Nature of Oxidative Metabolites of Dimethoate Formed in Rats, Liver Microsomes, and Bean Plants

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The oxidative metabolism of dimethoate-*N*-methyl-^{14}C and dimethoate-carbonyl-^{14}C was studied in bean plants, rats, and mammalian liver microsomes. Oxidative desulfuration to form the oxygen analog rapidly occurred in bean plants and rats. Both dimethoate and its oxygen analog also underwent *N*-demethylation coupled with the formation of *N*-hydroxymethyl intermediates. Rabbit and rat liver microsomes converted dimethoate to its oxygen analog and des-*N*-methyl derivatives, although the *N*-hydroxymethyl intermediates were not detected. The P=O derivatives were at least 10^3 times more potent inhibitors of fly head and human plasma cholinesterase than the P=S compounds, although dimethoate oxygen analog was only four times more toxic than dimethoate to houseflies *in vivo*. Des-*N*-methyl oxygen analog was the most toxic compound tested to houseflies. Sesamex synergized dimethoate oxygen analog toxicity to houseflies, but did not significantly affect the toxicity of dimethoate and the *N*-demethylated derivatives.

The fate of dimethoate [*O,O*-dimethyl-*S*-(*N*-methylcarbamoylmethyl) phosphorodithioate], a selective organophosphate insecticide and an effective plant and animal systemic, has been studied in several biological systems. Most reports have been concerned with the formation of hydrophilic, nonorganoextractable compounds which are relatively nontoxic to animals in comparison with the neutral phosphate ester metabolites. Plants deactivate dimethoate by enzymatic reactions at several different sites on the molecule. Des-*O*-methyl dimethoate [*O*-methyl *O*-hydrogen *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] (Dauterman *et al.*, 1960; Lucier and Menzer, 1968; Rowlands, 1966; Santi, 1961); dimethoate carboxylic acid (*O,O*-dimethyl *S*-carboxymethyl phosphorodithioate) (Hacskaylo and Bull, 1963; Lucier and Menzer, 1968; Rowlands, 1966; Santi and Giacomelli, 1962, Santi *et al.*, 1962); *O,O*-dimethyl phosphorodithioic acid; *O,O*-dimethyl phosphorothioic acid; *O,O*-dimethyl phosphoric acid; and phosphoric acid (Hacskaylo and Bull, 1963; Lucier and Menzer, 1968; Santi and Giacomelli, 1962) have been isolated and identified following plant treatments. Similar products have resulted from animal metabolism (Bull *et al.*, 1963; Chamberlain *et al.*, 1961; Dauterman *et al.*, 1959; Hassan *et al.*, 1969; Kaplanis *et al.*, 1959; Zayed *et al.*, 1968). Uchida *et al.* (1964) demonstrated that liver microsomes of several species were capable of metabolizing dimethoate through amidase and phosphatase activity.

The toxicity of dimethoate to animals depends on its conversion to the oxygen analog [*O,O*-dimethyl-*S*-(*N*-methylcarbamoylmethyl) phosphorothiolate], which is the only cholinesterase-inhibiting metabolite of dimethoate definitely characterized (Bull *et al.*, 1963; Chamberlain *et al.*, 1961; Dauterman *et al.*, 1960; Hacskaylo and Bull, 1963; Hassan *et al.*, 1969; Lucier and Menzer, 1968; Sanderson and Edson, 1964; Santi and Giacomelli, 1962; Uchida *et al.*, 1965; Zayed *et al.*, 1965). Sanderson and Edson (1964) isolated from animals three additional cholinesterase-inhibiting metabolites of dimethoate which were theorized to be *N*-hydroxymethyl derivatives. Metabolites resulting from the oxidative demethylation of the *N*-methylamide might be toxic, since they could be converted to P=O compounds which would have significant anticholinesterase activity. Lucier and Menzer (1968), working with bean plants, isolated two neutral phosphate ester metabolites following a foliar application of dimethoate-^{32}P and -^{14}C, which were possibly *N*-hydroxymethyl or des-*N*-methyl derivatives of dimethoate and its oxygen analog. Bidrin, Azodrin, and phosphamidon, other organophosphorus insecticides which have substituted amide moieties, have been shown to undergo oxidative dealkylation in plants and animals with the formation of *N*-hydroxyalkyl intermediates (Clemons and Menzer, 1968; Lucier and Menzer, 1970; Menzer and Casida, 1965). Many carbamate insecticides also undergo oxidative *N*-demethylation with the formation of *N*-hydroxymethyl intermediates (Oonithan and Casida, 1968).

The phenylurea herbicides are *N*-dealkylated in plants and rats. However, the corresponding *N*-hydroxyalkyl intermediates have not been isolated (Ernst and Böhme, 1965; Onley *et al.*, 1968; Smith and Sheets, 1967).

N-Dealkylation conversions occurring with a wide range of pesticidal compounds in both plants and animal systems suggest that the unknown organoextractable metabolites isolated by Sanderson and Edson (1964) and Lucier and Menzer (1968) could represent *N*-demethylated compounds of dimethoate and its oxygen analog and/or their *N*-hydroxymethyl intermediates. This study was conducted with the aim of isolating and characterizing the oxidative metabolites of dimethoate, which are potential anticholinesterases.

MATERIALS AND METHODS

Synthesis of Dimethoate-*N*-methyl-^{14}C. Dimethoate-*N*-methyl-^{14}C was prepared by reacting dimethoate carboxylic acid with labeled methyl isocyanate, prepared by the method of Krishna *et al.* (1962). Acetyl chloride-2-^{14}C (19.6 mg, 2 mCi/mmole) was the starting material. Dimethoate carboxylic acid (128 mg) in 2 ml benzene was placed in a clean, dry test tube in a nitrogen atmosphere. The tube containing methyl-^{14}C-isocyanate was immersed in crushed ice and then opened with an oxygen flame. The contents were quickly transferred to the dimethoate carboxylic acid solution with the addition of a few drops of triethylamine. The tube was sealed while still immersed in crushed ice and then placed in a 35° C water bath for 2 hr with constant shaking. The reaction tube was opened and the benzene evaporated under a stream of air to 0.5 ml, which was added directly to a Celite parti-

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tioning column. Elution with hexane and chloroform mixtures resolved dimethoate from its major reaction impurities. The product was further purified by silica gel thin-layer chromatography. The radioactive spot corresponding to unlabeled dimethoate contained only 2.2 mg (3.9% yield).

An alternative procedure for dimethoate *N*-methyl-^{14}C synthesis was developed from a modification of that used by Losco and Peri (1962). Methylamine-^{14}C-hydrochloride (6.7 mg, 1.01 mCi/mmole) obtained from Mallinckrodt Nuclear (St. Louis, Mo.) was used in the synthesis. The labeled material was mixed with 4 ml of water and the solution neutralized with 0.05*N* NaOH. The mixture was added dropwise to a chilled test tube containing 40 mg dimethoate carboxylic acid. The tube was sealed with an oxygen flame and shaken in a water bath for 72 hr at 50° C. The tube was opened and the contents extracted three times with an equal volume of chloroform. The water fraction still contained considerable radioactivity and was reacted again for 72 more hr after the addition of 40 mg dimethoate carboxylic acid. The chloroform extractions from the two reactions were pooled and evaporated under a heat gun to a small volume. The remaining solution was spotted on thin-layer plates, and the radioactive spot corresponding to unlabeled dimethoate extracted with 100 ml of chloroform. The chloroform was evaporated on a rotary evaporator leaving a residue of 3.1 mg of dimethoate *N*-methyl-^{14}C (13.5% yield).

Other Chemicals. Dimethoate carbonyl-^{14}C prepared previously (Lucier and Menzer, 1968) was purified on silica gel thin-layer plates prior to use.

O,O-Dimethyl *S*-(*N*-hydroxymethylcarbamoylmethyl) phosphorodithioate (*N*-hydroxymethyl dimethoate) was prepared by a procedure analogous to that used by Menzer and Casida (1965) in the synthesis of Bidrin *N*-hydroxymethyl analogs. *O,O*-Dimethyl *S*-(carbamoylmethyl) phosphorodithioate (des-*N*-methyl dimethoate) (215 mg) was dissolved in 15 ml of water in a 50 ml Erlenmeyer flask. One hundred μl of 37% formaldehyde, 50 μl formaldehyde-^{14}C, and 1.0 ml of saturated sodium bicarbonate solution were added to the reaction flask. The flask was shaken in a water bath at 40° C for 24 hr, and then extracted five times with equal volumes of chloroform. The chloroform extract was evaporated to a small volume and resolved on a Celite partitioning column. One radioactive peak was isolated from the column. An infrared spectrum of the peak showed the characteristic OH stretch band at 3400 cm^{-1}, and otherwise suggested the structure to be *N*-hydroxymethyl dimethoate. Isotope dilution analysis of the synthetic *N*-hydroxymethyl-^{14}C-dimethoate revealed that the radioactivity was recoverable as labeled formaldehyde, indicating the presence of —N—CH_2OH on the molecule. The total yield from the reaction was 22.1%. Similar synthesis, resolution, and structure confirmation was done in the preparation of *O,O*-dimethyl *S*-(*N*-hydroxymethylcarbamoylmethyl) phosphorothiolate (*N*-hydroxymethyl oxygen analog). *O,O*-Dimethyl *S*-(carbamoylmethyl) phosphorothiolate (des-*N*-methyl oxygen analog) prepared by a method adapted from Santi and de Pietri-Tonelli (1959) was used as the starting material in the synthesis. Des-*N*-methyl dimethoate (250 mg) was dissolved in 50 ml of 1% potassium permanganate solution and stirred with a magnetic stirrer at 25° C for 6 hr. The solution was extracted five times with equal volumes of chloroform. The solvent was evaporated to a small volume, and the des-*N*-methyl oxygen analog separated from unreacted starting material on a Celite partitioning column. The structure of the reaction product was proposed to be des-*N*-methyl oxygen analog from an analysis of infrared spectra and chromatographic properties. Des-*N*-methyl oxygen analog (45.6 mg) was reacted with formaldehyde by the above-mentioned procedure to give *N*-hydroxymethyl oxygen analog (20.8 mg, 45.5% yield). The purity of the *N*-hydroxymethyl derivatives of dimethoate and its oxygen analog was checked before use on silica gel thin-layer plates, since they are known to be extremely unstable (Menzer and Casida, 1965).

Unlabeled dimethoate, des-*N*-methyl dimethoate, dimethoate oxygen analog, and dimethoate carboxylic acid were supplied by American Cyanamid Company (Princeton, N.J.).

Treatment of Plants, Rats and Liver Microsomes. Bean plants (*Phaseolus vulgaris* L.) were treated by foliar application, as described by Lucier and Menzer (1968). It consisted of spreading 25 μl of dimethoate-^{14}C dissolved in water over the surface of each of two leaves, giving an approximate dosage of 15 ppm for each plant. Samples were taken at 1, 3, and 6 days. Three replications of two plants each (whole plant) were taken for each day. Plants were grown in vermiculite for 10 days in a plant growth chamber in which the daylight temperature was 90° F and the night temperature 80° F. The daily photoperiod consisted of 16 hr light and 8 hr dark.

Three female and three male white rats, Sprague-Dawley derived (Flow Labs, Inc., Bethesda, Md.), were treated through a stomach tube with 4.0 mg per kg of dimethoate-carbonyl-^{14}C or dimethoate-*N*-methyl-^{14}C in 250 μl water. Rat urine was collected in an apparatus designed to separate the urine and feces. Urine samples were taken at 6, 12, 24, and 72 hr. The feces were not assayed.

Rabbit and rat liver microsomes were prepared according to the procedure of Remmer *et al.* (1967). The rats were killed by a blow on the head and the rabbits by exsanguination, and the livers immediately removed and placed in chilled 0.25*M* sucrose. The prepared microsomes were resuspended in tris-HCl buffer at approximately 6 mg protein per ml. The prepared microsomes were used up to 2 days after preparation. Incubation mixtures consisted of 100,000 cpm of either dimethoate-carbonyl-^{14}C (17.6 μg) or dimethoate *N*-methyl-^{14}C (10.3 μg), 0.5 ml of microsome preparation, 2 μmoles of NADPH, and 5.0 ml of tris-buffer. In one experiment the rabbit was pretreated for 3 days with intraperitoneal injections of sodium phenobarbital at 75 mg per kg per day. The flasks were incubated for 4 hr at 37.5° C, after which the addition of 6 ml of chloroform stopped the reaction.

Extraction Procedures. Plants were frozen for at least 30 min immediately after harvesting and then extracted as previously described (Lucier and Menzer, 1968).

Rat urine at each sampling time was extracted five times with equal volumes of chloroform, and centrifuged for 15 min at 2000 rpm in a model CS International Centrifuge when necessary to separate the phases. The chloroform fraction was dried with anhydrous sodium sulfate.

After the addition of chloroform to the microsomal incubation flasks, the two layers were separated, followed by four or more extractions with equal volumes of chloroform. The chloroform extractable metabolites from plants, rat urine, and microsomes were resolved by column and thin-layer chromatography. The water fractions were assayed for radioactivity and stored at 4° C.

Chromatography. A previously described two-dimensional thin-layer chromatographic system was used to resolve dimethoate and its metabolites in the chloroform extracts (Lucier and Menzer, 1968).

Chloroform extracts were also chromatographed on Celite partitioning columns. The extracts were evaporated to approximately 0.5 ml, placed on Celite columns, and eluted with

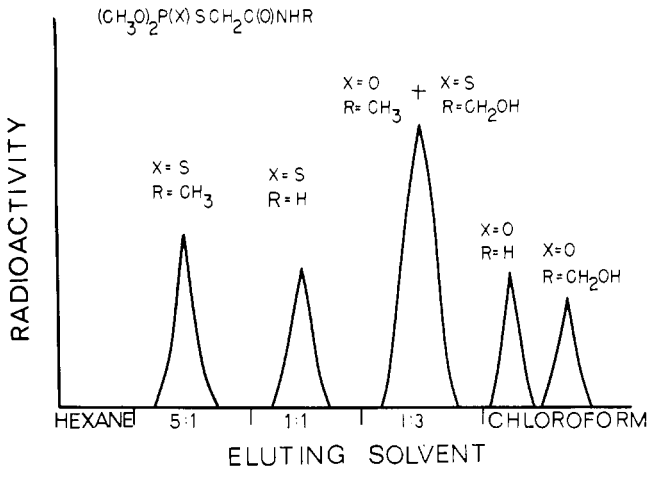

Figure 1. Representation of the separation of dimethoate metabolites achieved on a Celite column based on partitioning between water and hexane-chloroform mixtures

hexane-chloroform mixtures; the first solvent was pure hexane and the last pure chloroform. Twenty-milliliter fractions were collected in all cases. Each Celite column was prepared by thoroughly mixing 32 g Celite (Johns Manville's Analytical Filter Aid) and 28 ml distilled water. The Celite was slurried in hexane for packing in 19 mm i.d. columns.

Detection and Identification of Metabolites. Dimethoate-^{14}C and its metabolites were identified by spotting known, unlabeled standards with radioactive extracts or peaks isolated from Celite columns. After resolution of the metabolites, the location of the unlabeled standards was determined by spraying with a palladium chloride reagent prepared by diluting 5 ml of a 5% palladium chloride stock solution with 1 ml concentrated HCl and 94 ml of 95% ethanol. Dimethoate and its phosphorodithioate analogs gave yellow spots, while the phosphorothiolate derivatives gave a brownish-yellow color on a white background. Fifty μg of the standards gave easily detectable spots. The presence of radioactivity on thin-layer plates was determined by exposing the chromatograms to Kodak-No Screen medical X-ray film for a 1 to 7-day period, depending on the amount of radioactivity applied to the plates. The coincidental location of the radioactive spots and those resulting from the palladium chloride spray indicated their possible identity.

Twenty mg of unlabeled dimethoate and its metabolites were added to the radioactive extracts placed on the Celite partitioning columns. Co-chromatography of the unlabeled material with radioactive peaks was determined by phosphate analysis (Allen, 1940) of a 1-ml aliquot from the individual fractions collected from the column.

Radioactivity was assayed by liquid scintillation counting according to procedures previously described (Lucier and Menzer, 1968).

An isotope dilution procedure was employed to ascertain the nature of the *N*-methyl moiety of unknown metabolites (Menzer and Casida, 1965). The procedure utilized the formation of labeled dicoumarol from formaldehyde-^{14}C and 4-hydroxycoumarin after hydrolysis of *N*-hydroxymethyl-^{14}C compounds. The average yield of dicoumarol based on unlabeled formaldehyde was 74%.

Biological Activity of Dimethoate and its Metabolites. The biological activity of dimethoate and its metabolites was evaluated against housefly head and human plasma cholinesterase. In the fly head experiments, 0.4 ml of a $5 \times 10^{-2}M$ buffered solution of acetylcholine was added to the sidearm of the Warburg flask. Three- and 4-day old flies (C.S.M.A. 1948 strain, obtained from Entomology Research Division, U.S.D.A., Beltsville, Md.) of both sexes were used as the enzyme source. The flies were held for 30 min at $-20°$ C and decapitated. The heads were homogenized in bicarbonate buffer (0.374*M* sodium bicarbonate and 0.164*M* sodium chloride) and filtered through cheesecloth. The final enzyme solution represented 1.0 fly head per 0.81 ml. The main compartment of the Warburg flasks contained 1.3 ml of enzyme solution and 0.3 ml of the inhibitor solution. After equilibration at 37° C for 15 min, the sidearm contents were mixed with the contents of the main compartment, and the rate of hydrolysis of acetylcholine was measured by carbon dioxide evolution. The same procedure was used for the plasma determinations, except that 0.4 ml of a $5.85 \times 10^{-2}M$ buffered solution of butyrylcholine iodide was used for the substrate and 0.05 ml of human plasma (National Red Cross Blood Service, Washington, D.C.) in 1.25 ml of buffer as the enzyme source. The final volume in all flasks for the plasma and fly head experiments was 2.0 ml.

Toxicities of dimethoate and its metabolites to houseflies and white mice were also determined from LD_{50} values. Adult female houseflies (*Musca domestica* L.), 3 days after emergence, were treated with 1 μl of acetone containing dimethoate or its analogs. The treatments with each toxicant were done in the presence and absence of sesamex [2-(2-ethoxyethoxy) ethyl-3,4-(methylenedioxy)phenyl acetal of acetaldehyde]. The flies were treated with dimethoate and its metabolites on the tip of the abdomen. Those treated with sesamex received a dose of 10 μg in 1.0 μl of acetone on the abdomen immediately before application of the toxicant. Sesamex itself shows no toxic effect with a 10 μg dose (Menzer and Casida, 1965). Male albino mice, Swiss-Webster derived strain, 20 to 25 g live weight, were obtained from Dublin Laboratory Animals, Dublin, Va. Intraperitoneal injections of dimethoate and its metabolites were made to groups of animals, which were then held for 24 hr to observe mortality.

RESULTS

Chemical Nature of Metabolites. Six radioactive peaks from beans, five from rat urine, and four from liver microsomes were isolated on Celite columns following dimethoate-carbonyl-^{14}C treatment (Figure 1). Dimethoate, des-*N*-methyl dimethoate, oxygen analog, des-*N*-methyl oxygen analog, and *N*-hydroxymethyl oxygen analog were identified by co-chromatography with known standards on thin-layer plates and Celite columns. The R_f values for dimethoate and its metabolites are shown in Table I.

Dimethoate-*N*-methyl-^{14}C was administered to plants, rats, and microsomes to ascertain the nature of the amide moiety of metabolites. As expected, the demethylated compounds, des-*N*-methyl dimethoate and des-*N*-methyl oxygen analog, were not associated with radioactivity from the dimethoate-*N*-methyl-^{14}C applications. A portion of the peak representing the oxygen analog liberated formaldehyde-^{14}C upon acid hydrolysis. It was also found that nonradioactive oxygen analog and *N*-hydroxymethyl dimethoate had identical chromatographic properties on the Celite column and thin-layer systems used. From this evidence, the formaldehyde-^{14}C-liberating portion of the oxygen analog fraction was designated *N*-hydroxymethyl dimethoate. The identity of the peak co-chromatographing with unlabeled *N*-hydroxymethyl oxygen analog was further confirmed, since it evolved formaldehyde-^{14}C in the expected yield. The radioactive fraction which co-chromatographed with unlabeled di-

Table I. Common Name, Chemical Name, and R_f Values of Dimethoate and its Metabolites on Thin-Layer Chromatograms

Common Name	R_f[a] 1	R_f[a] 2	Chemical Name
Dimethoate	0.95	0.45	*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate
Des-*N*-methyl dimethoate	0.90	0.35	*O,O*-dimethyl *S*-(carbamoylmethyl) phosphorodithioate
N-Hydroxymethyl dimethoate	0.80	0.30	*O,O*-dimethyl *S*-(*N*-hydroxymethylcarbamoylmethyl) phosphorodithioate
Oxygen analog	0.80	0.30	*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate
Des-*N*-methyl oxygen analog	0.75	0.25	*O,O*-dimethyl *S*-(carbamoylmethyl) phosphorothiolate
N-Hydroxymethyl oxygen analog	0.65	0.15	*O,O*-dimethyl *S*-(*N*-hydroxymethylcarbamoylmethyl) phosphorothiolate
Unknown I	0.80	0.35	

[a] Thin-layer chromatography on silica gel G using 5 to 1 acetone-water in direction 1 and 3.3% methanol in chloroform in direction 2.

Table II. Percentages of Administered Dose of Labeled Compounds from Urine Following Treatment of Rats with 4.0 Mg per Kg of Dimethoate-Carbonyl-^{14}C

Metabolites $(CH_3O)_2P(X)SCH_2C(O)NHR$		Number of Hr: Males 0–6	Males 6–12	Males 12–24	Females 0–6	Females 6–12	Females 12–24
X=S	R=CH_3	0.15	0.13	0.02	0.21	0.09	0.01
X=S	R=CH_2OH	0.06	0.03	0.002	0.16	0.03	0.004
X=S	R=H	0.36	0.15	...	0.72	0.17	...
X=O	R=CH_3	0.61	4.58	0.11	1.44	3.86	0.16
X=O	R=CH_2OH	0.09	0.28	0.02	0.12	0.13	0.006
X=O	R=H	0.23	1.36	0.04	0.55	1.09	0.07
	Water Fraction	11.45	55.33	3.72	17.21	41.55	4.03
	Total excreted	12.95	61.86	3.91	20.41	46.92	4.28

Table III. Percentages of Administered Dose of Labeled Compounds Recovered from Plants Treated with 15.0 ppm of Dimethoate-Carbonyl-^{14}C

Metabolites $(CH_3O)_2P(X)SCH_2C(O)NHR$		Days After Treatment: 1	3	6
X=S	R=CH_3	69.67	29.34	7.42
X=S	R=CH_2OH	0.03	0.05	0.008
X=S	R=H	0.30	0.52	0.24
X=O	R=CH_3	3.01	7.33	7.12
X=O	R=CH_2OH	0.03	0.03	0.03
X=O	R=H	0.23	0.53	0.41
Unknown I		0.88	0.37	0.16
Hexane extract[a]		1.22	0.69	0.58
Water fraction		20.41	50.55	54.32
Total Recovery		95.78	89.41	70.29

[a] Dimethoate activity in hexane extract subtracted and included in dimethoate recovery above.

Table IV. Percentages of Administered Dose of Labeled Compounds from Liver Microsomes Treated with 17.6 μg of Dimethoate-Carbonyl-^{14}C

Metabolites $(CH_3O)_2P(X)SCH_2C(O)NHR$		Treatment: Rat	Rabbit: Control	Rabbit: Phenobarbital Treated
X=S	R=CH_3	71.64	59.33	60.67
X=S	R=H	0.21	0.61	0.47
X=O	R=CH_3	0.18	0.69	0.71
X=O	R=H	0.02	0.03	0.03
	Water fraction	20.69	36.54	34.53
	Total Recovery	92.74	97.20	96.41

methoate also contained the *N*-methyl portion of the molecule and did not liberate formaldehyde-^{14}C.

A metabolite, corresponding in chromatographic position to the unknown I reported by Lucier and Menzer (1968), was detected in bean plants but not rat urine or liver microsomes. The identity of the metabolite is still unknown, although it does liberate formaldehyde-^{14}C, but in less than the theoretical amount, and contains the *N*-methyl, carbonyl, and phosphorus portions of the molecule. The metabolite designated des-*N*-methyl oxygen analog corresponds chromatographically to the unknown II, previously reported.

Recovery of Administered Radioactivity. Good recovery of administered radioactivity was achieved with rats (Table II), beans (Table III), and liver microsomes (Table IV). Recoveries of dimethoate and its metabolites were calculated as dimethoate-carbonyl-^{14}C equivalents.

After 24 hr, an average of 78.92% of radioactivity was recovered from male rats and 71.60% from females. A similar sex difference in excretion has been observed with dimethoate (Dauterman *et al.*, 1959), Bidrin and Azodrin (Menzer and Casida, 1965), and phosphamidon (Clemons and Menzer, 1968). However, there was a delay in excretion of dimethoate such that the largest amount of radioactivity was recovered in the 6 to 12 hr period (Table II). Hydrolysis products predominated over chloroform-extractable metabolites at all sampling times, but the activity of the hydrolytic components was not chromatographed since this fraction would not include the neutral phosphorus esters that are potential anticholinesterase agents. The oxygen analog was the major organo-extractable metabolite, with most of it excreted in the 6 to 12 hr sampling period. It represented 5.39% of the administered radioactivity from males and 5.46% from females. Des-*N*-

Table V. Biological Activity of Dimethoate and its Metabolites

Compound $(CH_3O)P(X)SCH_2C(O)NHR$		LD_{50}, Mg/Kg			pI_{50}[a]	
			Housefly, ♀, topical			
		Mouse, ♂, i.p.	− Sesamex	+ Sesamex	Fly ChE	Plasma ChE
X=S	$R=CH_3$	151 (137–166)	0.83 (0.72–0.95)	0.71 (0.59–0.86)	1.2	<1[b]
X=S	$R=CH_2OH$	...	...	...	2.2	<1[b]
X=S	R=H	190 (168–215)[c]	0.69 (0.54–0.88)	0.84 (0.54–1.28)	2.2	<1[b]
X=O	$R=CH_3$	13 (11–16)	0.21 (0.17–0.25)	0.05 (0.03–0.07)	6.1	4.2
X=O	$R=CH_2OH$	...	...	...	5.4	3.7
X=O	R=H	10 (8–13)	0.09 (0.07–0.12)[d]	0.06 (0.04–0.08)	5.1	3.4

[a] Negative logarithm of molar organophosphate concentration for 50% inhibition. [b] Solubility limits of organophosphate in Warburg buffer prevented accurate determination of pI_{50}. [c] Numbers in parentheses are 95% confidence limits as calculated by method of Litchfield and Wilcoxon (1949). Value indicated by *c* is significantly different from value immediately above at 5% level. [d] Value is significantly different from value immediately above at 5% level.

methyl oxygen analog was the next most predominant metabolite accounting for about 1.5% of the administered radioactivity with each sex. Dimethoate was rapidly degraded by rats with only small quantities of the parent compound being recovered, even at the 6 hr sampling time. *N*-Hydroxymethyl dimethoate, des-*N*-methyl dimethoate, and *N*-hydroxymethyl oxygen analog were detected in small quantities. No additional *N*-hydroxymethyl derivatives were obtained after treatment of water fractions from rat urine with β-glucuronidase.

The half life of dimethoate in beans was 1.7 days. The oxygen analog was the major organoextractable metabolite at all harvest times, reaching 7.33% after 3 days. The entire oxidative sequence plus unknown I was detected at all harvest times, although no other metabolite accumulated as much as 1% (Table III). Hexane extracts contained little radioactivity, and steadily decreased at the later harvest times. A significant amount of the hexane extracts was found to be dimethoate, and was included as such in the recovery calculations. Hydrolytic products reached 54.4% of administered radioactivity after 6 days, but were not chromatographed. The total recovery from plants was very good, although incorporation of radioactivity into plant constituents was not measured. Only small quantities of des-*N*-methyl dimethoate, oxygen analog, and des-*N*-methyl oxygen analog were recovered from rabbit and rat liver microsome incubations (Table IV). The *N*-hydroxymethyl intermediates were not detected and there was no significant increase in oxidative metabolites from rabbits pretreated with phenobarbital. Rabbit livers were slightly more effective in degrading dimethoate than rat livers.

Biological Activity of Dimethoate and its Metabolites. Dimethoate, oxygen analog, des-*N*-methyl dimethoate, and des-*N*-methyl oxygen analog were tested for toxicity to houseflies, in the presence and absence of sesamex, and to white mice (Table V). The oxygen analog and its *N*-demethylated derivative were significantly more potent toxicants than their corresponding phosphorodithioate precursors (dimethoate and des-*N*-methyl dimethoate). Des-*N*-methyl oxygen analog was the most potent compound tested. Although sesamex should inhibit the oxidative conversion of P=S to P=O and therefore might be expected to antagonize the toxic action of dimethoate and its des-*N*-methyl derivative, no significant difference in toxicity to houseflies was observed with these compounds in the presence and absence of sesamex. The oxygen analog was the only compound tested which was significantly synergized by pretreatment with sesamex.

The phosphorodithioates, in comparison to the desulfurated derivatives, were extremely poor inhibitors of fly head and human plasma cholinesterase (Table V). The oxygen analog was 10^5 times more potent than dimethoate. The *N*-hydroxymethyl oxygen analog and des-*N*-methyl oxygen analog demonstrated 10^3 times the inhibition of the corresponding phosphorodithioates. All of the compounds tested inhibited fly head cholinesterase at lower concentrations than human plasma cholinesterase. The degree of inhibition decreased respectively with the *N*-hydroxymethyl and *N*-demethylated derivatives of the oxygen analog.

DISCUSSION

Metabolites comprising the complete oxidative sequence of dimethoate were detected in bean plants, rats, and liver microsomes, except that the *N*-hydroxymethyl intermediates were not isolated from microsomal incubations. The metabolic scheme is depicted in Figure 2. Oxidative desulfuration of dimethoate to form the oxygen analog represents the major pathway in both bean plants and rats. The oxygen analog may have increased stability to enzymatic hydrolysis, since it accumulates in higher levels than dimethoate in plants and animals, or its increased appearance in rat urine could be influenced by increased water solubility. Dauterman *et al.* (1959) reported that following treatment of male rats with dimethoate, 81% of the administered dose was excreted in the urine in 24 hr, while following treatment with dimethoate oxygen analog, only 19% was excreted in 24 hr. Lucier and Menzer (1968), using dimethoate-^{32}P and -^{14}C, reported that the oxygen analog accumulates to levels comparable to the levels of dimethoate by 10 days after treatment with foliar, excised leaf, stem injection, and root applications to bean plants. Dimethoate and its *N*-butyl homologs were degraded three times more rapidly than their corresponding oxygen analogs by tomato plants (Grimmer *et al.*, 1968).

The *N*-methylamide moiety of dimethoate and its oxygen analog were metabolized to produce additional anticholinesterases. Oxidation of the *N*-alkyl portion of a molecule theoretically takes place by the formation of a hydroxylalkyl compound which is dealkylated by liberation of the aldehyde (McMahon *et al.*, 1969; Menzer and Casida, 1965). Due to their instability, the *N*-hydroxymethyl intermediates con-

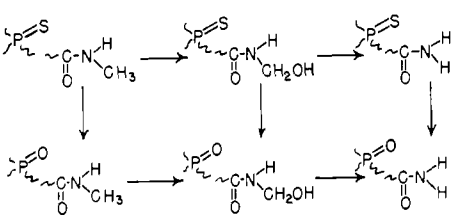

Figure 2. Proposed oxidative metabolic route of dimethoate in rats and bean plants

sistently occurred in lower concentrations in bean plants and rat urine compared to the des-*N*-methyl compounds. The prepared *N*-hydroxymethyl standards decompose completely when stored for 3 months at 4° C to yield des-*N*-methyl compounds with the evolution of formaldehyde.

Des-*N*-methyl oxygen analog and its hydroxymethyl precursor were recovered in higher concentrations from rat urine than the corresponding metabolites from dimethoate itself. The observed accumulation of the oxygen analog metabolites would result if desulfuration of dimethoate increased the stability of the resulting compound toward enzymatic degradation. In bean plants, the ratio of phosphorodithioates to phosphorothiolates in the *N*-demethylation scheme was approximately 1 to 1, again possibly indicating a reduced rate of hydrolytic metabolism for the desulfurated compounds. However, no kinetic data were obtained for the relative rates of metabolism in the present study. Indirect evidence for the occurrence of oxidative *N*-demethylation of dimethoate was reported by Hassan *et al.* (1969). These researchers traced the metabolism of methylamine liberated by rat amidase activity on dimethoate.

The concentration and chromatographic properties of metabolites from rat urine compare favorably with the compounds isolated by Sanderson and Edson (1964). This observation assumes that the chromatographic system employed by these workers would not resolve the oxygen analog and *N*-hydroxymethyl dimethoate. The des-*N*-methyl oxygen analog is identical to the unknown II found by Lucier and Menzer (1968), but the identity of unknown I and its location in the metabolic sequence still remain unclear.

Disappointing results were obtained from the microsomal incubations of rabbits pretreated with sodium phenobarbital. Phenobarbital is known to be an inducer of hepatic microsomal enzymes, which include the mixed function oxidases responsible for conversion of P=S to P=O and for *N*-dealkylation reactions (Vardanis, 1966). However, no increase in oxidative metabolites was observed in liver microsomes from rabbits pretreated with phenobarbital compared to controls. *In vivo* studies with mice showed dimethoate toxicity to be markedly increased by phenobarbital pretreatments, presumably through increased P=S to P=O conversion (Menzer and Best, 1968).

The housefly toxicity studies present some interesting relationships. Dimethoate and its des-*N*-methyl metabolite are less toxic to houseflies than the corresponding phosphorothiolates, which is in agreement with the cholinesterase inhibition data. Des-*N*-methyl oxygen analog is significantly more toxic than the oxygen analog. Similar results were obtained for houseflies and white mice with the series of *N*-dealkylated derivatives of Bidrin and phosphamidon (Clemons and Menzer, 1968; Menzer and Casida, 1965), with the unsubstituted amides being significantly more toxic than their alkyl or dialkyl precursors.

Sesamex, which inhibits the hepatic microsomal oxidations responsible for *N*-dealkylations, might theoretically antagonize the toxic action of dimethoate oxygen analog, rather than act as a synergist. But sesamex may enhance toxicity by inhibiting detoxication pathways. *O*-Demethylation, a deactivation, has been reported to be at least in part an oxidative process (Donninger *et al.*, 1966). Hall and Sun (1965) found sesamex effected a marked reduction in the formation of hydrolytic products of Bidrin metabolism.

Dimethoate and its des-*N*-methyl derivative retained the same level of toxicity to houseflies when treated with sesamex. Two competing systems of metabolism must be involved, apparently of equal magnitude. Sesamex inhibits P=S to P=O conversion, thus antagonizing the toxic action, but on the other hand, any oxygen analog formed is likely synergized by an inhibition of detoxication conversions.

An alternative explanation for the synergistic action of sesamex would be that sesamex, by inhibiting oxidative *N*-dealkylation, prevents formation of the unsubstituted amide metabolite, which could be more enzymatically labile. Therefore, the toxic neutral phosphate ester metabolites would accumulate to higher levels in animals treated with sesamex. However, this theory does not explain why the des-*N*-methyl oxygen analog is more toxic *in vivo* than the oxygen analog even though it is a less potent anticholinesterase agent. The same relation between *in vivo* and *in vitro* toxicity was observed for Bidrin and phosphamidon compared to their *N*-dealkylated derivatives (Ciba, 1968; Clemons and Menzer, 1968; Menzer and Casida, 1965). These results indicate that the unsubstituted amide compounds could exert their greater toxicity by being more resistant to enzymatic hydrolysis, although differential penetration to the target area may be important here. Information concerning the relative rates of enzymatic detoxication of the oxygen analog and its des-*N*-methyl metabolite would determine if the differential toxicity resulted from a metabolic factor.

The neutral phosphate ester metabolites represent less than 10% of the administered radioactivity from bean plants and rats, but must be considered in an evaluation of the toxic properties of dimethoate, since these compounds are potential anticholinesterases. Fortunately, dimethoate and its toxic metabolites are rapidly degraded in rats to innocuous nonorganoextractable compounds. Only trace amounts of organoextractable metabolites were present after the 12 hr sampling time. The systemic insecticidal activity of dimethoate in plants is retained by the enzymatic stability of the oxygen analog, with this metabolite accumulating to higher levels in the plant tissue than the less toxic parent compound.

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