Synthesis of ^{14}C- or ^{35}S-Labeled Dialkyl 4,4′-*o*-Phenylenebis(3-thioallophanate) (Thiophanates)

Yoshinori Soeda,*[1] Daigaku Takiguchi,[2] Shogo Kosaka, and Teruhisa Noguchi

In order to meet the need for the metabolic or degradative study of thiophanate fungicides [dialkyl 4,4′-*o*-phenylenebis(3-thioallophanate)], thiophanate-methyl was separately radiolabeled at four different positions of the molecule, giving methyl thiophanate *-thiocarbonyl-*^{14}C, *-thiocarbonyl-*^{35}S, *-methyl-*^{14}C, and *-phenyl-*$^{14}C(U)$. Likewise thiophanate was also separately radiolabeled at three different positions of the molecule, giving thiophanate-*thiocarbonyl-*^{14}C, *-thiocarbonyl-*^{35}S, and *-ethyl-1-*^{14}C. Among those, thiophanate-methyl-*phenyl-*^{14}C (237 mg, 1.0 mCi/mmol) was obtained in radiochemical purity of 97.2% by the reaction of methyl isothiocyanatoformate with *o*-phenylenediamine-$^{14}C(U)$, which was prepared *via* a series of reactions starting from aniline-$^{14}C(U)$ sulfate (270 mg, 2.1 mCi/mmol).

Thiophanate fungicides [dialkyl 4,4′-*o*-phenylenebis(3-thioallophanate)] developed by Nippon Soda Co., Ltd., are in worldwide use for the control of vegetable and orchard pests such as cecrospora leaf spot, powdery mildew, gray mold, and so forth (Noguchi *et al.*, 1969). Among the thiophanates, diethyl 4,4′-*o*-phenylenebis(3-thioallophanate) was developed first and named thiophanate. Later the methyl homolog, thiophanate-methyl, was found to be even superior in its efficacy (Ishii, 1970).

For degradative and metabolic studies of those two representative thiophanates, compounds suitably labeled at different positions have been synthesized. The positions of the labeling were determined from the structural features of thiophanates and a biochemical viewpoint. The general preparation scheme for these radiolabeled thiophanates is shown in Figure 1.

These radiolabeled compounds have been used in studies on the metabolism of thiophanates in or on some plants. Alkyl 2-benzimidazolecarbamates are found as major metabolites of thiophanates in or on bean plants (Soeda *et al.*, 1972a). The persistence of thiophanate-methyl and its metabolites on apple and grape leaves and glass plates is reported with their half-lives (Soeda *et al.*, 1972b).

EXPERIMENTAL

Syntheses with radiolabeled chemicals were preceded by a series of experiments with nonlabeled chemicals to optimize techniques and yields as discussed later. In every case, the identity and the purity of the products were checked by thin-layer chromatography (tlc) with authentic standards. Tlc was performed on a polyethylene film coated with a silica gel layer (Eastman chromagram sheet 6060). The developing solvents used for the separation of thiophanates were ethyl acetate–*n*-hexane–acetic acid (20:80:2) and ethyl acetate–chloroform–acetic acid (10:90:2). In both cases, the tlc plate was not saturated with the solvent prior to the development, since it gave better results. All radioactivity was measured by a liquid scintillation spectrometer (Packard Tri-Carb 3320). To determine the distribution of radioactivity on tlc, the area corresponding to autoradiographic zones was scraped off into scintillation vials and the radioactivity was counted. The chemical yields in weight and percent are described for each experiment and other data are summarized in Table I.

Starting Radiolabeled Chemicals. The following chemicals were used without further purification in this study. Potassium thiocyanate-^{14}C (16.5 mCi/mmol) and potassium thiocyanate-^{35}S (19.8 mCi/mmol) were purchased from The Radiochemical Centre (Amersham, England). Methanol-^{14}C (45.0 mCi/mmol) and aniline-$^{14}C(U)$ sulfate (30.9 mCi/mmol, 99% purity) were purchased from Daiichi Pure Chemicals, Japan. Ethanol-*1*-^{14}C (3.2 mCi/mmol) was purchased from New England Nuclear Corp., U.S.A.

Standard Nonlabeled Chemicals. Nonradioactive intermediates as authentic standards were either purchased from Wako Pure Chemical Industries, Ltd., or supplied from Fine Chemical Research Laboratories of Nippon Soda Co., Ltd. Alkyl 4-(*o*-aminophenyl)-3-thioallophanate (**1**) was synthesized from alkyl isothiocyanatoformate and a large excess of *o*-phenylenediamine (OPD); melting point of the ethyl homolog was 162.0–162.5° and of the methyl homolog was 184–185°. Thiophanates were synthesized from OPD and a large excess of alkyl isothiocyanatoformate; the melting point of thiophanate was 192–193° and of thiophanate-methyl was 177–178°. For the structural identity spectroscopic data of thiophanates are shown in Figures 2 and 3.

Syntheses of Thiophanates ^{14}C -or ^{35}S-Labeled in the Thiocarbonyl Moiety. THIOPHANATE-^{14}C (**2**, R = C_2H_5). Potassium thiocyanate (94.2 mg, 0.97 mmol, dried over phosphorus pentoxide) was added to K$S^{14}CN$ (0.5 mCi, 3 mg, 0.03 mmol) in its ampoule with 1 ml of acetone. Ethyl chloroformate (108.5 mg, 1.0 mmol) was then added to the mixture with a capillary tube. The ampoule, connected with an air condenser by a rubber stopper, was heated at 45° for 30 min with occasional shaking. After the ampoule had been cooled in an ice–water bath, ethyl 4-(*o*-aminophenyl)-3-thioallophanate (**1**) (287.3 mg, 1.2 mmol) was added into it. The insoluble crystals were crushed with a spatula. Then the mixture was again treated at 30° for 20 min. After leaving it at room temperature for 30 min and most of the acetone was evaporated, 0.5 ml of water and 1 ml of 1% aqueous hydrochloric acid were added under cooling in an ice–water bath. The precipitates which appeared were filtered and washed with water, 2 ml of 1% aqueous ammonium hydroxide and again water, and then dried. The crude material (281 mg) was dissolved in 3.5 ml of acetone at 55°, and then 8.8 ml of warm *n*-hexane were added. The clear solution was allowed to stand overnight.

Nisso Institute for Life Science, Nippon Soda Co., Ltd., Oiso, Kanagawa, Japan.
[1] Present address: Section of Neurobiology and Behavior, Langmuir Laboratory, Cornell University, Ithaca, New York 14850.
[2] Fine Chemical Research Laboratories, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan.

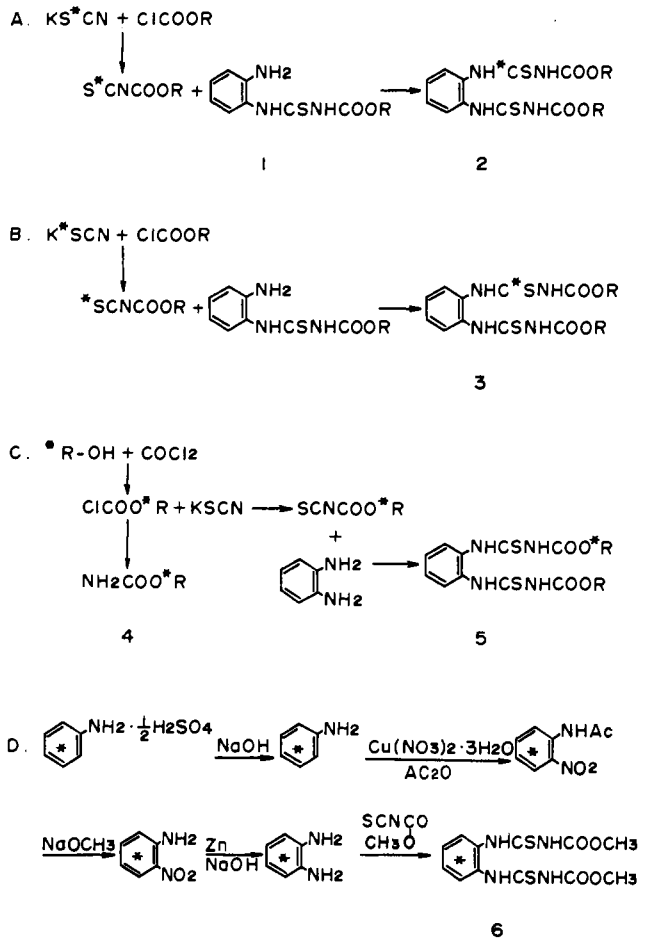

Figure 1. Scheme for the synthesis of radiolabeled thiophanates and carbamates; R = CH_3 or C_2H_5

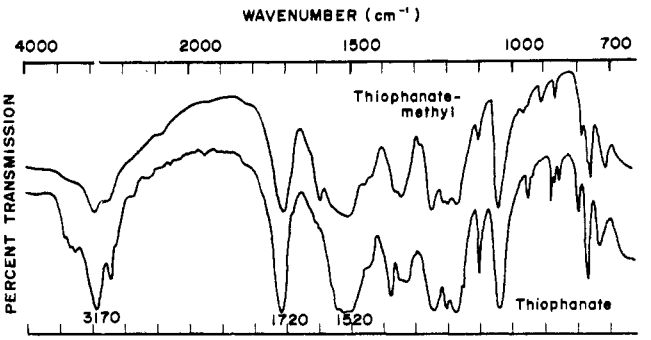

Figure 2. Infrared spectra of thiophanates. Measured in KBr (1.0%)

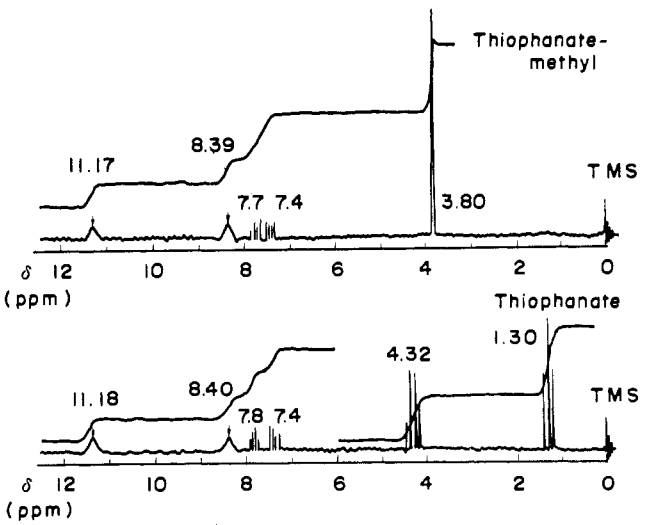

Figure 3. Nuclear magnetic resonance spectra of thiophanates. Measured in saturated solution of $CDCl_3$ at 60 MHz. Figures mean ppm (δ) from TMS. Signals indicated with (↓) disappeared by deuterium exchange in a few minutes

The crystals which precipitated were collected on a filter paper and weighed (227 mg, 61.3% for KSCN).

THIOPHANATE-METHYL-^{14}C (**2**, R = CH_3). Potassium thiocyanate (94.2 mg, 0.97 mmol, dried over P_2O_5) and $KS^{14}CN$ (0.5 mCi, 3.0 mg, 0.03 mmol) were combined in a flask (20 ml) with 1 ml of acetone. According to the method for thiophanate-^{14}C, methyl chloroformate (113 mg, 1 mmol) and methyl 4-(*o*-aminophenyl)-3-thioallophanate (270.5 mg, 1.2 mmol) were added in that order. After the reaction mixture had been allowed to stand at room temperature for 30 min, most of the acetone was evaporated and 3 ml of concentrated hydrochloric acid solution was added under cooling in an ice water bath. The mixture was agitated with a glass rod and diluted with water (3 ml). The precipitates were filtered and washed with a 20% aqueous hydrochloric acid followed by 1% aqueous ammonium hydroxide and water, and then dried. The crude material was recrystallized from acetone–*n*-hexane (4:8) to give pure crystals (199.8 mg, 58.5% for KSCN).

THIOPHANATE-^{35}S (**3**, R = C_2H_5). Potassium thiocyanate (86.4 mg. 0.88 mmol, dried over P_2O_5) and $K^{35}SCN$ (1.80 mCi, 10.8 mg, 0.12 mmol) were combined in a small test tube (10 ml) with 1 ml of acetone. According to the procedure used in the synthesis of thiophanate-^{14}C, ethyl chloroformate (108.5 mg, 1 mmol) and ethyl 4-(*o*-aminophenyl)-3-thioallophanate (287.2 mg, 1.2 mmol) were added in that order. The crude material (335 mg) was recrystallized from acetone–*n*-hexane (2:6) giving pure crystals (259.6 mg, 70.0% of KSCN).

THIOPHANATE-METHYL-^{35}S (**3**, R = CH_3). Potassium thiocyanate (37.8 mg, 0.39 mmol) and $K^{35}SCN$ (0.64 mCi, 10.8 mg, 0.11 mmol) were treated successively with methyl chloroformate (57 mg, 0.50 mmol) in 1 ml of acetone and methyl 4-(*o*-aminophenyl)-3-thioallophanate (136.0 mg, 0.60 mmol), according to the procedure used for thiophanate-methyl-^{14}C ex-

Table I. Data in Synthesis of Radiolabeled Thiophanates and Carbamates

Compound	Specific radioactivity, mCi/mmol	Radiochemical yield,[a] %		Chemical yield, %	Radiochemical purity,[b] %
Ethyl analog					
thiocarbonyl-^{14}C	0.532	62.8		62.5	99
thiocarbonyl-^{35}S	1.78	76.0		74.8	99
ethyl-1-^{14}C	0.606	34.0	47.6	35.5	99
carbamate-^{14}C	0.0785	13.6		96.5	99
Methyl analog					
thiocarbonyl-^{14}C	0.462	55.2		59.3	99
thiocarbonyl-^{35}S	0.914	41.5		39.8	99
methyl-^{14}C	0.285	23.0	31.5	21.8	99
carbamate-^{14}C	0.027	8.5		52.2	99
phenyl-^{14}C	0.996	40.4		38.1	97.2

[a] Sum of the first and second crops obtained by reverse dilution method. *Thiocarbonyl*-^{14}C and ^{35}S are based on potassium thiocyanate, *ethyl* and *methyl*-^{14}C on alcohol, carbamate-^{14}C on alkyl chloroformate, and *phenyl*-^{14}C on aniline sulfate. [b] Judged by tlc–autoradiography and measurement of radioactivity of spots. Developing solvent: ethyl acetate–*n*-hexane–acetic acid = 20:80:2 (unsaturated). R_f value: thiophanate, 0.60; thiophanate-methyl, 0.26; ethyl carbamate, 0.65; methyl carbamate, 0.41.

cept using a half volume of each solvent. Yield (66.5 mg, 38.9% for KSCN).

Syntheses of Thiophanates-^{14}C and Alkylcarbamates-^{14}C Labeled in the Alkyl Moiety. THIOPHANATE-^{14}C (**5**, R = C_2H_5) AND ETHYLCARBAMATE-^{14}C (**4**, R = C_2H_5). Phosgene must be used carefully in a good ventilated hood. Liquid phosgene (1.0 mmol) was added by the mixture of ethanol (0.19 g, 4 mmol) and ethanol-*1*-^{14}C (1.0 mCi, 0.014 g) in an ice cooled flask (10 ml). The reaction mixture was treated at 40° for 30 min. Evaporation of excess phosgene by the nitrogen stream gave resultant ethyl chloroformate-^{14}C (0.40 g, 90% for ethanol). Three-fourths of ethyl chloroformate-^{14}C (0.30 g, 3.0 mmol) was dissolved in acetone and the solution was dried over anhydrous potassium carbonate. After the desiccant had been filtered off, acetone was evaporated to adequate volume, and was treated with KSCN (0.40 g, 4 mmol) and *o*-phenylenediamine (OPD, 0.13 g, 2.4 mmol) in that order according to the method for thiocarbonyl labeling. Crude product was recrystallized from acetone–*n*-hexane (5:13) to give pure crystals (219 mg, 34.0% for ethanol).

On the other hand, one-fourth of ethyl chloroformate-^{14}C (0.1 g, 1 mmol) diluted with ethyl chloroformate (0.1 g) was dissolved in 1 ml of ethyl ether. Concentrated ammonium hydroxide (28%, 0.40 g) was added to the ethereal solution and the mixture was allowed to react with vigorous shaking. Ethylcarbamate-^{14}C (160 mg, 96.5% for ethyl chloroformate) was extracted with ether.

THIOPHANATE-METHYL-^{14}C (**5**, R = CH_3) AND METHYLCARBAMATE-^{14}C (**4**, R = CH_3). Phosgene must be used carefully in a good ventilated hood. Liquid phosgene (1.0 g, 10 mmol) was added to the mixture of methanol (0.16 g, 5 mmol) and methanol-^{14}C (1.0 mCi) in an ampoule. After evaporation of excess phosgene with nitrogen stream, resultant methyl chloroformate-^{14}C (0.40 g, 85.2% for methanol) was divided into two portions. Three-fourths of methyl chloroformate-^{14}C (0.30 g, 3 mmol) was treated with KSCN and OPD to give pure crystals of thiophanate-methyl-^{14}C (107.0 mg, 21.8% for methanol).

On the other hand, one-fourth of methyl chloroformate-^{14}C (0.1 g) diluted with nonlabeled methyl chloroformate (0.1 g) was reacted with ammonium hydroxide to give methylcarbamate-^{14}C (83.0 mg, 52.2% for methyl chloroformate).

Syntheses of Thiophanate-methyl-^{14}C Labeled in the Phenyl Moiety, and Intermediates. ANILINE-^{14}C. Aniline-$^{14}C(U)$ sulfate (2 mCi, 18.4 mg, 0.065 mmol) added with nonlabeled aniline sulfate (251.6 mg, 0.885 mmol) was converted into free base by the addition of 18 *N* sodium hydroxide (0.22 ml, 3 mmol) and by agitation with spatula under cooling. Anhydrous sodium sulfate (3.9 g) was added to the mixture and the free base of aniline was extracted three times with each 4 ml of ethyl ether. Ether was evaporated in a round-bottomed flask to give an oil (158 mg, 89.2%).

o-NITROACETANILIDE-^{14}C. Acetic anhydride (408 mg) was added to aniline-^{14}C (158 mg, 1.7 mmol) in a flask immersed in an ice bath. The mixture of cupric nitrate [$Cu(NO_3)_2 \cdot 3H_2O$, 530 mg, 1.3 mmol] and acetic anhydride (612 mg) in a small test tube was washed into the flask with additional amount of acetic anhydride (204 mg) under enough cooling. After larger granules in the reaction mixture had been crushed with a glass rod, the ice path was removed. The reaction mixture was gently warmed to initiate the reaction. After the exothermic reaction was finished, the temperature of the mixture was kept at 30–35° for 40 min. Precipitates which occurred by the addition of 6 ml of water followed by agitation were filtered, washed with water, and dried (217.4 mg). The filtrate was extracted with the mixture of ethyl ether and benzene. The extract was dried over anhydrous sodium sulfate and concentrated to solidify (114.2 mg). Total yield of *o*- and *p*-nitroacetanilide-^{14}C was 331.6 mg (96.4%).

The combined crystals were chromatographed over the silica gel column (Wako Gel C-200, 14 g, packed in 10 cm height with benzene) to separate *o* and *p* isomers. The solvents for elution were followed by 160 ml of benzene–chloroform (3:7), 50 ml of chloroform, 50 ml of chloroform–methanol (7:3), and 30 ml of methanol. Each fractionated eluate (10–15 ml) was checked by comparing the tlc-gram with that of authentic standards. R_f values of *o* isomer (fractions 4–16), *p* isomer (fractions 17–20), and other (fractions 21–23) were 0.77, 0.31, and 0.0, respectively, when benzene–methanol (95:5) was used for development. Each fraction containing *o* or *p* isomer was combined and concentrated to solidify. Yield of the *o* isomer was 210 mg (68.8%) and of the *p* isomer was 71 mg (23.2%). Specific activity was 1.92 mCi/mmol (calculated as 2.11 mCi/mmol). Radiochemical purity was 99%.

o-NITROANILINE-^{14}C. *o*-Nitroacetanilide-^{14}C (210 mg, 1.21 mmol) was dissolved in 1.7 ml of methanol and added by 0.19 ml of 0.2 *N* methanolic solution of sodium. The mixture was kept at 63–64° for 1 hr. After methanol was evaporated, 3.8 ml of water was added. Then the mixture was extracted with chloroform. The extract was dried over anhydrous sodium sulfate and condensed to give a yellow solid of *o*-nitroaniline-^{14}C (158 mg, 98.1%), whose R_f value on tlc-gram developed with benzene was 0.40.

o-PHENYLENEDIAMINE-^{14}C. To *o*-nitroaniline-^{14}C (158 mg, 1.19 mmol) in 0.49 ml of ethanol was added 0.097 ml of 20% aqueous solution of sodium hydroxide and 307 mg of zinc powder. The mixture was heated at 80° for 1 hr with frequent shaking. Because the mixture was not decolorized at that moment, additional alkali and ethanol were added. Resulting colorless solution was extracted with chloroform and the extract was concentrated after drying over anhydrous sodium sulfate. Brown deposit (121 mg) was dissolved in 5 ml of ethyl acetate. This solution was filtered to remove insoluble substance which showed a R_f value of 0.0 on tlc-gram. *o*-Phenylenediamine-^{14}C was obtained by evaporation of ethyl acetate in a test tube (94 mg, 76.0%). Radiochemical purity was 90%, as judged by alumina tlc (Eastman 6063) developed with ethyl acetate–methanol (6:2) (R_f 0.54).

THIOPHANATE-METHYL-^{14}C (**6**). In a 10-ml Erlenmeyer flask the mixture of potassium thiocyanate (641 mg, 6.6 mmol), methyl chloroformate (566 mg, 6.0 mmol), and 4.5 ml of acetone was warmed to 45° and allowed to stand for 40 min with frequent shaking. After having been cooled in an ice bath, the mixture containing methyl isothiocyanatoformate was transferred into *o*-phenylenediamine-^{14}C (94 mg, 0.96 mmol). It was then treated at 32° for 30 min with shaking. Then 5 ml of water was added and the mixture was cooled enough to give rise to the precipitates, which were collected on a filter paper and washed with 1% ammonium hydroxide and water. Dried crude methyl thiophanate-^{14}C (280 mg, 94.0% for OPD-^{14}C), having the radiochemical purity of 88.6%, was purified through alumina column chromatography (5 g, 5 cm in height) with chloroform as a solvent for elution. The fractions containing thiophanate-methyl-^{14}C were combined and concentrated to give the deposit, which was recrystallized from acetone–*n*-hexane (5:6). Yield was 237 mg (80.0% for OPD-^{14}C).

DISCUSSION

Thiophanates-*thiocarbonyl*-^{14}C and ^{35}S. Thiophanates are produced commercially from *o*-phenylenediamine (OPD) and

alkyl isothiocyanatoformate. This synthetic method, however, is not suitable for thiophanates-*thiocarbonyl*-^{14}C or -^{35}S, since it would give a mixture of double- and single-labeled compounds as is obvious from the structure of thiophanates. In addition, excess OPD, if used to minimize the loss of radioactivity, would give rise to a large amount of mono-substituted OPD (**1**) as a by-product. Therefore, compound (**1**), 4-(*o*-aminophenyl)-3-thioallophanate, was synthesized first and this was reacted with labeled isothiocyanates prepared from potassium thiocyanate and chloroformate.

From preliminary experiments with nonlabeled compounds, it was found that the best result could be obtained with a molar ratio of 1:1:1.2 (potassium thiocyanate–alkyl chloroformate–compound **1**). Since isothiocyanatoformate was subjected to further reaction without isolation, the *N*-alkoxycarbonyl derivative of compound **1** was formed as a by-product. The attempt to lower the amount of the by-product by using excess potassium thiocyanate *vs.* alkyl chloroformate was unsuccessful.

From the reaction mixture unreacted compound **1** was removed by washing with aqueous hydrochloric acid. Stronger acid was used for the methyl derivative because it is less soluble in dilute acid. Solubility of ethyl derivative in 10% aqueous hydrochloric acid was larger than 2.5% and that of methyl derivative was smaller than 0.5%.

Thiophanates-*alkyl*-^{14}C and Alkylcarbamate-^{14}C. Labeled ethyl or methyl alcohol was treated with phosgene to give alkoxycarbonyl chloride. A part of each chloride was converted to the corresponding carbamate which was necessary for the metabolic study of thiophanates. The rest of each chloride was reacted with potassium thiocyanate to give alkyl isothiocyanatoformate, which was treated with OPD to give thiophanates-*alkyl*-^{14}C with a higher specific radioactivity than thiophanates-*thiocarbonyl*-^{14}C. In these syntheses radiochemical yield of thiophanate-methyl was lower than that of thiophanate. This may be explained by the loss of chloride due to its higher volatility (bp was 71° for methyl chloroformate and 93–95° for ethyl chloroformate). Likewise the specific activities of thiophanate-methyl and methylcarbamate which synthesized were also lower than those of ethyl homologs.

Thiophanate-methyl-*phenyl*-$^{14}C(U)$. Among many reports on the nitration of aniline, the method of Menke (1925) was adopted because it appeared to give the highest ortho substitution (70.6%). In this reaction it was found to be necessary to dry cupric nitrate thoroughly before use and to chill the reaction mixture enough while the granules of cupric nitrate were being crushed with a glass rod. The reaction went quantitatively to give *o* and *p* isomers in a ratio of 74:26. Two isomers were separated by silica gel column chromatography, taking advantage of the differences of their solubility in solvent, as shown in Table II.

Table II. Solubility[a] of *o*- and *p*-Nitroacetanilide

Solvent system[b]	*o* isomer, mg/ml	*p* isomer, mg/ml
Chloroform (C)	553	0.68
C:B = 80:20	222	0.17
C:B = 50:50	167	0.14
Benzene (B)	111	0.073
B:H = 80:20	87	0
B:H = 50:50	35.7	0
B:H = 20:80	6.7	0
n-Hexane (H)	0	0

[a] Measured at 20° using 20–400 mg of *o* isomer and 1–5 mg of *p* isomer. [b] Abbreviated as follows: chloroform, C; benzene, B; and *n*-hexane, H.

o-Nitroacetanilide was deacetylated quantitatively to *o*-nitroaniline with sodium methoxide in methanol according to the method of Verkade and Witjens (1943). *o*-Nitroaniline was then reduced to OPD according to the method of Martin (1943). In this reaction it appeared important to use strong enough alkali to get good results.

Six times the amount of methyl isothiocyanatoformate *vs.* OPD in molar basis gave methyl thiophanate in quantitative yield, while molar ratio of 2:1 gave methyl 4-(*o*-aminophenyl)-3-thioallophanate (**1**) as a major product. Purified OPD afforded thiophanate-methyl as white precipitates in good purity, but crude ones gave colored precipitates which had to be purified with chromatography.

ACKNOWLEDGMENT

The authors are indebted to the following for assistance, discussion, and/or contribution of materials: Kazuhiko Ohkuma, Hideo Kamimura, Nisso Institute for Life Science, Oiso, and Kohshin Miyazaki and his coworkers, Fine Chemical Research Laboratories, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan.

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Received for review January 4, 1972. Accepted May 24, 1972.