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THIN-LAYER CHROMATOGRAPHY OF DIESTERS AND SOME MONOESTERS OF PHOSPHORIC ACID

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SUMMARY

Several monoesters and diesters of phosphoric acid and phosphorothioic acid are separated in two chromatographic systems which are described. A spray reagent, DQC (2,6-dibromobenzoquinone-4-chloroimide, 1%, in acetic acid), gives a yellow color with thiolo-sulfur and sulfhydryl groups, a red color with thiono-sulfur and a brown color with thioureas. The identity of phosphorothioate esters is often changed due to isomerization reactions. This has led to erroneous conclusions in previous reports concerning the color obtained with the DQC spray and the identity of the product formed by demethylation of the insecticide bromophos.

INTRODUCTION

In a recent publication¹, a simple procedure for the separation of some insecticide degradation products was reported. Scrutinizing the reference substances—many of them obtained from producers of insecticides—demonstrated, however, that their identities are not always in accordance with their label. This is mainly due to isomerization reactions.

In the present study a revised list of R_F values of compounds run in the acetonitrile-water system, and the color developed with the spray reagent (DQC) used for sulfur-containing phosphates, is given, and the isomerization and esterification reactions are discussed. Another chromatographic system, more suitable for the monoesters of phosphoric acid, is reported. The DQC spray has been tried for other sulfur-containing compounds.

EXPERIMENTAL

Substances for chromatography

AUTENRIETH AND HILDEBRAND² synthesized phenyl phosphorothionate by hydrolysis of phenyl phosphorothiodichloride in ethanolic alkali. The NMR spectrum of the product formed by this method in our laboratory showed the presence of phenoxy and ethoxy groups in a 1:1 proportion. The product actually formed was O-ethyl O-phenyl phosphorothionate. The use of other alcohols as solvents led to

other monoalkyl monophenyl phosphorothionates. In the same way, *p*-nitrophenyl phosphorothiodichloride (synthesized according to TOLKMITH³), gave O-alkyl O-*p*-nitrophenyl phosphorothionates.

Many O-alkyl O-aryl phosphorothionates could therefore easily be synthesized by this method. Small samples for TLC were prepared by mixing 10 mg aryl phosphorothiodichloride with 0.5 ml alcohol and 0.5 ml 0.2 M NaOH. The mixtures were heated at 37° overnight and 1 μ l samples were directly chromatographed. The O-aryl phosphorothionates were synthesized in the same way, using acetone instead of alcohol in the reaction mixture. Also, when 2-propanol was used, small amounts of monoesters were formed together with isopropyl aryl phosphorothionate diester. No confirmation by NMR was, however, performed for these monoesters because the reaction products were unstable and difficult to crystallize. Under similar reaction conditions, $(C_6H_5O)_2PSCl$ and $(CH_3O)_2PSCl$ were hydrolysed to give $(C_6H_5O)_2PSO_2Na$ and $(C_2H_5O)_2PSO_2Na$, respectively.

Dialkylesters were prepared according to methods described earlier^{4,5}. $C_2H_5S(C_2H_5O)PO_2NH_4$ and $C_2H_5S(C_6H_5O)PO_2Na$ were prepared from the O-ethyl compounds by isomerization. They were heated (as dry powder) in a Pyrex tube on the bunsen flame for 2 min. The isomerization of $(CH_3O)_2PSO_2NH_4$ to $CH_3S(CH_3O)PO_2NH_4$ also occurred spontaneously if kept refrigerated, but the sodium and potassium dimethyl phosphorothionate were stable.

Salts of S-methyl O-aryl phosphorothionates were prepared by refluxing equimolar amounts of KI, NaI or LiCl with O,O'-dimethyl O'-aryl phosphorothionates in butanone. Isomerization and demethylation take place as described by HILGETAG *et al.*⁶. Methyl aryl phosphates and dimethyl phosphate were synthesized by the same method.

The other substances used were purchased or obtained as gifts from different producers of insecticides. If doubtful, their identities were checked by NMR.

Chromatography

Layers (0.25 mm) of Silica Gel G were activated by heating at 110° for 1 h. No precautions were taken to prevent deactivation. Air drying was sufficient when the humidity of the air was moderate. 0.20-mm layers of microcrystalline cellulose (Sigmacell, Type 19) were prepared by blending 15 g with 85 ml water in a Servall Omnimixer at about 10000 r.p.m. for 1 min. Before spraying on glass plates, the mixture was de-aerated by applying vacuum. The plates were air-dried before use.

Technical acetonitrile (Merck) was used without any purification. The standard mixture used was 88% acetonitrile + 12% water, but other mixtures were also tried, using pure water or 1.2 N HCl⁷. Chromatograms on cellulose were developed in water-propionic acid-benzene (25:62:25)⁸.

Water solutions, 0.5% w/v of the esters and 0.1% w/v of the inorganic phosphates were made, and 1- μ l aliquots were chromatographed.

Spray reagents

The sulfur-containing compounds were detected by spraying with 1% DQC (2,6-dibromobenzoequinone-4-chloroimide) in acetic acid¹. The reagent was tried for other sulfur-containing compounds in spot tests. Phosphate was detected by the very sensitive method described previously¹, using Chrystall Violet in the second spray.

RESULTS AND DISCUSSION

The acetonitrile-water system is excellent for separating a wide variety of diesters of phosphoric- and phosphorothioic acid. The R_F values are given in Table I.

TABLE I

R_F VALUES OF PHOSPHATE AND PHOSPHOROTHIOATE ESTERS

(I) Layer, Silica Gel G; development, acetonitrile-water (88:12). (II) Layer, microcrystalline cellulose; development, water-propionic acid-benzene (25:62:25).

Substance	I	II
<i>Inorganic phosphate and monoesters</i>		
Na_3PO_4	0.0	0.2
Na_3PSO_3	0.10	0.2
$\text{CH}_3\text{OPO}_3\text{Na}_2$	0.01	0.36
$\text{C}_6\text{H}_5\text{OPO}_3\text{Na}_2$	0.03	0.50
4- NO_2 - $\text{C}_6\text{H}_4\text{OPO}_3\text{Na}_2$	0.0	0.46
β -naphthyl- OPO_3HNa	0.0	0.57
β -glycerophosphate	0.0	0.25
$\text{CH}_3\text{OPSO}_2\text{Na}_2$	0.03	0.30
$\text{C}_6\text{H}_5\text{OPSO}_2\text{Na}_2$	0.06	0.41
<i>p</i> - NO_2 - $\text{C}_6\text{H}_4\text{OPSO}_2\text{Na}$	0.10	0.38
<i>Phosphate diesters</i>		
$(\text{CH}_3\text{O})_2\text{PO}_2\text{Na}$	0.10	0.43
$(\text{C}_2\text{H}_5\text{O})_2\text{PO}_2\text{N}$	0.25	—
$(\text{C}_4\text{H}_9\text{O})_2\text{PO}_2\text{H}$	0.25	0.54
$\text{CH}_3\text{O}(4\text{-NO}_2\text{-C}_6\text{H}_4\text{O})\text{PO}_2\text{Na}$	0.35	0.58
$\text{CH}_3\text{O}(4\text{-Br-2,5-Cl}_2\text{-C}_6\text{H}_3\text{O})\text{PO}_2\text{Na}$	0.51	0.72
$\text{CH}_3\text{O}(\text{CHCl}=\text{C}(2,4,5\text{-Cl}_3\text{-C}_6\text{H}_2)\text{O})\text{PO}_2\text{Na}$	0.57	0.87
$(\text{C}_6\text{H}_5\text{O})_2\text{PO}_2\text{Na}$	0.55	0.67
$(4\text{-NO}_2\text{-C}_6\text{H}_4\text{O})_2\text{PO}_2\text{Na}$	0.72	0.60
<i>Thiolo diesters</i>		
$\text{CH}_3\text{S}(\text{CH}_3\text{O})\text{PO}_2\text{Na}$	0.18	0.45
$\text{C}_2\text{H}_5\text{S}(\text{C}_2\text{H}_5\text{O})\text{PO}_2\text{NH}_4$	0.22	0.65
$\text{CH}_3\text{S}(\text{CH}_3\text{O})\text{P}(\text{O})\text{NH}_2$	0.70	0.82
$\text{CH}_3\text{S}(\text{C}_6\text{H}_5\text{O})\text{PO}_2\text{Na}$	—	0.68
$\text{C}_2\text{H}_5\text{S}(\text{C}_6\text{H}_5\text{O})\text{PO}_2\text{Na}$	0.42	—
$\text{CH}_3\text{S}(4\text{-NO}_2\text{-C}_6\text{H}_4\text{O})\text{PO}_2\text{Na}$	0.54	0.59
$\text{CH}_3\text{S}(4\text{-NO}_2\text{-3-CH}_3\text{-C}_6\text{H}_3\text{O})\text{PO}_2\text{Na}$	0.54	0.65
$\text{CH}_3\text{S}(4\text{-Br-2,5-Cl}_2\text{-C}_6\text{H}_2\text{O})\text{PO}_2\text{Na}$	0.60	0.83
$\text{C}_2\text{H}_5\text{S}(4\text{-Br-2,5-Cl}_2\text{-C}_6\text{H}_2\text{O})\text{PO}_2\text{Na}$	0.62	0.86
<i>Thiono diesters</i>		
$(\text{CH}_3\text{O})_2\text{PSO}_2\text{Na}$	0.38	0.39
$(\text{C}_2\text{H}_5\text{O})_2\text{PSO}_2\text{Na}$	0.48	0.55
$\text{C}_2\text{H}_5\text{O}(\text{C}_2\text{H}_5)\text{PSO}_2\text{Na}$	0.34	0.82
$(\text{CH}_3\text{O})_2\text{PS}_2\text{K}$	0.74	0.30
$\text{CH}_3\text{O}(\text{C}_6\text{H}_5\text{O})\text{PSO}_2\text{Na}$	0.63	0.53
$\text{C}_2\text{H}_5\text{O}(\text{C}_6\text{H}_5\text{O})\text{PSO}_2\text{Na}$	0.65	0.58
<i>iso</i> - $\text{C}_3\text{H}_7\text{O}(\text{C}_6\text{H}_5\text{O})\text{PSO}_2\text{Na}$	0.67	0.62
$(\text{C}_6\text{H}_5\text{O})_2\text{PSO}_2\text{Na}$	0.71	0.64
$\text{CH}_3\text{O}(4\text{-NO}_2\text{-C}_6\text{H}_4\text{O})\text{PSO}_2\text{Na}$	0.70	0.53
$\text{C}_2\text{H}_5\text{O}(4\text{-NO}_2\text{-C}_6\text{H}_4\text{O})\text{PSO}_2\text{Na}$	0.70	0.58
<i>iso</i> - $\text{C}_3\text{H}_7\text{O}(4\text{-NO}_2\text{-C}_6\text{H}_4\text{O})\text{PSO}_2\text{Na}$	0.71	0.60
$\text{CH}_3\text{O}(4\text{-NO}_2\text{-3-CH}_3\text{-C}_6\text{H}_3\text{O})\text{PSO}_2\text{Na}$	0.72	0.59
$\text{CH}_3\text{O}(4\text{-Br-2,5-Cl}_2\text{-C}_6\text{H}_2\text{O})\text{PSO}_2\text{Na}$	0.75	0.80
2,4,5- $\text{Cl}_3\text{-C}_6\text{H}_3\text{O}(\text{C}_2\text{H}_5)\text{PSO}_2\text{Na}$	0.69	0.82

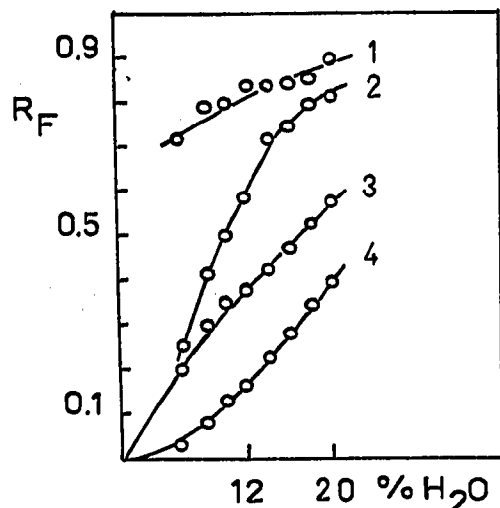


Fig. 1. The variation of R_F values, with the water content of acetonitrile-water mixtures used as the mobile phase. 1 = $\text{CH}_3\text{O}(4\text{-Br-2,5-Cl}_2\text{-C}_6\text{H}_2\text{O})\text{PSO}_2\text{Na}$; 2 = $\text{CH}_3\text{S}(4\text{-Br-2,5-Cl}_2\text{-C}_6\text{H}_2\text{O})\text{PO}_2\text{Na}$; 3 = $(\text{CH}_3\text{O})_2\text{PSO}_2\text{Na}$; 4 = $\text{CH}_3\text{S}(\text{CH}_3\text{O})\text{PO}_2\text{Na}$.

O-alkyl and S-alkyl phosphorothioates are separated from each other and from the phosphate esters. JAGLAN AND GUNTHER⁷ have used the system for separating the S- and the O-isomers of desmethyl methyl parathion. While HCl was added to their system, it was not found necessary to do so in the present study, and many of the other compounds were not separated in the acetonitrile-HCl system. The conflicting results may be due to the different procedures for activating plates or the different amounts of water present in the acetonitrile. The R_F values are highly influenced by the water content, as demonstrated in Fig. 1.

Many of the salts were available with different cations (sodium, potassium, lithium, ammonium or tetramethyl ammonium), but the R_F values did not seem to be significantly influenced by the cation. Therefore only the sodium salts are presented in Table I except when they were not available.

As it appears from their R_F values, the phosphomonoesters are not very well separated with acetonitrile. They are, however, well separated in water-propionic acid-benzene on microcrystalline cellulose.

This system is also suitable for many diesters and has quite different separation properties from the acetonitrile-water system, as thiono-sulfur seems to lower the R_F values compared to the corresponding esters with or without thio-sulfur. The time of development is about $2\frac{1}{2}$ h. Organophosphorus compounds are not so easily destroyed on an organic layer—a necessity when a detection method based upon color development with inorganic phosphate is used.

The spray reagent DQC gave a *yellow* color with S-alkyl phosphorothioates and a *reddish* color with O-alkyl phosphorothionates. Sulfhydryl groups, as in mercaptoethanol, glutathione (reduced and oxidized), cystein, cystine, etc., also gave *yellow* colors. The sensitivity of mercaptoethanol was about $0.2 \mu\text{g}$, and that of glutathione was about $1 \mu\text{g}$. Substituted thioureas gave a *brown* color. Therefore DQC may also be a sensitive spray for other sulfur-containing compounds. S-acetyl thiocholine and S-methyl glutathione gave *no* coloration.

These results concerning R_F values and color developments clearly indicates

that one of the products of bromophos (O,O'-dimethyl O''-4-bromo-2,5-dichlorophenyl phosphorothionate) metabolism has previously been erroneously identified as bis-desmethyl bromophos^{9,10}. The reference substance labeled "des-methyl bromophos" was actually the S-methyl derivative ($\text{CH}_3\text{S}(4\text{-Br-2,5-Cl}_2\text{-C}_6\text{H}_2\text{O})\text{PO}_2\text{Na}$), and those labeled O-aryl phosphorothionate monoesters were actually O-alkyl O-aryl phosphorothionates in these reports^{9,10}. These results prove that *one* methyl group of bromophos is split off, in agreement with the work of PLAPP AND CASIDA^{11,12}.

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