# A Semimicro Preparation of *O,O*-Diethyl *O*-(3,5,6-Trichloro-2-pyridyl-2,6-$C^{14}$) Phosphorothioate

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A semimicroprocedure is described which gives high yields of *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl-2,6-$C^{14}$) phosphorothioate. Using this six-step synthesis, 40 mc. of isotopic potassium cyanide were converted to 7.5 mc. of tracer having a specific activity of 1.5 mc. per millimole. It assayed better than 99.5% by thin-layer chromatography. The yield is one half of the expected amount based on results from pilot experiments. With the 1.7 mc. of recovered precursor, 3,5,6-trichloro-2-pyridinol-2,6-$C^{14}$, a radiochemical yield of 23% was obtained.

Dursban insecticide (Dow Chemical Co.) contains *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate as the principal ingredient. Its remarkable insecticidal properties (*2*, *4*, *5*) have prompted others to investigate its fate in plants and animals (*10*, *11*). To aid these studies, this thiophosphate ester was synthesized with radioactive carbon in the pyridine moiety.

Of the several synthesis routes investigated, the one chosen for the tracer preparation labeled the molecule in the 2- and/or 6-ring position. Glutarimide (VI) was prepared from isotopic potassium cyanide via glutaronitrile (V) (Figure 1, *B*) by established methods. This procedure requires more time than an alternate route (Figure 1, *A*) reported by Pichat, Baret, and Audinot (*7*), but it gives consistently high yields. The latter method needs carefully controlled conditions during the condensation of potassium cyanide with butyrolactone (I) to maintain acceptable yields of glutaric acid (III). The authors preferred the glutaronitrile route because it was easily prepared (*12*) from 1,3-dibromopropane (IV) and simply converted (*1*) to glutarimide with one less step. The product obtained in this manner was readily purified by recrystallization from alcohol and sublimation in vacuo.

## *Materials and Methods*

Potassium cyanide-$C^{14}$, 40 mc. at 25 mc. per millimole, was purchased from New England Nuclear Corp., Boston, Mass., and diluted with Mallinckrodt analytical reagent to 50.1 mmoles. Purification of this starting material was not necessary.

1,3-Dibromopropane (Eastman Kodak Co., Rochester, N. Y.) and *O,O*-diethyl phosphorochloridothioate (Victor Chemical Co., Detroit, Mich.) were distilled prior to use.

The chlorinated pyridines and pyridinols used in the chromatography studies were obtained from the E. C. Britton Laboratory, The Dow Chemical Co., Midland, Mich. These are research chemicals whose authenticity was proved by elemental analysis and one or more spectroscopic methods.

**Thin-Layer Chromatography.** Glass plates, 20 × 20 × 0.4 cm., were prepared for coating by careful degreasing in a solvent solution of Dowclean 10 (Dow Chemical Co., Midland, Mich.). The substrate was prepared by shaking together 30 grams of silica gel G (Brinkman Instruments, Westbury, N. Y.) and 60 to 70 ml. of distilled water. This suspension was applied to the plates immediately after mixing, using a Desaga-type spreader. Five plates were coated at once with a layer of absorbent 250 microns thick. After air drying overnight, they were activated by heating in an oven at 110° C. for 1 hour. The plates were cooled and stored in a cabinet over a desiccant prior to use.

Solutions of samples to be analyzed were spotted 1.5 to 2.0 cm. from the edge of the plate at 15- to 20-mm. intervals. The edge selected for the starting line was chosen so that the development would be in the same direction as the application of the substrate. The plates were developed in tanks containing benzene, dioxane, and acetic acid (90:10:4 v./v./v.) ascendingly without prior equilibration.

The location of compounds on the developed chromatograms was determined by exposing the air-dried plates to ultraviolet light and by autoradiography. The autoradiograms were made by exposing Kodak Type AA film (Eastman Kodak Co., Rochester, N. Y.) to the plates for a 24-hour period. The following $R_f$ values were determined from authentic samples: glutarimide, 0.32; 3,6-dichloro-2-pyridinol, 0.74; 3,5,6-trichloro-2-pyridinol, 0.82; *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate, 0.90.

Quantitative determinations of radioactivity on the developed chromatograms were made by removing segments of the substrate to counting sample bottles where they were suspended in liquid scintillator solution. Sections covering an area of 1 × 2 cm. were generally taken. To assure quantitative transfers, the plates were sprayed with Neatan (E. Merck AG, Darmstadt, Germany) and covered with transparent tape prior to removal. Assays were then made by comparing the activity of sections corresponding to the $R_f$ values of test samples with the sum of the activity of all the sections of a given chromatogram.

**Radiometric Determination.** Radioactivity determinations were made on a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Solid samples were dissolved in a suitable solvent (generally benzene) and counted in toluene scintillator solution. Chromatography samples were

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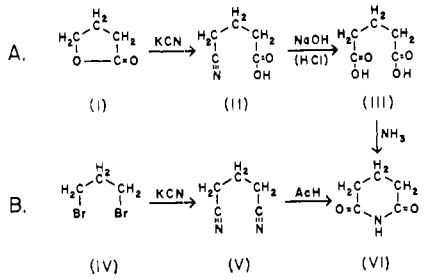

Figure 1. Synthesis routes to glutarimide

*A.* Butyrolactone. *B.* 1,3-Dibromopropane

counted in alcohol scintillator solution containing 3.5% Cab-O-Sil (Cabot Corp., Boston, Mass.). Both scintillator solutions were prepared from Liquiflour reagent (T. M. Pilot Chemical, Inc., Watertown, Mass.). Where possible, triplicate samples were prepared, one of which contained toluene-$C^{14}$ standard for counting efficiency determination.

*Experimental*

**Glutaronitrile-1,5-$C^{14}$.** Potassium cyanide-$C^{14}$ (3.265 grams, 50.1 mmoles), containing 40 mc. of activity, was dissolved in 4 ml. of water and stirred with a magnetic bar in a 100-ml. round-bottomed flask fitted with a reflux condenser. 1,3-Dibromopropane (5.051 grams, 25.1 mmoles), dissolved in 2 ml. of 2B absolute ethanol was added through the top of the condenser followed by three 1-ml. rinses of the same alcohol. The flask was heated in a 110° C. oil bath for 65 hours. After removal of the solvent by distillation in vacuo, the solid residue was extracted five times with 5-ml. portions of chloroform. Lyophylization of the extract left 2.32 grams (24.7 mmoles) of crude glutaronitrile-1,5-$C^{14}$ (Table I).

**Glutarimide-2,6-$C^{14}$.** The glutaronitrile-1,5-$C^{14}$ above was dissolved in 10 ml. of glacial acetic acid containing 2 drops of trifluoroacetic acid and transferred to a 60-ml. thick-walled Carius tube. After being cooled in a dry ice bath, the tube was evacuated and sealed. It was then heated in an oil bath maintained at 210° C. for 18 hours. The contents were next transferred to a sublimation flask where the acetic acid was removed by lyophylization. The solid residue was recrystallized from 10 ml. of methanol prior to sublimation at 100° to 110° C. and 1 mm. of mercury pressure. Boiling methanol was used to remove the sublimate from the cold finger. This was removed by evaporation in vacuo leaving a residue of pure glutarimide-2,6-$C^{14}$ which weighed 2.13 grams (18.85 mmoles) (Table I).

**2,3,6-Trichloropyridine-2,6-$C^{14}$.** Phosphorus pentachloride, 12 grams (56 mmoles), was mixed with the glutarimide-2,6-$C^{14}$ in a 100-ml. round-bottomed flask fitted with a reflux condenser and protected from atmospheric moisture by a calcium chloride drying tube. The flask was heated slowly in a water bath which was brought to 70° C. over a 20-minute period. Following the initial vigorous reaction, the mixture became a light yellow liquid. This was stirred and heated to 100° C. After 2 hours, the reaction was cooled in an ice bath, water was cautiously added to a volume of 50 ml., and the solid was slurried in the cold for 1 hour. The water phase was then removed via a filter stick. The remaining solid was dissolved in ether and dried over sodium sulfate. Removal of the ether gave 2.76 grams of a white crystalline solid.

**3,6-Dichloro-2-pyridinol-2,6-$C^{14}$.** The mixture of chloropyridines was dissolved in 20 ml. of aqueous *tert*-butyl alcohol (88% by volume) and transferred to a stainless steel bomb (5/8 inch i.d. × 10 inches long) containing 1.2 grams (30.2 mmoles) of sodium hydroxide. The bomb was heated in a rocker assembly for 18 hours at 150° to 160° C. After cooling, the reaction products were transferred to a 125-ml. round-bottomed flask with the aid of 50 ml. of hot water. The alcohol was removed by steam distillation through a Claisen head, leaving a clear, slightly colored water solution. This was filtered while still hot and acidified with 6*N* hydrochloric acid. The resulting slurry was agitated for 1 hour before cooling to 10° C. in an ice water bath.

**3,5,6-Trichloro-2-pyridinol-2,6-$C^{14}$.** Chlorine (15 mmoles) measured by a gas buret, was admitted to the pyridinol slurry through a glass tube submersed below the liquid level. By keeping the reaction temperature below 10° C. and with good stirring, complete absorption of the chlorine was achieved in 1 hour. After an additional 15 minutes, the solid product was collected on a filter and washed with two 15-ml. portions of distilled

**Table I. Comparison of Yields from Tracer and Pilot Preparations**

| | Mmoles of Product | | Yield for Step, % | | Yield from KCN, % | |
|---|---|---|---|---|---|---|
| | Pilot | Tracer | Pilot | Tracer | Pilot | Tracer |
| Potassium cyanide | 50.0 | 50.1 | ... | ... | 100 | 100 |
| Glutaronitrile | 23.7 | 24.7 | 94.8 | 98.4 | 94.8 | 98.4 |
| Glutarimide | 17.8 | 18.85 | 75.1 | 76.3 | 71.2 | 75.4 |
| Chloropyridines | 15.2 | 15.15 | 85.4 | 80.4 | 60.8 | 60.6 |
| 3,5,6-Trichloro-2-pyridinol | 10.9 | 6.40 | 71.6 | 42.2 | 43.4 | 25.6 |
| purified | | 5.39 | | 35.6 | | 21.6 |
| *O,O*-Diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate | 9.8 | 5.03 | 90.1 | 93.4 | 39.1 | 20.2 |

water. The crude trichloropyridinol was purified by recrystallization from aqueous methanol (75% by volume) and then from 10 ml. of benzene. The yield was 1.07 grams (5.39 mmoles) (Table I).

Additional tracer material was obtained by reworking the crude pyridinols (0.36 gram) recovered from the benzene and methanol mother liquors. Radiochemical analysis by TLC showed this to be about 85% trichloro- and 12% dichloropyridinol. These were dissolved in 20 ml. of 0.1*N* sodium hydroxide solution, filtered, and acidified with 6*N* hydrochloric acid. The resulting aqueous slurry was cooled and treated with 0.2 mmole of chlorine gas over 30 minutes. The product was collected and purified by recrystallization from 5 ml. of benzene containing 0.10 gram of carrier trichloropyridinol, and then from 4 ml. of 80% aqueous methanol. In this manner 0.28 gram (1.14 mmoles) was obtained having a reduced specific activity (0.97 mc. per mmole). A second crop of trichloropyridinol was obtained by recrystallizing 0.10 gram of carrier from the above mother liquors. This additional tracer material, amounting to 0.12 gram (0.62 mmole), had a much lower specific activity (0.47 mc. per mmole).

***O,O*-Diethyl *O*-(3,5,6-Trichloro-2-pyridyl-2,6-$C^{14}$) Phosphorothioate.** To 1.07 grams (5.39 mmoles) of 3,5,6-trichloro-2-pyridinol-2,6-$C^{14}$ dissolved in 10 ml. of freshly distilled dimethyl formamide was added 0.57 gram (5.39 mmoles) of anhydrous sodium carbonate and 1.12 grams (5.39 mmoles) of *O,O*-diethyl phosphorochloridothioate. This mixture was stirred at room temperature for 3 hours. Distilled water (50 ml.) was then added and the precipitated product collected and washed with water on a sintered glass filter. The crude product was dried in vacuo over anhydrous calcium sulfate before purification. After two recrystallizations from *n*-pentane (10 ml.), it weighed 1.76 grams (5.03 mmoles) and contained 1.5 mc. per mmole (Table I).

**2,3,5,6-Tetrachloropyridine.** 2,3,4,5,6-Pentachloropyridine (5.03 grams, 20 mmoles) was added to 20 ml. of 2-propanol and water (87:13 v./v.) and 1.31 grams (20 mmoles) of zinc dust in a 100-ml. round-bottomed flask. The flask was fitted with a reflux condenser and a dropping funnel containing 3.16 grams of concentrated hydrochloric acid dissolved in 15 ml. of the same alcohol-water mixture. The solution in the flask was heated in an oil bath at a temperature to maintain gentle refluxing of the solvent during the dropwise addition of the hydrochloric acid ($1\frac{1}{2}$ hours) and for 1 hour following. Distilled water (20 ml.) was then added to the cooled reaction mixture and the alcohol removed by azeotropic distillation. The product was recovered from the water by continuous extraction with *n*-pentane for 14 hours. Lyophilization of the extract left 4.16 grams of chlorinated pyridines.

The tetrachloropyridine was isolated from this mixture by VPC through a $\frac{3}{8}$-inch i.d. × 12-foot long stainless steel column packed with Silicone 410 gum (Dow Corning Corp., Midland, Mich.), 20 weight % on Chromosorb W (Johns-Manville Corp., New York, N. Y.). The chromatograph was operated at 265° C. and a helium flow rate of 60 cc. per minute. Repeated 100-mg. injections were made with the products collected in μ-tubes packed with glass wool and cooled to −70° C. In this manner 1.79 grams (8.26 mmoles) were obtained; this represented 41.3% of the theoretical amount.

*Discussion*

The glutarimide when treated with phosphorus pentachloride (*3*) produced a mixture of chlorinated pyridines (VII) (Figure 2). The composition, as determined by vapor phase chromatography, varied about the following values: 10% 2,6-dichloro-, 75% 2,3,6-trichloro-, and 15% 2,3,5,6-tetrachloropyridine. This is in good agreement with results reported by Meikle and Williams (*6*).

The mixture of halopyridines was hydrolyzed in aqueous butanol with two equivalents of sodium hydroxide to the corresponding 2-pyridinols (VIII). The reaction was carried out in a stainless steel reactor. When glass Carius tubes were used, the concentrated alkali was depleted by reaction with the glass, resulting in incomplete hydrolysis and lowered yields. The sodium salts of the halo-pyridinols are not very soluble in water or aqueous butanol and form heavy slurries in cold or concentrated solutions. Therefore, good agitation is required during this step.

The preparation of 3,5,6-trichloro-2-pyridinol (IX) was completed by chlorinating the hydrolyzate, after removal of the butanol by azeotropic distillation and acidification (*8*). One mole of elemental chlorine gave the best results. A reduction in yield occurred when more than one molar proportion was used. With less than one, the product contained dichloropyridinol and, to a lesser extent, monochloropyridinol. The method gave upon recrystallization, first from aqueous methanol and then from anhydrous benzene, a product from which no impurities were detectable either by thin-layer chromatography or infrared spectroscopy.

An alternate route to 3,5,6-trichloro-2-pyridinol was tried. This involved chlorination of VII to pentachloropyridine (X) followed by reductive dehalogenation

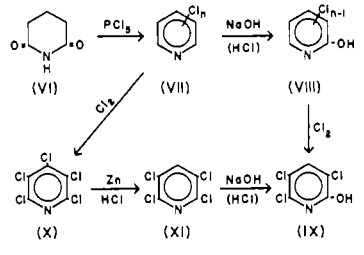


Figure 2. Schemes for converting glutarimide to 3,5,6-trichloro-2-pyridinol

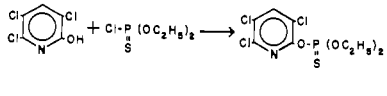

Figure 3. *O,O*-Diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate preparation from 3,5,6-trichloro-2-pyridinol

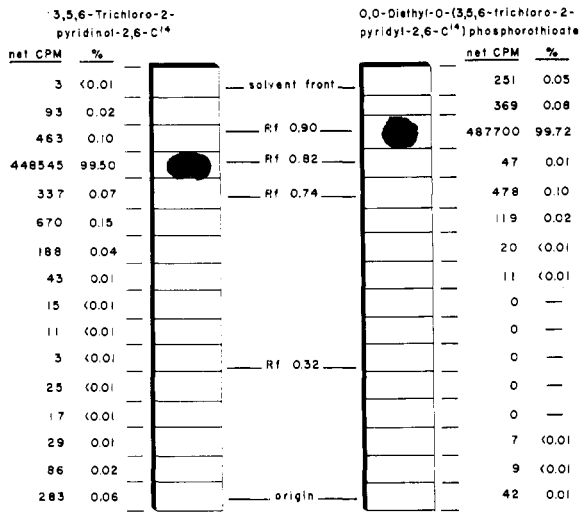


Figure 4. Analysis of sections from thin-layer chromatograms

to 2,3,5,6-tetrachloropyridine (XI). The desired 2-pyridinol (IX) was obtained upon hydrolysis. Of the several procedures considered, photochlorination was best for our purposes. A carbon tetrachloride solution of VII at the boiling point was treated with gaseous chlorine and irradiated with a W4 tube (General Electric Co., Schenectady, N. Y.). Complete conversion was obtained after 72 hours as determined by vapor phase chromatography. Some black insoluble tar was also produced during this long reaction period.

Conditions for the dehalogenation were explored for optimizing the tetrachloropyridine yield. The highest conversion was obtained when equal molar quantities of pentachloropyridine and zinc dust in isobutyl alcohol were treated with 2 equivalents of concentrated hydrochloric acid. The product consisted of a mixture of dichloropyridine (3.6%), trichloropyridine (9.1%), tetrachloropyridine (58.2%), and pentachloropyridine (29.1%). This is nearly a fourfold increase in tetrachloropyridine content, but the procedure was abandoned because of over-all low yields and difficulty in separating the variously chlorinated pyridines.

The final step in the tracer preparation was carried out in dimethyl formamide (*9*). Excellent yields were obtained when the trichloropyridinol was treated with a slight excess of *O,O*-diethyl phosphorochloridothioate in the presence of anhydrous sodium carbonate (Figure 3). At ambient temperature the reaction was rapid and nearly quantitative. The tracer was purified by recrystallization from *n*-pentane and analyzed by thin-layer chromatography (Figure 4).

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