which was liberated by electrolysis of tritiated water (130 mc./ml.), was introduced into the cell and electric discharge (8000 V.) was struck at 40 mmHg for 10 min.

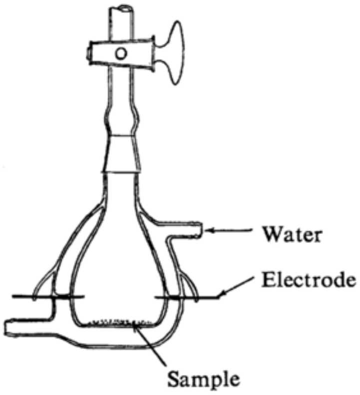

Fig. 1. Discharge cell.

During the discharge, the electrodes were overheated. In order to avoid over-heating, the electrodes were cooled by passing of cold water around the outside of the cell and by stopping further discharge. After the discharge the cell was degassed and air was introduced into the cell. As it was presumed that tritium labeled alanine was produced by this method, the discharged sample was extracted with ether. Almost all α-hydroximinopropionic

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* Part III; Y. Sato, T. Meshi and T. Takahashi, This Bulletin, 33, 1146 (1960).

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

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

acid being soluble in ether, was removed from the discharged sample by filtration. The amount of residue was about 5 mg. and was assayed for tritium in a gas flow counter. Its specific activity was 0.66 μc./mg.; on the other hand, the specific activity of ether soluble part was 0.71 μc./mg.

Paper chromatography of the residue, using *n*-butanol-acetic acid-water (4 : 1 : 1) as developing solvent, was performed. Owing to the low energy of the β-rays from tritium, the radioactivity from tritium could not be measured directly on the paper chromatogram. The paper was cut into pieces, and the active products were extracted with water. Water was evaporated in stainless steel dishes, and the activity of the extracted products was measured in a gas flow counter. Fig. 2 shows the results of the paper chromatogram and the radioactivity. As a comparison, parallel runs were made with ordinary DL-alanine and α-hydroximinopropionic acid.

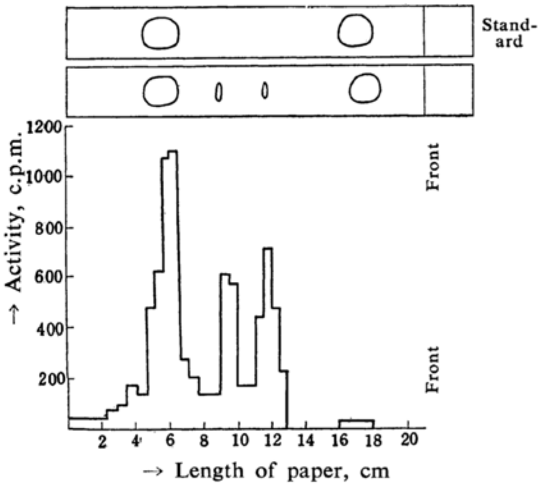

Fig. 2. Paper chromatography and the radioactivity of ether insoluble part. For comparison spots obtained with pure DL-alanine and α-Hydroximinopropionic acid are shown.

Four hundred μg. of ether insoluble part of sample was employed as a sample of paper chromatography. DL-Alanine, the unidentified product A and B and, α-hydroximinopropionic acid were obtained in the amounts and with activity values as shown in Table I.

TABLE I. SPECIFIC ACTIVITY OF THE PRODUCTS SEPARATED BY PAPER CHROMATOGRAPHY

| Compound | Activity c. p. m. | Amount μg. | Sp. act. μc./mg. | $R_f$ |
|---|---|---|---|---|
| DL-Alanine | 15600 | 20* | 4.4 | 0.28 |
| Unidentified product A | 4600 | 2* | 12.9 | 0.36 |
| B | 6450 | 2* | 17.2 | 0.45 |
| α-Hydroximino-propionic acid | 100 | 350** | 0.0016 | 0.80 |

* Determination by spraying with ninhydrin.
** Determination by spraying with Bromophenyl Blue.

## Discussion

In the previous reports[3], it was reported that specific activity of tritium labeled DL-alanine obtained by means of electric discharge was only about 0.02 μc./mg. when DL-alanine was used as the sample. However, in this paper, tritium labeled DL-alanine with specific activity of 4.4 μc./mg. was obtained by using α-hydroximinopropionic acid as the sample. A higher specific activity can be expected by using carrier free tritium gas.

## Summary

To obtain tritium labeled alanine of a high specific activity by means of electric discharge, α-hydroximinopropionic acid was employed in place of alanine. After electric discharge, tritium labeled α-hydroximinopropionic acid was removed by ether extraction and the residue was chromatographed. Tritium labeled alanine was obtained with a specific activity of 4.4 μc./mg. which was two hundred times as great as that obtained by the use of alanine.

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[3] Y. Sato, T. Meshi, T. Takahashi and N. Sugimoto, *Tanabe Seiyaku Kenkyû Nempô*, 5, (1) 57 (1960).