Table III. Parts per Million Equivalents of Ordram in the Blood of Rats 7 Days after Administration of Oral Doses of [*ring*-^{14}C]Ordram

Rat	Sp act. dose dpm/mg	Dose, mg/kg	Equiv in blood, ppm	Equiv, dose, ppm mg^{-1} kg^{-1}
1	3.24×10^7	5	0.9	0.18
2	8.10×10^6	20	8.9	0.45
3	2.03×10^6	80	40.1	0.50

constant over this period. The elevated blood level may also contribute to rather high 7-day values of other "blood-rich" tissues such as liver, kidney, lungs, heart, and spleen.

As shown in Table III, the amount of ^{14}C present in blood 7 days after treatment is dose related. The ppm Ordram equivalent value is roughly 4.5 times greater after the 80 mg/kg dose as compared with that after the 20 mg/kg dose. At a dose of 5 mg/kg, the 7-day value is less than 1 ppm. Precipitation of erythrocytes by centrifugation showed that virtually all of the blood ^{14}C was associated with the precipitated cellular fraction.

Since the blood was analyzed 7 days after administration of the herbicide, it is doubtful that the ^{14}C residues are due to the presence of intact parent compound. Studies by Casida et al. (1975) and Lay et al. (1975) demonstrated that, after sulfoxidation, certain thiocarbamates are capable of carbamoylating nucleophilic sites such as sulfhydryl groups in biological molecules. A similar mechanism may be responsible for the persistent blood residues observed in these studies after the administration of relatively high doses.

Expressed as percent administered ^{14}C, the combined gastrointestinal tract contents accounted for an average of 3.9, 0.1, and 0.03% at the 1-day, 3-day, and 7-day intervals, respectively. The whole-body residues, including the gastrointestinal tract contents, accounted for an average of 13.8% (1 day), 5.2% (3 day), and 3.7% (7 day) of the administered ^{14}C. Blood collected during the decapitation procedure accounted for roughly 25% of this 7-day value. Residual blood not removed from the tissues during decapitation may make a significant contribution to the 7-day, whole-body residue.

These data show that, even at a relatively high dose of 72 mg/kg, a single oral dose of [*ring*-^{14}C]Ordram is rapidly excreted, resulting in the elimination of approximately 97% of the administered ^{14}C in 48 h. A second report (DeBaun et al., 1978) shows that extensive metabolism of Ordram occurred in these animals. Unchanged Ordram accounted for only 0.1% of the urinary ^{14}C.

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Metabolism of [*ring*-^{14}C]Ordram (Molinate) in the Rat. 2. Urinary Metabolite Identification

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Ordram (*S*-ethyl hexahydroazepine-1-carbothioate) is readily degraded by the rat to more polar products which are excreted primarily in the urine. Unchanged Ordram accounts for only 0.1% of the urinary ^{14}C after an oral dose (72 mg/kg) of [*ring*-^{14}C]Ordram. The major metabolic pathway involves sulfoxidation and conjugation with glutathione, giving rise ultimately to a mercapturic acid derivative which accounts for 35.4% of the urinary ^{14}C. Ring hydroxylation to give the 3- and 4-hydroxy-Ordram derivatives (0.8% free, 26.1% as *O*-glucuronides) represents another major metabolic route. Hydroxylation in the 2 position of the ring and subsequent ring cleavage represent a minor pathway. Hexamethyleneimine (14.6%) and 3- and 4-hydroxyhexamethyleneimine (10.3%) are major metabolites presumably formed by hydrolysis of sulfoxidized Ordram and its hydroxy derivatives. Although there are small quantitative differences, the metabolism of [*ring*-^{14}C]Ordram in female and male rats is qualitatively the same.

Ordram (molinate) is a selective thiocarbamate rice herbicide used throughout the world for weed control in rice culture (Ashton and Crafts, 1973; Tweedy and Houseworth, 1976).

Recently, several definitive studies were published delineating the rapid degradation of thiocarbamate herbicides, including Ordram, in plants and mammals (Casida et al., 1975a,b). Lay and Casida (1976) have shown that in rats the thiocarbamates and corresponding sulfoxides form *S*-(*N*,*N*-dialkylcarbamoyl)-*N*-acetylcysteine derivatives. Hubbell and Casida (1977) have shown that rats treated ip with 1.0 mmol/kg of Ordram excreted 2% of the dose as *N*-acetyl-*S*(hexahydroazepine-1-carbonyl)-L-cysteine, (mercapturic acid conjugate) in the urine. Larger amounts of this conjugate were recovered from thiocarbamates and thiocarbamate sulfoxides containing *N*,*N*-dialkylcarbamoyl moieties.

This report describes rat metabolism studies with [*ring*-^{14}C]Ordram which were designed to elucidate the total biotransformation of this thiocarbamate herbicide in a mammalian species, based on identification of urinary

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metabolites. Results of a balance and tissue residue study were reported by DeBaun et al. (1978).

MATERIALS AND METHODS

Chemicals. [^{14}C]Ordram (*S*-ethyl hexahydro-[2-^{14}C]-azepine-1-carbothioate) (8.2 mCi/mM), Ordram sulfoxide, Ordram sulfone, *S*-ethyl 5-formylpentylthiocarbamate, and hexamethyleneimine were provided by Stauffer Chemical Co., Richmond, Calif. (RRC). [^{14}C]Ordram sulfone was prepared by reacting [^{14}C]Ordram with 2 molar equiv of *m*-chloroperbenzoic acid in chloroform. The product was purified by preparative thin-layer chromatography (TLC) using hexane–ether (7:3) as the developing solvent to give a final radiopurity of 95%. An Ordram-cysteine derivative, *S*-(hexahydro[2-^{14}C]azepine-1-carbonyl)-L-cysteine, was prepared by reacting 0.11 mM of [^{14}C]Ordram sulfone with 0.11 mM L-cysteine·HCl in 0.6 mL of ethanol, 0.2 mL of water, and 40 μL of triethylamine for 2 h at ambient temperature. Attempts to prepare the corresponding mercapturic acid analogue by direct acetylation of the Ordram-cysteine derivative using acetic anhydride in triethylamine yielded only *N*-acetylhexamethyleneimine. Accordingly, the mercapturic acid was prepared using the same procedure described for the Ordram-cysteine derivative except that *N*-acetyl-L-cysteine was substituted for L-cysteine. Another derivative, *S*-(hexahydro[2-^{14}C]azepine-1-carbonyl)-γ-glutamylcysteinylglycine, was prepared using the same procedure except that glutathione was substituted for L-cysteine in the reaction mixture. Using this procedure, reaction of [^{14}C]Ordram sulfone with L-cysteine, *N*-acetyl-L-cysteine, or glutathione produced only one major reaction product, in each case, with increased polarity as determined by TLC using a butanol–acetic acid–water (15:8:3) solvent system.

Treatment of Urine. Urine for this study was collected from the six male and six female Simonsen albino rats (200 g) utilized for the [^{14}C]Ordram balance and tissue residue studies (DeBaun et al., 1978). The animals had been orally dosed with [^{14}C]Ordram (72 mg/kg) and were housed individually in metabolism cages designed for the separate collection of urine and feces. Urine was collected at 8, 24, and when available, 48 h after dosing and was frozen prior to analysis. Ten-milliliter aliquots of 0–48 h urine from the female and male rats used for the balance study were individually lyophilized, and the residues were extracted with equal volumes of methanol. Extractability of ^{14}C was greater than 97% in both cases. These extracts were used for determining the comparative distribution of metabolites in the urine from female and male rats.

For the isolation of metabolites, 0–24 h urine from the eight tissue residue study rats and the remainder of the 0–48 h urine from the balance study rats (four rats) were combined for extraction. A portion of this combined urine (150 mL, 2.57×10^8 dpm) was diluted with an equal volume of water, adjusted to pH 7, and extracted with ether in a continuous liquid–liquid extractor for 24 h. The pH of the extracted urine was then adjusted to ~2 with HCl and it was continuously extracted with ether for another 24 h. Finally, the pH was adjusted to ~10 with NaOH and the extraction with ether was repeated. These extracts were concentrated by rotary vacuum evaporation for further isolation of metabolites.

After readjustment of the pH to 7, the extracted aqueous phase was lyophilized, and the residue was extracted with ethanol. After evaporation of the ethanol and reconstitution of the residue in 20 mL of water, this fraction was chromatographed on Sephadex G-10 (Pharmacia, Piscataway, N.J.). Ten-milliliter aliquots were placed on a 5.5 × 87 cm column and eluted with water at a flow rate of 156 mL/h. Twenty-five-milliliter fractions were collected after the passage of one void volume (550 mL).

Enzymatic Hydrolysis. A. *Analytical.* One-milliliter aliquots of the lyophilized 0–48 h urine, methanol extract were evaporated to dryness under argon in test tubes, and the residues were dissolved in 2 mL of 0.1 M sodium acetate, pH 5. The following additions were then made to individual tubes: (1) control, no addition; (2) β-glucuronidase, 5 mg, bovine liver (Calbiochem, 34743); (3) β-glucuronidase–aryl sulfatase, 50 μL, *Helix pomatia* (Calbiochem, 30100); (4) same as 3 plus 1 mg of glucosaccharo-1:4-lactone.

After the additions, all tubes were incubated at 37 °C for 24 h. The contents were then lyophilized to dryness, and the residues were resuspended in methanol for TLC analysis.

B. *Preparative.* Isolated glucuronide conjugates and 5 mg of β-glucuronidase (Worthington, GLOIA, 17 U/g) were dissolved in 5 mL of 0.1 M sodium acetate, pH 5, and were incubated at 37 °C for 48 h. After ether extraction, another 5 mg of β-glucuronidase was added to the aqueous phase and the incubation was continued for another 24 h, followed by ether extraction. These extracts were combined for further purification of the aglycons by TLC.

Thin-Layer Chromatography (TLC). Silica gel GF chromatoplates (Analtech Inc.) were developed in one or more of the following solvent systems: (1) 2,2,4-trimethylpentane–*p*-dioxane (2:1), (2) benzene–ether (7:3), (3) butanol–ethanol–water (4:1:1), (4) butanol–acetic acid–water (15:8:3), (5) ethyl acetate–formic acid (30:1), (6) isopropyl alcohol–1% NH_4OH–water (10:1:1), (7) ether–benzene (1:1), (8) chloroform–ethyl acetate (4:1), (9) ether–benzene (7:3), (10) ether–toluene (1:1), (11) chloroform–ether (9:1), (12) hexane–ether (7:3), (13) 2-butanone–water–pyridine–acetic acid (55:30:15:2), (14) benzene–acetone (9:1), (15) chloroform–ether (1:1).

For routine analytical determinations, 0.25-mm chromatoplates were used. One-millimeter chromatoplates were used for preparative applications, and metabolites were eluted from the silica gel with methanol. Radiolabeled regions were visualized by autoradiography, while unlabeled standards were detected by UV fluorescence quenching. Percent distribution of labeled metabolites was determined by scraping gel regions from the glass support and direct liquid scintillation counting (LSC).

Radioanalysis. All samples were analyzed by LSC in a Packard Model 3375 TriCarb scintillation counter using Insta-Gel scintillation fluid (Packard Instrument Co.) and internal standardization with [^{14}C]toluene.

Mass Spectral (MS) and Nuclear Magnetic Resonance (NMR) Analyses. Mass spectral analyses were obtained using the computerized Finnigan Model 1015 gas chromatograph–mass spectrometer (GC–MS) operated at 70 eV. Samples were introduced via a direct insertion probe or by utilizing the GC–MS combination, both operated over a programmed temperature range. Operating parameters are described in the text figures. NMR analyses were performed using a Varian XL-100-15 spectrometer. Samples were dissolved in deuterated solvents containing tetramethylsilane as an internal reference standard.

RESULTS AND DISCUSSION

Metabolite Distribution. As shown in Figure 1, [^{14}C]Ordram was extensively metabolized to more polar products, five of which (designated as M-1, -2, -3, -7, and -8) accounted for approximately 87% of the urinary ^{14}C. Although there is a somewhat greater proportion of the less polar metabolites M-7 and M-8 in the urine from the

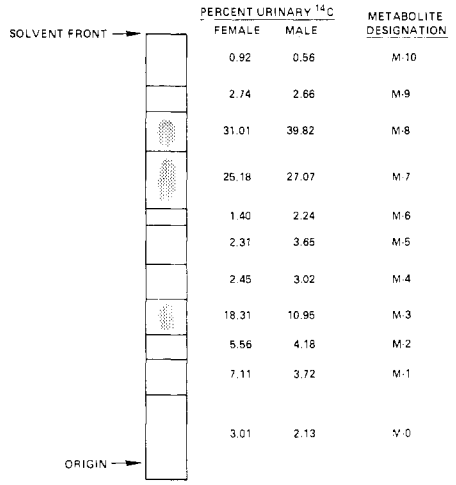


Figure 1. Thin-layer chromatographic distribution of metabolites in 0–48 h urine of female and male rats orally dosed with [*ring*-^{14}C]Ordram (72 mg/kg). Development was with solvent system 4. Horizontal lines denote areas scraped for LSC.

male rats, no significant qualitative differences were observed in the excretion of urinary metabolites from the males and females.

Extraction of neutral urine with ether in the liquid–liquid extractor removed only 2.4% of the urinary ^{14}C. Subsequent ether extraction of the urine after adjustment of the pH to 2 removed another 29.4%. Readjustment of the pH to 10, followed by ether extraction, removed an additional 14.5%. Under the same TLC conditions as in Figure 1, the radiolabeled products present in the neutral urine ether extract migrated primarily above region M-8. Ninety percent of the ^{14}C in the acidic urine ether extract was associated with the M-8 region, and virtually all of that in the basic urine ether extract migrated with M-1, -2, and -3.

Enzymatic Hydrolysis. Results of the enzymatic hydrolysis of the methanol extract of lyophilized female rat urine are depicted in Figures 2 and 3. As shown in Figure 2, the distribution of ^{14}C was virtually the same for the untreated extract and the incubated control. However, incubation with β-glucuronidase resulted in the disappearance of M-7 and the release of less polar products. The addition of arylsulfatase did not result in any further hydrolytic degradation, and glucosaccharo-1:4-lactone, a specific inhibitor of β-glucuronidase, prevented the hydrolysis of M-7 by β-glucuronidase. Inhibition of β-glucuronidase-mediated hydrolysis of M-7 by glucosaccharo-1:4-lactone provides definitive evidence that the M-7 region contains glucuronide conjugates. The lack of hydrolysis by arylsulfatase indicates that [^{14}C]Ordram sulfate conjugates are not present in the urine. As shown in Figure 3, two hydrolytic products, H-1 and H-2, are released by the action of β-glucuronidase. A third product, H-3, appears to be artifactual since it was also produced to the same extent during the control incubation. Results of the enzymatic hydrolysis of urine from the male rats were the same as those described here for the female.

Metabolite Identification. Numbers in parentheses after TLC solvent systems indicate R_f values. Standards were not available for several key metabolites, necessitating identification primarily from their mass and NMR spectra. Metabolite codes refer to those designated in Figure 1.

A. *Lyophilized Methanol Extract.* M-1 (7.1% female, 3.7% male), 4-hydroxyhexamethyleneimine [4-OH-HMI]; M-2 (5.6% female, 4.2% male), 3-hydroxyhexamethyleneimine [3-OH-HMI]; M-3 (18.3% female, 11.0% male), hexamethyleneimine [HMI]. These three metabolites,

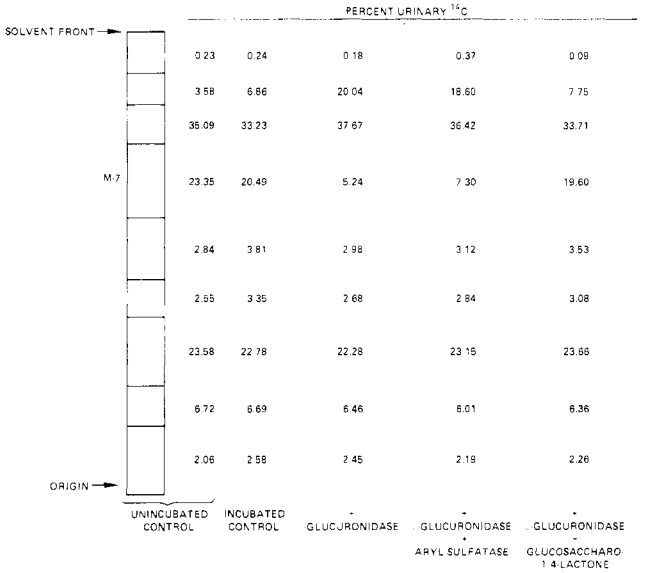


Figure 2. Effect of enzymatic hydrolysis on the thin-layer chromatographic distribution of metabolites in the 0–48 h urine of rats orally dosed with [*ring*-^{14}C]Ordram (72 mg/kg). Development was with solvent system 4. Horizontal lines denote areas scraped for LSC.

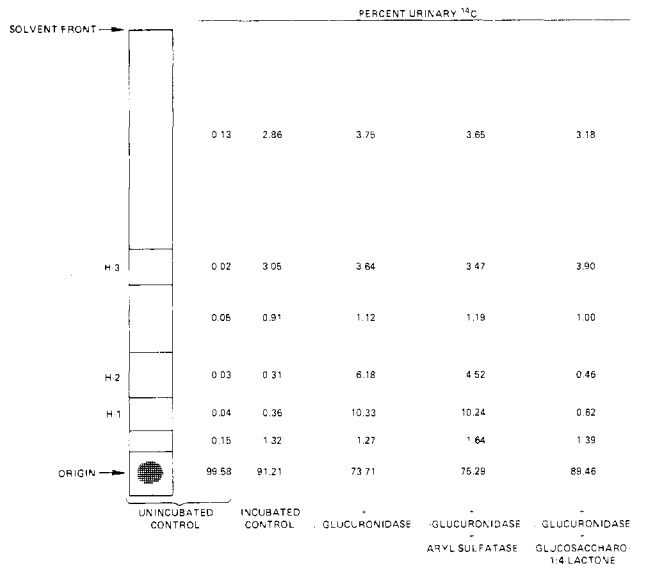


Figure 3. Same as Figure 2 except development was with solvent system 2.

which accounted for approximately 31 and 19% of the urinary ^{14}C from female and male rats, respectively, were purified by preparative TLC of the ether extract of basic urine using solvents 3, 4, and 6. Additional M-1 was also obtained from the second of the two major peaks which were eluted from Sephadex G-10 column chromatography of the ether-extracted urine. The elution volume/void volume ratio (V_e/V_o) for this peak was 2:1.

M-3 cochromatographed with standard HMI in solvent systems 2 (0.08), 4 (0.34), 6 (0.31), and 13 (0.77). Mass spectral data also support this structural assignment (see Figure 4).

Basic hydrolysis (1% NaOH, 20 h, 95 °C) of aglycon H-1 (subsequently identified as 4-hydroxy-Ordram) gave rise to one ether-soluble product which cochromatographed with M-1 in solvent system 4 (2× developed, 0.30) and 6 (2× developed, 0.10). The same basic hydrolysis of H-2 (subsequently identified as 3-hydroxy-Ordram) produced one ether-soluble product which cochromatographed with

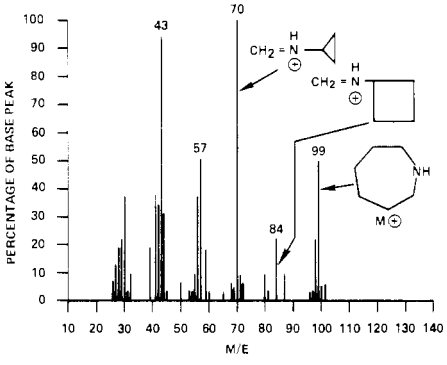


Figure 4. Mass spectrum of metabolite M-3. GC conditions: Tenax-GC 60–80 mesh (2 ft); helium flow rate, 24 mL/min; column temperature, 150 °C; retention time, 0.7 min.

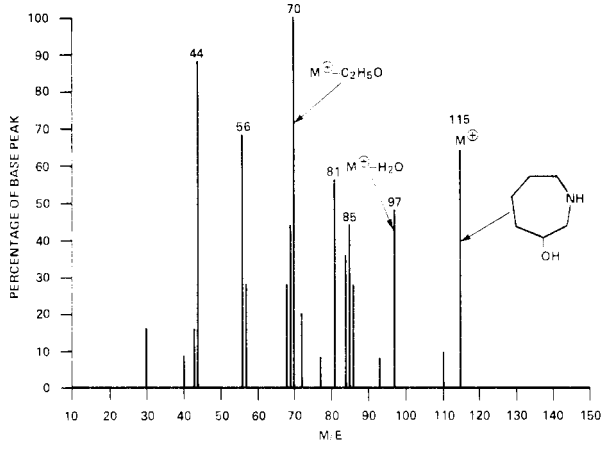


Figure 5. Mass spectrum of metabolite M-2. GC conditions: Tenax-GC 60–80 mesh (2 ft); helium flow rate, 24 mL/min; column temperature, 150 °C; retention time, 5.0 min.

M-2 in solvent systems 4 (2× developed, 0.41) and 6 (2× developed, 0.43). Basic hydrolysis of [^{14}C]Ordram under the same conditions liberated HMI as the only ether-soluble product. This latter reaction established that under these hydrolytic conditions the imine moiety was liberated from the parent thiocarbamate. Based on the structures of the metabolites H-1 and H-2, from which they were liberated by basic hydrolysis, M-1 has been identified as 4-OH-HMI and M-2 as 3-OH-HMI. The greater polarity of M-1 and M-2 relative to that of HMI is consistent with the introduction of a hydroxyl group onto the HMI ring.

Mass spectral analysis (GC–MS) of M-2, which was isolated by ethyl acetate extraction of basidified urine, further supports the ring-hydroxylated structure (Figure 5). The intense base peak (*m/e* 115) together with the loss of water (*m/e* 97) and loss of the C_2H_5O fragment (*m/e* 70) are consistent with the proposed structure. Sufficient quantities of purified metabolite M-1 were not available for GC–MS analysis. However, conformation of the structure of M-2 by GC–MS provides further evidence that M-1 is also a ring-hydroxylated HMI derivative.

M-4, -5, and -6, unidentified (6.2% female, 8.9% male). Because of the small amounts of material in these regions and because of their polar characteristics, no identification was achieved.

M-7 (25.2% female, 27.1% male), 3- and 4-hydroxy-Ordram-*O*-glucosiduronic acid. Metabolites corresponding to the M-7 region were isolated from the first of the two major peaks (V_e/V_o = 1.8) to be eluted during Sephadex G-10 column chromatography of the ether-extracted urine. This fraction was subsequently purified by preparative

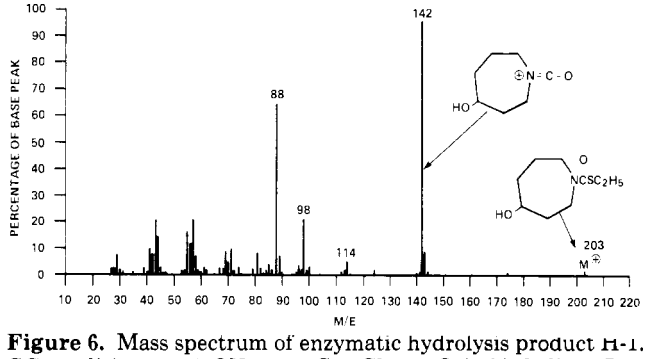


Figure 6. Mass spectrum of enzymatic hydrolysis product H-1. GC conditions: 3% OV-17 on Gas-Chrom Q (4 ft); helium flow rate, 24 mL/min; column temperature, 20–180 °C (15 °C/min); retention time, 9.8 min.

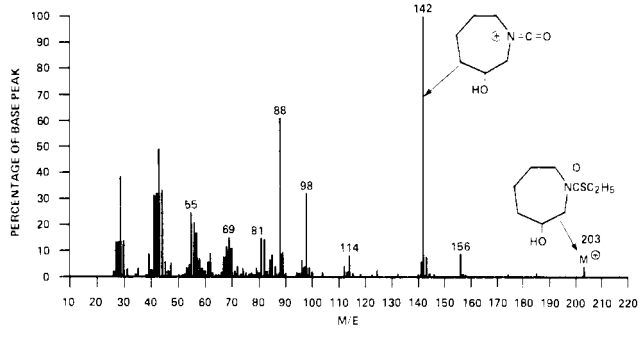
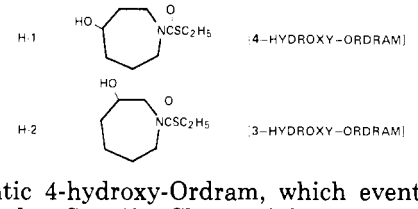


Figure 7. Mass spectrum of enzymatic hydrolysis product H-2. GC conditions: 3% OV-17 on Gas-Chrom Q (4 ft); helium flow rate, 24 mL/min; column temperature, 25–180 °C (15 °C/min); retention time, 10.0 min.

TLC using solvents 3 and 4. Preparative enzymatic hydrolysis of this fraction with β-glucuronidase gave rise to the same two aglycons shown in Figure 3 and in the same proportions. Since M-7 accounts for approximately 26% of the urinary ^{14}C, the conjugates of H-1 and H-2 represent 16 and 10%, respectively, based on the ratio of these two products (Figure 3). The aglycons were purified by preparative TLC using solvent systems 2, 7, and 11 prior to analysis.

The mass spectra of H-1 and H-2, shown in Figures 6 and 7, are consistent with structures in which a hydroxyl group has been introduced onto the Ordram ring. The base peaks at *m/e* 142 are definitive evidence for the ring hydroxylation. The NMR spectrum of H-1 (run in chloroform-d_1) had δ 1.29 (t, 3 H, methyl), 1.50 (s, 1 H, hydroxyl), ~1.80 (m, 6 H, methylene), 2.92 (q, 2 H, CH_3CH_2), 3.4–3.7 (bm, 4 H, NCH_2), and 3.93 (m, 1 H, *CH*OH). The NMR spectrum of H-2 had δ 1.32 (t, 3 H, methyl), 1.52 (s, 1 H, hydroxyl), ~1.62 (m, 6 H, methylene), 2.95 (q, 2 H, CH_3CH_2), 3.20–4.20 (m, 5 H, NCH_2 and *CH*OH). The small chemical shift difference in the two CH_2N groups in H-1 and the large shift difference in those protons in the case of H-2 favor the following structure assignments:



Authentic 4-hydroxy-Ordram, which eventually was synthesized at Stauffer Chemical Company, Richmond, Calif., was not initially available as a TLC standard. However, comparison of the NMR spectrum of this standard with that of H-1 confirms the proposed structure.

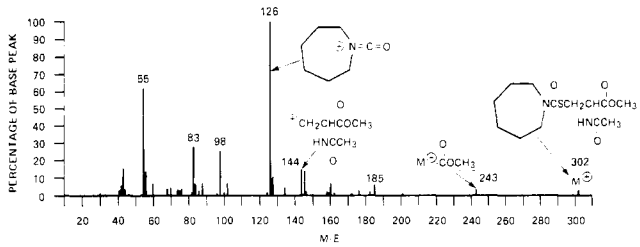


Figure 8. Mass spectrum of the methyl ester of metabolite M-8. Direct solid probe.

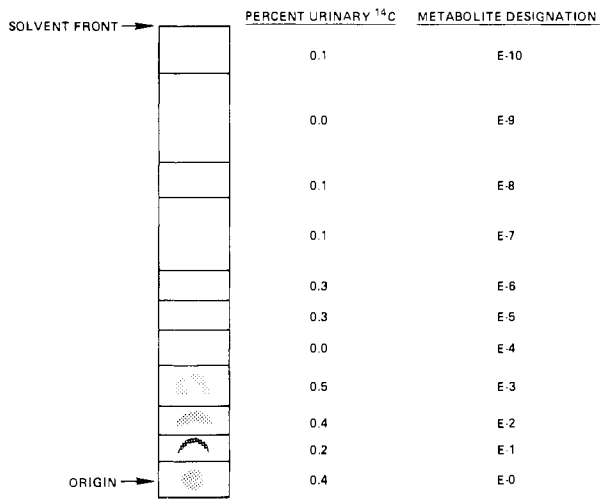
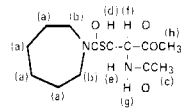


Figure 9. Thin-layer chromatographic distribution of metabolites in the ether extract of neutral 0–48 h urine from rats orally dosed with [*ring*-^{14}C]Ordram (72 mg/kg). Development was with solvent system 7. Horizontal lines denote areas scraped for LSC.

M-8 (31.0% female, 39.8% male), *N*-acetyl-*S*-(hexahydroazepine-1-carbonyl)-L-cysteine [Ordram mercapturate].

M-8, the major urinary metabolite, was isolated by preparative TLC of the acidic urine ether extract using solvents 2, 4, and 5. M-8 cochromatographed (TLC) with synthetic Ordram mercapturate in solvent systems 4 (0.78), 5 (0.59), and 3 (0.46). Prior to spectral analysis, M-8 was methylated by reaction with ethereal diazomethane to produce a less polar derivative which was further purified by preparative TLC in solvents 9 and 10. The mass spectrum of this derivative (Figure 8) is consistent with the structure proposed above.

The NMR spectrum of methylated M-8 (run in acetone-d_6) also supports this structural assignment.



δ a, 1.4–2.0 (bm, 8 H) δ e, ~3.5 (m, 1 H)
δ b, 3.4–3.7 (bm, 4 H) δ f, 4.51 (m, 1 H)
δ c, 1.95 (s, 3 H) δ g, 7.45 (b, 1 H)
δ d, ~3.2 (m, 1 H) δ h, 3.74 (s, 3 H)

B. *Neutral Ether Extract.* As mentioned previously, radiolabeled metabolites present in the neutral urine ether extract chromatographed for the most part in regions M-9 and M-10. The distribution of metabolites in this extract, which accounted for only 2.4% of the combined male and female urinary ^{14}C, is shown in Figure 9. Metabolites present in regions E-1, -3, -5, -6, and -8 were further purified by preparative TLC in solvent systems 7 and 14. Percentage of these metabolites in whole urine was determined by multiplying 0.024 × percent distribution in the extract.

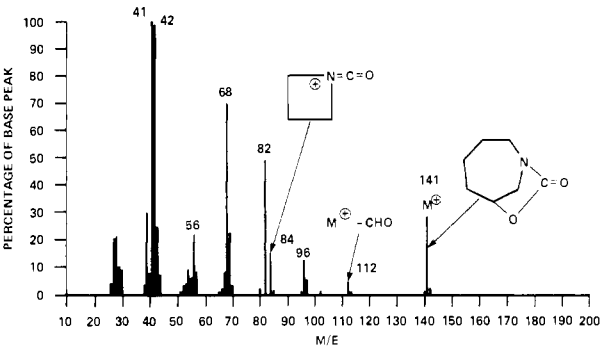


Figure 10. Mass spectrum of metabolite E-6. GC conditions: 3% OV-17 on Gas-Chrom Q (4 ft); helium flow rate, 25 mL/min; column temperature, 100–290 °C (15 °C/min); retention time, 6.1 min.

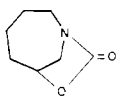
E-1 (0.2%), Ordram sulfoxide. The radiolabeled product isolated from this region cochromatographed with standard Ordram sulfoxide in solvents 3 (0.62), 4 (0.63), and 14 (0.51).

E-2 (0.4%), not identified.

E-3 (0.5%), 4-OH-Ordram. This metabolite cochromatographed with H-1 in solvent systems 7 (0.16), 8 (0.18), and 15 (0.39).

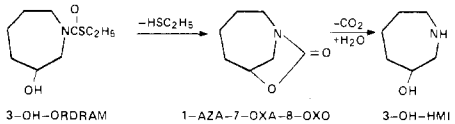
E-5 (0.3%), 3-OH-Ordram. This metabolite cochromatographed with H-2 in solvent systems 7 (0.32), 8 (0.27), and 15 (0.62).

E-6 (0.3%).



1-AZA-7-OXA-8-OXO BICYCLO[4.2.1]NONANE

Mass spectral data (Figure 10) support the proposed cyclic carbamate structure. Furthermore, basic hydrolysis of E-6 (1% NaOH, 96 °C, 2 h) produced only one ether-soluble radiolabeled product which cochromatographed with M-2 (3-OH-HMI) in solvent 4 (0.29). As described previously, basic hydrolysis of H-2 (3-OH-Ordram) also produced M-2 as the only ether-soluble product. Of significance was the observation that the hydrolysis of E-6 and H-2 to yield M-2 was essentially complete after 2 h. On the other hand, hydrolysis of Ordram and H-1 (4-OH-Ordram) to the corresponding imines was only ~3% complete after 24 h. The more facile hydrolysis of E-6 and H-2 is further support that, with these metabolites, hydroxylation of the ring has occurred at a position such that an interaction of the hydroxyl group with the thiocarbamyl moiety is facilitated. Involvement of the two position on the ring is highly unlikely in this case since the HMI cyclic carbamate would then contain a severely strained four-membered ring system. Involvement of the 4 position is ruled out since the hydrolytic product of E-6 and H-2 is different than that obtained from the hydrolysis of H-1 (4-OH-Ordram). These data are consistent with the proposal that H-2 is 3-OH-Ordram, and E-6 appears to be the cyclic carbamate derived from it. The reactions which interrelate the aforementioned metabolites are depicted as follows:



The ability to form the cyclic intermediate, E-6, may account for the more facile hydrolysis of the 3-hydroxy

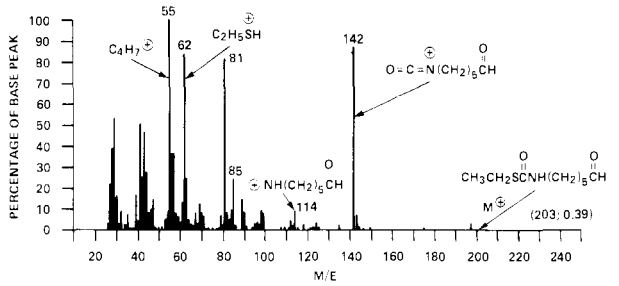


Figure 11. Mass spectrum of metabolite E-8. GC conditions: 3% OV-17 on Gas-Chrom Q (4 ft); helium flow rate, 27 mL/min; column temperature, 30–150 °C (15 °C/min); retention time, 5.9 min.

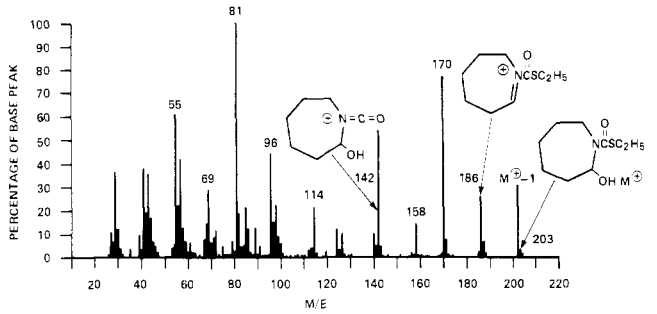


Figure 12. Mass spectrum of metabolite A-1. GC conditions: 3% OV-17 on Gas-Chrom Q (4 ft); helium flow rate, 27 mL/min; column temperature, 30–150 °C (15 °C/min); retention time, 8.5 min.

derivative as compared to that of Ordram and the 4-hydroxy derivative. Although this intermediate is stable in vivo, it is not detected under the more rigorous in vitro hydrolytic conditions.

E-7 (0.1%), not identified.

E-8 (0.1%), $CH_3CH_2SC(=O)NH(CH_2)_5C(=O)H$, *S*-ethyl 5-formylpentylthiocarbamate. Figure 11 shows the mass spectrum of E-8. Although this metabolite produced a rather weak parent ion at *m/e* 203, the major fragment at *m/e* 142 is consistent with the proposed structure. The NMR spectrum (run in chloroform-d_1) had δ 1.30 (t, 3 H, methyl), 1.53 (bs, 6 H, methylene), 2.46 (d of t, 2 H, CH_2CH_2CHO), 2.93 (q, 2 H, CH_3CH_2), 3.30 (q, 2 H, $NHCH_2CH_2$), 5.35 (b, 1 H, NH), 9.79 (t, 1 H, CH_2CHO). TLC cochromatography was achieved with the standard compound in solvent systems 1 (0.51), 2 (0.61), and 15 (0.81).

E-10 (0.1%), Ordram. The product in this region cochromatographed with Ordram in systems 1 (0.91), 2 (0.83), and 12 (0.61).

C. *Acidified Ether Extract.* As described above, acidification of the 0–48 h urine and extraction with ether removed 29.4% of the urinary ^{14}C. Although the majority of this extracted ^{14}C was identified as Ordram mercapturate, a nonpolar metabolite, A-1 (TLC R_f 0.94 in solvent system 2), which accounted for 4.8% of the extracted ^{14}C, was also present in this extract.

Metabolite A-1 was purified by preparative TLC in solvent system 2 and was analyzed by GC–MS (Figure 12). Although complete interpretation of the mass spectrum has not been accomplished, the parent ion at *m/e* 203, the M^+ (−17) peak at *m/e* 186, and the intense peak at *m/e* 142 are all consistent with a ring-hydroxylated Ordram metabolite. Metabolite A-1 is considerably less polar than the 3- and 4-hydroxy-Ordram derivatives which have R_f's of 0.23 and 0.13, respectively, in system 2. This suggests that, with A-1, hydroxylation has occurred at the 2 position in which case hydrogen bonding with the carbonyl oxygen could decrease the polarity of the hydroxyl group. Further evidence for hydroxylation at the 2 position is provided by the finding that, during preparative TLC of metabolite A-1, a more polar product was detected which cochromatographed with the *S*-ethyl 5-formylpentylthiocarbamate standard in solvent systems 1, 2, and 15. This latter product appeared to be formed whenever purified A-1 was stored at 4 °C for extended periods of time.

Metabolite A-1 does not appear to be present in the organic extract of neutral urine. This suggests that this metabolite may be excreted in a conjugated form which hydrolyzed upon acidification of the urine.

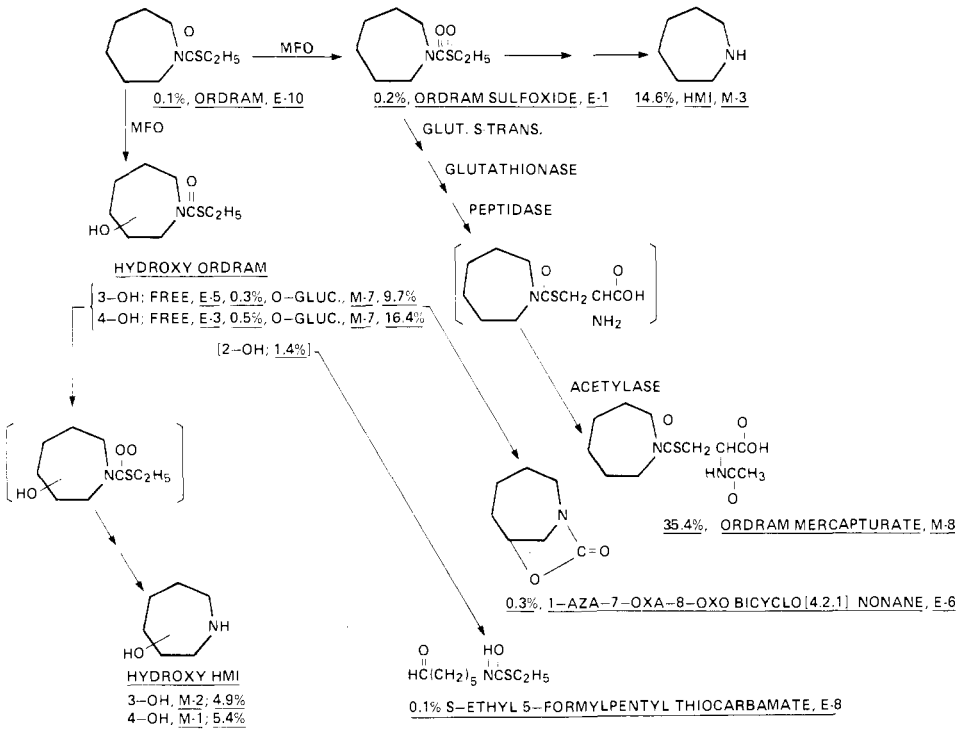


Figure 13. Proposed metabolism of [*ring*-^{14}C]Ordram in the rat. Percentages are average values for female and male 0–48 h urine after oral dosing with 72 mg/kg. Expressed as percent urinary ^{14}C.

These data support a structure in which oxidation of the ring at the 2 position is followed by ring scission to yield an aldehyde. Formation of E-8 may be depicted by the reaction sequence which follows:

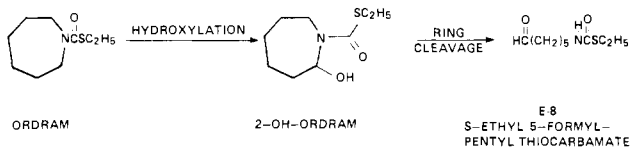


Figure 13 shows the proposed metabolism of [^{14}C]Ordram by the rat based on the identification of metabolites present in 0–48 h urine. The major route of metabolism appears to involve sulfoxidation and conjugation with glutathione to ultimately give rise to the mercapturic acid derivative.

This mercapturic acid accounted for 35.4% of the urinary ^{14}C in the present study as compared to only 2% in the study reported by Hubbell and Casida (1977). This discrepancy may be due to the different routes of administration (oral vs. ip) and/or doses (72 vs. 187 mg/kg) employed for the two studies.

No metabolites which cochromatographed with the Ordram-glutathione or Ordram-cysteine derivatives were detected in these studies. Inclusion of Ordram sulfoxide in this pathway is based on studies by Casida et al. (1975a, b) and Lay et al. (1975). The sulfoxide can also presumably undergo hydrolysis to yield HMI.

Another significant degradative pathway involves ring hydroxylation to yield the 2-, 3-, and 4-hydroxy derivatives, excreted predominantly as the glucuronide conjugates. No sulfate conjugates were detected.

Two basic metabolites which cochromatographed with hydrolysis products from 3- and 4-hydroxy-Ordram were identified as 3- and 4-hydroxy-HMI. As indicated above, sulfoxidation is presumed to occur prior to liberation of the hydroxyimine moiety. Another minor metabolite which appears to be a cyclic carbamate may be formed by internal rearrangement of 3-hydroxy-Ordram or its sulfoxide.

The data show that Ordram is readily degraded by the rat to more polar products which are excreted primarily in the urine. Only 0.1% of the urinary ^{14}C was identified as unchanged Ordram after an oral dose of 72 mg/kg. Major metabolic pathways include sulfoxidation, conjugation with glutathione, ring hydroxylation, and liberation of the unmodified and hydroxylated cyclic imine moiety.

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Insecticidal Properties of Phosphonamidothioate Esters and Derivatives

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A series of *S*-alkyl methyl- or ethylphosphonamidothioate esters analogous to methamidophos (*O,S*-dimethyl phosphoramidothioate) and acephate (*O,S*-dimethyl *N*-acetylphosphoramidothioate) was synthesized and evaluated for toxicological properties. Although *S*-methyl methyl- and ethylphosphonamidothioate were more effective against the housefly than methamidophos, the acylated derivatives showed variable toxicity but all were substantially less effective than acephate. The acylated derivatives, however, showed improved mouse toxicity. *S*-Methyl methyl- and ethylphosphonamidodithioate were less active against the housefly than methamidophos.

Acephate or *O,S*-dimethyl *N*-acetylphosphoramidothioate is one of the most interesting new insecticides discovered during the past decade (Magee, 1974). Compared to many organophosphorus insecticides, acephate has the virtue of being relatively simple in structure and of low mammalian toxicity (rat oral LD_{50} = 900 mg/kg). On the other hand, methamidophos (*O,S*-dimethyl phosphoramidothioate), a compound which differs from acephate only in the replacement of the acetyl moiety by a hydrogen atom, is relatively toxic to mammals (rat oral LD_{50} = 20 mg/kg) although of about equal insecticidal activity. Recent studies have indicated that acephate and related esters are converted in vivo to methamidophos in insects and it is methamidophos which is responsible for intoxication (Kao and Fukuto, 1977; Khasawinah et al., 1978). In mammals, relatively little methamidophos is formed, thus accounting for the safety of acephate.

In an earlier study (Quistad et al., 1970), we described the outstanding insecticidal activity of a few phosphon-

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