Gas-Liquid Chromatographic Determination of Methyl Parathion and Metabolites

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Gas-liquid chromatography (glc) was used to isolate and identify methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate), its oxygen analog methyl paraoxon, and their primary ionic metabolic products. Methyl parathion and methyl paraoxon were separated and detected directly by glc but the ionic diesters could not be eluted from different glc columns without first treating the diesters with diazomethane or by dissolving them in methanolic HCl prior to injection. Desmethyl parathion was analyzed in glc as *S*-methyl methyl parathion, desmethyl paraoxon as methyl paraoxon, dimethylphosphoric acid as trimethyl phosphate, and dimethyl phosphorothioic acid as *S*-methyl dimethyl phosphorothioate. The feasibility of using glc to investigate the thermal isomerization of methyl parathion also was demonstrated.

Because of its effectiveness to a wide range of insects and its lower mammalian toxicity compared to the ethyl analog parathion, methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate) has become one of the most widely used organophosphorus insecticides. Its metabolism in biological systems is well documented (Hollingworth *et al.*, 1967a,b; Mengle and Casida, 1960) and the major means of isolation and characterization of the metabolites of organophosphorus compounds have been through the use of radiotracers and chromatographic methods, including paper (Chamberlin, 1964 a,b), thin-layer (Giang and Beckman, 1969; Stenersen, 1968), and ion exchange chromatography (Hollingworth *et al.*, 1967a,b; Plapp and Casida, 1958).

Gas-liquid chromatography (glc) provides capabilities of simultaneous separation and detection, and with recent advancements in thermionic and flame photometric detectors, methods have been developed in which less than 1 ng of organophosphorus compound can be detected because of enhancement of thermionic response (without concomitant increase in response to carbon-containing compounds) and flame photometric response, thus making glc a promising tool for analysis of biological extracts without necessitating extensive cleanup.

Although methods for the determination of parent phosphorothioate esters by glc have been described, which undoubtedly are useful to residue chemists for monitoring and surveillance, the procedures generally are not applicable for the analysis of polar, ionic metabolites of questionable thermal stability. For example, Bowman and Beroza (1968) were able to determine fenthion and several of its intact metabolites but not the dimethyl phosphate or monodemethylated metabolites which are formed by splitting of the ester moiety. Giang and Beckman (1968) attempted glc determination of the dealkylated metabolites of Azodrin and Bidrin and were partially successful after reesterification of their silver salts with methyl iodide. St. John and Lisk (1968), however, determined dialkyl derivatives of phosphoric and phosphorothioic acids by glc after reesterification with diazomethane.

This paper describes a glc method for the determination of methyl parathion and its first-stage metabolites. Primarily this paper is concerned with the behavior of methyl parathion and its principal metabolites in glc and particular attention is given to the reaction between diester derivatives of methyl parathion and alkylating reagents. The application of this glc method for elucidation of the metabolism of methyl parathion in houseflies will be described in another paper.

MATERIALS AND METHODS

Methyl parathion, m.p. 35.5° C, and methyl paraoxon (dimethyl *p*-nitrophenyl phosphate), b.p. 156–58° C (0.3 mm), n_D^{26} 1.5236 were prepared according to Hollingworth *et al.* (1967a). The sodium salt of *O*-methyl *O*-*p*-nitrophenyl phosphorothioic acid (desmethyl methyl parathion) was prepared by the action of sodium benzenethiolate on methyl parathion as described by Hollingworth *et al.* (1967b). The product was purified by preparative thin-layer chromatography (tlc) using silica gel plates and acetonitrile—1.2*N* HCl (90:10) as the solvent system (Jaglan, 1969). The sodium salt of methyl *p*-nitrophenyl phosphoric acid (desmethyl methyl paraoxon) was prepared in the same manner from methyl paraoxon using sodium iodide as the cleaving reagent (Hollingworth *et al.*, 1967b). The sodium salt of *S*-methyl *O*-*p*-nitrophenyl phosphorothioic acid (desmethyl *S*-methyl parathion) was prepared from methyl parathion using sodium iodide. Monomethyl- and dimethylphosphoric acid were prepared as barium salts by fractional crystallization of the product obtained from phosphorus pentoxide and methyl alcohol (Cherbuliez and Weniger, 1945; Harlay, 1935). *O,O*-Dimethylphosphorothioic acid was available from a previous preparation (Hollingworth *et al.*, 1967b) and trimethyl phosphate was obtained from Matheson, Coleman, and Bell. *O,O,O*-Trimethyl phosphorothioate was prepared according to Emmett and Jones (1911), elemental analysis calculated for $C_3H_9O_3PS$, C 23.22, H 5.81; found C 23.16, H 6.41. Tlc of the product, however, showed that *O,O*-dimethyl *S*-methyl phosphorothioate was a major contaminant. Each of these materials was purified after repeated separation on tlc. The list of relevant compounds along with their numbers for identification purposes are given in Table I.

General Procedure. Stock solutions of the relevant compounds listed in Table I were prepared in methanol or acetone at a concentration of 1 μg/μl. Methyl parathion and methyl paraoxon was dissolved directly in solvent but the diesters were dissolved as their sodium or potassium salts and equivalent amounts of methanolic HCl were added to liberate the acid of the diester. These stock solutions were stored at 0° C and prepared every other day to minimize the possibility of decomposition. The standard solutions were diluted to appropriate concentrations of the test samples just prior to

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Table I. Identification of Compounds by Compound Numbers

1	Methyl parathion
2	Methyl paraoxon
3	Desmethyl methyl parathion
4	Desmethyl methyl paraoxon
5	Dimethylphosphorothioic acid
6	Dimethylphosphoric acid
7	*S*-Methyl methyl parathion (*O*-methyl *S*-methyl *O*-*p*-nitrophenyl phosphorothioate)
8	Trimethyl phosphate
9	*O,O*-Dimethyl *S*-methyl phosphorothioate
10	*O,O,O*-Trimethyl phosphorothioate

injection in the glc. Injections on the column were made with a 10 μl Hamilton syringe as follows. A small amount of solvent was drawn into the syringe, followed by a small amount of air. The sample was then drawn into the barrel and its volume was determined by reading both ends of the liquid sample. Injection into the glc was then made and the solvent first drawn into the barrel flushed the sample completely.

The diester derivatives of methyl parathion, paraoxon were realkylated with diazomethane as follows. Diazomethane was generated from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Stanley, 1966) or from nitrosomethylurea (Werner, 1919) in ether solution and was used immediately or stored below $-20°$ C for short periods. The sample to be esterified was prepared in a mixture of methanol and ether (1:9 by volume) and a small drop of methanolic HCl (1:1) was added. Pure diazomethane was swept from stock solution by bubbling a stream of nitrogen saturated with ether into the sample solution. The solution was allowed to react for exactly 15 min and excess diazomethane was removed by a jet of nitrogen or reaction with acetic acid. The final concentration was then adjusted as desired for glc analysis by either concentration or dilution.

Glc analyses were carried out (Jaglan *et al.*, 1969) with a Hewlett-Packard Model 402 high efficiency gas chromatograph equipped with hydrogen flame detector. The detector was modified for thermionic detection of phosphorus by mounting a potassium chloride pellet on the burner jet. The following glass columns (T series) were used. Column T_1 contained diethyleneglycol succinate (DEGS, Hewlett-Packard) 1.4% coated on 80–100 mesh Gas Chrom Q in a column 61 cm × 4 mm i.d. The columns, unless specified otherwise, contained the same solid support and were operated at column, injector, and detector temperatures of 180°, 210°, and 200° C, respectively, and flow rates of nitrogen, hydrogen, and air at 40, 21, and 300 ml per min, respectively. Column T_2 contained 12% QF-1, a polysiloxane (Varian Aerograph) and the column temperature was 100° C. Column T_3, 183 cm in length, contained 20% DC-710, a polysiloxane (Hewlett-Packard) and was operated at a column temperature of 190° C. Columns T_4 and T_5, each 61 cm long, contained 5% Ucon 50 HB 280X, a polypropylene glycol (Hewlett-Packard) and 5% Apiezon L (Associated Electrical Industries, Ltd.), respectively.

Column F_1, 244 cm × 4 mm i.d., was stainless steel packed with 5% Carbowax 20M, a polyethyleneglycol (Varian Aerograph) on 80–100 mesh Gas Chrom Q fitted on an Aerograph Model 1520B gas chromatograph, equipped with the flame photometric detector of Brody and Chaney (Microtek Instrument Corp.) with two replaceable filters of 526 and 394 mμ for specific detection of phosphorus and sulfur, respectively. The temperatures of the column, injector, and detector were 190°, 210°, and 200° C, respectively, and flow rates of nitrogen, hydrogen, and oxygen were 100, 160, and 25 ml per min, respectively.

For preparative separation, high-loaded columns were used and the effluent was split with an effluent splitter. The effluents were collected in Teflon tubing closed with glass wool soaked in acetone. The compounds were individually collected by monitoring the remainder of the effluent with the detector and were removed from the collection tubes with acetone or methanol.

RESULTS AND DISCUSSION

Previous papers from this laboratory (Hollingworth *et al.*, 1967b,c) showed by means of P^{32}-labeled material that the principal metabolites obtained from the white mouse and the housefly upon treatment with methyl parathion (**1**) were methyl paraoxon (**2**), desmethyl methyl parathion (**3**), desmethyl methyl paraoxon (**4**), dimethylphosphorothioic acid (**5**), and dimethylphosphoric acid (**6**). Other metabolites, *e.g.*, phosphoric acid, methylphosphoric acid, and another metabolite of unknown structure, also were detected but in minor quantities. Owing to the need for a more convenient and complementary method for the isolation and identification of the principal metabolic products of organophosphorus esters for toxicological and mode of action studies, attention was turned to glc as a means of analysis.

Although glc may be used for the separation and identification of the intact esters **1** and **2**, all attempts to elute the ionic diesters **3**, **4**, **5**, and **6** without alteration of the structure proved to be unsuccessful on a wide variety of columns. Injection of solutions containing **3**, **4**, **5**, or **6** obtained by acidification of the sodium salts of these compounds with methanolic HCl gave eluates which later proved to be the reesterified triesters, presumably formed by acid-catalyzed esterification of the acid with methanol.

Desmethyl Methyl Parathion (3) and Desmethyl Methyl Paraoxon (4). An earlier note (Jaglan *et al.*, 1969) briefly described the behavior of **3** and **4** after injection of these compounds in methanolic HCl into a variety of glc columns. Injection of **3** produced a single peak which was shown to be *S*-methyl methyl parathion **7** (*O*-methyl *S*-methyl *O*-*p*-nitrophenyl phosphorothioate). The gas chromatographic response was satisfactory, as little as 10 ng of the **3** being detected as **7**. Injection of **4**, again in methanolic HCl, gave a single peak which was identical to methyl paraoxon **2**, although in this case the response was less satisfactory, with sensitivity equal to about 100 ng of **4**.

The initial results with methanolic HCl indicated the feasibility of using reesterification procedures for metabolite analysis and the use of diazomethane as an esterifying agent was investigated. Combined glc results of **3** and **4** after treatment with diazomethane are given in Figure 1. Esterification of **3** with diazomethane and injection into column T_1 at 180° C produced two peaks, the first at retention time 1.20 min and identical to **1**, and the second at retention time 2.34 min and identical to **7**. The relative proportion of **1** to **7** was 10:90. Unlike on-column methylation with methanolic HCl which gave only **7**, diazomethane produced the isomeric **1** and **7** by *O*-methylation and *S*-methylation with the latter predominating. Treatment of **4** with diazomethane gave a single peak with retention 1.50 min, identical to **2**. However, unlike on-column methylation with methanolic HCl which would not produce a response at quantities of **4** less than 100 ng which indicates inefficient conversion of **4** to **2**, the use

Table II. Observed Glc Retention Times on Columns of Dialkyl Acids in Methanolic HCl or after Esterification with Diazomethane

Column	Reagent	Retention Time (Min) Trimethyl phosphate	*O,O,O*-Trimethyl phosphorothioate	*O,O*-Dimethyl *S*-methyl phosphorothioate	Dimethyl-phosphoric acid	*O,O*-Dimethyl-phosphorothioic acid
T_2	Diazomethane	1.60	0.85	3.50	1.6	0.85 (**10**)[a] 1.60 (**8**) 3.50 (**9**)
T_3	Diazomethane	1.15	1.15	1.90	1.15	1.15 (**8**) 1.90 (**9**)
T_3	Methanolic HCl	1.15	1.15	1.90	1.15	1.90 (**9**)
T_4	Methanolic HCl	1.60	1.60	5.00	1.60	5.00 (**9**)
F_1	Diazomethane	0.95	0.95	2.05	0.95	0.95 (**8**) 2.05 (**9**)

[a] Parenthetical numbers correspond to compound numbers in Table I.

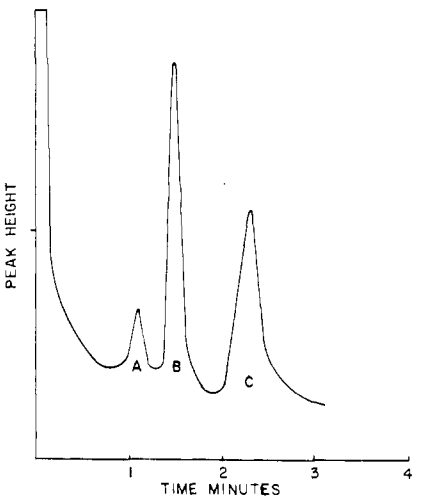

Figure 1. Chromatogram (column T_1) of methyl parathion metabolites after esterification with diazomethane. Peaks (A) and (C) desmethyl methyl parathion, (B) desmethyl methyl paraoxon

of diazomethane gave excellent results and less than 5 ng of **4** could be detected by glc as **2**.

The difference in quantitative response with **4** and in number of products with **3** after treatment with methanolic HCl as compared to diazomethane undoubtedly is due to the poorer alkylating ability of methanolic HCl. The mechanism of the alkylation process with both reagents is probably similar, involving nucleophilic attack of the **3** or **4** as either the acid or anion on the carbon atom of the reagent. In methanolic HCl the methyloxonium cation is attacked preferentially by the more nucleophilic sulfur atom of the phosphorothioate diester in either the thiolate or thionate form giving the *S*-methyl isomer **7** as the sole product. Apparently attack by oxygen to give methyl parathion **1** proceeds at a much slower rate and, therefore, only one product is obtained. Direct evidence for the lower nucleophilicity of oxygen toward the methyloxonium cation under the conditions of the column is provided by the inefficient methylation of **4** to **2**. The two products, **1** and **7**, obtained from **3** by treatment with diazomethane is explained by the much stronger alkylating property of this reagent.

Dimethylphosphorothioic Acid (5) and Dimethylphosphoric Acid (6). The results with the two dialkyl acids (**5** and **6**) were similar to those for **3** and **4**. Injection of either **5** or **6** in methanolic HCl or after prior treatment with diazomethane gave peaks of the trimethyl esters as indicated below with sensitivities in the order of 0.1 to 0.4 ng. Evidently, trimethyl phosphate and the two isomer trimethyl phosphorothioates are very responsive to thermionic detection. The observed retention times on a number of columns for **5** and **6** in methanolic HCl or after treatment with diazomethane are given in Table II. As expected, injection of **6** produced a single peak identical to trimethyl phosphate (**8**) on all columns. The behavior of **5** was more complex, producing more than one peak in glc columns T_2, T_3, and F_1, as shown in Table II. The major peak, 90% or more of the eluate on all columns, was identical to that for *S*-methyl *O,O*-dimethyl phosphorothioate (**9**). On the T_2 column, two other minor peaks were detected, the peak at retention time 0.85 min corresponding to that for *O,O,O*-trimethyl phosphorothioate (**10**) and the peak at retention time 1.6 min corresponding to **8**. None of these minor metabolites combined to total more than 10% of the eluate. Except for the T_2 column, the retention times for **8** and **10** were identical (Table II). On the Carbowax column (CF_1) using flame photometry as a means of detection, both **8** and **10** were observed in the phosphorus mode with identical retention times. However, using the flame photometric detector in the sulfur mode, only **10** was observed with the same retention time. The different detectors, therefore, may be used to distinguish **8** from **10** in columns in which retention times are identical.

The appearance of small amounts of **8** as an eluent after injection of **5** is difficult to explain since methyl paraoxon (**2**) was not observed after similar treatment of desmethyl methyl parathion (**3**). This point needs further investigation. Direct injection of **5** in methanolic HCl without prior treatment with diazomethane gave a single peak corresponding to **9**. This is directly analogous to the behavior of **3** which gave only **7** in methanolic HCl.

The above results with dimethylphosphorothioic acid (and also with desmethyl methyl parathion) in which *S*-alkylation occurs preferentially to *O*-alkylation are consistent with observations made by others. Hoffman and Moore (1958) and Cadogen (1962) also have observed that alkylation of dialkyl phosphorothioic acid gives the *S*-alkyl isomer as the major product along with minor amounts of the phosphorothionate. Mastryukova *et al.* (1964) found that treatment of the sodium salt of diethyl phosphorothioic acid with triethyl oxonium fluoroborate in chloroform at 40° to 50° C resulted in 4.5% triethyl phosphorothionate and 61.7% *S*-ethyl isomer.

Thin-Layer Chromatography. Thin-layer chromatography (tlc) also was used in conjunction with glc for affirmation of the identity of the various compounds. Tlc of the original

Table III. Tlc Characterization of Methyl Parathion and Metabolites and Their Glc Eluates[a]

Compound	R_f	Color with Reagents
Methyl parathion	0.56	Red with DCQ
Methyl parathion glc eluate	0.56	Red with DCQ
Methyl paraoxon	0.21	Blue with molybdate
Methyl paraoxon glc eluate	0.21	Blue with molybdate
Desmethyl methyl parathion	0.00	Red with DCQ
Desmethyl methyl parathion glc eluate	0.23	Yellow, not red, with DCQ
Desmethyl methyl paraoxon	0.00	Blue with molybdate
Desmethyl methyl paraoxon glc eluate	0.21	Blue with molybdate
Desmethyl methyl parathion *S*-isomer	0.00	Blue with molybdate
Desmethyl methyl parathion *S*-isomer glc eluate	0.22	Blue with molybdate
Dimethylphosphoric acid	0.00	Blue with molybdate
Dimethylphosphoric acid glc eluate	0.22	Blue with molybdate
Trimethylphosphate	0.22	Blue with molybdate
Dimethylphosphorothioic acid	0.00	Red with DCQ
Dimethyl phosphorothioic acid glc eluate	0.22	Yellow, not red, with DCQ
O,O,O-Trimethyl phosphorothioate	0.51	Red with DCQ
O,O-Dimethyl *S*-methyl phosphorothioate	0.22	Yellow with DCQ

[a] Silica gel tlc plates 20 × 20 cm (250 μ thick) with ether:hexane (7:3) at room temperature.

compounds and their glc eluates are shown in Table III. Methyl parathion has the same R_f value as its eluate and both give the same (red) color reaction with 2,6-dichloroquinone-4-chloroimide (DCQ) reagent indicating that P = S is present in both cases. The areas and the intensities of the spots were semiquantitatively estimated to be equal. This suggests that methyl parathion does not isomerize in the gas chromatograph under these conditions. Methyl paraoxon behaves similarly; both the eluate and the original compound have the same R_f value and give the same color with ammonium molybdate. In addition, they give a yellow color with DCQ reagent and the areas and the intensities of the spots are comparable.

Desmethyl methyl paraoxon remains at the origin, while its glc eluate has an R_f value identical to the methyl paraoxon. Both give a blue color with ammonium molybdate. The area and intensity of the spot of the eluate were less, however. Desmethyl methyl parathion remains at the origin and gives a red color with DCQ reagent, as expected. Its eluate has an R_f value very near to that of methyl paraoxon, gives not a red color but yellow with DCQ reagent, and a blue color with ammonium molybdate. These results provide additional evidence that the eluate is methyl parathion *S*-isomer.

Dimethylphosphoric acid remains at the origin while its glc eluate has the same R_f as of trimethyl phosphate and gives a blue color with ammonium molybdate. Dimethylphosphorothioic acid remains at the origin and gives a red color with DCQ, while its eluate gives a single spot at R_f 0.22, producing yellow color with DCQ and a blue color with molybdate. Diazomethane treatment of dimethyl phosphorothioic acid produced two compounds, a predominant spot at R_f 0.22 and a minor spot at R_f 0.51. These results provide additional evidence that the eluates at R_f's 0.22 and 0.51 are **9** and **10**, respectively.

Isomerization of Methyl Parathion. The thermal isomerization of methyl parathion to the *S*-methyl isomer (**7**) was examined to demonstrate the feasibility of using glc for isomerization studies and to determine if it could be used as a method

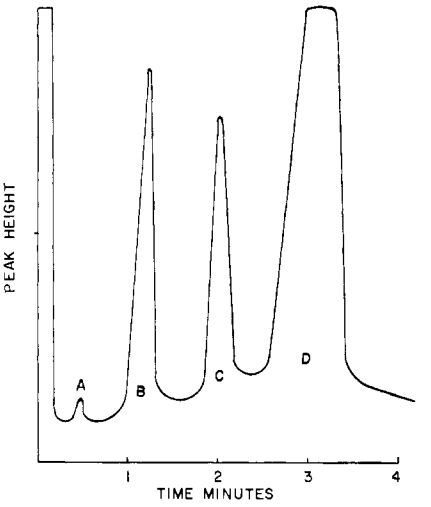

Figure 2. Chromatogram (column T_5) of reaction products from heating 1 g of methyl parathion at 125° C for 7 hr. Peak (A) unknown, (B) methyl paraoxon, (C) methyl parathion, and (D) methyl parathion *S*-isomer

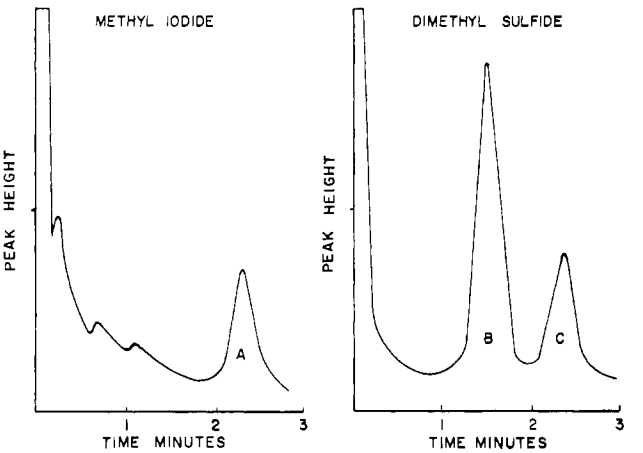

Figure 3. Chromatogram (column T_5) of products obtained from the action of methyl iodide and dimethyl sulfide on methyl parathion. Peaks (A) and (C) methyl parathion *S*-isomer, (B) methyl parathion

for the preparation of **7**. Methyl parathion heated for 7 hr in a sealed ampule at 125° C provided the chromatogram shown in Figure 2. The major isomerization product was **7** although **2** also was produced and some of the original methyl parathion remained unchanged. Nmr spectrum of the material corresponding to **7** in deuteriochloroform with 2% TMS showed a doublet at δ 2.25, 2.52 ppm for the CH_3S-protons, and a doublet at δ 3.85, 5.41 ppm for CH_3O-protons. The peak integrals of the two doublets were identical, indicating equal numbers of CH_3S- and CH_3O-protons, thus providing support for **7** as the principal heat product of **1**.

The effect of dimethyl sulfide and methyl iodide on the isomerization of methyl parathion to **7** also was examined. Both these reagents have been used to isomerize *O*-methyl phosphorothioates to *S*-methyl phosphorothioates (Hilgetag and Teichman, 1965). Figure 3 shows the effect of heating 1 g of methyl parathion at 58° C with excess methyl iodide and dimethyl sulfide. Although methyl parathion itself is not isomerized to any significant extent at this temperature, the figure shows appreciable conversion to **7** with dimethyl sulfide after 18 hr and essentially complete conversion with methyl iodide to **7** and other products after 36 hr.

These results indicate the feasibility of using heat to prepare the *S*-methyl isomer of methyl parathion. Thermal isomerization actually proved to be the most convenient method for the preparation of **7**. Purification of the product may be accomplished by glc or thin-layer chromatography.

CONCLUSIONS

Glc may be used to isolate and quantify methyl parathion and its various metabolic and isomerization products. Methyl parathion (**1**), methyl paraoxon (**2**), and *S*-methyl methyl parathion (**7**) may be separated and detected directly by glc. However, in the case of the diesters (**3**, **4**, **5**, and **6**) prior treatment with an alkylating reagent such as diazomethane or injection in methanolic HCl is necessary. Desmethyl methyl parathion (**3**) may be analyzed in glc as *S*-methyl methyl parathion (**7**), desmethyl methyl paraoxon (**4**) as methyl paraoxon (**2**), dimethylphosphoric acid (**6**) as trimethyl phosphate, and dimethylphosphorothioic acid (**5**) as *S*-methyl dimethyl phosphorothioate. Compounds **1** and **2** may be detected in the presence of **3** and **4** by first separating **1** and **2** from **3** and **4** by solvent partition between water and organic solvent, then analyzing each phase separately after prior treatment of **3** and **4** with diazomethane or a comparable esterifying agent. Thus, a mixture of compounds **1** through **6** may be analyzed for each component by use of glc in conjunction with alkylating agents and solvent partitioning. The use of these methods in investigating the metabolism of methyl parathion in the housefly and the details for the determination of a mixture of **1** through **6** will be discussed in a second paper.

LITERATURE CITED

Bowman, M. C., Beroza, M., J. AGR. FOOD CHEM. **16,** 399 (1968).
Cadogen, J. I. G., *J. Chem. Soc.* **18** (1962).
Chamberlin, W. F., *J. Econ. Entomol.* **57,** 119 (1964a).
Chamberlin, W. F., *J. Econ. Entomol.* **57,** 329 (1964b).
Cherbuliez, E., Weniger, H., *Helv. Chim. Acta* **28,** 1584 (1945).
Emmett, W. G., Jones, H. O., *J. Chem. Soc.* **99,** 713 (1911).
Giang, B. Y., Beckman, H. F., J. AGR. FOOD CHEM. **16,** 899 (1968).
Giang, B. Y., Beckman, H. F., J. AGR. FOOD CHEM. **17,** 63 (1969).
Harlay, V., *J. Pharm. Chim.* **20,** 160 (1934); Chem. Abstr. **29,** 2506 (1935).
Hilgetag, G., Teichman, H., *Angew. Chem. Intern. Ed.* **4,** 914 (1965).
Hoffman, F. W., Moore, T. R., *J. Amer. Chem. Soc.* **80,** 1150 (1958).
Hollingworth, R. M., Fukuto, T. R., Metcalf, R. L., J. AGR. FOOD CHEM. **15,** 235 (1967a).
Hollingworth, R. M., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **15,** 242 (1967b).
Hollingworth, R. M., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **15,** 250 (1967c).
Jaglan, P. S., Ph.D. dissertation, University of California, Riverside, Calif., 1969.
Jaglan, P. S., March, R. B., Gunther, F. A., *Anal. Chem.* **41,** 1671 (1969).
Mastryukova, T. A., Shipov, D. E., Abalyaeva, V. V., Popovand, E. M., Kabachnik, M. I., *Dokl. Akad. Nauk S.S.S.R.* 158, 1372 (1964).
Mengle, D. C., Casida, J. E., J. AGR. FOOD CHEM. **8,** 431 (1960).
Plapp, F. W., Casida, J. E., *Anal. Chem.* **30,** 1622 (1958).
St. John, L. E., Lisk, D. J., J. AGR. FOOD CHEM. **16,** 48 (1968).
Stanley, C. W., J. AGR. FOOD CHEM. **14,** 321 (1966).
Stenersen, J., *J. Chromatogr.* **38,** 538 (1968).
Werner, E. A., *J. Chem. Soc.* **115,** 1093 (1919).

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