Biochemical Pharmacology, Vol. 17, pp. 1339–1347. Pergamon Press. 1968. Printed in Great Britain

METABOLISM OF ORGANOPHOSPHORUS INSECTICIDES—IX.*

DISTRIBUTION, EXCRETION AND METABOLISM OF DIMETHOATE *PRODENIA LITURA F.*

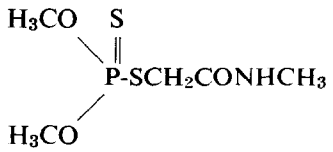
S. M. A. D. ZAYED, A. HASSAN and I. M. I. FAKHR

National Research Centre, Dokki, Cairo and Department of Radiobiology, Atomic Energy Establishment, Cairo, U.A.R.

(*Received* 11 *December* 1967; *accepted* 31 *January* 1968)

Abstract—The distribution and degradation of dimethoate in the adult larva of *Prodenia litura F.* has been studied using ^{32}P labelled insecticide. About 40 per cent of the topically applied dose was metabolized in 20 hr. An apparent initial oxidation of P=S to P=O constitutes a major metabolic pathway for the insecticide. Seven hydrolytic metabolites have been identified.

THE ORGANOPHOSPHORUS insecticide Dimethoate (*O,O*-dimethyl S-(*N*-methylcarbamoyl methyl)phosphorodithioate I which is an anticholinesterase agent[1] has found practical application in the fields of plant protection[2] and veterinary medicine.[3, 4]



I

It possesses systemic insecticidal properties in both animal[5, 6] and plant[7, 8] and has at the same time a low order of mammalian toxicity.[9] Dimethoate has been successfully used for controlling insects attacking olive and cherry trees.[10, 11]

The metabolism of dimethoate in mammals has been extensively studied.[12–14] Its degradation in housefly and American cockroach was examined by Krueger *et al.*,[15] but the nature of the metabolic products was not established. Studies on the metabolism of dimethoate in some cotton pests, namely boll worm larvae (*Heliothis Zea*), boll weevils[16] (*Anthonomus grandis*) and locusts[17] have been recently carried out.

The present work was undertaken to gain insight into the mode of action and metabolic fate of ^{32}P-dimethoate in larvae of *Prodenia litura*, which is a serious cotton pest.

MATERIALS

^{32}P-O,O-*Dimethyl hydrogen phosphorodithioate* (*dithioate*) *II*

Dithioate of sp. act. $7{\cdot}2 \times 10^5$ cpm/mg was prepared by the interaction of $^{32}P_2S_5$ with absolute methanol at 40° according to March *et al.*[18] and the product purified by vacuum distillation (b.p.$_{11}$ 88–91°; Lit. b.p.$_{0.5}$ 42–44°)[18] $^{32}P_2S_5$ was prepared by

* For part VIII, cf. A. Hassan, S. M. A. D. Zayed and I. Y. Mostafa, *Z. Naturforsch.* **21b** 498 (1966).

an exchange reaction between non-labelled phosphorus pentasulphide and ^{32}P-dibasic sodium phosphate in a sealed tube at 450° for 2 hr.[19] The product was used for the preparation of dithioate after crystallisation from carbon disulphide.

^{32}P-O,O-Dimethyl-S(N-methylcarbamoylmethyl)phosphorodithioate (Dimethoate) I

To an aqueous solution of *N*-methyl-α-chloro acetamide[20] (4 g in 10 ml water) an equivalent volume of chloroform was added and the mixture heated to 85°. The potassium salt of dithioate (8 g neutralized with a solution of 3·413 g potassium carbonate in 16 ml water) was added over 30 min to the stirred reaction mixture. after further 15 min the chloroform layer was separated and the aqueous phase exhaustively extracted with chloroform. The combined chloroform extracts were evaporated under reduced pressure to remove chloroform and the crude product was purified on a silica gel column[13] using hexane-chloroform mixtures for elution (yield about 80%). Dimethoate had a sp. act. of 5×10^5 cpm/mg.

For the distribution and metabolism experiments, healthy laboratory reared larvae of *Prodenia litura* (5th–6th instars) were used after 3 hr starvation.

METHODS

Distribution

For each gram of insect 200 μg of the insecticide (100,000 cpm) in 20 μl. acetone were uniformly applied on the dorsal side. At specified time intervals after treatment, the unabsorbed insecticide was removed by acetone. Samples of the hemolymph, gut and fat were freshly weighed and measured for their radioactivity.

In vivo *experiments*

^{32}P-Dimethoate, dissolved in acetone was applied topically at a dose level of 750 μg/g of insect; equivalent to 360,000 cpm. The larvae were left in a beaker containing little sawdust for 20 hr, after which the excreta were exhaustively extracted with distilled water. Insects were homogenized with least amount of acetone–water (19:1) and homogenates were chilled at − 10° and centrifuged. From the supernatant, acetone was driven off under nitrogen atmosphere and the rest extracted with a little water. The combined aqueous extracts (from the worms and excreta) were then shaken several times with chloroform. Both the chloroform extracts and the aqueous layer were analyzed for possible metabolites.

Chloroform extracts

The combined chloroform extracts were concentrated under reduced pressure at room temperature. Samples of the chloroform solution were used for paper chromatographic analysis in different solvent systems (Table 2). The chloroform concentrate was also analyzed by partition chromatography with silica gel column,[2, 13] using hexan–chloroform mixtures for elution. Fractions (3 ml each) collected from the column as well as the paper chromatograms were measured for their ^{32}P-activity.

Aqueous extracts

The aqueous extracts were concentrated to about 10 ml under reduced pressure at room temperature to avoid any possible decomposition of the metabolites. The water concentrate was applied on an anion exchanger column (Dowex 1-X8, Cl^-, 100–

200 mesh (15 × 1 cm)) for the resolution of the water soluble metabolites. The column was first washed with 200 ml of distilled water to remove the unadsorbed radioactivity.

The acidic metabolites were eluted with the following solutions (75 ml each): HCl pH 1·5; HCl pH 0·5; N HCl + methanol (1:3); conc. HCl + water + acetone (1:1:6).

3 ml Fractions were measured for their ^{32}P-activity and analyzed by paper chromatography.

Spots were made visible by spraying with Hanes–Isherwood reagent.[21] To locate the phosphorothioate compounds, the papers were sprayed with a 2% solution of cupric chloride and then with a 0·5% solution of potassium ferricyanide, where red brown spots on a yellow-green background were obtained.[22]

In one experiment, the collected fractions were analyzed for total phosphorus according to Casida *et al.*[23]

Radio-measurements

^{32}P-Activity eluted from column chromatographic analysis was determined in solution. All measurements were carried out in an end window Tracer-lab G-M tube under uniform geometrical conditions. The data were corrected for decay and background and no allowance was made for self absorption.

Radiometric assay for paper chromatograms was carried out with a similar device.

RESULTS

Distribution

Table 1 shows the distribution of ^{32}P-activity among hemolymph, gut, and fat after topical application of ^{32}P-dimethoate. After 1 hr the larva was found to contain 9·8 per cent of the applied radioactivity. The radioactivity in the different organs reached its maximum after 1 hr and decreased gradually with time. The ^{32}P-activity in the fat showed a definite progressive increase with time.

TABLE 1. DISTRIBUTION OF ^{32}P-ACTIVITY IN ADULT *Prodenia* LARVA AFTER TOPICAL APPLICATION OF 200 μG OF ^{32}P-DIMETHOATE/G INSECT

Organ	cpm/g fresh wt.* after			
	30 min	1 hr	3 hr	5 hr
Hemolymph	10100	20150	13000	6500
Gut	8500	15300	11000	3300
Fat	2800	3600	6900	9000
Total insect	5700	9800	8400	6300

* Values are mean of six determinations.

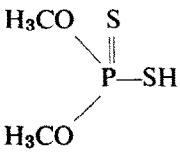
In vivo *metabolism*

For this experiment, a pool of 10 g insects was used (3,600,000 cpm). After 20 hr, 81·5 per cent of the applied ^{32}P-activity could be recovered in the chloroform extracts. From several chromatograms and from partition chromatography on silica gel, it has been estimated that unchanged dimethoate contributed to about 54 per cent of the chloroform extractable activity.

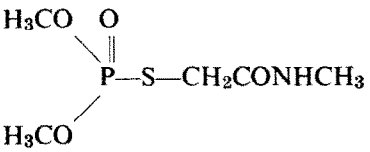
The combined aqueous extracts contained 10·4 per cent of the applied dose (375,000 cpm), and the ^{32}P-activity remaining in the larvae was about 5 per cent. Analysis of the sawdust showed that it retained about 1 per cent of the applied radiodose, thus demonstrating an excellent recovery of the radioactivity (about 98 per cent).

Chloroform layer

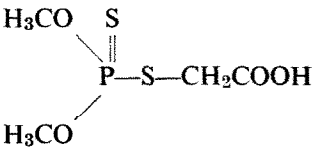
Partition chromatography of the chloroform extractable radioactivity on silica gel[12, 13] showed the presence of unchanged dimethoate and the oxygen analog (Dimethoxon III). The former was eluted with hexane–chloroform (4:1) and the latter with chloroform. The R_f values of dimethoate and some of its degradation products in different solvent systems are listed in Table 2.



II



III



IV

TABLE 2. R_f-VALUES OF DIMETHOATE AND SOME OF ITS DEGRADATION PRODUCTS IN SEVERAL SOLVENT SYSTEMS*

Substance	System				
	A†	B‡	C†	D†	E†
I. Chloroform layer					
Dimethoate (I)	0·87	0·99	0·95	0·93	0·93
Dimethoxon (III)	0·73	0·85	0·87	0·83	0·85
II. Aqueous layer					
Carboxy derivative (IV)	0·51	0·59	0·69	0·71	0·61
O,O-Dimethylphosphorothioic acid (V)	0·06	0·35	0·68	0·64	0·51
O,O-Dimethylphosphorodithioic acid (II)	0·14	0·51	0·71	0·69	0·62
Thiophosphoric acid	0·28	0·10	0·03	0·02	0·01
Dimethyl phosphate	0·04	0·24	0·57	0·50	0·17
Monomethyl phosphate	0·45	0·05	0·11	0·04	0·04
Phosphoric acid		0·00	0·05	0·00	0·29

* For paper chromatography Schleicher & Schüll paper 2043b has been used, temperature 30°.

(A) Papers were impregnated with 0·2 M. potassium acetate buffer (pH 3·6) elution was achieved with iso-butanol: 0·2 M. acetate buffer (pH 3·6), 5:1[24].

(B) Acetonitrile : water : ammonia (40 : 9 : 1)[16, 25].

(C) Iso-propanol : water : ammonia (75 : 24 : 1).[22]

(D) Iso-propanol: ammonia (75 : 25).[22]

(E) *n*-Butanol : Pyridine : water (12 : 8 : 6).[26]

† Development time 16 hr.

‡ Development time 4 hr.

Aqueous extracts

Fig. 1 shows an elution curve of the acidic ^{32}P-labelled metabolites using hydrochloric acid and mixtures of hydrochloric acid–methanol or acetone for elution. The identity and percentage of the acidic ^{32}P-labelled substances recovered from the

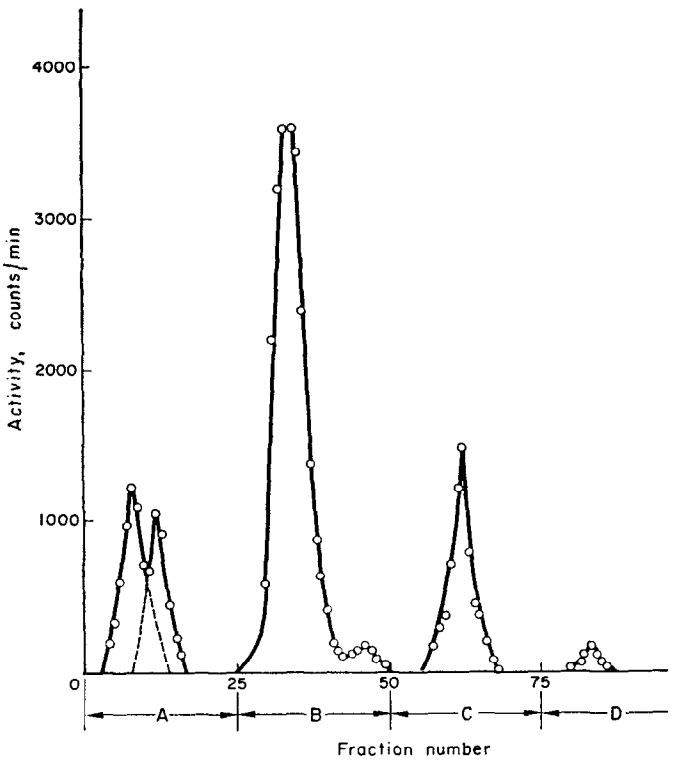


FIG. 1. Fractionation of the water soluble metabolites of ^{32}P Dimethoate on Dowex 1.
(A) Elution with HCl pH 1·5; (B) Elution with HCl pH 0·5; (C) Elution with *N* HCl + methanol (1:3); (D) Elution with conc. HCl + water + acetone (1 : 1 : 6).

TABLE 3. ^{32}P-LABELLED SUBSTANCES RECOVERED FROM THE ANION EXCHANGER

Eluent	cpm	Percentage*	Metabolite
HCl; pH 1·5	92625	24·8	Phosphoric acid, monomethyl- and dimethyl phosphate (1 : 1 : 2)
HCl; pH 0·5	20700	55·2	Thiophosphoric acid
N HCl + methanol	63000	16·8	Carboxy derivative (IV) and dimethyl phosphorothioic acid (2 : 3)
Conc. HCl + water + acetone (1 : 1 : 6)	10500	2·8	Dimethyl phosphorodithioic acid (II)

* Total hydrolytic product = 100%.

anion exchanger are shown in Table 3. The elution curve (Fig. 1) obtained from radioactivity measurements was found to be identical with that obtained from total phosphorus determination.

Radio-paper chromatography of the water soluble hydrolytic products confirmed the presence of seven metabolites given in Table 3. Authentic substances were run alongside for identification purposes.

Paper chromatographic analysis of the original water soluble metabolites, revealed the presence of at least two more minor products, which could not be detected by analysis on the anion exchanger. The nature of these metabolites is still unknown.

DISCUSSION

Distribution studies showed that dimethoate ^{32}P-activity did not tend to accumulate in high concentrations within the larva.

After 1 hr from topical application, the larva was found to contain about 10 per cent of the applied radiodose. This value decreased gradually with time, and after 20 hours the ^{32}P-activity remaining in the larva was about 5 per cent. This may indicate effective excretion of the insecticide and/or metabolites by the adult *Prodenia* larva. Such rapid elimination of the insecticide and its metabolites has been observed in dimethoate treated bollworm larvae (*Heliothis Zea* Boddi) where about 50 per cent of the injected radiodose was excreted after 8 hr.[16]).

The products of metabolism suggest that the dimethoate molecule undergoes two main metabolic reactions in the adult *Prodenia* larva. One consists of oxidation of P=S to P=O, the second is hydrolysis involving four possible bonds. The initially formed products may suffer further degradation leading to a variety of hydrolytic products; the ultimate product being inorganic phosphate or thiophosphate.

From metabolism experiments it was found that about 37–42 per cent of the topically applied dose is metabolized during 20 hr. The major metabolite (about 7 per cent of the total metabolites) is a product of oxidation of dimethoate to the oxygen analog (III). It cannot be, however, excluded that some of the obtained dimethoxon (III) originated from partial non-enzymic oxidation of dimethoate which might have taken place during analysis. Oxidation of dimethoate was found to occur in other insects such as American cockroach, house flies[27] and boll worm larvae.[16] Dimethoxon (III) is presumably more toxic than the parent compound.[28] It is worth mentioning that oxidation rather than hydrolysis constituted the major detoxification mechanism of Sevin in *Prodenia* larvae.[29]

Of the water soluble acidic substances, seven main metabolites have been identified (Table 3). At pH 1·5 monomethyl-, dimethyl and inorganic phosphate were eluted.

The paper chromatographic analysis of the acidic eluates recovered from the anion exchanger revealed always the presence of variable amounts of dimethyl-phosphate; the percentage of which increased with prolonged staying of the metabolites in the acidic solution. This may be attributed to the relative instability of many of these metabolites in strong acid solutions.[22, 30] The actual percentage of the metabolites was, therefore, difficult to determine. Decomposition of the metabolites could be minimized by a short and rapid separation on the anion exchanger followed by quick extraction of the metabolites from the acid eluates with amyl alcohol.[22]

Many of the dimethoate metabolites obtained from *Prodenia* larvae were similar to those previously reported for insects,[16, 27] mammals,[12–14] plants,[2, 7, 31, 32] and stored grains.[33]

Fig. 2 illustrates the possible sites of metabolic attack of dimethoate. The formation of the hydrolytic metabolites provides evidence that the dimethoate molecule is attacked at least at the following sites: P—S, C—N and at the alkoxy-groups.

A major site of enzymatic attack on dimethoate seems to occur at the P—*O*— alkyl ester bonds, followed by hydrolysis of the P—S bond, leading to the formation of

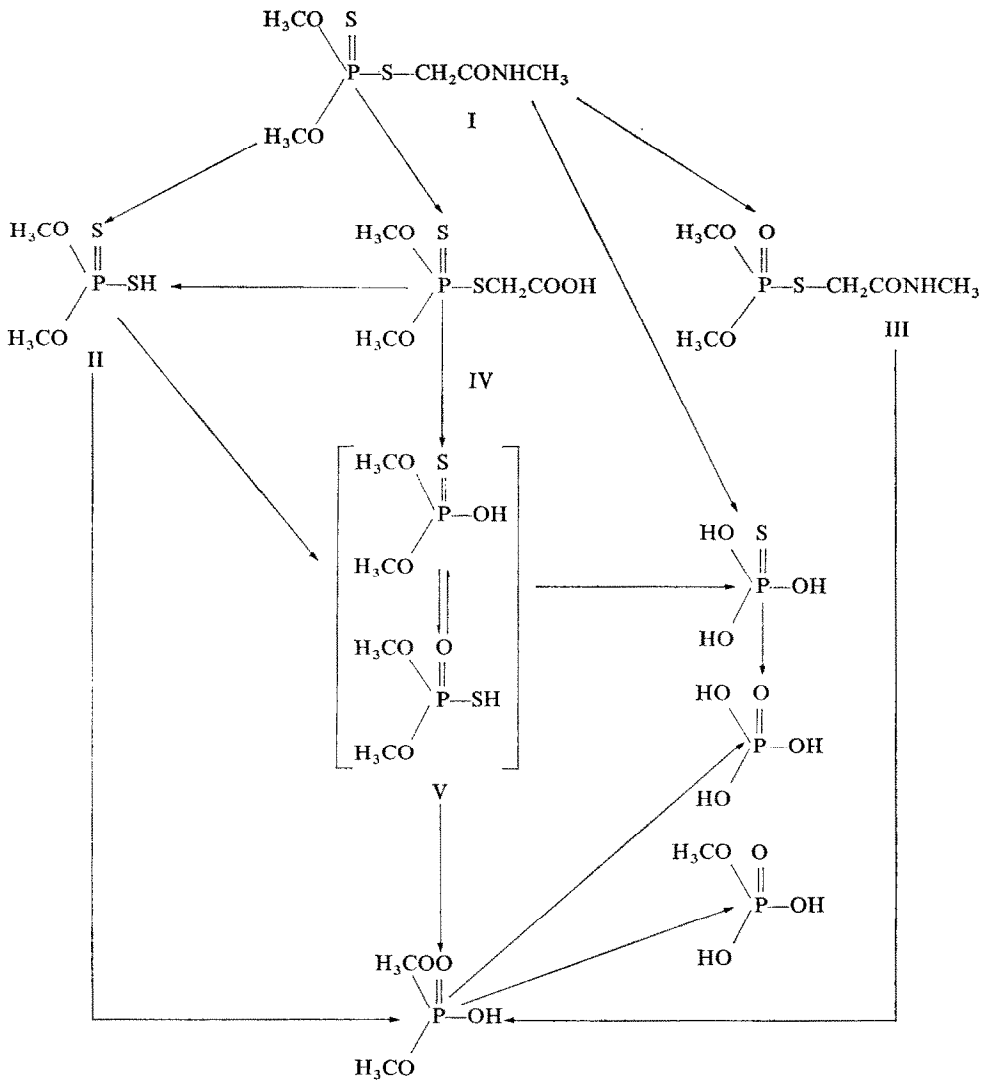


FIG. 2. Suggested scheme for the metabolic pathway of Dimethoate in *Prodenia* larva.

thiophosphoric acid. It constitutes the major acidic metabolite and contributes to about 50% of the total hydrolytic components.

Recently, it has been found that preferential methyl ester cleavage constitutes the major process in the enzymic degradation of Dipterex by the cotton leaf worm, *Prodenia litura F.*[26,34] The formation of *O,O*-dimethyl-S-carboxymethyl phosphorodithioate (IV) may be attributed to the presence of a carboxyamidase in *Prodenia* larvae. A similar amidase scission of the $\overset{O}{\overset{\|}{C}}$—N bond of dimethoate has been reported to take place in insect larvae, e.g. boll worm larvae.[16]

In general the results suggest that phosphatase action constitutes the major hydrolytic mechanism. This is in good agreement with the general belief that the phosphatase action in insects is of greater importance than carboxyamidase for the degradation of organophosphates containing a carboxyamide group.[35]

O,O-Dimethylphosphorodithioic acid (II) could be isolated in relatively small percentage from the anion exchanger. It could be produced by a phosphatase attack of dimethoate or the thiocarboxy-derivative, at the S — C linkage.

The formation of *O,O*-dimethylphosphorothioic acid may be attributed either to enzymic oxidation of dithioate or to hydrolysis of P — S bond of dimethoate or the S — C bond of dimethoxon.

The apparent high percentage of dimethylphosphate may be attributed to the possible oxidation of *O,O*-dimethylphosphorothioic acid and/or dithioate. It may also be formed by cleavage of the P — S bond of the oxygen analog.

A large number of the formed metabolites may undergo further reactions to give monomethyl phosphate and/or phosphoric acid.

Neither des-methylated nor monodemethylated Dimethoate could be identified in the present investigation.

Acknowledgements—The authors are indebted to Mr. John E. Boyd, group leader, Metabolism Laboratory and Mr. N. E. Schafer, Manager, Plant Product Research and Development, American Cyanamide Company, New Jersey for kindly supplying dimethoate carboxylic acid and dimethoxone.

REFERENCES

1. D. C. MENGELE and J. E. CASIDA, *J. econ. Ent.* **51**, 750 (1958).
2. W. C. DAUTERMAN, G. B. VIADO, J. E. CASIDA and R. D. O'BRIEN, *J. agr. Fd Chem.* **8**, 115 (1960).
3. F. W. KNAPP, C. J. TERHAAR and C. C. ROAN, *J. econ. Ent.* **53**, 541 (1960).
4. R. HEWITT, A. BREBBIA and E. WALETZKY, *J. econ. Ent.* **51**, 126 (1958).
5. R. O. DRUMMOND, *J. econ. Ent.* **52**, 1004 (1959).
6. W. C. MARQUARDT and S. A. LOVELACE, *J. econ. Ent.* **54**, 250 (1961).
7. D. A. LINDQUIST, J. HACSKAYLO, J. C. CLARK and T. B. DAVICH, *J. econ. Ent.* **54**, 1132 (1961).
8. M. ZAKI and H. T. REYNOLDS, *J. econ. Ent.* **54**, 568 (1961).
9. R. SCHUPPON, *Chimica ind.* **85**, 421 (1961).
10. R. SANTI and P. PIETRI-TONELLI, *Nature, Lond.* **183**, 398 (1959).
11. P. PIETRI-TONELLI, *Olivicoltura* **12**, 3 (1957).
12. W. F. CHAMBERLAIN, P. E. GATTERDAM and D. E. HOPKINS, *J. econ Ent.* **54**, 733 (1961); *Chem. Abstr.* **55**, 26252 h (1961).
13. W. C. DAUTERMAN, J. E. CASIDA, J. B. KNAAK and T. KOWALCZYK, *J. agric. Fd Chem.* **7**, 188 (1959).
14. J. N. KAPLANIS, W. E. ROBBINS, D. I. DARROW, D. E. HOPKINS, R. E. MONROE and G. TREIBER, *J. econ. Ent.* **52**, 1190 (1959).
15. H. R. KRUEGER, R. D. O'BRIEN and W. C. DAUTERMAN, *J. econ. Ent.* **53**, 25 (1960).
16. D. L. BULL, D. A. LINDQUIST and J. HACSKAYLO, *J. econ Ent.* **56**, 129 (1963).
17. D. M. SANDERSON and E. F. EDSON, *Br. J. ind. Med.* **21**, 52 (1964)
18. R. B. MARCH, T. R. FUKUTO, R. L. METCALF and M. G. MAXON, *J. econ. Ent.* **49**, 185 (1956).
19. J. E. CASIDA, *Acta chem. scand.* **12**, 1691 (1958).
20. W. A. JACOBS, M. HEIDELBERGER and I. P. ROLF, *J. Am. chem. Soc.* **41**, 472 (1919).
21. C. S. HANES and F. A. ISHERWOOD, *Nature, Lond.* **164**, 1107 (1949).
22. F. W. PLAPP and J. E. CASIDA, *Analyt. Chem.* **30**, 1622 (1958).
23. J. E. CASIDA, R. K. CHAPMAN and T. C. ALLEN, *J. econ. ent.* **45**, 568 (1952).
24. T. UCHIDA, W. C. DAUTERMAN, and R. D. O'BRIEN, *J. agric. Fd Chem.* **12**, 48 (1964).
AYLO and D. L. BULL, *J. agric. Fd Chem.* **11**, 464 (1963).

26. S. M. A. D. ZAYED and A. HASSAN, *Can. J. Biochem.* **43**, 1257 (1965).
27. U. E. BRADY Jr. and B. W. ARTHUR, *J. econ. Ent.* **56**, 477 (1963).
28. R. D. O'BRIEN, *Toxic Phosphorus Esters*. Academic Press, London (1960).
29. S. M. A. D. ZAYED, A. HASSAN and T. M. HUSSEIN, *Biochem. Pharmac.* **15**, 2057 (1966).
30. G. M. KOSOLAPOFF, *Organophosphorus Compounds*, p. 232. Wiley, New York (1950).
31. J. HACSKAYLO, D. A. LINDQUIST and T. B. DAVICH, *J. econ. Ent.* **54**, 1206 (1961).
32. R. SANTI and R. GIACOMELLI, *J. agric. Fd Chem.* **10**, 257 (1962).
33. D. G. ROWLANDS, *J. Sci. Fd Agric.* **17**, (2), 90 (1966).
34. A. HASSAN, S. N. A. D. ZAYED and F. M. ABDEL-HAMID, *Biochem. Pharmac.* **14**, 1577 (1965).
35. R. D. O'BRIEN, *Toxic Phosphorus Esters*, p. 334. Academic Press, London (1960).