Derivatization of Pesticide-Related Acids and Phenols for Gas Chromatographic Determination

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Organic acids can be readily esterified with diazomethane or diazoethane, and the resultant esters can be separated by gas chromatographic methods. The diazoalkane can be quickly prepared from its more stable precursor by alkaline hydrolysis followed by extraction into ether. Conditions have been developed for the gas chromatographic separation of the methyl and ethyl derivatives of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 2-(2,4,5-trichlorophenoxy)propionic acid, α-(2,4-dichlorophenoxy)butyric acid, pentachlorophenol, o-phenylphenol, and 4,6-dinitro-o-sec-butylphenol from each other on one column and the methyl, ethyl, and butyl phosphoric acids from each other on another column.

A NUMBER of papers on the isolation and determination of herbicide residues in plant materials by gas chromatographic methods have been published recently (*2, 4, 7–10, 12, 21*). Since many herbicide residues are acids which are difficult to chromatograph, the free acids have usually been converted to the methyl esters, which may be readily chromatographed. In most instances, the acids have been esterified with diazomethane, which is unstable and must be prepared from a more stable precursor, usually by distillation of an ether solution of diazomethane from *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide or a similar precursor (*1, 6*) or by an extraction method, using as a precursor nitrosomethylurea, which is not very stable and is not commercially available (*7, 11*). Other esterification methods for organic acids offer no clear cut advantages over the diazomethane method and are slower or more cumbersome to use (*8, 14, 16, 19*). This paper gives a convenient method for the preparation of diazomethane and diazoethane, so that either the methyl or the ethyl esters of acids may be rapidly and easily prepared. It also gives the conditions for the gas chromatographic separation of the chlorinated carboxylic acid esters and phenol ethers from each other and of the phosphate esters from each other.

Experimental

Apparatus. The gas chromatographs used were Micro-Tek Models 2500R, 2000R, and 2000MF (Micro-Tek Instruments, Inc.) all equipped with both flame ionization and electron affinity detectors and with glass inlet systems. In some work, simultaneous detection of a split exit stream with a flame ionization detector, and an electron affinity detector was used. Nitrogen was the carrier gas in all work.

Reagents. Diethyl ether, reagent grade.

Potassium hydroxide, reagent grade.

Hydrochloric acid-ethyl acetate (1:1), reagent grade chemicals.

N - Methyl - *N'* - nitro - *N* - nitrosoguanidine, Aldrich Chemical Co.

N - Ethyl - *N'* - nitro - *N* - nitrosoguanidine, Aldrich Chemical Co. or K & K Laboratories, Inc.

2,4-Dichlorophenoxyacetic acid (2,4-D), Eastman Organic Chemicals.

2,4,5-Trichlorophenoxyacetic acid (practical) (2,4,5-T), Eastman Organic Chemicals.

2 - (2,4,5 - Trichlorophenoxy)propionic acid (practical), [2-(2,4,5-T)P], Eastman Organic Chemicals.

α-(2,4-Dichlorophenoxy)butyric acid, [α-(2,4-D)B], K & K Laboratories, Inc.

2-Methyl-4-chlorophenoxyacetic acid (MCPA), K & K Laboratories, Inc.

Pentachlorophenol, Eastman Organic Chemicals.

o-Phenylphenol, Eastman Organic Chemicals.

4,6-Dinitro-*o*-*sec*-butylphenol, Dow Chemical Co.

Trimethyl phosphate, [$(MeO)_3PO$], K & K Laboratories, Inc.

Triethyl phosphate [$(EtO)_3PO$], City Chemical Corp.

Mono- and dimethyl phosphate, Victor Chemical Division, Stauffer Chemical Co. [A mixture of *O*-methyl phosphoric acid, $(MeO)(HO)_2PO$, and *O,O*-dimethyl phosphoric acid $(MeO_2(HO)PO$.]

Mono- and diethyl phosphate, Victor Chemical Division, Stauffer Chemical Co. [A mixture of *O*-ethyl phosphoric acid, $(EtO)(HO)_2PO$, and *O,O*-diethyl phosphoric acid, $(EtO)_2(HO)PO$.]

Mono- and dibutyl phosphate, Victor Chemical Division, Stauffer Chemical Co. [A mixture of *O*-butyl phosphoric acid, $(BuO)(HO)_2PO$, and *O,O*-dibutyl phosphoric acid, $(BuO)_2(HO)PO$.]

Preparation of Diazomethane or Diazoethane. This procedure has been adapted from a method for the preparation of diazohydrocarbons (*15*). Dissolve 2.3 grams of potassium hydroxide in 2.3 ml. of distilled water in a 125-ml. Erlenmeyer flask, cool the solution to room temperature, and add 25 ml. of ethyl ether. Cool the flask in a refrigerator or an ice bath. Carry out the following preparation step in a hood. Add either 1.5 grams of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine for diazomethane and or 1.6 grams of *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine for diazoethane in small portions over a period of a few minutes to the flask, and shake the flask vigorously after each addition. Decant the ether layer from the aqueous slurry that has formed into a bottle capped with a "Poly-Seal" cap (A. H. Thomas Catalog No. 2849-E) and store in a freezer. Do not use a ground glass stoppered bottle (*6*). While moisture apparently does not interfere in the derivatization reaction, the diazoalkane solution may be dried over potassium hydroxide pellets if a dry solution is desired (*6*). If it is kept in a tightly capped bottle, the diazoalkane solution may be stored at −20° C. for over a week. This procedure gives about 16 ml. of ether solution. Do not allow the nitrosoguanidine or the diazoalkane solution to come in contact with the skin, as these compounds may cause skin rashes. The diazoalkane may be prepared in a larger quantity by increasing the amounts of chemicals, but the proportions must not be changed; a higher diazoalkane concentration may result in an explosion. The diazoalkanes are toxic and potentially explosive. Etched or scratched glassware and strong light should be avoided. Only ethyl ether should be used as the solvent for the diazoalkane (*6*).

Preparation of Methyl or Ethyl Esters. Transfer up to 0.5 ml. of sample into a 2-dram vial, add a small drop of hydrochloric acid-ethyl acetate

(1:1), and mix well. Add 2 ml. of the diazoalkane-ether solution. If the color of the ether solution is discharged, add more ether solution until a color remains. Allow the solution to stand for a few minutes, and evaporate it to less than the original volume in a stream of air. Adjust the volume to the original volume of the sample for gas chromatography.

Chlorinated Carboxylic Acids. Esterification of the chlorinated carboxylic acids appeared to be complete within a few minutes.

Esters of the chlorinated carboxylic acids were separated in 6-mm. O.D. glass columns with a packing of 2% methyl silicone gum rubber SE-30 (General Electric Co.), 0.2% Versamid 900 (Applied Science Laboratories, Inc.) on 100/110 mesh Anakrom ABS (Analab, Inc.) that has been used for the separation of insecticides (*3*). Relative retention times are given in Table I. The carrier gas from the 0.5-meter column was passed through an 8 to 1 exit splitter for simultaneous detection in a flame ionization detector and an electron affinity detector. The retention times shown in Tables I and II for the 0.5-meter column are for the major peak in both detectors. Only the electron affinity detector was used with the 1-meter column. Carrier gas flow was 85 ml. per minute for the 0.5-meter column and 120 ml. per minute for the 1-meter column.

Phenols. Ethers of phenols were separated on the same columns and under the same conditions as the chlorinated carboxylic acids. Under the reaction conditions, *o*-phenylphenol is incompletely reacted, and free *o*-phenylphenol will be present along with the methyl or the ethyl ether. The other phenols appeared to be completely reacted. Relative retention times are given in Table II.

Phosphorus Acids. Esterification of the phosphoric acids appeared to be complete within a few minutes.

Trimethyl phosphate and triethyl phosphate were readily separated from each other by 1-meter long, 6-mm. O.D. copper columns packed with 10% PEG 20M (F & M Scientific Corp.) on 100/110 mesh Anakrom ABS (Analab, Inc.), or a polyester phase such as LAC-1-R 296 (Applied Science Laboratories), or Reoplex 400 (Wilkens Instrument and Research, Inc.). However, a 3-meter long, 6-mm. O.D. copper column packed with the PEG 20M liquid phase was required to separate the mixed esters *O,O*-dimethyl *O*-ethyl phosphate [$(MeO)_2(EtO)PO$] and *O*-methyl *O,O*-diethyl phosphate [$(MeO)(EtO)_2PO$]. In Table III, relative retention times for the methyl and ethyl esters of the methyl, ethyl, and butyl phosphoric acids are given. While tributyl phosphate is not a common constituent of pesticide formulations, it is a frequently used solvent, and it or its decomposition products might be found in samples for residue analysis. The 1-meter column is recommended for the butyl phosphoric acid esters while the 3-meter column is required for the mixed methyl and ethyl phosphates. The flame ionization detector was used for the phosphate esters. Carrier gas flow was 120 ml per minute for all columns.

Table I. Relative Retention Times for Chlorinated Carboxylic Acids

	Relative Retention Times[a]			
	0.5-Meter Column, 115° C.		1-Meter Column, 140° C.	
	Ester		Ester	
Acid	Methyl	Ethyl	Methyl	Ethyl
2,4-D	1.00	1.23	1.00	1.34
2-(2,4,5-T)P	1.65	2.08	1.69	2.09
2,4,5-T	2.23	2.88	2.03	2.63
α-(2,4-D)B	2.88	3.80	2.69	3.69

[a] Retention times are relative to 2,4-D methyl ester, retention time 2.6 minutes on 0.5-meter column and 3.2 minutes on 1-meter column.

Table II. Relative Retention Times for Phenols

	Relative Retention Times[a]					
	0.5-Meter Column, 115° C.			1-Meter Column, 140° C.		
	Ether			Ether		
Phenol	Methyl	Ethyl	Free	Methyl	Ethyl	Free
o-Phenylphenol	0.596	0.654	0.924	0.406	0.500	0.969
Pentachlorophenol	1.31	1.69	...	1.34	1.78	...
Dinitro-*o*-*sec*-butylphenol	2.58	3.15	...	2.66	3.16	...

[a] Retention times relative to 2,4-D, methyl ester, retention time 2.6 minutes on 0.5-meter column and 3.2 minutes on 1-meter column.

Table III. Relative Retention Times for Phosphate Esters

	Relative Retention Times[a]	
Phosphate Ester	1-Meter column, 130° C.	3-Meter column, 150° C.
$(MeO)_3PO$	1.00	1.00
$(MeO)_2(EtO)PO$	...	1.147
$(MeO)(EtO)_2PO$	...	1.246
$(EtO)_3PO$	1.41	1.29
$(MeO)_2(BuO)PO$	1.43	...
$(MeO)(BuO)_2PO$	2.94	...
$(EtO)_2(BuO)PO$	2.84	...
$(EtO)(BuO)_2PO$	5.08	...

[a] Retention times relative to $(MeO)_3PO$, retention time of 3.7 minutes on 1-meter column, 5.5 minutes on 3-meter column.

Discussion

The preparation of diazomethane by the extraction method was unsuccessful in several attempts, when the other commercially available diazomethane precursors, *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide and *N,N'*-dimethyl-*N,N'*-dinitrosoterephthalimide were used. Since diazomethane was readily prepared from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, no further attempt was made to prepare diazomethane from other precursors.

When 2,4-D methyl ester was chromatographed at the level at which 2,4-D would be expected in pesticide work (up to 100 nanograms), the response of the electron affinity detector increased with repeated injections unless the column was loaded by injections of relatively massive amounts of the ester. Reproducible results were obtained after five injections of 10 micrograms each of 2,4-D ester; this loading of the column had to be repeated each day. A new column gave a sensitivity of about two-thirds that obtained after loading. A column which was used for other pesticide work gave a much lower sensitivity before loading. The response of the electron affinity detector to 2,4-D methyl ester, was linear up to 50 nanograms of 2,4-D.

Figure 1 is a chromatogram of a sample of 25 nanograms of 2,4-D, methyl ester, in an extract of oranges, separated by a procedure under development, but similar to some published procedures (*20*). A blank sample showed no peaks after the first two minutes.

Interfering peaks appeared in the gas chromatograms of MCPA and *o*-phenylphenol derivatives; peaks resulting from the diazoalkane were comparable in magnitude and overlapped peaks from the esters or ethers of these two compounds. Simultaneous detection techniques on MCPA esters showed that the peak which was most likely the compound of interest gave a smaller peak in the electron affinity detector than other, later peaks. The small size of this peak was a result of the low response of the electron affinity detector to these esters (*2, 9*). *o*-Phenylphenol was incompletely reacted; the free phenol was present in all samples. The free phenol is preferably detected rather than the ether.

Minor peaks were present in the chromatograms of several compounds; these peaks were assumed to be isomers (*16*) or manufacturing impurities. The minor peaks are not indicated in the tables.

Initially, the carboxylic acids were dissolved in methanol; the free acids were partially or completely converted to the methyl ester, and so the solutions

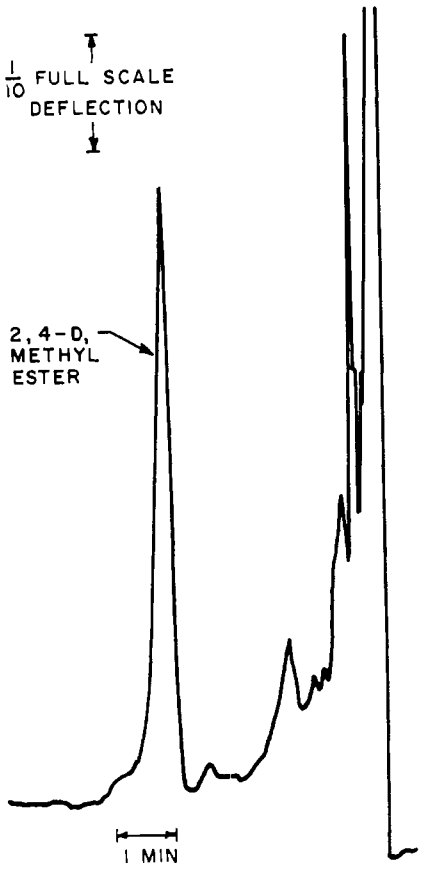

Figure 1. Chromatogram of 2,4-D methyl ester in oranges extract

Instrument: 2500R Detector: electron affinity
Column: 1-meter glass, 2% SE-30, 0.2% Versamid 900
Attenuator settings: input × 10^2, output × 4

were not suitable for the preparation of the ethyl esters. When the acids were dissolved in ethyl acetate, there was no evidence of any interaction between acid and solvent. Since the phenols did not react with methanol, methanol would be suitable as a solvent for the phenols.

The methyl esters of the butyl phosphoric acids have been prepared with diazomethane and, thus, separated by gas chromatography on the silicone liquid phases DC 200 and E301 (*11*). The diazomethane precursor nitrosomethyl urea, which was used in this work, is not readily available and was not considered in the present investigations. The gas chromatographic liquid phases did not offer any advantage over the liquid phases which were used in the present investigations.

While there have been investigations on the acids which result from degradation of phosphate pesticides using paper chromatography (*5*, *17*), and thin-layer chromatography (*13*) techniques, apparently none have used gas chromatographic techniques. Since the esters may be readily prepared with diazomethane, the acids which are degradation products may be easily detected with gas chromatographic techniques. The esters also may be more readily chromatographed on thin-layer chromatographic plates than the free acids (*18*).

With both the methyl and the ethyl esters of the acids being available for chromatography, the acids may be identified definitively, using a single gas chromatographic column. The availability of the second ester also affords an opportunity for the avoidance of any interferences which may be in the sample. The extraction method for the preparation of diazomethane and diazoethane provides a quick, easy method for the preparation of the esters of acids for chromatographic analysis.

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