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# SYNTHESIS OF CARBON-14 LABELED 3-(*p*-CHLOROPHENYL)-1,1-DIMETHYLUREA

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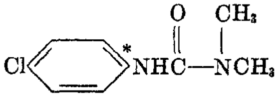
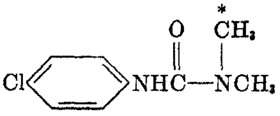
Discovery of the new herbicide 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) (1) is being followed by an investigation of its mode of behavior in plants. To facilitate this study, two radioactive species of CMU labeled with carbon-14 have been synthesized. One was labeled in the benzene ring at the carbon attached to nitrogen (I); the other at a methyl carbon (II).



I



II

The ring-labeled CMU was prepared from aniline-1-$C^{14}$, obtained commercially as the hydrochloride with an activity of 0.38 mc./mM. The method comprised chlorination of the acetyl derivative with N-chlorosuccinimide, deacetylation, phosgenation of the *p*-chloroaniline to the isocyanate, and reaction of the latter with dimethylamine. The over-all yield was 56% of recrystallized 3-(*p*-chlorophenyl-1-$C^{14}$)-1,1-dimethylurea. The activity level indicated one molecule in about 200 to be radioactive.

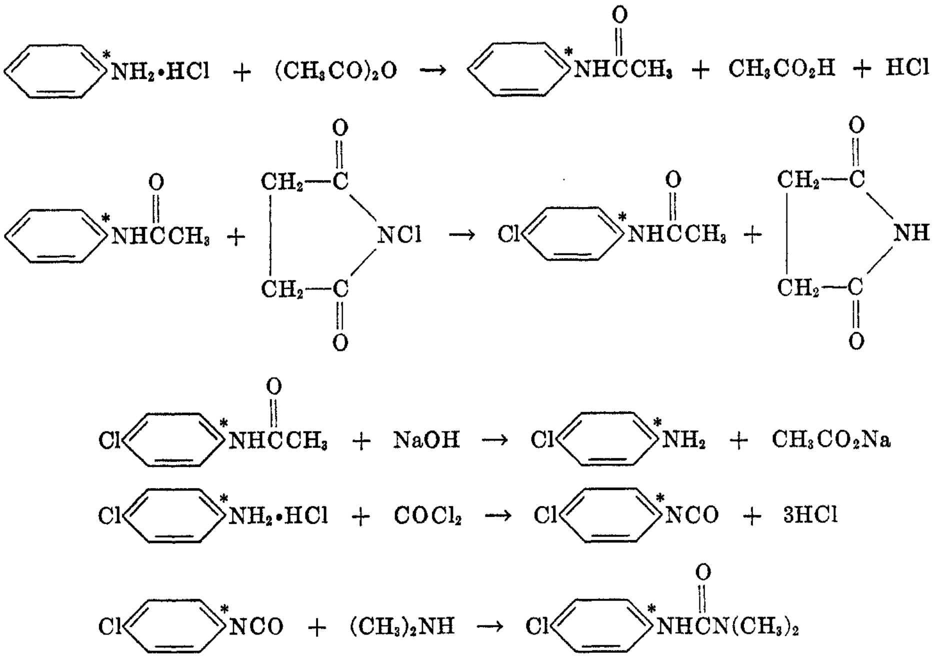
The synthesis of CMU labeled in a methyl group required radioactive dimethylamine. This compound was prepared by methylating N-tosylmethylamine with methyl-$C^{14}$ iodide followed by hydrolysis with aqueous hydrobromic acid. The dimethylamine was liberated from its salt and reacted with *p*-chlorophenyl isocyanate. The over-all yield was 36% of 3-(*p*-chlorophenyl)-1-methyl-1-(methyl-$C^{14}$)urea with an activity level of 1.06 mc./mM. Thus, one molecule in about 70 was labeled.

Both preparations were aimed at obtaining as high a level of activity as possible from convenient, commercially available starting materials. This was necessary because the extremely high phytotoxicity of CMU toward some plants severely restricted concentrations that could be employed in experiments of more than a few days' duration. The ring-labeled CMU of specific activity 0.38 mc./mM. (1.9 mc./g.) enables the detection of as little as 0.25 p.p.m. in live plant parts. With the methyl-labeled material of specific activity 1.06 mc./mm. (5.3 mc./g.) the lower limit can be extended to approximately 0.1 p.p.m. A recent paper by Logan and Odell (2) describes the synthesis of carbonyl-$C^{14}$ labeled CMU with a specific activity of approximately 0.5 mc./g. Thus, the three principal segments of the CMU molecule have now been labeled with carbon-14 for use in plant investigations.

## EXPERIMENTAL

### A. SYNTHESIS OF CARBON-14 RING-LABELED CMU

The steps employed in the synthesis of the ring-labeled CMU were as follows:



1. *Acetylation of aniline-1-$C^{14}$ hydrochloride.* Aniline-1-$C^{14}$ hydrochloride (sp. activity 0.39 mc./mM.) (1.36 g., 10.5 mM.), obtained from Tracerlab Inc., was rinsed with 16 ml. of carbon tetrachloride into a 50-ml. reaction flask fitted with a short column topped with a cold finger condenser. Acetic anhydride (3 g.) was added and the mixture was heated at the reflux with agitation by a magnetic stirrer for 25 minutes. An additional 3 g. of acetic anhydride was added and the refluxing was continued for 20 minutes. Hydrogen chloride was evolved during the reaction. After cooling, 10 ml. of methanol was added to esterify the excess acetic anhydride and acetic acid. Methyl acetate was slowly distilled off and water was removed by azeotropic distillation with 30 ml. of carbon tetrachloride introduced in several portions.

The use of carbon tetrachloride as a solvent in the acetylation of aniline hydrochloride was found to circumvent formation of diacetanilide, an oily contaminant that resulted when acetylation was attempted with acetic anhydride alone. In pilot experiments, the above procedure gave quantitative yields of essentially pure acetanilide directly from aniline hydrochloride.

2. *Chlorination of acetanilide-1′-$C^{14}$.* To the dry radioactive acetanilide remaining in the reaction flask was added 1.55 g. (11.6 mM.) of recrystallized N-chlorosuccinimide (m.p. 147.5–148.5°, white scales from acetic acid) together with 7 ml. of acetic acid. Gentle warming initiated an exothermic reaction, which carried the temperature to 60° while the flask was being cooled in an ice bath. The mixture was then maintained at 82° with stirring until only a trace of unreacted N-chlorosuccinimide remained (45 minutes), as indicated by a faintly positive test with moist starch-potassium iodide paper.

The reaction product, containing both the *ortho* and *para* isomers of the radioactive chloroacetanilide, was concentrated to a paste by carefully distilling the acetic acid with magnetic stirring under reduced pressure. Most of the *ortho* isomer was separated by adding 10 ml. of *n*-hexane and withdrawing the solution through a small, fine porosity filter stick. This extraction was repeated and the final traces of *n*-hexane removed from the flask by heating at 70° while passing through a slow stream of nitrogen. Evaporation of the combined *n*-hexane extracts gave 0.35 g. (20% yield) of crude 2′-chloroacetanilide-1′-$C^{14}$. The succinimide by-product was removed from the 4′-chloroacetanilide-1′-$C^{14}$ remaining in the flask by mixing with 5 ml. of water and aspirating through a filter stick.

The 10% excess of N-chlorosuccinimide used in this chlorination was found in pilot experiments to give the best yield of 4′-chloroacetanilide. A study of solvents, which included carbon tetrachloride, chloroform, chlorobenzene, dimethylformamide and acetic acid, indicated that the latter combined the most desirable properties.

Complete removal of the chlorinating agent prior to the hydrolysis step was found to be essential. The presence of any unreacted N-chlorosuccinimide resulted in highly colored aniline oxidation products. Traces of N-chlorosuccinimide can be detected easily with moist starch-potassium iodide paper, and can be eliminated with sodium bisulfite. An alternative method consists of treating the reaction mixture with isopropyl alcohol and warming slightly. The isopropyl alcohol is oxidized to acetone.

Separation of most of the 2′-chloroacetanilide in this step depends upon the relatively greater solubility of the *ortho* isomer in *n*-hexane. 4′-Chloroacetanilide is essentially insoluble.

3. *Hydrolysis of 4′-chloroacetanilide-1′-$C^{14}$.* To the aqueous paste of 4′-chloroacetanilide-1′-$C^{14}$ remaining in the reaction flask was added a solution of 2.1 g. (5.25 mM.) of sodium hydroxide in 4 ml. of water and 10 ml. of methanol. A few drops of 5% aqueous sodium bisulfite solution was added to decompose a trace of N-chlorosuccinimide and the mixture was heated at the reflux for 1.5 hrs. After the mixture had stood for an additional 1.5 hrs., 10 ml. of water was added and the methanol was distilled over. Upon cooling, the oil phase crystallized.

The *p*-chloroaniline-1-$C^{14}$ was isolated by extraction with methylene chloride in a small separatory-funnel. The combined methylene chloride extracts were returned to the reaction flask and concentrated. Then 25 ml. of dioxane was added and the remaining traces of methylene chloride were removed by distillation.

An alternative procedure for isolating the chloroaniline comprised separation of the aqueous alkali from the crystallized product by aspiration through a filter stick followed by washing with water.

4. *Phosgenation of p-chloroaniline-1-$C^{14}$ and reaction of the radioactive p-chlorophenyl isocyanate with dimethylamine.* Removal of traces of water from the dioxane solution of the *p*-chloroaniline-1-$C^{14}$ was accomplished by distilling until the vapor temperature reached 100°. The solution was cooled to 5° and dry hydrogen chloride was passed in until present in excess. The *p*-chloroaniline hydrochloride precipitated as fine fluffy crystals. It is essential that the *p*-chloroaniline be completely converted to its hydrochloride to avoid formation of 4,4′-dichlorocarbanilide during phosgenation. The slurry of crystals then was heated and dry, chlorine-free phosgene passed in with magnetic stirring while maintaining the temperature at 75° for 20 minutes. The phosgene was purified by bubbling through cotton seed oil and sulfuric acid (3). At the end of the phosgenation, a clear, pale amber solution of *p*-chlorophenyl-1-$C^{14}$ isocyanate resulted. Excess phosgene and hydrogen chloride were removed by slowly distilling a small portion of the solvent.

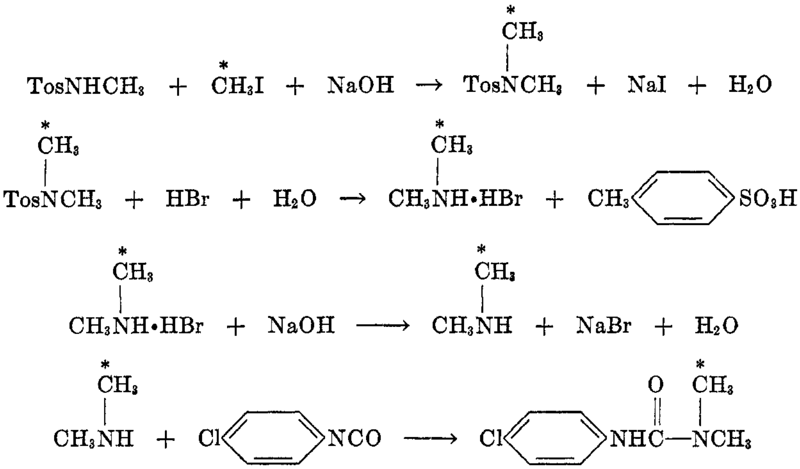
The isocyanate solution was cooled to 10° and anhydrous dimethylamine was passed in until present in excess. The reaction mixture was heated for a short time to 55°, and the dioxane together with excess dimethylamine was removed by evaporation under reduced pressure. The crystalline residue was dissolved in 75 ml. of methylene chloride, and the solution treated with Darco. After filtering through a sintered glass funnel, the solution was evaporated to a volume of about 5 ml. by warming under a jet of nitrogen. To the resulting slurry of crystals, 20 ml. of *n*-hexane was added and the mixture was cooled.

The crystals were drained on a sintered glass filter, washed with 10 ml. of cold *n*-hexane, and dried under a heat lamp while drawing through a slow stream of air. The yield of 3-(p-chlorophenyl-1-$C^{14}$)-1,1-dimethylurea was 1.164 g. (56%) of fine white needles. The capillary melting point was 170.2–171.3° after shrinking at 169.5°. Since no dilution of radioactivity had occurred in this procedure, the molar specific activity was the same as that of the starting material, *i.e.*, 0.38 mc./mM.

Evaporation of the *n*-hexane washes gave 0.242 g. of an oil which crystallized on standing. This by-product appeared to be crude 3-(*o*-chlorophenyl)-1,1-dimethylurea (12% yield). The total radioactive products isolated accounted for 88% of the aniline-1-$C^{14}$ hydrochloride used.

### B. SYNTHESIS OF METHYL-$C^{14}$ LABELED CMU

The following reaction sequence was employed for the preparation of methyl-labeled CMU.



1. *Methylation of N-tosylmethylamine with methyl-$C^{14}$ iodide.* Methyl-$C^{14}$ iodide with a specific activity of 1.06 mc./mM. was supplied by Tracerlab in ampoules equipped with a break-off seal inside a delivery tube. Four ampoules containing a total of 0.543 g. (3.83 mM.) of methyl-$C^{14}$ iodide were used in this experiment. A plunger made from iron rod sealed in glass tubing was placed inside the delivery tube of each ampoule and the tubes were sealed to a manifold leading into a 50-ml. reaction flask. The ampoules were cooled with liquid nitrogen and the seals were broken by manipulating the plunger with a magnet. A slow stream of nitrogen was passed through the manifold and by alternately warming and cooling the ampoules the methyl iodide was transferred to the reaction flask, which contained a solution of 0.814 g. (4.4 mM.) of N-methyl-*p*-toluenesulfonamide (4) and 0.176 g. (4.4 mM.) of sodium hydroxide in 5.2 ml. of water and 4 ml. of methanol. During the addition, the reaction mixture was held at 25° and was stirred with a magnetic stirrer coated with "Teflon" tetrafluoroethylene resin. The effluent gas from the reaction flask was passed through a U-tube cooled with liquid nitrogen to trap any unreacted methyl iodide. After the transfer from the ampoules was completed, the U-tube trap was attached to the gas inlet tube. A second trap was attached to the effluent side of the flask and the condensate was passed back into the reaction mixture. This procedure was repeated five times with progressive increase in the reaction temperature up to 55° to insure complete utilization of the radioactive methyl iodide.

One milliliter of 10% aqueous sodium hydroxide was added to the reaction mixture and the methanol was removed by distillation. The distillate contained essentially no radioactivity, indicating complete utilization of the methyl iodide. The residue was cooled until the oil crystallized, and the aqueous liquid phase containing the excess N-methyl-*p*-toluenesulfonamide was removed through a small filter stick. The crystals were washed twice with 2% aqueous sodium hydroxide solution in the same manner. After washing twice with water, the N-tosylmethyl-$C^{14}$-methylamine was hydrolyzed.

2. *Hydrolysis of N-tosylmethyl-$C^{14}$-methylamine.* To the crystals of N-tosylmethyl-$C^{14}$-methylamine remaining in the flask was added 8 ml. of 48% hydrobromic acid. The mixture was heated at the reflux for 1.5 hours under an atmosphere of nitrogen. The toluenesulfonamide gradually dissolved giving a clear, pale yellow solution containing the radioactive dimethylamine hydrobromide and *p*-toluenesulfonic acid.

3. *Liberation of the radioactive dimethylamine and reaction with p-chlorophenyl isocyanate.* The reaction flask containing the radioactive dimethylamine hydrobromide solution was connected through a reflux condenser and a small drying tower packed with solid potassium hydroxide to a second 50-ml. reaction flask containing 6 ml. of dry *n*-hexane. The apparatus was flushed with dry nitrogen and protected from atmospheric moisture. The second reaction flask was cooled with a Dry Ice-acetone bath and connected to a U-trap cooled with liquid nitrogen.

With ice-cooling and magnetic stirring, 20 g. of 50% aqueous sodium hydroxide was added to the dimethylamine hydrobromide solution in the first reaction flask. The liberated radioactive dimethylamine was swept out by a slow stream of nitrogen, while the contents of the flask were gradually heated to the reflux temperature over a period of 1.5 hours. Refluxing was continued for 15 minutes to insure complete removal of the amine. A solution of 0.82 g. (5.35 mM.) of freshly distilled *p*-chlorophenyl isocyanate in 9 ml. of dry *n*-hexane was then added to the methyl-$C^{14}$-methylamine solution in the second reaction flask. Condensate in the U-trap was swept back into the reaction zone with nitrogen and the mixture was warmed gradually to 45°. The radioactive CMU precipitated in the form of fine white crystals.

The slurry of crystals was cooled to room temperature and the liquid phase was drawn off through a filter stick. Because of the presence of excess *p*-chlorophenyl isocyanate, exposure of the reaction mixture to atmospheric moisture must be prevented to avoid contamination of the product with 4,4′-dichlorocarbanilide. The crystals were washed twice in the same manner with *n*-hexane and finally rinsed into a small sintered glass funnel. After drying over phosphoric anhydride, there was obtained 0.275 g. of fine white crystals which melted at 170–171° as compared with 170.5–171.5° for a standard CMU sample. The yield of 3-(*p*-chlorophenyl)-1-methyl-1-(methyl-$C^{14}$)urea was 36.3% of the theoretical, which was less than one-half of the expected 80–85% yield obtained in the pilot runs. An accidental mechanical loss occurred in transferring the methyl iodide from the ampoules to the reaction flask.

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## SUMMARY

Samples of 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) containing carbon-14 in two different positions have been synthesized. One was labeled with $C^{14}$ in the benzene ring and was obtained with an activity of 1.9 mc./g. from aniline-1-$C^{14}$ in 56% yield. The other, labeled with methyl-$C^{14}$, was prepared in 36% yield with an activity of 5.3 mc./g. starting with methyl-$C^{14}$ iodide.

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(4) Lukes and Preucil, *Collection Czechoslov. Chem. Commun.*, **10**, 384–398 (1938); *Chem. Abstr.*, **33**, 983 (1939).