

Characterization of Alkylation Products of Diethyl Phosphorothioate

The methylation of *O,O*-diethyl phosphorothioate (DETP) produced a minor component identified as the *O,O*-diethyl-*O*-methyl phosphorothionate (DEMMTP) (15%) and a major product identified as the *O,O*-diethyl-*S*-methyl phosphorothiolate (DEMMPT_h) (85%). The ethylation also produced two compounds which were identified as *O,O,O*-triethyl phosphorothionate (TETP) (37.5%) and *O,O,S*-triethyl phosphorothiolate (TEPT_h) (62.5%). Alkylation of *O,O*-dimethyl phosphorothioate has never produced a detectable amount of trialkylated

thiolate. The compounds were identified by comparing the gas chromatograms obtained with an instrument equipped with a flame photometric detector and the mass spectra with those of the purified standards TETP, TEPT_h, DEMMTP, and DEMMPT_h. In a previous publication concerning the analysis of hydrolytic and metabolic products of organophosphorus pesticides, the compounds labeled as ethylated or methylated DETP should be labeled ethylated or methylated *O,O*-diethyl phosphorothiolate (DEPT_h).

The gas chromatographic analysis (glc) of the methyl esters of *O,O*-dimethyl or *O,O*-diethyl phosphate, phosphorothioate, and phosphorodithioate has been discussed in the recent publications mentioned below. The reported order of elution of the methylated compounds by glc using a 6-ft column packed with 2% Ucon polar on 80- to 100-mesh Gas Chrom Q was: *O,O*-dimethyl phosphate (DMP); *O,O*-diethyl phosphate (DEP); *O,O*-dimethyl phosphorodithioate (DMDTP); *O,O*-dimethyl phosphorothioate (DMTP); *O,O*-diethyl phosphorodithioate (DEDTP); and *O,O*-diethyl phosphorothioate (DETP) (St. John and Lisk, 1968).

Askew *et al.* (1969) described another gas chromatographic technique based on the detection of methylated hydrolysis products of organophosphorus compounds. In this work, the dialkyl phosphate standards were prepared by hydrolysis of the parent organophosphorus pesticides which, upon methylation with diazomethane, produced the various trialkyl phosphates. The methylated dialkyl phosphates were separated on silica gel thin-layer plates, eluted with chloroform, and examined by glc to show that one component was present. The structure of the phosphate esters was confirmed by infrared spectroscopy. The reported glc elution pattern of the methylated compounds employing a 150-cm column packed with 4% Versamid 900 was in the following order: DMP, DEP, DMTP, and DETP.

A recent publication describes the use of a 12-ft column packed with 20% Versamid 900 for the simultaneous determination of six major metabolites of organophosphorus and thiophosphorus insecticides (Shafik and Enos, 1969). The suggested order of elution of the methylated or ethylated compounds was: DMTP, DMP, DEP, DMDTP, DEDTP, and DETP.

Recent mass spectral data obtained in this laboratory (Biros *et al.*, 1970) on purified standard materials suggested that the glc peak assignments for the methyl and ethyl derivatives of DETP reported previously were erroneous, and that these compounds were, in fact, *S*-alkylated derivatives of *O,O*-diethyl phosphorothiolate (DEPT_h). The purpose of this investigation was to verify the structure of the alkylated diethyl phosphorothioates obtained from the reactions of the free acids with diazomethane and diazoethane, and to determine the relative proportion of *O*-alkylation and *S*-alkylation which may occur during the alkylation procedure.

EXPERIMENTAL

Standards

<i>O,O</i> -dimethyl phosphorothioate potassium salt	(DMTPK)
<i>O,O,O</i> -trimethyl phosphorothionate	(TMTP)
<i>O,O</i> -dimethyl phosphorodithioate	(DMDTPK)
<i>O,O,S</i> -trimethyl phosphorodithioate	(TMDTP)
<i>O,O</i> -diethyl phosphorothioate potassium salt	(DETPK)
<i>O,O,O</i> -triethyl phosphorothionate	(TETP)
<i>O,O</i> -diethyl phosphorodithioate potassium salt	(DEDTPK)
<i>O,O,S</i> -triethyl phosphorodithioate	(TEDTP)
<i>O,O,S</i> -triethyl phosphorothioate	(TEPT _h)
<i>O,O</i> -diethyl- <i>O</i> -methyl phosphorothionate	(DEMMTP)
<i>O,O</i> -diethyl- <i>S</i> -methyl phosphorothiolate	(DEMMPT _h)

The potassium salts of the dialkyl phosphates were obtained from American Cyanamid Co., Princeton, N.J. The symmetrical trialkyl phosphates, phosphorothionates, phosphorothiolates, and phosphorodithioates were prepared and purified by Vero Beach Laboratories, Inc., Vero Beach, Fla.

The DEMMTP and DEMMPT_h were obtained in purified form from the reaction products of the methylation of *O,O*-diethyl phosphorothioate by silica gel column chromatography. The experimental details of the column chromatography procedure, useful as a combination cleanup and separation, are discussed in a separate publication (Shafik *et al.*, 1970).

The reagents, extraction and alkylation procedures, and gas chromatographic analysis were the same as those used in the procedure for the determination of the metabolic and hydrolytic products of organophosphorus pesticide chemicals (Shafik and Enos, 1969). The apparatus was the same except that the aluminum gas chromatographic column (1/4-in. o.d. × 12 ft) was replaced with a glass column of the same size.

RESULTS AND DISCUSSION

The methylation reaction of DMTPK and DMDTPK with diazomethane resulted in the formation of two compounds which produced identical glc retention times, and mass spectra as the purified standards TMTP and TMDTP, respectively.

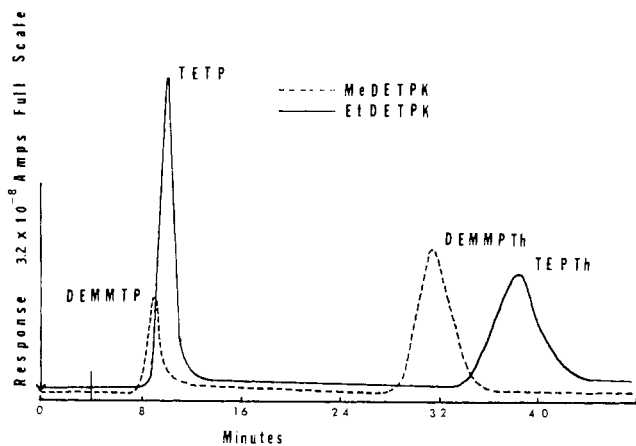


Figure 1. Chromatograms of methylated and ethylated products of 25 ng of DETPK, using the phosphorus filter, 526 m μ

No evidence of internal rearrangement was noted for these compounds. The ethylation of DEDTPK produced a trialkyl phosphate that had a mass spectrum and glc retention time identical to those of the purified standard TEDTP.

The alkylation of the available DETPK standard was examined after methylation and ethylation. Glc of the methylated standard showed the presence of two compounds; the early eluting derivative was a minor component and the later eluting derivative was a major component. The ethylated standard produced two major components (Figure 1). Glc of the two purified standards TETP and TEPTTh, whose identities have been verified by mass spectrometry (Biros *et al.*, 1970), produced peaks which coincided with the two major components of the ethylated DETPK; the first was determined to be TETP and the second TEPTTh.

The quantitation of the resulting two components after the ethylation of DETPK indicated that the reaction produced 37.5% TETP and 62.5% TEPTTh.

Alkylation of DETPK with higher diazoalkanes, such as *n*-propyl, isobutyl, amyl, and isoamyl, produced one major component DETPR and a minor derivative DEPTThR (R = *n*-propyl, isobutyl, amyl, or isoamyl). The amount of the minor component DEPTThR decreased as the size of the R group increased. *S*-alkylation predominates on methylation of DETPK, but *O*-alkylation occurs preferentially with the higher diazoalkanes.

The methylation of DETPK produced two compounds in an 85 to 15 ratio. These components were separated by silica gel column chromatography and, by analogy with the mass spectra and relative glc retention times of the ethylated derivatives of DETPK, were shown to be DEMMPTh (85% isomer) and DEMMTP (15% isomer). The minor component DEMMTP cannot be quantitated under actual sample conditions as it will be masked by the large amount of inorganic phosphates which may be present in reagents and in urine samples. The inorganic phosphate is converted to TMP in the analytical procedure.

After methylation, the DETP is determined as DEMMPTh. Thus, in our previous publication (Shafik and Enos, 1969) the compound labeled DETP should be labeled DEPTTh. At the present time it is impossible to determine the origin of *O*,*O*-diethyl-*S*-methyl phosphorothiolate by this method.

Quantitation of standards and unknown samples is based on the methylated DEP, DMDTP, DMDTP, DETP, and ethylated DMTP and DMP, using the phosphorus mode of the flame photometric detector. The chromatograms of the six

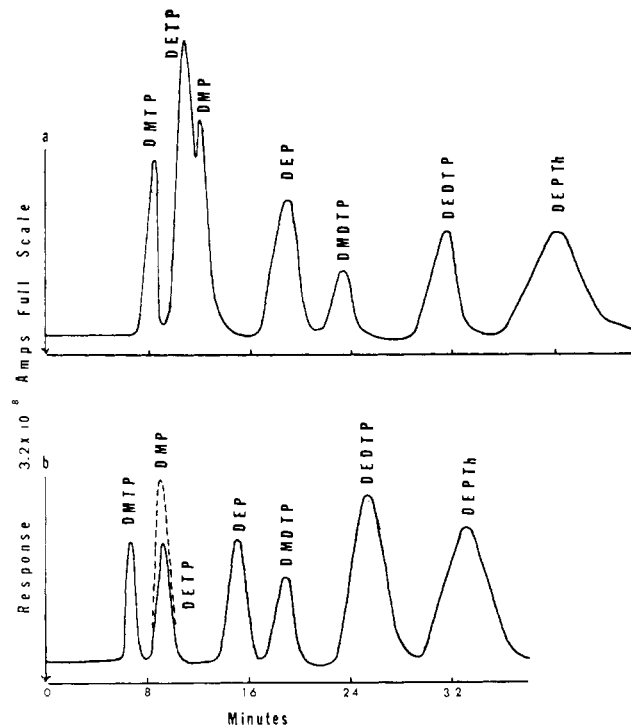


Figure 2. Chromatograms of trialkyl phosphate esters using the phosphorus filter, 526 m μ . (a) Ethylated: DMTP, 10 ng; DETP, 5 ng; DMP, 10 ng; DEP, 5 ng; DMDTP, 50 ng; DEDTP, 10 ng; DEPTTh, 5 ng. (b) Methylated: DMTP, 10 ng; DETP, 10 ng; DMP, 10 ng; DEP, 5 ng; DMDTP, 50 ng; DEDTP, 5 ng; DEPTTh, 5 ng

methylated and ethylated alkyl phosphates are shown in Figure 2. The two derivatives DEMMTP and TMP that have identical retention times can be separated quantitatively by solvent partitioning. The details of the separation are presented, together with a cleanup procedure for the determination of low levels of alkyl phosphates in urine (Shafik *et al.*, 1970).

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