CHROM. 3855

POLYAMIDE LAYER CHROMATOGRAPHY OF ORGANOPHOSPHORUS PESTICIDES

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SUMMARY

The separation of nineteen organophosphorus pesticides by one-dimensional and two-dimensional chromatography and their detection on polyamide layers were investigated. A comparison was made between the detection limits on polyamide and silica gel layers, and this showed the advantages of polyamide layer chromatography.

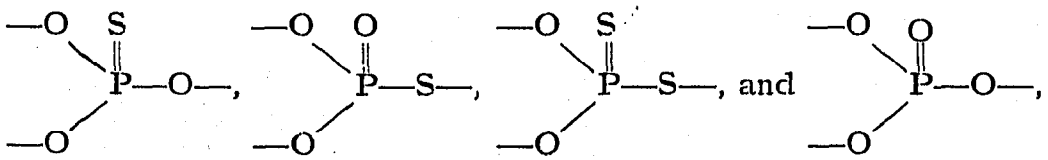
Some correlations between the chemical structure of the samples and their R_F values on polyamide layers and their connection with the type of solvent system used were discussed.

INTRODUCTION

Polyamide has been used as a chromatographic substrate by a number of workers[1–20]. Recent developments include its use for the separation and identification of plant constituents[1–6], biochemical substances[7–11], medicinals[12–14] and food additives[2,15,16].

In column chromatography on polyamide the separation process was found to be highly affected by hydrogen bonding forces between the hydroxyl groups in phenolic compounds and the –CONH– groups in the polyamide molecules[6,23]. It was also reported that the specific interaction between aromatic nitro groups or quinone structures and the polyamide molecules participates in the separation on polyamide[19,20]. Accordingly, it has been generally assumed that in thin-layer chromatography on polyamide layers hydrogen bonding likewise would play a predominant role[2,21,22].

The chemical structures of most organophosphorus pesticides have fundamental skeletons such as



which are composed of linkages such as —O—, —S—, P = S and P = O. Each oxygen and sulfur atom in these linkages could be concerned, more or less, in hydrogen bonding with the —CONH— groups in the polyamide molecules, and this is the reason for selecting organophosphorus pesticides for study by polyamide layer chromatography. The nineteen organophosphorus pesticides (shown in Table I) which have been in practical use were analyzed on $CaSO_4$-bound polyamide layers in the solvent systems listed in Table II. It was found that thin-layer chromatography on polyamide is a suitable method for the micro-analysis of these compounds, and some correlation exists between the chemical structure and the R_F value of the compounds in various types of solvent system.

EXPERIMENTAL

Preparation of the polyamide chromatoplates

Fourteen grams of polyamide powder "Wakō Polyamide B-10"* were mixed with 55 ml of distilled water to give a homogeneous suspension. This suspension was spread evenly on each of five glass plates (20 × 20 cm), held horizontally, with a suitable applicator, pre-set to give an applied layer of 0.25 mm thickness. The coated plates were kept horizontal and dried at 60° for 1 h and stored in a desiccator containing silica gel.

Application of samples and development of chromatoplates

The samples (1 μl of 0.5% w/v solution in ethanol) were spotted on a line 3 cm from the bottom edge. The plates were then eluted in the developing chamber (24 × 24 × 11 cm) by an ascending technique, at 24–26°. The solvent front was allowed to travel a distance of 12 cm from the starting line.

Detection

Most of the samples containing a phenyl group in their structures (*cf.* Table I) gave dark spots in transmitted U.V. light. All of the samples except for Nos. 18 and 19 could be detected by spraying with an 0.4% ethanolic solution of Congo Red or an 0.25% ethanolic solution of fluorescein after exposure to bromine vapor. Samples of Nos. 10–17 could also be detected by spraying with 10% HCl containing 0.5% of $PdCl_2$.

Organophosphorus pesticides

All nineteen organophosphorus pesticides used by us were supplied by Nippon Tokushu-Nōyaku K.K., Nissan Chemical Co. Ltd., Sankyo Co. Ltd., and Sumitomo Chemical Co. Ltd., (Table I).

Solvents

The solvents used in this experiment were purified by conventional methods to meet with chromatographic requirements.

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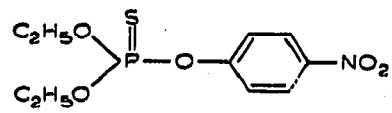
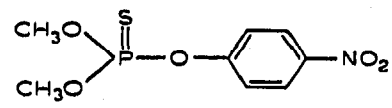
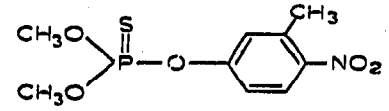
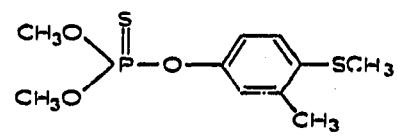
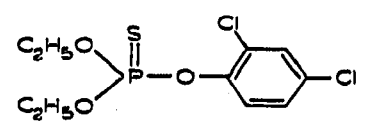
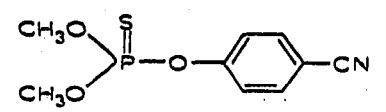
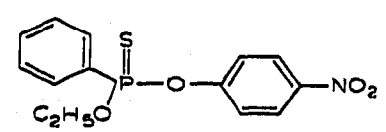
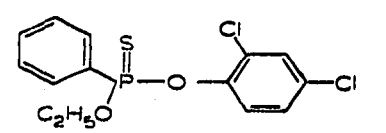
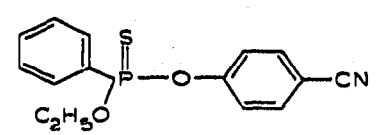
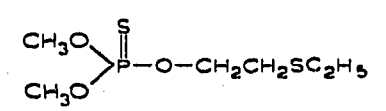
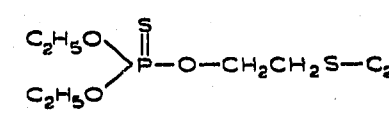
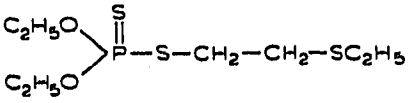
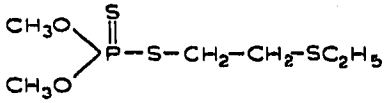
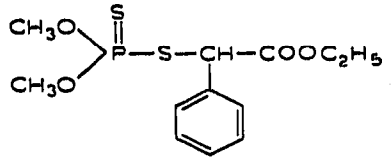
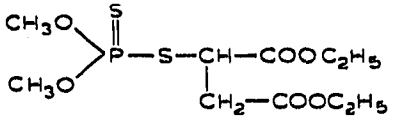
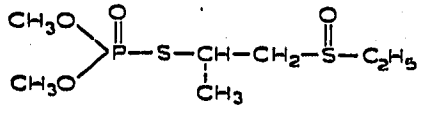
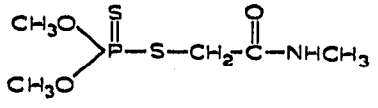
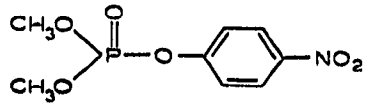
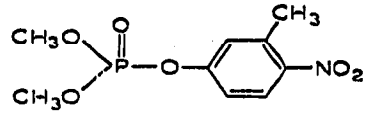
* "Wakō Polyamide B-10" is composed of 90% polyamide powder and 10% anhydrous calcium sulfate as a binder, and available from Wakō Pure Chemical Industries, Ltd., Nihonbashi-honchō, Chuō-ku, Tokyo, Japan.

TABLE I

ORGANOPHOSPHORUS PESTICIDES

Sample No.	*Name*	*Chemical structure*	
1	Parathion		O,O-Diethyl-O-(4-nitrophenyl) phosphorothioate
2	Methyl parathion		O,O-Dimethyl-O-(4-nitrophenyl) phosphorothioate
3	Sumithion (MEP)		O,O-Dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate
4	Baycid		O,O-Dimethyl-O-(3-methyl-4-methylthiophenyl) phosphorothioate
5	ECP (VC-13)		O,O-Diethyl-O-(2,4-dichlorophenyl) phosphorothioate
6	Cyanox (S-4084)		O,O-Dimethyl-O-(4-cyanophenyl) phosphorothioate
7	EPN		O-Ethyl-O-(4-nitrophenyl)phenyl phosphonothioate
8	S-seven		O-Ethyl-O-(2,4-dichlorophenyl) phenyl phosphonothioate
9	Surecide (CYP)		O-Ethyl-O-(4-cyanophenyl)phenyl phosphonothioate
10	Methyl demeton (Metasystox)		O,O-Dimethyl-O-(2-ethylthio)ethyl phosphorothioate
11	Demeton (Systox)		O,O-Diethyl-O-(2-ethylthio)ethyl phosphorothioate

12	Disyston (Dithio-Systox)		O,O-Diethyl-S-(2-ethylthio)ethyl phosphorodithioate
13	Thiometon (Ekatin)		O,O-Dimethyl-S-(2-ethylthio)ethyl phosphorodithioate
14	Cidial		O,O-Dimethyl-S-(phenyl carboethoxy) methyl phosphorodithioate
15	Malathion		O,O-Dimethyl-S-(1,2-diethoxy carbonyl ethyl) phosphorodithioate
16	Estox		O,O-Dimethyl-S-(2-ethylsulfoxy-1-methyl)ethyl phosphorothiolate
17	Dimethoate		O,O-Dimethyl-S-(N-methyl carbamoyl methyl) phosphorodithioate
18	Methyl paraoxon		O,O-Dimethyl-O-(4-nitrophenyl) phosphate
19	Oxo-Sumithion		O,O-Dimethyl-O-(3-methyl-4-nitrophenyl)phosphate

RESULTS AND DISCUSSION

One-dimensional chromatography

The solvent systems suitable for the separation of organophosphorus pesticide on polyamide layers are summarized in Table II. They are roughly classified into tw types, group I and II according to the polarity of the constituent solvents. Table II shows the R_F values of these samples in the above solvent systems. It is observe that R_F values of most of the samples conflict with each other when the use of an two systems, one from Group I and one from Group II, are compared and such tendency is remarkable in the cases of Nos. 16–19 each of which has a highly pola functional group such as $=S \rightarrow O$, $-CONHCH_3$ and $\equiv P=O$. Among the solver systems listed in Table II, d, g and j are the most suitable for the separation of thes organophosphorus pesticides.

TABLE II

SOLVENT SYSTEMS FOR CHROMATOGRAPHY OF ORGANOPHOSPHORUS PESTICIDES ON POLYAMIDE LAYERS

Symbol	*Group*	*Components*	*Ratio (v/v)*
a	I	H_2O–AcOH	9:1
b	I	H_2O–AcOH	8:2
c	I	H_2O–AcOH	5:5
d	I	H_2O–MeOH	5:5
e	I	H_2O–MeOH	6:4
f	I	H_2O–acetone	5:5
g	I	H_2O–acetone	6:4
h	I	H_2O–AcOH–MeOH	5:1:4
i	II	*n*-Hexane–AcOH	9:1
j	II	*n*-Hexane–AcOH	95:5
k	II	Petroleum ether–AcOH	95:5
l	II	Cyclohexane–AcOH	95:5

Each spot is detected by using any of the methods described above as in the case of silica gel layers. In Table IV, a comparison is made between the detection limit of the spots on polyamide layers and on silica gel layers. It can be seen from this that the detection of organophosphorus pesticides on polyamide layers is superior to that on silica gel layers except in the case of the $PdCl_2$–HCl method. To avoid severe damage of polyamide layers, the detection of the spots with the $PdCl_2$–HCl reagent has to be performed without heating and this results in a lower sensitivity. Because polyamide has an extreme affinity for bromine, in the case of methods

TABLE III

R_F VALUES OF ORGANOPHOSPHORUS PESTICIDES ON POLYAMIDE LAYERS

Sample No.	*Solvent system*											
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*
1	0.03	0.07	0.58	0.27	0.13	0.60	0.35	0.61	0.89	0.82	0.87	0.93
2	0.08	0.15	0.63	0.33	0.20	0.66	0.43	0.65	0.62	0.59	0.73	0.76
3	0.04	0.10	0.61	0.30	0.17	0.65	0.39	0.63	0.74	0.69	0.82	0.85
4	0.01	0.03	0.49	0.23	0.10	0.61	0.33	0.55	0.91	0.87	0.91	0.95
5	0.00	0.00	0.40	0.16	0.05	0.53	0.21	0.50	1.00	1.00	1.00	1.00
6	0.12	0.22	0.70	0.45	0.32	0.73	0.51	0.71	0.52	0.46	0.63	0.60
7	0.00	0.01	0.41	0.12	0.03	0.47	0.16	0.42	0.82	0.76	0.84	0.93
8	0.00	0.00	0.23	0.05	0.01	0.37	0.06	0.32	1.00	0.97	0.96	1.00
9	0.00	0.02	0.50	0.22	0.08	0.56	0.25	0.54	0.75	0.68	0.79	0.88
10	0.63	0.68	0.89	0.81	0.75	0.88	0.81	0.89	0.63	0.46	0.61	0.79
11	0.45	0.57	0.81	0.79	0.69	0.85	0.77	0.84	0.85	0.70	0.82	0.89
12	0.04	0.08	0.57	0.36	0.20	0.64	0.40	0.65	1.00	1.00	1.00	1.00
13	0.10	0.17	0.63	0.41	0.28	0.69	0.48	0.67	1.00	0.94	0.96	1.00
14	0.03	0.07	0.57	0.36	0.18	0.68	0.41	0.67	0.94	0.87	0.91	1.00
15	0.19	0.30	0.75	0.59	0.46	0.75	0.62	0.80	0.81	0.78	0.88	0.87
16	0.95	0.91	0.93	0.95	0.94	0.97	0.95	0.95	0.10	0.03	0.11	0.07
17	0.60	0.65	0.82	0.76	0.69	0.86	0.78	0.84	0.09	0.03	0.09	0.07
18	0.50	0.61	0.85	0.73	0.65	0.87	0.77	0.86	0.25	0.15	0.31	0.26
19	0.39	0.53	0.83	0.69	0.59	0.85	0.76	0.85	0.34	0.22	0.40	0.38

TABLE IV

DETECTION LIMITS OF SPOTS OF ORGANOPHOSPHORUS PESTICIDES ON POLYAMIDE AND SILICA GEL LAYERS

Sample No.	*Method of detection*							
	U.V. absorption		*HCl–$PdCl_2$*		*Br_2–fluorescein*		*Br_2–Congo Red*	
	*PA**	*Si**	*PA*	*Si*	*PA*	*Si*	*PA*	*Si*
1	1γ	5γ	5–10γ	1γ	1γ	2.5γ	2.5–5γ	5γ
2	1γ	5γ	5–10γ	1γ	1γ	2.5γ	2.5–5γ	5γ
3	1γ	5γ	5–10γ	1γ	1γ	2.5γ	2.5–5γ	5γ
4	1γ	5γ	5–10γ	1γ	1γ	2.5γ	2.5	5–10
5	2.5	5–10	5–10γ	1γ	1γ	2.5γ	2.5–5	5–10
6	2.5	5–10	5–10γ	1γ	1γ	2.5γ	2.5	2.5
7	2.5	5–10	5–10γ	1γ	1γ	2.5γ	2.5–5	5
8	2.5	5–10	5–10γ	1γ	1γ	2.5γ	2.5	5
9	2.5	5–10	5–10γ	1γ	1γ	2.5γ	2.5	5
10	—**	—	2.5	1γ	1γ	2.5γ	2.5–5	2.5
11	—	—	2.5	1γ	1γ	2.5γ	2.5–5	2.5
12	—	—	2.5	1γ	1γ	2.5γ	1–2.5	1–2.5
13	—	—	2.5	1γ	1γ	2.5γ	1–2.5	1–2.5
14	2.5	5–10	2.5	1γ	1γ	2.5γ	1–2.5	1–2.5
15	—	—	2.5	1γ	1γ	2.5γ	2.5	2.5
16	—	—	2.5	1γ	1γ	2.5γ	2.5	1–2.5
17	—	—	2.5	1γ	1γ	2.5γ	1–2.5	1–2.5
18	1	5	—	—	—	—	—	—
19	1	5	—	—	—	—	—	—

* PA = polyamide layer; Si = silica gel layer.
** Symbol — means that no spot was observed by these detection methods.

involving bromine treatment (*viz.* the Br_2–fluorescein reagent and the Br_2–Congo Red reagent) a developed polyamide plate must be left to stand for 20–24 h at room temperature after exposing to bromine vapour to free the polyamide surface from bromine. U.V. absorption of the spots on polyamide layers is much more distinct than those on silica gel layers because of brightness of the background on the polyamide layers.

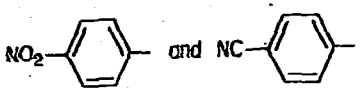
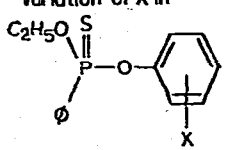
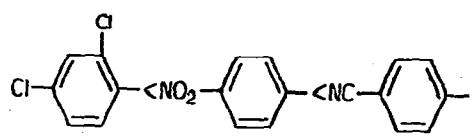
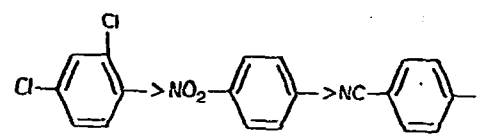
Some correlations between the variation of functional groups in the chemical structure of the samples and their R_F values are summarized in Table V. This Table also shows a reversal of the effect on the R_F values of these compounds if a group II solvent system is used instead of a group I solvent system.

Two-dimensional chromatography

Fig. 1 shows the two-dimensional separation of a mixture containing the nineteen organophosphorus pesticides using a combination of solvent systems d and j. Of all the possible combinations of solvent systems listed on Table II, this combination gives the best distribution of spots on the chromatogram. From the relationship between the chemical structures of the samples tested and the positions of their spots, these compounds are classified into three groups, A, B and C. In the group A compounds (Nos. 10–15) the structure is such that all three substituents through an —O— or —S— linkage in each compound are alkyl groups. Each group B compound

TABLE V

EFFECT OF THE VARIATION OF FUNCTIONAL GROUPS IN ORGANOPHOSPHORUS PESTICIDES ON THEIR R_F VALUES

Functional group	*Example of organophosphorus pesticide*	R_F *value*	
		Solvent	
		*Group I (a–h)**	*Group II (i–l)*
CH_3O- and C_2H_5O-	1** and 2, 12 and 13	$CH_3O- > C_2H_5O-$ lowering R_F value	$CH_3O- < C_2H_5O-$ elevating R_F value
Introduction of CH_3 group into phenyl group	2 and 3, 18 and 19		
P=S and P=O	2 and 18, 3 and 19	P=S < P=O	P=S > P=O
P—S—R and P—O—R	10 and 13	P—S—R < P—O—R	P—S—R > P—O—R
	2 and 6	NO_2- < NC—	NO_2- > NC—
Variation of X in 	7, 8 and 9		

* Symbols, see Table II.

** Sample numbers, see Table I.

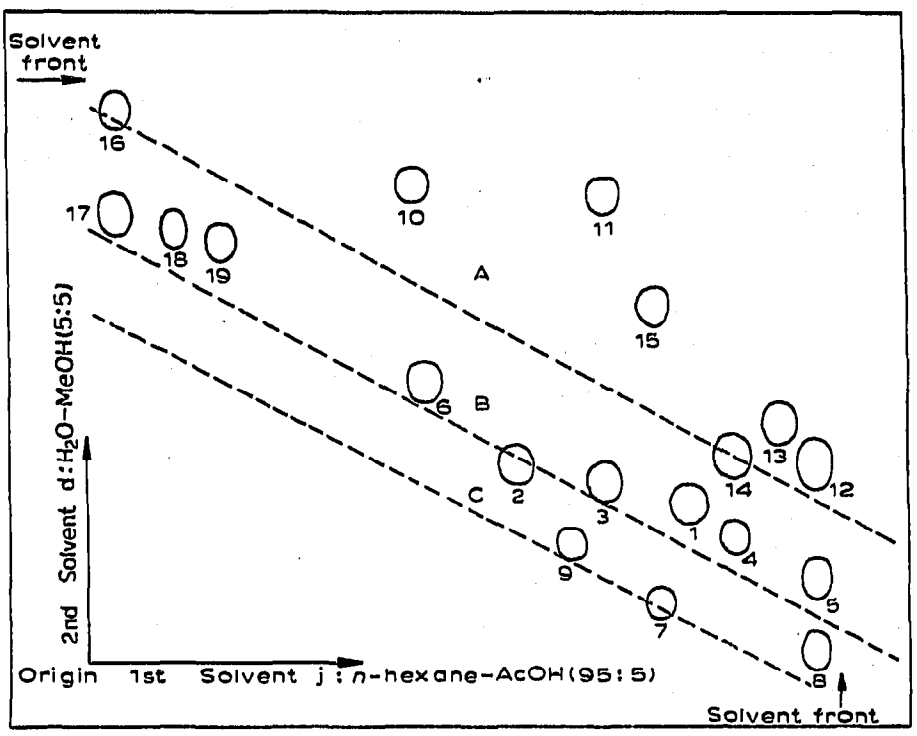


Fig. 1. Two-dimensional chromatogram of organophosphorus pesticides. For sample numbers, see Table I.

(Nos. 1–6, 18 and 19) is a derivative of O,O-dialkyl-O-phenyl phosphorothioate or phosphate, and all group C compounds (Nos. 7–9) are phenyl phosphonic acid derivatives. Both compounds No. 16 and 17 show irregular chromatographic behavior which is attributed in the presence of a polar functional group such as $=S \rightarrow O$ or $—CONHCH_3$ in their structures.

CONCLUSION

The thin-layer chromatography of organophosphorus pesticides on polyamide was investigated on the basis of the assumption that hydrogen bonding between particular groups in the pesticide and the polyamide layer would occur. However, it was found that in these compounds hydrogen bonding is not of great importance, but the polar or non-polar character of the solvent used decisively affects their chromatographic behavior. Consequently, the results of this investigation lend some support to the suggestion that the polyamide surface has a dual character depending on the nature of the eluent system[15,24].

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