

## SYNTHESIS OF SOME USEFUL TRITIUM LABELLED AUXINS

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### SUMMARY

The synthesis of six useful auxins labelled with tritium is described. The following compounds were prepared: 3-indoleacetic acid-5-<sup>3</sup>H (28.9 Ci-1.07 TBq/mmol), 3-indolebutyric acid-5-<sup>3</sup>H (7.3 Ci-270 GBq/mmol), 1-naphthylacetic acid-4-<sup>3</sup>H (27.6 Ci-1.02 TBq/mmol), 2,4-dichlorophenoxyacetic acid-5-<sup>3</sup>H (18.5 Ci-685 GBq/mmol), 2(2,4-dichlorophenoxy-5-<sup>3</sup>H)-propionic acid (20.7 Ci-766 GBq/mmol), 2(2,4-dichlorophenoxy)-propionic acid-3-<sup>3</sup>H (0.39 Ci-14.4 GBq/mmol), and 4-chlorophenoxyacetic acid-2-<sup>3</sup>H (13.3 Ci-492 GBq/mmol).

Key words: tritium, auxins, labelling, plant growth hormones

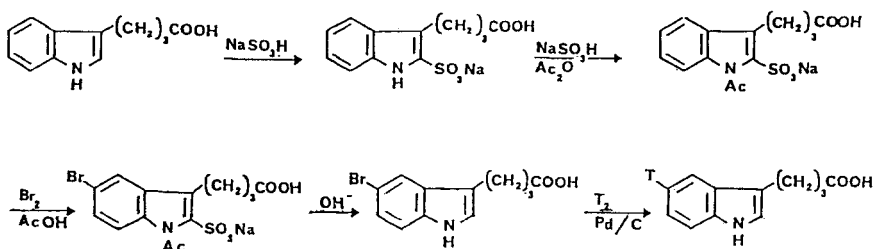
### INTRODUCTION

Auxins are known as chemical messengers influencing many patterns of plant development. Almost every dynamic part of plant growth and development seems to be affected by auxin<sup>(1-3)</sup>. The range of chemical materials which possess growth-stimulating abilities may be listed in four main chemical groups: indole, naphthyl, and phenoxy derivatives of C<sub>2</sub> to C<sub>4</sub> carboxylic acids, and benzoic acid derivatives. The indole and naphthyl derivatives are used principally for rooting and fruit set, the phenoxy derivatives are fine herbicides and modifiers of fruit growth, and the benzoic derivatives are herbicides<sup>(1-3)</sup>. In this work we have prepared, by appropriate methods of synthesis, some of the most active auxins labelled with tritium, to be used as tracers by investigators interested in reaching a better understanding of the effects of these growth hormones.

## RESULTS AND DISCUSSION

3-Indoleacetic acid-5- $^3\text{H}$  was tritiated, starting from the commercially available 5-bromo derivative, giving a product with a specific activity of 28.9 Ci-1.07 TBq/mmol.

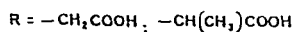
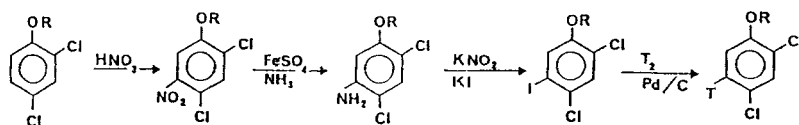
5-Bromo-3-indolebutyric acid<sup>(2)</sup> (Scheme I) and 4-bromo-1-naphthylacetic acid<sup>(3)</sup> were synthesized as precursors for the preparation of the tritium labelled compounds 3-indolebutyric acid-5- $^3\text{H}$  (7.3 Ci-270 GBq/mmol), and 1-naphthylacetic acid-4- $^3\text{H}$  (27.6 Ci-1.02 TBq/mmol), respectively.



Scheme I

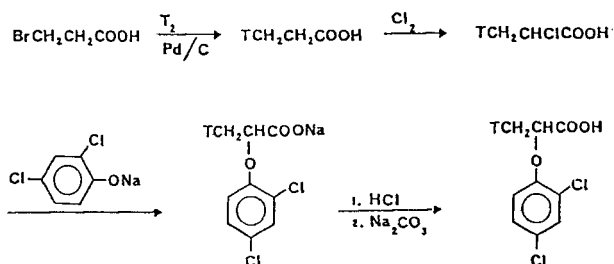
The relatively low specific activity achieved for indolebutyric acid is probably due to a dilution effect of the final tritiated product by the unreacted starting material (Scheme I) which has not been separated during the different steps of synthesis.

Iodinated precursors for the preparation of 2,4-dichlorophenoxyacetic acid-5- $^3\text{H}$  and 2(2,4-dichlorophenoxy-5- $^3\text{H}$ )-propionic acid were synthesized following Scheme II<sup>(4)</sup>, giving final radioactive products of 18.0 Ci-666 GBq/mmol and 20.7 Ci-766 GBq/mmol, respectively. The advantage of applying deiodination instead of debromination as the tritiation step, was the complete deactivation of Pd catalyst occurring simultaneously with the formation of HI/TI. The poisoning of the catalyst after the iodine atom has reacted results in a selective dehalogenation step, avoiding any further undesired dechlorination. Such deactivation of the catalyst is not observed when HBr/TBr is formed.



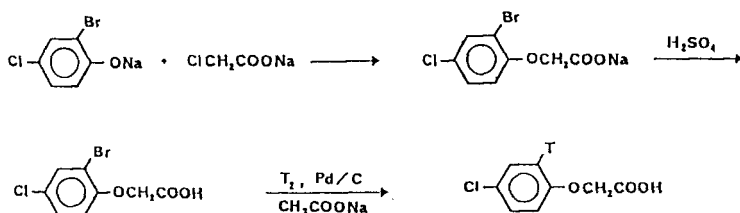
Scheme II

Labelling of 2,4-dichlorophenoxypropionic acid in the alkyl moiety giving 2(2,4-dichlorophenoxy)-propionic acid-3- $^3\text{H}$  was achieved (Scheme III) starting from tritiated propionic acid (18.9 Ci-699 GBq/mmol), which was diluted about 30 fold by unlabelled propionic acid before further steps<sup>(4)</sup> were performed, resulting in a final product of specific activity 0.39 Ci-14.4 GBq/mmol.



Scheme III

In the case of 4-chlorophenoxyacetic acid-2- $^3\text{H}$ , the precursor was prepared<sup>(5)</sup> as in Scheme IV, and the final product was obtained by tritioderobromination in the presence of sodium acetate<sup>(6)</sup>, which enabled selective debromination and avoided dechlorination. The final product was obtained with a specific activity of 13.3 Ci-492 GBq/mmol.



Scheme IV

## EXPERIMENTAL

General

Chemicals were from commercial sources (Aldrich); tritium gas (> 98%) was purchased from Radium Chemie, Teufen (Switzerland). Silica gel thin-layer chromatography (tlc) plates (Merck) of 0.25 mm thickness were used for preparative purification of radioactive products. Radiochemical purity was determined by radioscanning of analytical tlc plates or analytical  $\text{C}_{18}$  reverse phase (RP) plates (Merck), developed with different solvent systems. The amount of labelled compound for

specific activity calculations was determined by UV absorption as referred to a known standard. Ultra-violet spectra were recorded on a Gilford 2600 spectrophotometer and NMR spectra were performed in  $\text{CDCl}_3$  with TRMS as internal standard on a Varian EM 360 spectrometer. Radiochemical purity was determined by radiochromatogram scanning on tlc plates on a Berthold Duennschicht Scanner II, LB 2722; total and specific activity were measured on a Packard Tri-Carb 1000 Liquid Scintillation Analyzer. All the reductive tritiodehalogenations were performed on a capillary vacuum manifold, using usual techniques for tritium gas transfers, freezing of solutions in liquid nitrogen before gas evacuation, and methanol washes for discarding of tritium labiles.

3-Indoleacetic acid-5- $^3\text{H}$  - 12.2 mg (0.048 mmol) 5-bromoindoleacetic acid are dissolved in 0.5 mL dioxane and 0.1 mL triethylamine, 12.7 mg Pd/C 10% and a magnetic stirrer bar are added, the reaction vessel is connected to the manifold, and 30 Ci-1.11 TBq are introduced. After 1/2 hour, 4 Ci-148 GBq are consumed, the reaction is stopped and the products are worked up as usual, giving 555 mCi-20.5 GBq of chemically and radiochemically pure indoleacetic acid-5- $^3\text{H}$ , 28.9 Ci-1.07 TBq/mmol; t.l.c. solvent system: isopropanol:ammonium hydroxide:water (80:5:15)  $R_f$ : 0.58.

3-Indolebutyric acid-5- $^3\text{H}$  - The reaction is performed as for 3-indoleacetic acid-5- $^3\text{H}$ . Quantities of reactants are: 40 mg (0.14 mmol) 5-bromo-3-indolebutyric acid, 1 mL dioxane, 1 mL triethylamine, 20 mg Pd/C 10%, 25 Ci-925 GBq tritium gas. The reaction is left overnight and 8 Ci-296 GBq are consumed. Purification is performed by tlc using isopropanol:ethyl acetate:ammonium hydroxide:water (35:45:10:10) as the solvent system, and the product is extracted with ethanol giving 0.44 Ci-16.3 GBq of > 98% pure 3-indolebutyric acid-5- $^3\text{H}$ . The solvent systems used are: chloroform:acetic acid (95:5),  $R_f$ : 0.71; isopropanol:ammonium hydroxide:water (80:5:15),  $R_f$ : 0.79; isopropanol:ethyl acetate:ammonium hydroxide:water (35:45:10:10),  $R_f$ : 0.48.

1-Naphthylacetic acid-4- $^3\text{H}$  - The reaction is performed as for 3-indoleacetic acid-5- $^3\text{H}$ . Quantities of reactants are: 18 mg (0.068 mmol) 4-bromo-1-naphthylacetic acid, 0.75 mL dioxane, 0.2 mL triethylamine, 20 mg Pd/C 10%, 35 Ci-1.3 TBq tritium gas. After 1 hour, 8 Ci-296 GBq are consumed and 1.4 Ci-51.8 GBq of > 98% pure product obtained without further purification. Tlc solvent systems used are: ethyl acetate,  $R_f$ : 0.36; carbon tetrachloride:ethyl acetate (3:7),  $R_f$ : 0.22.

2,4-Dichlorophenoxyacetic acid-5-<sup>3</sup>H - The reaction is performed as for 3-indoleacetic acid-5-<sup>3</sup>H. Quantities of reactants are: 30 mg (0.086 mmol) 2,4-dichloro 5-iodophenoxyacetic acid, 1 mL water, 0.5 mL triethylamine, 20 mg Pd/C 10%, and 30 Ci-1.11 TBq tritium gas. After 220 min., 6 Ci-22 GBq are consumed and 1.65 Ci-61.1 GBq of crude product obtained. Purification is performed by tlc using isobutanol saturated with 10% ammonium hydroxide as the solvent system, and the product is extracted with methanol giving 0.706 Ci-26.1 GBq of > 98% pure 2,4-dichlorophenoxyacetic acid-5-<sup>3</sup>H with a specific activity of 18.5 Ci-685 GBq/mmol. Tlc solvent systems used are: isobutanol saturated with 10% ammonium hydroxide, R<sub>F</sub>: 0.35; tert.butanol:methyl-ethylketone:ammonium hydroxide:water (40:30:15:15), R<sub>F</sub>: 0.75; chloroform:acetic acid (95:5), R<sub>F</sub>: 0.50.

2(2,4-Dichlorophenoxy-5-<sup>3</sup>H)-propionic acid - The reaction is performed as for 3-indoleacetic-5-<sup>3</sup>H. Quantities of reactants are: 36 mg (0.1 mmol) 2(2,4-dichloro-5-iodophenoxy)-propionic acid, 1 mL dioxane, 0.5 mL triethylamine, 20 mg Pd/C 10%, and 20 Ci-740 GBq tritium gas. After 1 hour, 6 Ci-222 GBq are consumed and 1.46 Ci-54.0 GBq of crude product obtained. Purification is performed by tlc using tert.butanol: methylethylketone:ammonium hydroxide:water (40:30:15:15) as solvent system and the product is extracted with methanol giving 1.2 Ci-44.4 GBq of > 98% pure 2(2,4-dichlorophenoxy-5-<sup>3</sup>H)-propionic acid with a specific activity of 20.7 Ci-766 GBq/mmol. Tlc solvent systems used are: isobutanol saturated with 10% ammonium hydroxide, R<sub>F</sub>: 0.40; chloroform:acetic acid (95:5), R<sub>F</sub>: 0.73; tert.butanol:methylethylketone:ammonium hydroxide:water (40:30:15:15), R<sub>F</sub>: 0.84; methanol:water (7:3) on C<sub>18</sub> RP plate, R<sub>F</sub>: 0.45.

2(2,4-Dichlorophenoxy)-propionic acid-3-<sup>3</sup>H - To 62 mg (0.4 mmol) 3-bromopropionic acid dissolved in 0.7 mL dioxane + 0.35 mL triethylamine, 20 mg Pd/C 10% are added and 30 Ci-1.11 TBq tritium gas are introduced. The reaction is left overnight and 28 Ci-1.04 TBq are consumed giving 8 Ci-296 GBq of crude propionic acid-3-<sup>3</sup>H with a minimum calculated specific activity of 18.9 Ci-699 GBq. Dioxane is carefully removed by evaporation. The radioactive propionic acid is then dissolved in 0.6 mL POCl<sub>3</sub>, diluted with 0.9 mL (888 mg, 12 mmol) unlabelled propionic acid, and the solution is transferred into a 1 L chlorination reactor equipped with a magnetic stirrer. The solution is left overnight under a chlorine atmosphere at 60°C, and protected from light to avoid chlorination of the methylene function. The mixture is cooled to -5°C and 7.5 mL of 10N NaOH is added. 1.4 mL

solution of 10N NaOH containing 2.3 gr (14 mmol) of 2,4-dichlorophenol are added slowly, the reaction mixture is heated to reflux for 4 hours and cooled thereafter overnight. The precipitate is filtered and discarded. The aqueous solution is acidified with conc. HCl to pH 1 and extracted with ether. The ether fraction is concentrated and extracted with an aqueous solution of Na<sub>2</sub>CO<sub>3</sub>. The aqueous phase contains the sodium salt of the final crude product (4.5 Ci-167 GBq), while the unreacted phenol derivative remains in the ether phase. The aqueous phase is acidified again with HCl to pH 1 and the crystalline precipitate is filtered, washed with a minimum volume of water, and dried in a dessicator under PCl<sub>5</sub>, giving 2.95 Ci-109 GBq of > 98% pure 2(2,4-dichlorophenoxy)-propionic acid-3-<sup>3</sup>H with a specific activity of 390 mCi-14.4 GBq/mmol. The solvent systems used are: isobutanol saturated with 10% ammonium hydroxide, R<sub>F</sub>: 0.33; chloroform :acetic acid (95:5), R<sub>F</sub>: 0.56; methanol:water (7:3) on C<sub>18</sub> RP plate, R<sub>F</sub>: 0.62.

4-Chlorophenoxyacetic acid-2-<sup>3</sup>H - The reaction is performed as for 3-indoleacetic-5-<sup>3</sup>H. Quantities of reactants are: 26.4 mg (0.1 mmol) 2-bromo-4-chlorophenoxyacetic acid, 1 mL dioxane, 8.1 mg (0.1 mmol) sodium acetate anhydrous, 16.2 mg Pd/C 10%, 25 Ci-925 GBq tritium gas. After 2 hours, 5 Ci-185 GBq are consumed and 725 mCi-2.69 GBq of radiochemically > 98% pure product obtained, together with NaBr salt which is removed by tlc using butanol:acetic acid:water (50:25: 25) as solvent system. The product is extracted (R<sub>F</sub>: 0.79) with methanol and a specific activity of 13.3 Ci-492 GBq/mmol is reached.

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