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METABOLISM OF ORGANOPHOSPHOROUS INSECTICIDES—X*

DEGRADATION OF ^{14}C-DIMETHOATE IN THE ADULT LARVA OF THE COTTON LEAF WORM

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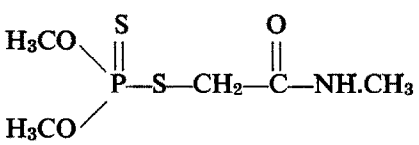
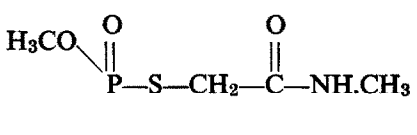
Abstract—The metabolic fate of Dimethoate in the adult larva of cotton leaf worm has been investigated *in vivo* using ^{14}C-labelled insecticide, labelled at two different sites. The enzymatically liberated methylamine and methanol underwent oxidative degradation to $^{14}CO_2$ which was eliminated in the expired air and ^{14}C-formate which could be detected in the excreta. The major metabolite of Dimethoate II contributed 75–85 per cent of the water soluble ^{14}C-metabolites. This metabolite is believed to be α-hydroxy *N*-methylacetamide and is excreted as glucuronide.

THE SYSTEMIC insecticide *O,O*-dimethyl S-(*N*-methylcarbamoylmethyl) phosphorodithioate (Dimethoate)[1–4] has been effectively used in the field of plant protection[5] and Veterinary medicine.[6,7] The *in vivo* metabolism of ^{32}P-Dimethoate in the adult larva of the boll worm[8] (*Heliothis zea*) and the cotton leaf worm[9] (*Prodenia litura*) has been recently investigated.



Dimethoate



Dimethoxon

The results indicate that Dimethoate is mainly degraded in these larvae by two main metabolic reactions, one consists of oxidation to dimethoxon and the other involves hydrolysis of the insecticide to yield a variety of hydrolytic products.

For completion of our knowledge of rate and mechanism of degradation of the insecticide, the fate of the methyl groups has been investigated using ^{14}C-labelled Dimethoate. For this investigation two types of radioactive insecticide, in which both —OCH_3 groups (Dimethoate I) or the —$NHCH_3$ group (Dimethoate II) is ^{14}C-labelled have been used.

* For part IX, S. M. A. D. ZAYED, A. HASSAN and I. M. I. Fakhr, *Biochem. Pharmac.* **17**, 1339 (1968).

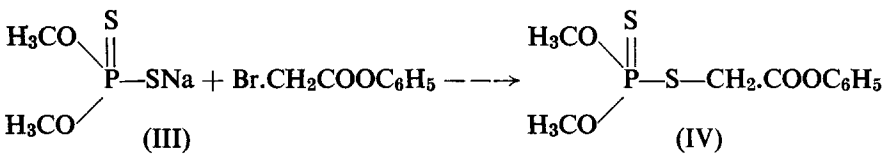
MATERIALS

Dimethoate I

A mixture of 2·5 g phosphorus pentasulphide and 1·6 ml ^{14}C-methanol* was refluxed gently for 45 min. After the pentasulphide had almost completely disappeared, ammonia gas was passed into the reaction mixture for 1 min. To the residue, toluene (10 ml), α chloroacetamide (2·4 g) and water (5 ml) were added and the mixture was heated on a water bath for 30 min. The product was extracted five times with toluene and the extracts were washed with 5% aqueous sodium bicarbonate solution, then with water. After removal of the solvent, 2·5 g of pure Dimethoate were obtained; specific activity $3{\cdot}6 \times 10^4$ cpm/mg.

Dimethoate II

To an aqueous solution of the sodium salt of *O,O*-dimethylphosphorodithioic acid (7 g) (Dithioate, III), a solution of monobromo-acetic acid phenyl ester (8 g) in 10 ml acetone was added during 2 hr while cooling and stirring. The product *O,O*-dimethyl-S(carboxyphenyl-methyl) dithiophosphate (IV) was isolated by extraction with chloroform and used without further purification for the preparation of Dimethoate II;[10] yield about 80%.



To a solution of 5 g of the ester in 20 ml aceton, ^{14}C-methylamine hydrochloride† (1·18 g in 1 ml water) and 2 g crystalline sodium acetate were added at 0°. The reaction flask was stoppered and shaken for 3 hr. Dimethoate was isolated and purified as in Dimethoate I; yield 2·9 g, specific activity $2{\cdot}4 \times 10^4$ cpm/mg.

METHODS

Laboratory reared *Prodenia* larvae (5th–6th instar) were treated topically with an acetone solution of the radioactive insecticide. The insects were left for 20 hr in a metabolic cage containing little sawdust. The respiratory $^{14}CO_2$ evolved during 20 hr, was trapped by 1N sodium hydroxide solution[11] and determined as $Ba^{14}CO_3$. The radioactivity was measured in an end-window counter under uniform geometrical conditions and corrected for background and self absorption.

The excreta was exhaustively extracted with water and the aqueous extract was shaken several times with chloroform.[9] Both water and chloroform extracts were analyzed for ^{14}C-metabolites. The ^{14}C-activity in aqueous and organic media was determined (after drying over P_2O_5) as described by Aronoff[12] using Van Slyke–Folch reagent.[13]

The ^{14}C-metabolites were analyzed by ascending paper chromatography using Schleicher and Schüll paper 2043b,[9] and the chromatograms were assayed radiometrically using a Frieske and Höpfner radioscanner. For the isolation and determina-

* 0·6 mc/methanol was diluted with 1·6 ml non labelled methanol.
†0·3 mc ^{14}C-CH_3NH_2.HCl was diluted with 1·18 g non labelled-compound.

tion of ^{14}C-formate in the excreta the inverse isotope dilution technique[14] was used.

Characterization of the isolated radioformate was achieved by paper chromatography.

RESULTS

For metabolism studies, a pool of 10 g insects was used. The percentage of ^{14}C-activity recovered in the aqueous and chloroform extracts and the inspired air are shown in Table 1. Analysis of sawdust showed that it contained only a negligible amount of ^{14}C-activity. From paper chromatographic analysis[9] and from partition

TABLE 1. ^{14}C-ACTIVITY RECOVERED AFTER 20 hr FROM TOPICAL APPLICATION OF ^{14}C-DIMETHOATE

No. of Dimethoate	Appl. (mg)	Dose (cpm)	Percentage of ^{14}C-activity (100% = applied dose)				No. of metab. in aqu. extract
			Chloroform extract	Aqueous extract	Larva	Expired air	
Dimethoate I	12·5	450,000	63·4	25·8	7·4	3·2	6
Dimethoate II	9·0	216,000	57·4	13·4	13·8	12·7	2

TABLE 2. R_f VALUES OF WATER SOLUBLE C^{14}-METABOLITES OF DIMETHOATE

Metabolite	System						
	A	B	C	D	E	F	G
Dimethoate I							
Carboxy derivative	0·51	0·59	0·69	0·71	0·61	—	—
O,O-Dimethylphosphorothioic acid	0·06	0·35	0·68	0·46	0·51	—	—
O,O-Dimethylphosphorodithioic acid	0·14	0·51	0·71	0·69	0·62	—	—
Dimethyl phosphate	0·04	0·24	0·57	0·50	0·17	—	—
Monomethyl phosphate	0·45	0·05	0·11	0·04	0·04	—	—
Formate	—	—	0·53	—	—	0·50	—
Dimethoate II							
Formate	—	—	0·53	—	—	0·50	0·50
Conjugated metabolite	—	—	0·30	—	—	0·44	0·34

(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.
(B) Acetonitrile: water: Ammonia (40:9:1)
(C) Iso-propanol: Ammonia (75:25)
(E) *n*-Butanol: Pyridine: Water (12:8:6)
(F) Butanol: Acetic acid: Water (4:1:5)
(G) Butanol: Acetic acid: Water (4:1:5). Paper impregnated with sodium acetate (0·2%).

chromatography on silica gel[5, 15] it has been estimated that Dimethoate contributed to 50–70 per cent of the chloroform extractable activity. The rest of ^{14}C-activity in the chloroform was due to the presence of dimethoxon.

The origin of Dimethoate recovered in the chloroform extract is uncertain. It could have been lost in surroundings during the movement of the larvae or/and excreted by the organism in an unchanged form.

A part of the aqueous extract was investigated for the presence of ^{14}C-formate, which was found to contribute 4–7 per cent and 0·3–0·5 per cent of the total ^{14}C-metabolites from Dimethoate I and Dimethoate II, respectively. The isolated radioformate was further characterized by radiopaper chromatography (Table 2).

The water soluble ^{14}C-metabolites were analyzed by radiopaper chromatography in different solvent systems (Table 2). Five metabolites of Dimethoate I could be identified. Dimethoate II was mainly metabolized to a coupled ^{14}C-metabolite which contributed 75–85 per cent of the water soluble radioactivity.

Characterization of the coupled metabolite from Dimethoate II

Experiments for the characterization of this metabolite were carried out on purified samples obtained by preparative paper chromatography in system C (Table 2) followed by elution of the radioactive substance with acetone. The metabolite does not contain phosphorus, since it gives no blue colour with Hanes–Isherwood reagent.[16] The radioactivity was only slightly decreased when the metabolite was heated with diluted hydrochloric acid for 20 min at 100°.

On the other hand alkaline hydrolysis (2 N sodium hydroxide at 100°), followed by acidification led to the disappearance of the ^{14}C-activity. The ether extract of the acidified solution gave positive Molisch's test and blackened ammoniacal silver nitrate solution; indicating the presence of a reducing sugar. The sugar-rest was identified as glucuronic acid by paper chromatography in *n*-butanol–acetic acid–water[17] (4:1:5; R_f 0·12) and in pyridine–ethyl acetate–acetic acid–water (5:5:1:3; R_f 0·3). The spots were made visible by spraying with ammoniacal silver nitrate or with methyl orange.

In order to demonstrate the presence of the structure C-*N*-^{14}C, a part of the water extract of known radioactivity was incubated with 2 N sodium hydroxide solution for 4 hr at room temperature. The liberated ^{14}C-labelled amine was trapped in sulphuric acid. About 50 per cent of the original radioactivity could be recovered as basic material.

DISCUSSION

Dimethane I has been prepared by a method similar to that of Uchida[19] for the preparation of ^{3}H-Dimethoate. The preparation of Dimethoate II is based on the interaction of the phenylester of the *O,O*-dimethyldithiophosphroyl acetic acid IV with methylamine.[10]

The data presented in this investigation demonstrated that ^{14}C-Dimethoate is fairly rapidly metabolized in *Prodenia* larvae and the metabolic products are eliminated in the excreta and the expired air. Following topical application, 51–59 per cent of Dimethoate I and 44–53 per cent of Dimethoate II is metabolized during 20 hr.

The results obtained from metabolism experiments are generally in line with those obtained from previous studies with ^{32}P-labelled insecticide.[9] Both oxidation of Dimethoxon and hydrolysis of the insecticide to yield a number of hydrolytic products occur. Oxidation rather than hydrolysis constitutes the major metabolic reaction; since the percentage of Dimethoxon represents 40–50 per cent of the total metabolites. The high percentage of Dimethoxon previously reported[9] may be due to partial non-enzymic oxidation of the insecticide.

The radioactive CO_2 eliminated from Dimethoate I in the expired air accounts only for a small percentage of the total ^{14}C-metabolites (about 8 per cent). ^{14}C-formate has been identified as a second metabolite of the enzymatically liberated methanol and constitutes about 5·7 per cent of the total ^{14}C-metabolites.

Previous studies with ^{32}P-labelled Dimethoate[9] showed that thiophosphoric acid

contributes about 50 per cent of the hydrolytic components. This indicates that phosphatase action constitutes a major hydrolytic mechanism of Dimethoate in *Prodenia* larva. It may, therefore, be concluded that $^{14}CO_2$ and ^{14}C-formate account only partly for the fate of the enzymatically liberated methanol. In this connection it is worthy mention that the production of $^{14}CO_2$ by *Prodenia* larvae is not a major pathway for methanol enzymatically liberated from ^{14}C-Dipterex labelled at the two carbon atoms of the methyl groups.[11, 20, 21]

The oxidative degradation of methanol to carbon dioxide in *Prodenia* larvae [11, 21] is believed to proceed in the same manner as in the rat.[22]

$$CH_3OH \longrightarrow HCHO \longrightarrow HCOO^- \longrightarrow CO_2$$

The $^{14}CO_2$ eliminated from Dimethoate II accounts for about 22 per cent of the total ^{14}C-metabolites. This indicates an oxidative degradation of methylamine, liberated by the C—N bond scission of Dimethoate under the influence of carboxyamidase.[9] A small percentage of ^{14}C-formate has been detected in the excreta (about 0·4 per cent of the applied dose). A similar oxidative degradation of methylamine in *Prodenia* larvae was found to occur with the carbamate insecticide Sevin.[23]

$$CH_3NH_2 \longrightarrow HCHO \longrightarrow HCOO^- \longrightarrow CO_2$$

The oxidative degradation of the methyl groups of the Dimethoate molecule receives support from the finding that larval hemolymph contains catalase, aldehyde oxidase[11] and amineoxidase.[24]

The coupled ^{14}C-metabolite has been detected as a metabolite of Dimethoate II. This metabolite is believed to be α-hydroxy-*N*-methyl acetamide (V), or a compound of similar structure with a modified methyl group (e.g. —CH_2OH). In the latter case, coupling with glucuronic acid might take place at either or both OH groups.

$$HO—CH_2—CONHCH_3$$

V

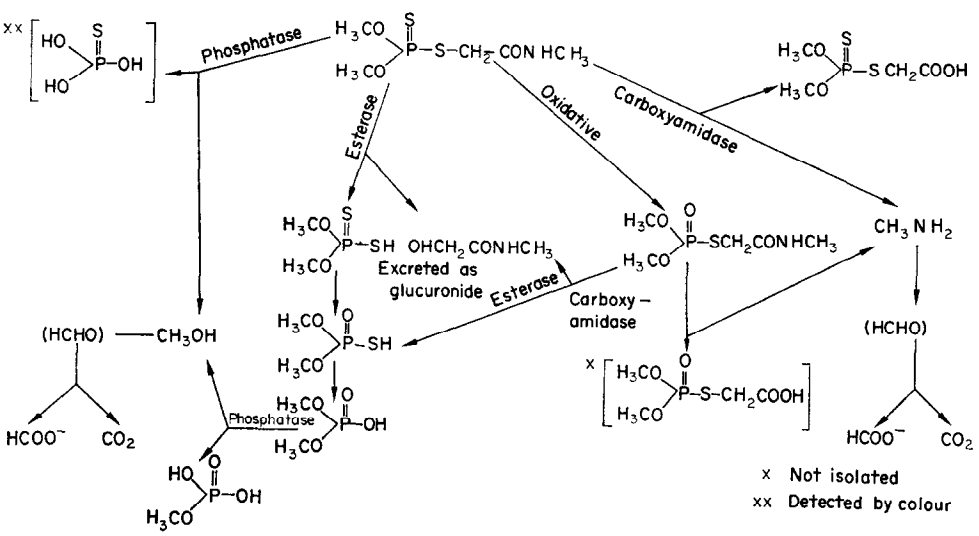


FIG. 1. Suggested scheme for the fate of the methyl groups of Dimethoate.

The formation of V may be attributed to enzymic hydrolysis of S—C bond of the Dimethoate molecule and/or of the oxygen analogue Dimethoxon. The possible metabolic pathways of Dimethoate are illustrated in Fig. 1.

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