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## *NOTES*

# Synthesis of N-(Mercaptomethyl)-Phthalimide-Carbonyl-$^{14}$C-S-(*O*,*O*-Dimethylphosphorodithioate)*

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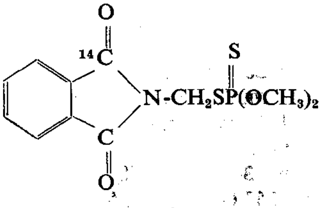
## SUMMARY

*N-(mercaptomethyl)-phthalimide-carbonyl-$^{14}$C-S-(O,O-Dimethylphosphorodithioate) has been synthesized. The starting material was carboxyl labelled* o-*toluic acid which was oxidized to* o-*phthalic acid. Phthalimide (carbonyl-$^{14}$C) was prepared from* o-*phthalic acid by subliming the ammonium salt of the acid. When $^{14}$C phthalimide was reacted with aqueous formaldehyde, N-hydroxymethyl phthalimide was formed. The final product was obtained when the N-hydroxymethyl derivative was reacted with thionyl chloride, followed by reaction with sodium dimethyl phosphorodithioate.*

## INTRODUCTION.

The biological activity of N-(Mercaptomethyl)phthalimide-S-(O,O-dimethylphosphorodithioate) (VI) was reviewed in a paper by Menn *et al.* (1). Studies on the metabolism of VI were conducted in plants by Menn and McBain (2), in rats by Ford *et al.* (3), in a steer by Chamberlain (4) and in insects by Menn *et al.* (5).

This paper reports the synthesis of VI labeled with $^{14}$C in one of the carbonyl groups.



VI

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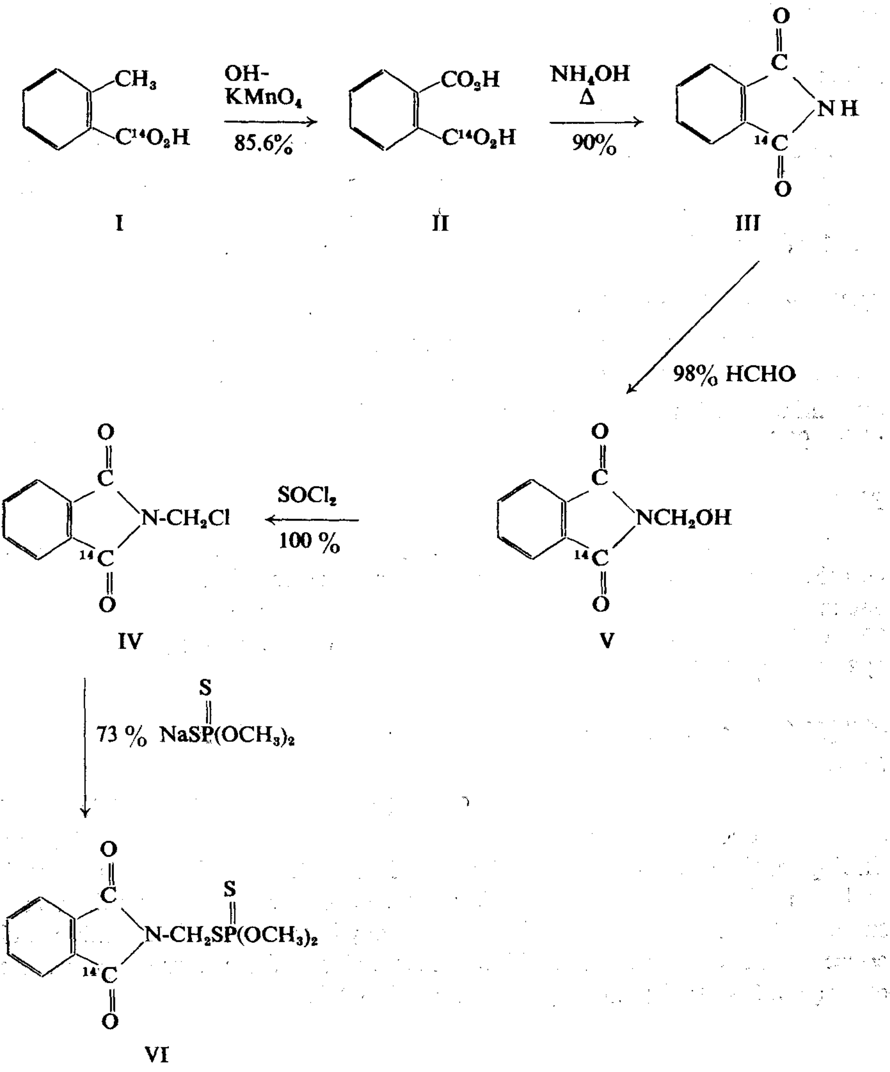
* Imidan, registered trademark of Stauffer Chemical Company.

The radioactive insecticide was subsequently utilized in the metabolism studies cited above (2-3-4-5).

In the synthetic scheme used, the formation of N-hydroxymethyl phthalimide and its subsequent conversion to N-chloromethyl phthalimide was anticipated to present no difficulties since adequate methods have been described (6, 7). The thionyl chloride route to the N-chloromethyl phthalimide was judged to be better adapted to the small scale synthesis. The final step involving the condensation of the N-chloromethyl phthalimide with sodium-dimethyl phosphorodithioate to give N-(Mercaptomethyl)-phthalimide-carbonyl-$^{14}$C-S-(O,O-dimethylphosphorodithioate) was essentially that of a patented procedure (8).



## EXPERIMENTAL.

### o-*Toluic acid (carbonyl-$^{14}$C) (I).*

This compound was purchased from Hazelton Nuclear Science Corporation, Palo Alto, California. A paper chromatogram (Whatman No. 1) developed with isopropanol : conc. ammonium hydroxide : water (8 : 1 : 1) showed that the compound was free of radioactive impurities.

### o-*Phthalic acid (carbonyl-$^{14}$C) (II).*

The labeled Toluic acid (2.160 g, 27.8 mc) was oxidized by the method of Stevens and Holland (9a) and Barker and Christian (9b). The yield of II was 85.6%.

### *Phthalimide (carbonyl-$^{14}$C) (III).*

*o*-Phthalic acid (2.25 g, 24.8 mc) was dissolved in 2.5 ml of concentrated ammonium hydroxide. To the clear solution was added 410 ml of cold dioxane. After chilling overnight at 0-5° C, the resulting ammonium salt was filtered.

The solid was sublimed at 300° C at atmospheric pressure affording 1.794 g (21.2 mc) of the desired phthalimide : yield, 90%.

### *N-Hydroxymethyl phthalimide (carbonyl-$^{14}$C) (V).*

Phthalimide (1.58 g) was refluxed with 4.7 ml of 37% formaldehyde and the solution was allowed to stand overnight at room temperature. The white, crystalline, solid which formed was filtered; 1.936 g (19.1 mc) m.p. 140-3° C, yield, 98%.

### *N-Chloromethyl Phthalimide (carbonyl-$^{14}$C) (IV).*

N-Hydroxymethylphthalimide (1.89 g) was refluxed with 6 ml of thionyl chloride for twenty minutes. The thionyl chloride solution was removed by vacuum evaporation, treated with two portions of benzene, and again dried. The white solid was dried under high vacuum; weight 2.05 g (18.2 mc); m.p. 128-131° C, yield, 100%.

### $^{14}$C-[*N-(mercaptomethyl)phthalimide-S-(O,O-Dimethylphosphorodithionate)*] *(VI).*

N-Chloromethylphthalimide (1.98 g, 10.5 mM) was dissolved in 25 ml of benzene and the solution added to an aqueous solution of sodium dimethyl dithiophosphate (27.4 mM, Stauffer Chemical Co.). The mixture was stirred, and refluxed for 2 hours. After cooling, the benzene layer was removed, washed twice with 200 ml of water, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The solid was recrystallized from benzene-petroleum ether (b.p. 30-60° C); 2.37 g (73%); m.p. 68-70° C.

An infrared scan of $^{14}$C-VI was identical to a nonradioactive recrystallized sample (m.p. 72-72.7° C). Thin layer chromatography was performed on 8 × 8-inch plates coated with Silica Gel G (Merck and Co., Darmstadt, Germany) 0.25 mm thickness. Compound $^{14}$C-VI and nonradioactive VI were spotted 2.5 cm from the edge of the dried, coated plate and developed in a solvent system consisting of 95% benzene and 5% ethyl acetate. The developed plates were sprayed with 5% ammonium molybdate in 10% sulfuric acid. The nonradioactive and radioactive VI gave identical blue spots with an $R_f$ 0.64. Radiochemical purity was determined as follows : Compound $^{14}$C-VI (30,000 DPM/3 μl acetone) was spotted on a thin-layer plate (8 × 8 inch) coated with Silica Gel G, a parallel spot was chromatographed with 50 μg of nonradioactive VI. The plate was developed in 90% ethyl acetate and 10% benzene v/v. The developed plate was exposed to Kodak No-screen, Medical X-ray film for two days and developed in the usual manner. The spot containing the non labeled compound VI was visualized by spraying it with DCQ (2,6-dibromo-N-chloro-*p*-benzo quinone imine) [(10)]. The colored spot coincided with the radioactive spot on the autoradiogram. The radioactive spots representing Compound $^{14}$C-VI, the minor impurity streaked below it and the trace spots at the origin were removed separately and radioassayed by liquid scintillation counting. By means of this procedure it was shown that the discrete spot representing Compound $^{14}$C-VI contained 91.5% of the radioactivity, the area immediately below it contained 8.4%, at least part of which could represent streaked $^{14}$C-VI and the spot of application at the origin contained 0.1% of the radioactivity. The specific activity of the $^{14}$C-VI was 1.53 mc/mmole as determined by liquid scintillation counting.

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## REFERENCES

1. MENN, J. J., MCBAIN, J. B. ADELSON, B. J. and PATCHETT, G. G. — *J. Econ. Entomol.*, **58** : 875 (1965).
2. MENN, J. J. and MCBAIN, J. B. — *J. Agr. Food Chem.*, **12** : 162 (1964).
3. FORD, I. M., MENN, J. J. and MEYDING, G. D. — *J. Agr. Food Chem.*, **14** : 83 (1966).
4. CHAMBERLAIN, W. F. — *J. Econ. Entomol.*, **58** : 51 (1965).
5. MENN, J. J., MCBAIN, J.B. and FORD, I. M.— Proc. XIIth International Cong. Entomol., London, 1964, p. 486 (1965).
6. NEFKENS, G. H. L. — *Nature*, **193** : 974 (1962).
7. RUCKER, G. W. and JOHNSON, T. B. — *J. Amer. Chem. Soc.*, **44** : 817 (1922).

8. Fancher, L. W. — U. S. Pat. 2,767,194 (1956).
9. (*a*) Stevens, W. H. and Holland, D. H. — *Science*, **112** : 718 (1950).
(*b*) Barker, D. Y. and Christian, J. E. — *J. Amer. Pharm. Assoc.*, **44** : 105 (1955).
10. Menn, J. J., Erwin, W. R. and Gordon, H. T. — *J. Agr. Food Chem*, **5** : 601 (1957).

# Synthesis of Nitrilotriacetic Acid-2-$^{14}$C

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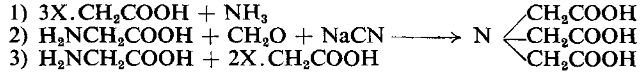
Nitrilotriacetic acid is a well known chelating agent and gives complexes of both theoretical and practical interest with many cations (1, 2). In the framework of a larger investigation the preparation of labelled nitrilotriacetic acid became necessary.

Different syntheses of nitrilotriacetic acid have been reported in the literature (3-12) and may be summarized in three main groups :



$$\left.\begin{array}{l} 1)\ 3X.CH_2COOH + NH_3 \\ 2)\ H_2NCH_2COOH + CH_2O + NaCN \\ 3)\ H_2NCH_2COOH + 2X.CH_2COOH \end{array}\right. \longrightarrow N \begin{array}{l} \diagup CH_2COOH \\ - CH_2COOH \\ \diagdown CH_2COOH \end{array}$$

In the first reaction considerable amounts of glycine and iminodiacetic acid were obtained together with the desired nitrilotriacetic acid. Although glycine could be precipitated easily by addition of methanol, fractional recrystallization with considerable loss of material was necessary to isolate nitrilotriacetic acid from the remaining mixture.

The second method gave good yields, as reported (11-12); however the preparation and especially the purification of the product were found rather cumbersome.

The third method, reported by Kazarinova and co-workers (5), gave satisfactory results and was adopted for the preparation of the labelled compound, although some modifications proved necessary.

Thus interaction of glycine-2-$^{14}$C in alkaline solution with excess chloroacetic acid gave upon acidification a 95% yield of nitrilotriacetic acid-2-$^{14}$C. After recrystallization, the chemical purity of the (inactive) sample was tested by infrared spectroscopy, thin-layer chromatography and elementary analysis.

The infrared spectrum was in agreement with that reported by Duval and co-workers (13); it showed the carboxyl absorption at 1 740 cm$^{-1}$. Radioactive purity was assayed by radiochromatogram scanning in thin layer, which gave one peak corresponding to an R*f* value of 0.35-0.42 in agreement with that reported by Hartley and Lawson (14). The same R*f* values were obtained by this method in chemical purity tests.

Nitrilotriacetic acid does not dissolve in toluene, xylene, anisole or dioxane, the common solvents of scintillating solutions. However, it dissolves well in