A Thin-Layer Chromatographic Procedure for Separating Desmethyl Methyl Parathion (O-methyl O-p-nitrophenyl Phosphorothionate) and its S-isomer (S-methyl O-p-nitrophenyl Phosphorothionate)

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There is no published method for separating desmethyl methyl parathion from its S-isomer. However, there is need for this separation, because in the laboratory-scale preparation of desmethyl methyl parathion a 10-15% isomerization always occurs. This biologically active "impurity" is undesirable in toxicological, metabolic, and residue persistence evaluations and in the development of reliable residue analytical methods for these and other applications. In addition, highly purified samples of these types of compounds are essential for the elucidation of mechanisms of reactions and to follow the kinetics of reactions.

This communication describes a simple, fast, and high-resolution system for the clean separation of these two isomeric compounds. This system may be useful for related isomeric compounds, an aspect still under investigation.

Experimental

Desmethyl methyl parathion plus its S-isomer in admixture were prepared as sodium salts by the reaction of benzenethiol with methyl parathion and the pure S-isomer was prepared by the action of sodium iodide on methyl parathion (1). The silica-gel plates, 250 u thick, were spotted with the desired amounts and developed in 90:10 spectrograde acetonitrile: 1.2N HCl. The resulting spots were visualized under ultraviolet light at 253.7 nm and identity was fixed by spraying with 0.5% DCQ (2,6-dibromobenzoequinone-4-chloro-imide) in cyclohexane (3) for P=S (red) and 1% ammonium molybdate in 10N HCl plus 72% $HClO_4$ for P=O (blue) by the method of Hanes and Isherwood as described by March et al. (2). An acidified DCQ reagent lowered the limit of minimum detectability from about 1 µg to about 0.2 µg of the P=S isomer; the P=O isomer gave a yellow product with DCQ.

Results and Discussion

These isomeric materials have the same elemental analysis, and also the infrared spectra do not show clear-cut differences due to lack of absorption of CH_3S- in the conventional region from 4000-650 cm^{-1}. Nuclear magnetic resonance spectroscopy of the methylthio and methoxy protons shows differences, however. In a normally synthesized mixture of these two isomers four peaks at 2.0, 2.25, 3.55, and 3.75 ppm were observed with reference to tetramethyl-

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silane (TMS). The doublet at δ 3.55 and 3.75 ppm is due to CH_3O- while that at δ 2.0 and 2.25 ppm is due to CH_3S-. Both doublets are due to splitting by phosphorus. This NMR evaluation was carried out on three separate syntheses and showed 10-15% S-isomer of desmethyl methyl parathion.

The R_f values in the solvent system described were 0.422 and 0.236 for desmethyl methyl parathion and its S-isomer, respectively. Semiquantitative visualization agreed with the percentage of S-isomer found by NMR.

Because the NMR was free from interference, the purities of the compounds were felt to be established and the percentages of the isomers were thought reliable. The NMR spectrum of the pure (separately synthesized) S-isomer also showed a single doublet at δ 2.0 and 2.25 ppm with respect to TMS. With co-chromatography, the thin-layer R_f value 0.236 was assigned to the S-isomer. The characteristic red color given by the compound at R_f 0.422 with DCQ indicated that it is the desmethyl methyl parathion (P=S). The spot at R_f 0.236 gave a blue color with the mixed ammonium molybdate reagent and a yellow color with the DCQ reagent, thus demonstrating that it was the S-isomer (P=O). These two spots on parallel chromatograms were marked, scraped off, and eluted with methanol. After concentrating, they were re-chromatographed separately; single spots resulted and the R_f values were identical with those reported above.

The NMR, thin-layer chromatography, and characteristic colors with the reagents were felt adequate for the present characterization.

A similar system using technical grade acetonitrile and water (88:12) has recently been reported by Stenersen (4) for the separation of some degradation products of dialkylaryl phosphorothionates. He found silica gel activated at 100°C for 30 minutes desirable for high resolution and used 1% DCQ reagent in acetic acid for detection. However, he did not report any separation of isomeric material. In his acid-free chromatographic system our compounds were not resolved or remained at the base. Use of HCl rather than acetic acid has the added advantage of releasing the sodium salts of our compounds (from the normal syntheses) to free acids.

Stenersen (4) mentioned that with 1% DCQ reagent he observed the well-known red color with thionates, but reported a yellow color with monoalkyl phosphorothionates. This observation is complicated by the fact that this red color is due to the formation of a complex with P=S compounds. It would appear that he had compounds of doubtful purity because these degradation products of thionates are notorious for their isomerization. Thus, we have found 0.2% DCQ in acetic acid to be most effective, and by heating at 100°C for 5 minutes we again observed two spots: red at R_f 0.422 for the desmethyl parathion (P=S) and yellow at R_f 0.236 for the S-isomer (P=O). We observed this yellow color with other P=O compounds as well. This interesting observation will help to

resolve P=S and P=O compounds with only a single spray. Under our conditions, p-nitrophenol gives a blue color with this reagent, but this observation is not new.

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