Thin-Layer Chromatographic Determination of Bidrin, Azodrin, and Their Metabolites

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A method for the determination of Bidrin and Azodrin, a major metabolite, and eight other metabolites of these two insecticides is described. The organophosphorus compounds are analyzed by a modified version of thin-layer chromatography in which the chromatogram is scraped to make narrow straight strips. Two adsorbents, silica gel G and silica gel H, are employed for preparing the chromatographic plates. Twenty-seven solvent systems are tested, and two chromogenic reagents are employed. Good separations are obtained for most compounds; however, no single solvent system can separate all of the organophosphorus compounds. The practical minimum detectable amount is 2 μg. when the chromatogram is treated with bromine vapor and silver nitrate solution; this is lowered to 1 μg. or less when the chromatogram is treated with 4-(*p*-nitrobenzyl)-pyridine and tetraethylenepentamine solutions. Thirty-eight dyes are included in this study.

A number of papers have been published on the thin-layer chromatographic analysis (TLC) of organophosphorus pesticides. Walker and Beroza (1963) reported a single and simple procedure for the determination of 62 organophosphorus and organochlorinated insecticides with 19 different solvent systems. In addition, the R_f values of a number of dyes were included. Bunyan (1964) compared the behavior of 17 organophosphorus insecticides toward three methods of detection. The thin-layer chromatographic behavior of 10 organophosphorus esters in 16 different solvent systems was described by Salame (1964), who noted that no single solvent system would separate all insecticides but each could be identified by two successive separations. Stanley (1964) published the R_f values of 31 organophosphorus pesticides in six solvent systems on coated microscope slides. A thin-layer chromatographic screening test for organophosphorus pesticide residues was developed by Abbott *et al.* (1967). A new chromogenic spray reagent, 4-(*p*-nitrobenzyl)pyridine, was reported by Watts (1965), and was employed by Ragab (1967a) in his study of organophosphorus pesticides and some of their breakdown products on thin-layer chromatograms. In other work, Ragab (1967b) employed TLC for the separation of 47 organothiophosphorus pesticides and breakdown products. The spots developed on the TLC plates were further analyzed by a direct fluorescent method. Bidrin was mentioned only by the Ragab, while the new Azodrin and the other metabolites were not mentioned by any of the above workers.

In this investigation, attempts were made to develop a TLC method for Bidrin and its metabolites. The important metabolic products and the parent compounds are listed in Table I (Bull and Linquist, 1964; 1966; Menzer and Casida, 1965; Porter, 1967). The TLC spots of these organophosphorus esters were not visible when exposed under either ordinary light or ultraviolet radiation. Various chromogenic reagents were employed to develop these spots. Also included in this study were 38 dyes which could be used as standards for locating spots when development with chromogenic reagents became undesirable. To prevent the spreading and diffusion of spots, an improved TLC technique—i.e., thin-strip thin-layer chromatography (Beckman and Winterlin, 1966)—was employed in the preparation of TLC plates.

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[1] Deceased.

EXPERIMENTAL

Apparatus and Reagents. THIN-LAYER CHROMATOGRAPHIC PLATES. Thoroughly washed glass plates (20 × 20 cm.) were rubbed with alcohol prior to application of the adsorbents. Five plates were prepared each time. Two types of adsorbents were used, silica gel G and silica gel H. The 0.25-mm. silica gel G plates were prepared by the standard method of spreading evenly onto the plates a slurry made of 30 grams of the adsorbent and 60 ml. of water. The plates were air-dried for 5 minutes and were activated in an oven at 130° C. for at least 30 minutes, after which they were placed in a desiccator for cooling to room temperature and storage.

Preparation of the 0.25-mm. silica gel H plates followed a modified method. The slurry used for these plates consisted of 30 grams of the adsorbent and 70 ml. of water. After application of slurry, the plates were air-dried for at least 1 hour, then activated in an oven at 130° C. for an hour prior to storage in a desiccator.

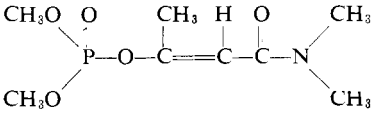
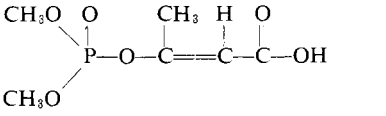
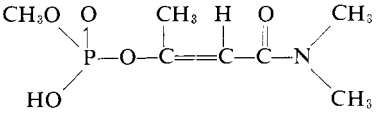
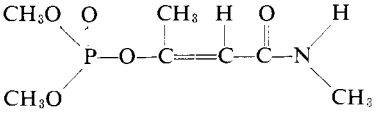
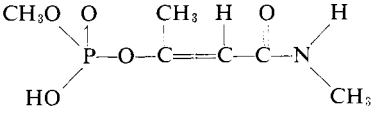
Standard Solutions. Standards of Bidrin and its metabolites were used without further purification.

Standard solutions of 1 μg. per μl. were prepared by weighing an exact amount of each compound and diluting to an exact volume with solvent. Benzene was used to dissolve Bidrin, Azodrin, and *N,N*-dihydrogen Bidrin; chloroform was used to dissolve Bidrin acid, *N*-hydroxymethyl Bidrin, *N*-hydroxymethyl Azodrin, and the two glucosides; methanol was used to dissolve the sodium salts of des-*O*-methyl Bidrin and des-*O*-methyl Azodrin.

The dyes (Table IV) were purchased from commercial chemical companies. Their standard solutions (1 μg. per μl.) were prepared by the technique described for the organophosphorus esters and ethanol was used as the solvent.

Solvents. Reagent grade solvents were redistilled in this laboratory. Most of the solvent systems used consisted of 90% (v./v.) of dichloromethane, acetonitrile, ethyl acetate, or benzene mixed with 10% of methanol, ethyl ether, ace-

Table I. Names and Formulas of

Shell No.	Abbreviated Name	Chemical Name	Formula
SD3562	Bidrin	3-(Dimethoxyphosphinyloxy)-*N,N*-dimethyl-*cis*-crotonamide	
SD4455	Bidrin acid	3-(Dimethoxyphosphinyloxy)-*cis*-crotonic acid	
SD8777	Des-*O*-methyl Bidrin[a]	3-(Methoxy, hydroxyphosphinyloxy)-*N,N*-dimethyl-*cis*-crotonamide	
SD9129	Azodrin	3-(Dimethoxyphosphinyloxy)-*N*-methyl-*cis*-crotonamide	
SD11191	Des-*O*-methyl Azodrin[a]	3-(Methoxy, hydroxyphosphinyloxy)-*N*-methyl-*cis*-crotonamide	

[a] Standards used in this laboratory in the form of their sodium salts.

Table II. R_f Values of Bidrin, Azodrin, and Their Metabolites

Organophosphorus Esters	CH_2CN + CH_3OH, 9 + 1	CH_3CN + Ether, 9 + 1	CH_3CN + Acetone, 9 + 1	CH_3CN + EtOAc, 9 + 1	CH_3CN + HOAc, 9 + 1	CH_3CN + H_2O, 9 + 1	CH_2Cl_2 + CH_3OH, 9 + 1	CH_2Cl_2 + Acetone, 9 + 1
Bidrin	0.40	0.18	0.15	0.17	0.31	0.49	0.83	0.05
Bidrin acid	0.05	[b]	[b]	[b]	0.59	0.14	0.08	0.04
Azodrin	0.48	0.29	0.21	0.23	0.39	0.51	0.71	0.03
N,N-Dihydrogen Bidrin	0.43	0.23	0.19	0.18	[b]	0.56	0.54	0.03
N-Hydroxymethyl Bidrin	0.47	0.31	0.32	0.23 0.07	[b]	[b]	0.78 0.69	0.03
N-Hydroxymethyl Azodrin	0.39	0.07	0.06	0.07	[b]	0.58	0.61 0.49	[b]
Des-*O*-Methyl Bidrin	Strips	Strips	Strips	[b]	[b]	0.10	0.27	[b]
Des-*O*-Methyl Azodrin	Strips	Strips	Strips	[b]	0.21	0.16	0.88 0.63	0.09
Azodrin glucoside	0.13	Strips	Strips	Strips	0.12	0.28	Strips	0.06
Bidrin glucoside	0.53 0.19	Strips	Strips	0.45	0.17	[b]	Strips	0.66 0.11

[a] No movement of CH_2Cl_2 + ether, CH_2Cl + EtOAc, CH_2Cl_2 + H_2O, C_6H_6 + ether, C_6H_6 + acetone, or C_6H_6 + EtOAc (all 9 + 1) observed. [b] No movement observed.

tone, ethyl acetate, acetic acid, or water. Two solvent systems were slightly different from the above systems (Table II).

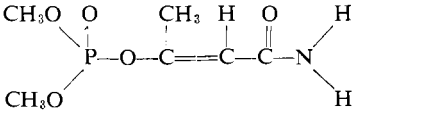
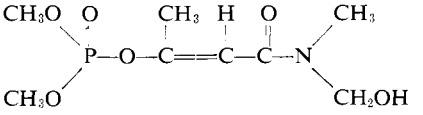
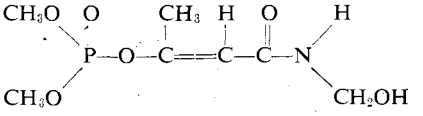
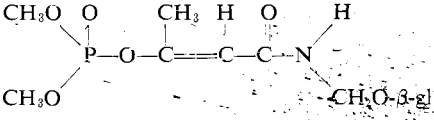
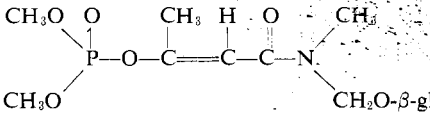
Chromogenic Reagents. Two sets of chromogenic reagents were employed. The first consisted of bromine vapor from a 5% solution of reagent grade bromine in carbon tetrachloride, and the silver nitrate solution (Mitchell, 1958) prepared by mixing a solution of 4.2 grams of reagent grade silver nitrate in 12.5 ml. of distilled water with 25.0 ml. of phenoxyethanol and diluting to 500 ml. with acetone.

The second set (Watts, 1965) consisted of a 2% solution of 4-(*p*-nitrobenzyl) pyridine (NSP) in acetone and a 10% solution of tetraethylenepentamine in acetone. The latter must be prepared fresh daily.

Ultraviolet Radiation Source. A Gates Raymaster multipurpose lamp equipped with a General Electric germicidal lamp (~2537 A.) was used for developing the spots.

Procedure. The TLC plates were scraped prior to the application of the samples by using a modified window cleaner (Beckman and Winterlin, 1966) to make 11 pairs of straight strips each 0.5 cm. wide. Then, 1- and 2-μl. (1- and 2-μg.) samples of each of the 10 organophosphorus ester solutions were applied to the first 10 pairs of the strips in a row 2 cm. from the bottom edge of the plates with a Hamilton 10-μl. syringe, and 1- and 2-μl. samples of a mixture containing 1-μg. per μl. of each of the compounds under investigation were applied to the last pair of strips. Two microliters (2 μg.) of each dye solution were also

Bidrin, Azodrin, and Their Metabolites

Shell No.	Abbreviated Name	Chemical Name	Formula
SD11311	*N,N*-Dihydrogen Bidrin	3-(Dimethoxyphosphinyloxy)-*cis*-crotonamide	
SD12210	*N*-Hydroxymethyl Bidrin	3-(Dimethoxyphosphinyloxy)-*N*-methyl, *N*-hydroxymethyl-*cis*-crotonamide	
SD12657	*N*-Hydroxymethyl Azodrin	3-(Dimethoxyphosphinyloxy)-*N*-hydroxymethyl-*cis*-crotonamide	
SD13311	Azodrin glucoside	3-(Dimethoxyphosphinyloxy)-*N*-β-glucosemethylenyl-*cis*-crotonamide	
SD14493	Bidrin glucoside	3-(Dimethoxyphosphinyloxy)-*N*-methyl, *N*-β-glucosemethylenyl-*cis*-crotonamide	

Obtained from TLC Analysis on Silica Gel G Plates[a]

CH_2Cl_2 + HOAc, 9 + 1	EtOAc + EtOH + HOAc, 9 + 9 + 1	EtOAc + CH_3OH, 9 + 1	EtOAc + Ether, 9 + 1	EtOAc + Acetone, 9 + 1	EtOAc	EtOAc + HOAc, 9 + 1	C_6H_6 + H_2O, 9 + 1	C_6H_6 + CH_3OH, 9 + 1	C_6H_6 + HOAc, 9 + 1	C_6H_6 + H_2O, 9 + 1	Hexane + CH_2Cl_2 + CH_3OH, 7 + 2 + 1
0.12	0.56	0.27	0.04	0.09	0.04	0.15	0.12	0.33	0.03	0.71	0.11
0.42	0.78	0.06	[b]	0.03	0.03	0.56	0.04	0.07	0.08	0.18	0.05
0.11	0.64	0.35	0.05	0.11	0.05	0.21	0.16	0.27	0.03	0.72	0.09
0.14	0.66	0.29	0.03	0.08	0.03	0.29	0.16	0.19	0.04	0.55	0.09
0.11 0.05	0.64	0.33	0.05	0.11	0.05	0.19	0.16	0.27	0.03	0.62	0.10
[b]	0.60	0.20	[b]	0.03	[b]	0.05	0.06	0.17	[b]	0.59	0.09
[b]	0.21	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	0.09	0.05
0.17	0.55 0.29	0.32	0.07	0.15	0.05	0.21	[b]	0.22	0.05	0.09	0.11 0.06
0.15	0.68 0.48	0.40 0.07	0.10	0.20 0.09	0.08	0.28 0.19	0.22	0.31 0.18	0.05	Strips	0.12 0.07
[b]	0.66 0.46	0.40 0.08	0.12 0.07	Strips	0.09	0.28 0.26	0.22	0.43 0.33	0.07 0.05	0.67	0.14 0.07

tested on the same TLC plate along with the organophosphorus esters. Nineteen dyes were placed on each plate, and two portions of a mixture containing these dyes were also applied.

Two hundred milliliters of mixed solvents were used for each solvent system. The mixture was placed in a TLC tank and was allowed to equilibrate for at least 1 hour. A large piece of filter paper which surrounded the inner wall of the tank with its bottom dipped in the solvents was introduced to help the vapor of the solvents reach saturation rapidly. After the chamber was saturated, two plates each containing 10 insecticides and 19 dyes were placed in the tank and allowed to develop. When the solvent front reached approximately 15 cm., the plates were removed from the tank, and the solvent front was marked immediately. Then the plates were air-dried for 5 minutes, and the positions of the dyes were marked.

The silica gel G plates, which were tested earlier than the silica gel H plates, were treated with the first set of chromogenic reagents. After each plate was treated with bromine vapor in a chamber for 30 seconds, it was removed, sprayed with silver nitrate reagent, then exposed to ultraviolet rays for 4 minutes. As the spots appeared they were marked. A Xerox copy of each plate was made for evaluation of data, and for permanent record.

The silica gel H plates were treated with the second set of chromogenic reagents. The plates were sprayed heavily with the NSP solution. After the solvent evaporated,

Table III. R_f Values of Bidrin, Azodrin, and Their Metabolites Obtained from TLC Analysis on Silica Gel H Plates

Organophosphorus Esters	CH_3CN + CH_3OH, 9 + 1	CH_2Cl_2 + CH_3OH, 9 + 1	CH_2Cl_2 + HOAc, 9 + 1	EtOAc + CH_3OH, 9 + 1	C_6H_6 + CH_3OH, 9 + 1	EtOAc + EtOH + HOAc, 9 + 9 + 1	C_6H_6 + EtOH + H_2O, 6 + 5 + 1
Bidrin	0.48	0.49	0.08	0.27	0.24	[a]	0.55
	0.47	0.50	0.08	0.29	0.24	[a]	0.55
Bidrin acid	0.06	0.08	0.33	0.08	0.07	0.80	0.21
	0.06	0.07	0.33	0.07	0.07	0.80	0.21
Azodrin	0.53	0.48	0.08	0.32	0.17	[a]	0.57
	0.52	0.47	0.08	0.34	0.18	[a]	0.56
N,N-Dihydrogen Bidrin	0.47	0.31	0.10	0.27	0.13	[a]	0.51
	0.47	0.31	0.10	0.27	0.12	[a]	0.51
N-Hydroxymethyl Bidrin	0.53	0.38	0.10	0.13	0.15	[a]	0.53
	0.52	0.38	0.10	0.13	0.14	[a]	0.52
N-Hydroxymethyl Azodrin	0.48	0.33	[b]	0.19	0.07	[a]	0.55
	0.48	0.32	[b]	0.20	0.07	[a]	0.55
Des-*O*-methyl Bidrin	[a]	[a]	[a]	[a]	[a]	[a]	[a]
	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Des-*O*-methyl Azodrin	[a]	[a]	[a]	[a]	[a]	[a]	[a]
	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Azodrin glucoside	0.16	0.01	[b]	0.04	[b]	[a]	0.26
	0.16	0.01	[b]	0.06	[b]	[a]	0.26
Bidrin glucoside	0.19	0.02	[b]	0.05	[b]	[a]	0.27
	0.19	0.02	[b]	0.07	[b]	[a]	0.27

[a] No spot developed.
[b] No movement observed.

the plates were placed in a 110° C. forced-draft oven for 5 minutes. Then they were removed and sprayed lightly with the tetraethylenepentamine solution until the development of blue spots. The outlines of these spots were marked, and Xerox copies of the plates were made.

RESULTS AND DISCUSSION

Thirty-eight dyes and 10 organophosphorus esters were analyzed on silica gel G plates with 26 solvent systems. The organophosphorus compounds were also analyzed on silica gel H plates with seven solvent systems. With only one exception the solvent systems used for the silica gel H plates were the same as some of the solvent systems used for the silica gel G plates. Four "major" solvents were employed, and their polarities ranged from very polar to nonpolar. These solvents were mixed with different "minor" solvents with polarities ranging from very polar to nonpolar.

The R_f values of Bidrin, Azodrin, and their metabolites on silica gel G plates are listed in Table II. In most cases, these compounds developed well defined dark brown spots against a light brown background, and in a few cases—e.g., spots of Bidrin and Azodrin plates developed with dichloromethane and methanol—well defined white spots against a light brown background were observed. Long exposure of the plates under ultraviolet radiation was undesirable because it darkened the background. An exposure time of 4 minutes using new light tubes gave best results. If no spots were visible after 5 minutes of exposure, the plates were placed under house light until the spots became visible.

With silver nitrate and ultraviolet treatments, the practical minimum detectable amount was observed to be 2.0 μg. for most compounds. In a few cases, 1.0 μg. was detected; however, the results were not reproducible. When the glucosides of Bidrin and Azodrin were analyzed on silica gel G plates, they tended to streak very badly and it often became impossible to determine the R_f values of these compounds from the chromatograms.

The R_f values of the organophosphorus esters on silica gel H are listed in Table III. Blue spots were observed for most compounds. No spots were observed for the sodium salts of des-*O*-methyl Bidrin and des-*O*-methyl Azodrin, regardless of the solvents used. Apparently, these chromogenic reagents would not work for inorganic salts of phosphates. Also, except for Bidrin acid, no spots were observed when the chromatographic plates were eluted with a mixture of ethyl acetate, ethanol, and acetic acid. Since the chromogenic reagents worked well for the plates eluted with mixed solvents such as ethyl acetate and methanol, or dichloromethane and acetic acid, no satisfactory explanation could be found for the phenomenon observed. Heating the plates after spraying with the NBP solution was a critical factor for obtaining good results. To develop satisfactory chromatograms, the plates must be heated in an oven operating at a temperature above 100° C. for sufficient time.

With the NBP treatment, the minimum detectable quantity was 1.0 μg. or less. The results were reproducible with both silica gel H and silica gel G plates. No streak was observed when Bidrin, Azodrin, and their metabolites were analyzed on silica gel H plates, and the spots were well defined. Chromatograms on silica gel H plates are far better

Table IV. R_f Values of Dyes from TLC Analysis on Silica Gel G Plates

Name	CH_3CN + CH_3OH, 9 + 1	CH_3CN + Ether, 9 + 1	CH_3CN + Acetone, 9 + 1	CH_3CN + EtOAc, 9 + 1	CH_3CN + HOAc, 9 + 1	CH_3CN + H_2O, 9 + 1	CH_2Cl_2 + CH_3OH, 9 + 1	CH_2Cl_2 + Ether, 9 + 1	CH_2Cl_2 + Acetone, 9 + 1	CH_2Cl_2 + EtOAc, 9 + 1	CH_2Cl_2 + HOAc, 9 + 1	CH_2Cl_2 + H_2O, 9 + 1	EtOAc + EtOH + HOAc, 9 + 9 + 1
Alizarin Yellow R	[a]	[a]	[a]	[a]	0.41-Y	0.37-Y	[a]	[a]	[a]	[a]	[a]	0.43-Y	0.34-Y
Bismarck Brown R	0.07-Y	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Bromocresol green	0.05-B	[a]	[a]	[a]	0.66-Y	0.40-B	[a]	[a]	[a]	[a]	[a]	[a]	0.17-Y
Bromophenol blue	0.03-B	[a]	[a]	[a]	0.66-B	0.32-B	[a]	[a]	[a]	[a]	[a]	[a]	0.77-B
Bromophenol red	0.18 0.10-P	[a]	[a]	[a]	0.24-P	0.43-P	[a]	[a]	[a]	[a]	[a]	[a]	0.57-P
Bromothymol blue	0.68-B	[a]	[a]	[a]	0.63-B	[a]	[a]	[a]	[a]	[a]	[a]	[a]	0.78-B
Chrysoidan V-special	0.74-Y	0.82-Y	0.68-Y	0.61-Y	0.08-Y	0.76-Y	0.70-Y	0.31-Y	0.50-Y	0.41-Y	[a]	0.11-Y	0.60-Y
Corallin yellow	0.51-Y	0.16-Y	[a]	0.10-Y	[a]	0.56-Y	0.33-Y	[a]	[a]	[a]	[a]	0.81-Y	[a]
Cresol red	0.18-P	[a]	[a]	[a]	0.24-Y	0.39-Y	[a]	[a]	[a]	[a]	[a]	0.30-P	0.75-Y
Darrow red	0.07-P	[a]	[a]	[a]	0.05-P	0.28-P	0.09-P	[a]	[a]	[a]	[a]	[a]	0.09-O
5′5′′-DBCSP[b]	[a]	0.09-Y	[a]	0.06-Y	[a]	0.52 0.09-Y	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Dibromofluorescein	0.08 0.06-Y	[a]	0.63-Y	[a]	0.72-Y	0.59 0.38-P 0.27 0.21-Y	0.13 0.07-Y	[a]	0.23 0.14-Y	[a]	0.88 0.75 0.66-Y 0.36	[a]	0.98-Y
Dichlorofluorescein	[a]	[a]	[a]	[a]	0.71-Y	0.13-Y	[a]	[a]	[a]	[a]	0.57-Y	0.74-Y	0.83-Y
DuPont Orange	[a]	[a]	[a]	[a]	0.61-Y	0.14 0.10-Y	[a]	[a]	[a]	[a]	[a]	[a]	0.30-Y
Eosine Y	[a]	[a]	0.74-P	0.75-P	[a]	0.20 0.19-P	[a]	[a]	0.22 0.15-P	[a]	[a]	[a]	0.91 0.86-P
Fluorescein	0.06-Y	[a]	[a]	[a]	[a]	0.51-Y	0.05-Y	[a]	[a]	[a]	0.13-Y	0.89-Y	[a]
Martius yellow	0.40-Y	0.20-Y	0.23-Y	0.11-Y	0.69-Y	0.67-Y	[a]	[a]	[a]	[a]	0.17-Y	[a]	0.73-Y
Metanil yellow	0.11-Y	[a]	[a]	[a]	0.24-Y	0.53-Y	[a]	[a]	[a]	[a]	[a]	0.39-Y	0.57-Y
Methylene blue	0.03-B	[a]	[a]	[a]	0.05-B	0.18-B	0.07-B	[a]	[a]	[a]	[a]	[a]	[a]
Methyl orange	0.05-Y	[a]	[a]	[a]	0.13-O	0.51-Y	[a]	[a]	[a]	[a]	[a]	[a]	0.49-O
Methyl red	0.13-Y	0.08-Y	0.06-Y	0.08-Y	0.50-P	0.55-Y	0.59-Y	[a]	0.09-Y	0.05-Y	0.21-P	0.20-Y	0.73-P
Methyl violet base	0.08-L	[a]	[a]	[a]	0.09-Y	0.47 0.41-L	0.26-L	[a]	[a]	[a]	[a]	[a]	0.05-Y
Natural black	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
α-Naphthol	0.66-Br	[a]	[a]	[a]	[a]	[a]	0.73-Br	0.54-Br	0.79-Br	0.67-Br	0.95-Br	0.23-Br	0.94-Br
β-Naphthol	[a]	[a]	[a]	[a]	[a]	[a]	[a]	0.48-Br	0.73-Br	0.61-Br	0.90-Br	[a]	0.94-Br
Nile Blue A	0.09-B	[a]	[a]	[a]	0.10-B	0.38-B	0.18-B	[a]	[a]	[a]	[a]	[a]	0.06-B
p-Nitrophenol	0.61-Y	[a]	[a]	0.65-Y	[a]	0.74-Y	0.67-Y	0.37-Y	0.58-Y	0.41-Y	[a]	[a]	[a]
Oil Blue N	0.79-B	0.80-B	0.67-B	0.71-B	0.70-B	0.77-B	0.87-B	0.88 0.81-B	0.96 0.85-B	0.88 0.85-B	[c]	0.77 0.70-B 0.62	0.81-B

Continued on Next Page

Table IV. R_f Values of Dyes from TLC Analysis on Silica Gel G Plates (*Continued*)

Name	CH_3CN + CH_3OH, 9 + 1	CH_3CN + Ether, 9 + 1	CH_3CN + Acetone, 9 + 1	CH_3CN + EtOAc, 9 + 1	CH_3CN + HOAc, 9 + 1	CH_3CN + H_2O, 9 + 1	CH_2Cl_2 + CH_3OH, 9 + 1	CH_2Cl_2 + Ether, 9 + 1	CH_2Cl_2 + Acetone, 9 + 1	CH_2Cl_2 + EtOAc, 9 + 1	CH_2Cl_2 + HOAc, 9 + 1	CH_2Cl_2 + H_2O, 9 + 1	EtOAc + EtOH + HOAc, 9 + 9 + 1
Oil Red O	0.80-P	0.82-P	0.67-P	0.73-P	0.72-P	0.82-P	0.89-P	0.89-P	0.90-P	0.87-P	[c]	0.83-P	0.82-P
Oil yellow	0.78-Y	0.81-Y	0.67-Y	0.72-Y	0.71-Y	0.79-Y	0.87-Y	0.87-Y	0.89-Y	0.89-Y	0.68-Y	0.84-Y	[a]
Orange IV	0.09-Y	[a]	[a]	[a]	[a]	0.57-Y	[a]	[a]	[a]	[a]	[a]	[a]	0.56-P
Sudan II	0.76-P	0.80-P	0.67-P	0.72-P	0.75-P	0.80-P	0.87-P	0.85-P	0.88-P	0.87-P	[c]	0.82-P	0.81-P
Sudan III	0.77-P	0.83-P	0.67-P	0.73-P	0.75-P	0.80-P	0.88-P	0.84-P	0.89-P	0.81-P	[c]	0.81-P	0.82-P
Sudan IV	0.80-P	0.84-P	0.68-P	0.75-P	0.75-P	0.81-P	0.89-P	0.80-P	0.96-P	0.88-P	[c]	0.85-P	0.83-P
Sudan Black B	0.80-B	0.91-B	0.78-B	0.80-B	0.76 0.68-B	0.84-B	[c]	0.76 0.52-B	0.80 0.67-B	0.89 0.69-B	[a]	0.89 0.63-B	0.91-B
Sudan yellow	0.80-Y	0.80-Y	0.67-Y	0.72-Y	0.72-O	0.78-Y	0.87-Y	0.81-Y	0.88-Y	0.84-Y	[c]	0.86-Y	0.83-O
Thymol blue	0.39-B	0.05-B	0.07-B	[a]	0.47-B	0.47-B	[a]	[a]	[a]	0.76-B	[a]	[a]	0.85-B
Toluidine Blue O	0.06-B	[a]	[a]	[a]	0.06-B	0.23-B	[a]	[a]	[a]	[a]	[a]	[a]	[a]

Name	EtOAc + CH_3OH, 9 + 1	EtOAc + Ether, 9 + 1	EtOAc + Acetone, 9 + 1	EtOAc	EtOAc + HOAc, 9 + 1	EtOAc + H_2O, 9 + 1	C_6H_6 + CH_3OH, 9 + 1	C_6H_6 + Ether, 9 + 1	C_6H_6 + Acetone, 9 + 1	C_6H_6 + EtOAc, 9 + 1	C_6H_6 + HOAc, 9 + 1	C_6H_6 + H_2O, 9 + 1	Hexane + CH_2Cl_2 + CH_3OH, 7 + 2 + 1
Alizarin Yellow R	[a]	[a]	[a]	[a]	0.46-Y	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Bismarck Brown R	[a]	[a]	0.52-Y	[a]	[a]	0.14-Y	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Bromocresol green	[a]	[a]	[a]	[a]	0.22-Y	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Bromophenol blue	[a]	[a]	[a]	[a]	0.10-B	0.05-B	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Bromophenol red	[a]	[a]	[a]	[a]	[a]	0.11-P	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Bromothymol blue	[a]	[a]	[a]	[a]	0.28-B	0.12-B	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Chrysoidan V-special	0.72-Y	0.68-Y	0.62-Y	0.62-Y	0.08-Y	0.68-Y	0.47-Y	0.44-Y	0.27-Y	[a]	[a]	0.32-Y	0.26-Y
Corallin yellow	0.48-Y	0.14-B	[a]	0.14-Y	[a]	0.42-Y	0.12-Y	[a]	[a]	[a]	[a]	[a]	0.07-Y
Cresol red	[a]	[a]	[a]	[a]	0.06-P	[a]	[a]	[a]	[a]	[a]	[a]	[a]	0.05-Y
Darrow red	0.09-P	[a]	[a]	[a]	[a]	0.14-P	0.09-P	[a]	[a]	[a]	[a]	[a]	0.05-P
5′5″-DBCSP[b]	[a]	[a]	[a]	[a]	0.13-Y	[a]	[a]	[a]	[a]	[a]	[a]	0.31-Y	[a]
Dibromofluorescein	0.13 0.04-Y	[a]	[a]	[a]	0.81-Y	0.28 0.12-Y	[a]	[a]	[a]	[a]	0.33 0.24 0.16-Y 0.08	0.34 0.25-Y 0.20	[a]
Dichlorofluorescein	[a]	[a]	0.08-Y	[a]	0.81-Y	0.10-Y	[a]	[a]	[a]	[a]	0.11-Y	0.47-P 0.40-Y	[a]
DuPont Orange	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[b]	[a]	0.47-P 0.36-L	[a]
Eosine Y	0.13-P	[a]	[a]	[a]	0.84-P	[a]	[a]	[a]	[a]	[a]	0.33 0.24-P 0.16	0.41 0.26-Y 0.20	0.03-P
Fluorescein	0.22-Y	[a]	[a]	[a]	[a]	0.43-Y	0.05-Y	[a]	[a]	[a]	0.03-Y	0.24-Y	0.06-Y
Martius yellow	0.05-Y	[a]	[a]	[a]	[a]	0.13-Y	0.03-Y	[a]	[a]	[a]	[a]	0.23-Y	[a]
Metanil yellow	[a]	[a]	[a]	[a]	0.04-Y	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Methylene blue	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Methyl orange	[a]	[a]	0.10-Y	[a]	0.03-P	[a]	[a]	[a]	[a]	[a]	[a]	0.41-Y	[a]
Methyl red	0.13-Y	0.03-Y	[a]	0.07-Y	0.50-P	0.44-Y	0.23-Y	[a]	0.07-Y	[a]	0.05-Y	0.37-Y	0.14-Y
Methyl violet base	[a]	[a]	0.66-L	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	0.16-L	[a]

Natural black	[a]	[a]	[a]	[a]	0.81-Br	0.88-Br	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
α-Naphthol	[a]	0.61-Br	0.69-Br	0.63-Br	0.81-Br	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	0.28-Br
β-Naphthol	[a]	0.61-Br	[a]	0.63-Br	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	0.27-Br
Nile Blue A	[a]	[a]	[a]	[a]	[a]	0.03-Br	[a]	[a]	[a]	[a]	[a]	[a]	[a]	0.48-B	[a]	
p-Nitrophenol	0.60-Y	[a]	0.56-Y	[a]	[a]	[a]	0.37-Y	0.33-Y	0.31-Y	0.19-Y	[a]	[a]	[a]	0.87-Y	0.17-Y	
										0.62					0.74	
Oil Blue N	0.79-B	0.80	0.73	0.68	0.88	0.93	0.76-B	[a]	[a]	0.52-B	[a]	[a]	0.83-Y		0.70-B	
		0.74-B	0.67-B	0.63-B	0.81-B	0.90-B				0.40						
		0.67														
Oil Red O	0.83-P	0.81-P	0.72-P	0.72-P	0.88-P	0.94-P	0.81-P	0.60-P	0.71-P	0.64-P	0.60-P		[c]	0.78-P		
Oil yellow	0.78-Y	0.75-Y	0.72-Y	0.67-Y	0.79-Y	0.89-Y	0.77-Y	0.55-Y	0.67-Y	0.62-Y	0.37-Y		[c]	0.74-Y		
Orange IV	[a]	[a]	0.66-Y	[a]	0.06-P	[a]	[a]	[a]	[a]	[a]	[a]		[a]	[a]		
Sudan II	0.81-P	0.78-P	0.72-P	0.70-P	0.87-P	0.91-P	0.77-P	0.59-P	0.68-P	0.61-P	0.54-P		[c]	0.80-P		
Sudan III	0.82-P	0.78-P	0.72-P	0.71-P	0.87-P	0.91-P	0.78-P	0.58-P	0.68-P	0.60-P	0.56-P		[c]	0.74-P		
Sudan IV	0.82-P	0.79-P	0.72-P	0.71-P	0.89-P	0.91-P	0.78-P	0.60-P	0.70-P	0.60-P	0.58-P		[c]	0.82-P		
Sudan Black B	0.70-B	0.69	0.81	0.65	0.87	0.75	0.74	0.51	0.54	0.55		0.88		0.51		
		0.62-B	0.76-B	0.58-B	0.76-B	0.69-B	0.64-B	0.19-B	0.32-B	0.25-B	0.44-B	0.81-B		0.20-B		
Sudan yellow	0.74-Y	0.70-Y	0.68-Y	0.68-Y	0.87-O	0.91-Y	0.77-Y	0.60-Y	0.69-Y	0.58-Y	0.56-Y	0.36-Y		0.82-Y		
Thymol blue	[a]	[a]	[a]	[a]	0.11-B	[a]	[a]	[a]	[a]	[a]		0.28-B		0.05-B		
Toluidine Blue O	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]		0.29-B		[a]		

[a] No movement.
[b] 5',5''-Dibromo-*O*-cresolsulfonephthalein indicator. Color code: B: blue; Br: brown; L: lavender; O: orange; P: pink; Y: yellow.
[c] Traveled with solvent front.

than the chromatograms obtained using silica gel G plates.

The results showed that R_f values were high when the polarity of the solvent system was high, and vice versa. In the case of Bidrin acid, little movement was observed when the chromatographic plates were eluted with neutral solvents, and much movement was observed when acetic acid was present in the solvent. No single solvent system is observed to be capable of separating all of the organophosphorus compounds studied. However, it is postulated that by eluting with two different solvent systems consecutively, one may further separate some of the compounds from each other. Because of their close resemblance, it is very difficult to separate *N*-hydroxymethyl Bidrin from Azodrin.

Slight changes in conditions could change the R_f values. R_f values of the esters changed when the plates were run at different times in the same solvents. This could be caused by changes in the ratio between the "major" and "minor" solvents, in chamber temperature, or in relative humidity. However, the R_f values of certain dyes were similar to the R_f values of certain esters despite the variation in the solvent system. Not all of the esters could be accompanied by a dye of exactly identical R_f values, but there were dyes which could locate the front or the back end of a spot. Therefore, these dyes are reliable standards for locating compounds when treatments with chromatogenic agents are not desired (Table IV).

Since it is a standard practice to determine the separated components in a thin-layer chromatogram quantitatively by other methods, the organophosphorus esters separated by TLC may be determined quantitatively by GLC. The authors have proposed a GLC method for the determination of Bidrin, Azodrin, and the metabolites (Giang and Beckman, 1968). The use of the dyes as standards is helpful for this type of work, as the presence of the chromogenic reagents may be undesirable.

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