

## Ring Labeling of 2,4,5-T with Tritium<sup>1</sup>

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**P**HENOXY HERBICIDES are not commonly ring-labeled with  $C^{14}$  due to the difficulty of synthesis and consequent high cost per unit of radioactivity. Instead, phenoxy herbicides are usually given a  $C^{14}$ -carboxyl tag. A low-cost ring label is needed, however, for studies in which decarboxylation limits the usefulness of the carboxyl tag. The use of tritium has not been fully explored because of the tendency to exchange with other hydrogen, which could lead to erroneous conclusions regarding the material being traced. The purpose of this study was to develop a ring-tritium labeled preparation of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) which would not lose radioactive components during exposure to plant metabolism and extraction processes.

In order to insure that the label be restricted to the ring, the procedure for tritiation was applied to sodium 2,4,5-trichlorophenate (Na-2,4,5-TCP). This material was then condensed with chloroacetic acid to form the 2,4,5-T. The tritiation process employed was similar to that used by Olsen and Melander (1954), consisting of acid exchange with  $T_2O$  and  $H_2SO_4$ . The  $T_2O$  used in this experiment was of very low activity, having 0.5 mc/ml. One hundred milligrams of 2,4,5-TCP were refluxed with 2 ml  $T_2O$  and 20 ml 20 per cent fuming  $H_2SO_4$  ( $SO_3$ ) for 72 hours. This solution was then extracted with Skelly Solvent B and the extract filtered. The Skelly B was then distilled off in the presence of NaOH and  $H_2O$ , and the aqueous phase was filtered. The product, Na-2,4,5-TCP, was then condensed with sodium monochloroacetate at pH 10 to form 2,4,5-T(3,6-t). The aqueous solution was then acidified, cooled to  $0^\circ C$ . and filtered. The precipitate was then washed in ice cold dilute HCl and dried. The yield of 2,4,5-T was 123.5 mg, which was slightly over 95 per cent. Based on an approximate counter efficiency of 5 per cent with toluene with PPO (2,5-diphenyl oxazole) and POPOP (1,4-bis-2(5 phenyloxazole) benzene) as phosphors, the yield of radioactivity was roughly 1.2 microcuries, or 96 per cent of the theoretical maximum.

The test of lability of the tritium label was conducted with cotyledon-stage

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Norway maple seedlings. One hundred mg of the labeled 2,4,5-T as the sodium salt was dissolved in 200 ml water and used to water roughly 600 grams of cut seedlings which had been placed in vials. After seven days, the seedlings had consumed all of the radioactive solution plus the wash water. All seedlings showed signs of severe herbicide effect at this time, hence the assumption was made that the herbicide had been exposed to a certain amount of metabolic activity. The plants were then ground and heated with strong sodium hydroxide solution to extract the herbicide residue. Acidification of the basic extract to form the ether-soluble acid and liquid-liquid extraction with ether removed the acid and some other aromatic compounds of similar properties from the plant material. This plant extract was then chromatographed, with  $C^{14}$ 2,4,5-T as a control band. The extract from the band containing the 2,4,5-T was shown by gas chromatography to be 19.4 per cent 2,4,5-T, the remainder being of naturally occurring compounds with similar properties.

Since further isolation of the 2,4,5-T appeared impractical, a sample of the plant residue mixture was made up for counting. A small sample was used in order to minimize the quenching effect of the contaminants which were present in the counting samples in about four times the concentration of the 2,4,5-T. From the calculated weight of the 2,4,5-T in the extracted sample of .784 mg there was a net counting rate of 960.4 cpm, or a specific activity of 1225 cpm/mg. From the original preparation of 2,4,5-T, a sample of 2.485 mg made up in the same counting solution gave 3041.9 cpm, or 1224 cpm/mg. The negligible difference of one count per milligram per minute indicates that the tritium-labeled 2,4,5-T synthesized in this manner is non-labile as treated in these tests. While it is possible that some tritium might have been present in the contaminants, it seems unlikely that the activity supplied by the small fraction of the total exchange opportunities would contribute significantly to the specific activity of the mixture.

The only compound recovered and tested for radioactivity in this experiment was the 2,4,5-T itself. Degradation products were not isolated, and some caution may be necessary when evaluating the distribution of the metabolic products of 2,4,5-T until the stability of the tritium label on the degradation products can be demonstrated. It appears quite likely, in view of the stability of the ring label of toluene (Ramsey and Winkle, 1959), that the ring label will remain relatively stable as long as the ring remains intact. Non-aromatic degradation products may be relatively non-labile, also, since carbon-tritium bonds have been shown to be relatively stable (Melander, 1960; Olsen and Melander, 1954). If the original degradation proceeds

according to Moses and Calvin (1959), the tritium may be transferred to an exchangeable bond.

It is acknowledged that the process of labeling in this experiment was relatively cumbersome and yielded a product which was of low specific activity. However, the synthesis of higher activity materials should be practical if higher activity  $T_2O$  were used or if  $T_2SO_4$  were used as the source of tritium. Any extraneous hydrogen source used in this process serves further to dilute the tritium, hence to lower the specific activity of the product.

Considering the low cost of tritium coupled with the increasing use of liquid-scintillation counting, this preparation of non-labile, ring-tagged phenoxy compounds should prove of practical significance. Moreover, the knowledge that the ring label with tritium is non-labile means that more practical methods for the production of this class of compound can be sought with the assurance that a useful product will result.

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#### *Literature Cited*

- Melander, L. 1950. On the mechanism of electrophilic substitution; an investigation by means of the isotopic mass effect on reaction velocity. *Arkiv for Kemi*, 2: 213-292.
- Moses, B., and M. Calvin. 1959. Photosynthesis studies with tritiated water. *Biochim. et Biophys. Acta*, 33: 397.
- Olsen, S., and L. Melander. 1954. Acid-catalyzed H-T exchange in toluene. *Acta Chem. Scand.*, 8: 523-525.
- Ramsey, J. C., and W. Winkle. 1959. II. Preparation of tritiated benzene. Unpublished typescript. Oregon State University, Department of Chemistry, Corvallis, 10 p.