a mechanism other than the one proposed by Smith and Loeppky (1967) becomes operative. In the latter mechanism (Figure 1), the tertiary amine undergoes nitrosative dealkylation to the corresponding secondary amine which reacts with nitrite to form the *N*-nitrosamine. If this mechanism were the only operative one, it would be difficult to rationalize the production of more DMNA from TMA than from an equimolar amount of DMA. In this regard, Lijinsky *et al.* (1972) suggested and Keefer and Roller (1973) demonstrated that the *N,N*-dimethylformaldiammonium ion initially produced can undergo nucleophilic attack by free nitrite with the adduct thus formed, collapsing to DMNA and formaldehyde. Keefer and Roller also demonstrated the catalytic effect of formaldehyde in the conversion of various secondary amines to nitrosamines in the pH range 6.4 to 11.0. Perhaps the catalytic effect of formaldehyde produced by nitrosative dealkylation of TMA (Figure 1) explains the increased yields of DMNA at the higher TMA levels considered in this study.

Figure 2 shows the dependence of DMNA formation from TMA on pH. The optimum appears to be pH 3.2–3.3 under the conditions of time and temperature employed and is the same as the pH optimum observed by Schweinsberg and Sander (1972) for nitrosation of TMA and triethylamine. Table II shows the amount of DMNA formed from equimolar amounts of TMA and DMA at various acidities and temperatures. It may be significant that DMNA can be formed from TMA as well as from DMA at pH 3.2, 37°, since these conditions are similar to stomach pH and body temperature. Because relatively large amounts of TMA can form in refrigerated seafood (Gulan, 1972; Keay and Hardy, 1972, Miller *et al.*, 1972), the dietary intake of TMA may have to be reevaluated if *in vivo* formation of DMNA from TMA does occur.

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Synthesis of ^{14}C-Labeled *O,S*-Dimethyl Phosphoroamidothioate

^{14}C-Labeled *O,S*-dimethyl phosphoroamidothioate (Tamaron, Monitor) was synthesized and characterized by gas chromatography, nuclear magnetic resonance, and mass spectra data. The phosphoroamidothioate labeled in the methoxy position had a specific activity of 86 μCi/mmol.

O,S-Dimethyl phosphoroamidothioate (Tamaron, from Bayer Chemical Co., Germany; Monitor, from Chevron Chemical Co., U. S. A.) a new systemic insecticide, was earlier (Khasawinah, 1970) labeled with ^{32}P, but in order to study the mode of decomposition and residues on industrially processed fruits, a ^{14}C-labeled compound was required. Thus, [O-^{14}C]methyl *S*-methyl phosphoroamidothioate as well as the intermediates by preparing apprority 96.2%), as shown in Figure 1, by using reactions that gave few undesired side products.

EXPERIMENTAL SECTION

A high-resolution Varian XL-100 MHz spectrometer was used to study the structure of the phosphoroamidothioate as well as the intermediates by preparing appropriate solutions in $CDCl_3$. Mass spectra were taken with a Hitachi Perkin-Elmer RMU-6H mass spectrometer. Purity studies of the phosphoroamidothioate were made by glc utilizing a Hewlett-Packard 5750 gas chromatograph provided with a flame ionization detector and a 6 × 2 mm glass column (1:1 mixture of 3% Reoplex 400 on Gas Chrom Q, 80–100 mesh and 3% QF-1 on Gas Chrom Q, 80–100 mesh). Critical parameters used were: column oven, 132°; carrier gas and flow rate, nitrogen, 45 ml/min; air and hydrogen flow rates, 325 and 35 ml/min; injector temperature, 215°; detector temperature, 250°; retention time, 4.7 min.

***O*-Methyl Phosphorodichloridothioate (II).** Acridine (0.18 g, 1.0 m*M*) and pulverized calcium oxide (56.1 g, 1 mol) were added to distilled phosphorus thiochloride (84.5 g, 0.5 mol) in benzene (300 ml). Then, methanol (30 ml, 0.75 mol) was added over a period of 1 hr while stirring during the addition and for 3 hr thereafter. Solids were then filtered from the reaction mixture and washed repeatedly with benzene to remove traces of entrained II. Acridine was removed by washing the benzene phases twice with 2% HCl cooled to 2°. Evaporation of the solvent and distillation *in vacuo* gave II (67.5 g, 82.0%, bp 60° at 55 mm). The nmr spectrum of II showed a doublet centered at δ 4.00 ppm (d, 3 H, CH_3O-P, J = 19 Hz).

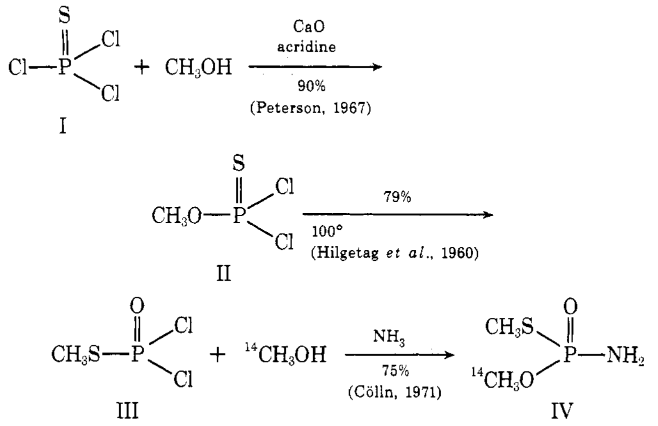

Figure 1. Scheme for the synthesis of [O-^{14}C]methyl-*S*-methyl phosphoroamidothioate.

***S*-Methyl Phosphorodichloridothioate (III).** *O*-Methyl phosphorodichloridothioate (II) (60 g, 0.36 mol) was heated for 5 hr at 100°, during which nmr spectra were used as control since III also yields a doublet but at higher field strength. The resulting product was distilled *in vacuo* (46.8 g, 78.0%, bp 75.5° at 10 mm).

[*O*-^{14}C]Methyl-*S*-methylphosphoroamidothioate (IV). [^{14}C]Methanol (specific activity 59 mCi/m*M*, 1.6 mg, 0.051 m*M*) diluted with nonlabeled methanol (0.793 g, 24.7 m*M*) was added to III (2.76 g, 16.7 m*M*) in methylene chloride (150 ml) at 2°. After 5 min, the solution was saturated with dry ammonia gas and the reaction mixture was then filtered to eliminate NH_4Cl. The methylene chloride was removed in a rotary vacuum evaporator and the slightly colored hygroscopic oil was lyophilized to remove water, giving IV as a white solid (1.35 g, yields 57.2 and 39% based on methanol). The product had identical nmr and mass spectra, as compared with an authentic sample (Bayer Chemical Co., Leverkusen, Germany). The purity of the labeled material was determined on solutions of IV in 2-methoxyethanol, containing about 1 μg/ml, by utilizing glc conditions previously described. Purity data were obtained by interpolating peak areas from a calibration curve obtained with standards. The weighed labeled material contained 94.6% of the phosphoroamidothioate. The nmr spectrum of IV yields doublets for each methyl group due to the proton-phosphorus interaction. The *O*-methyl protons yield a doublet centered at δ 3.69 ppm (d, 3 H, CH_3OP, J = 12 Hz), while the *S*-methyl protons yield a doublet centered at δ 2.32 ppm (d, 3 H, CH_3SP, J = 14 Hz). These values are similar to those previously reported (Fahmy *et al.*, 1972): doublets centered at δ 2.2 (d, 3 H, CH_3OP, J = 11 Hz) and at 3.7 ppm (d, 3 H, CH_3SP, J = 12 Hz). The mass spectrum of IV showed a molecular ion at *m/e* of 141, as well as ions at *m/e* 126 [$O_2P(SCH_3)(NH_2)$], 111 [*m*-($CH_2{=}O$)], 110 [*m*-(CH_3O)], 95 [*m*-($S{=}CH_2$)], 94 [*m*-(SCH_3)], 80 [*m*-(SCH_2 + CH_3)], 79 [*m*-(SCH_3 + CH_3)], and 64 [*m*-(SCH_3 + CH_2O)].

Radiochemical purity was determined by thin-layer chromatography on neutral aluminum oxide plates (Merck F-254, type E) developed for 30 min in 2-methoxyethanol, and then treated with ammoniacal silver nitrate solution (Mitchell, 1960). Under exposure to ultraviolet radiation the R_f was 0.60 and identical for the one obtained from the authentic material. Plates spotted with the labeled compound were scanned with a Packard radiochromatograph scanner model 7200 provided with a windowless G.M. counter. Area integration measurements of the radiochromatograms gave a radiochemical purity of 96.2% (average of three determinations). Since the chemical purity of the compound was 94.6%, it was possible to count directly a 50-μl aliquot of a solution, containing 13.6 mg/ml, in a liquid scintillation spectrometer. This count yielded a specific activity of 82.6 μCi/m*M* (counting efficiency 82.0%).

CONCLUSIONS

In the reaction of III with [^{14}C]methanol it was necessary to add an excess (48%) of methanol to maintain the weight ratio of dichloro compound to methanol close to 3.48. Increasing this ratio gave a major unidentified impurity in IV which showed a broad singlet (δ 5.3 ppm) in the nmr spectrum. However, maintaining the correct ratio gave an essentially pure product. The sample and versatile reactions used in this work can be used to synthesize the phosphoroamidothioate labeled with ^{32}P by synthesizing $PSCl_3$ from commercially available phosphorus-labeled reagents.

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