for use was 525 nm due to the wavelength dial calibration on the Hatachi 100-60 spectrophotometer. The developed color was very stable for at least 30 min.

Physical interferences include coloration, turbidity, and excessive temperature, above 85 °C. Filtering samples of AN usually removes turbidity. Chemical interferences from some primary amines and monosodium dimethylnaphthalenesulfonate have been noted. These compounds form an orange-colored complex.

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COMMUNICATIONS

Glutathione Conjugate of the Pyrethroid Tetramethrin

Tetramethrin and its cleavage product tetrahydrophthalimide readily undergo Michael addition with thiols. In the case of glutathione (GSH), the resulting tetramethrin–GSH conjugate is less stable than the mercapturic acid conjugates of tetramethrin and tetrahydrophthalimide. The tetramethrin–GSH conjugate is formed under physiological conditions in the presence of mouse liver and housefly abdomen homogenate fractions but probably as a nonenzymatic reaction. The mouse liver soluble thiol level is diminished by intraperitoneal (ip) administration of tetrahydrophthalimide. Mercapturic acid and GSH conjugates of tetramethrin are not evident in the bile or urine of ip-treated rats and mice. Although conjugation with GSH is not a significant factor in the metabolism of tetramethrin, it is interesting to speculate that reversible Michael addition with a critical thiol in the pyrethroid receptor site might contribute to the unique potency and transient character of the neuroactivity of tetramethrin.

Pyrethroid insecticides are biodegraded by hydrolysis and/or oxidation to form acids and alcohols undergoing conjugation (Casida and Ruzo, 1980). Tetramethrin (Figure 1) may be unusual since, by analogy with *N*-ethylmaleimide (NEM) (Kosower, 1976), it has the possibility of undergoing Michael addition with glutathione (GSH) to form **2A** (Figure 2). In addition, tetramethrin may form related conjugates (Figure 2) by cleavage to tetrahydrophthalimide (**1B**) (Miyamoto et al., 1968; Suzuki and Miyamoto, 1974), followed by GSH addition to give **2B** and by subsequent metabolism of GSH conjugates **2A** and **2B** to yield mercapturic acids **3A** and **3B** for urinary excretion. Tetramethrin is unique among the pyrethroids in its high knockdown activity (Kato et al., 1964) and exquisite potency and brief duration of action on cockroach cercal sensory nerves (Gammon et al., 1981). These observations together with the large number of polar, unidentified tetramethrin metabolites (Miyamoto et al., 1968) prompted the present study to evaluate the possible role of GSH and other sulfur nucleophiles in tetramethrin metabolism and action.

MATERIALS AND METHODS

Preparation of GSH and Mercapturic Acid Conjugates. **1A** or **1B** was mixed with an equimolar amount of either GSH or *N*-acetyl-L-cysteine in methanol containing triethylamine (10 equiv). After the mixture was stirred overnight at 25 °C, the solvent was removed under vacuum, and the requisite product (**2A**, **3A**, or **3B**) was isolated in 20–30% yield by TLC (MTBW, Table I). Methanol solutions of **3A** and **3B** were methylated by addition of excess ethereal diazomethane at 0 °C to obtain **3A**-Me and a mixture of **3B**-Me and **3C**-Me. (Compound **2A** decomposed on attempted methylation.). Each compound was pure based on TLC (BAW and MTBW, Table I). Mass spectral data confirmed compound identities: chemical ionization with methane as the reagent gas, **3A**-Me 509 (16%, M + 1), 341, and 178, **3B**-Me 329 (100%, M + 1), 178, 176, and 152, and **3C**-Me 343 (36%, M + 1), 192 and 152; fast atom bombardment with sodium as the ionizing source [instrument configuration similar to that described by Barber et al. (1981)], **2A** 661 $(M + Na)^+$, 683 $(MNa + Na)^+$, and 705 $(MNa_2 + Na)^+$. ^1H NMR ($CDCl_3$, 90 MHz) for **3A**-Me, **3B**-Me, and **3C**-Me δ 3.77 (s, OCH_3), 3.19 (d,

Table I. Thin-Layer Chromatographic Properties of the Glutathione Conjugate of Tetrametrhin (2A) and Related Compounds

compd	R_f[a] BAW	R_f[a] MTBW
1A	0.71	0.97
2A	0.14	0.66
3A	0.51	0.69
3B	0.41	0.56
GSH	0.11	0.33
N-acetylcysteine	0.41	0.49

[a] Silica gel F254 chromatoplates, 0.25-mm gel thickness, developed with 1-butanol–acetic acid–water (6:1:1) (BAW) or methanol–toluene–1-butanol–water (10:5:5:4) (MTBW).

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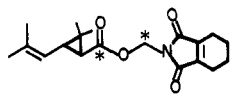

Figure 1. Structure of (1*RS,trans*)-tetramethrin used in chemical studies. Asterisks designate positions of ^{14}C labeling in the 1*R*,trans compound used in metabolism studies.

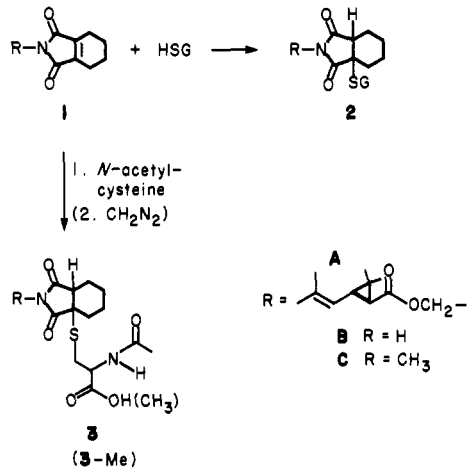

Figure 2. Reaction of tetramethrin (**1A**) or tetrahydrophthalimide (**1B**) with glutathione or *N*-acetylcysteine to obtain various compounds including the glutathione conjugate of tetramethrin (**2A**) and the mercapturic acid conjugate of tetrahydrophthalimide (**3B**) and its dimethyl derivative (**3C-Me**).

SCH_2), 2.69 (m, $CHCH_2CH_2$), 2.05 (s, $COCH_3$), 2.0–1.2 [m, $(CH_2)_4$]; for **3C-Me** also 3.00 (s, NCH_3).

Rate of Reaction of Tetramethrin and Related Compounds with GSH. The reaction mixture (10 mL) consisted of a stirred solution of GSH (10 μmol) in a 1:1 mixture of methanol and phosphate buffer (pH 8; 0.1 M) to which was added 1 equiv of **1A**, **1B**, or NEM in 2-propanol (0.15 mL). The rate of GSH depletion at 25 °C was determined on aliquots (0.25 mL) diluted to 3 mL with buffer containing 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (1.5 μmol) by absorbance measurements at 412 nm (Ellman et al., 1961).

Reaction of [*acid*-^{14}C]Tetramethrin with GSH in Biological Preparations. Reactions were performed in phosphate buffer (pH 7.4; 0.15 M; 1.2 mL) containing [^{14}C]tetramethrin (0.2 μmol) (Yamamoto and Casida, 1968), GSH (0 or 12 μmol), and biological material [100000*g* soluble and microsomal fractions from homogenates of housefly abdomens (insecticide susceptible strain) (3 and 0.9 mg of protein, respectively) and mouse liver (19 and 7 mg of protein, respectively)]. Incubations were for 1 h at 30 °C (housefly) or 0.5 h at 37 °C (mouse). Relevant controls lacked either GSH or enzyme or were heat denatured (100 °C for 30 min and then cooled). In parallel studies the microsomal fractions were preincubated for 10 min with an esterase inhibitor (10^{-5} M 2-phenyl-1,3,2-benzodioxaphosphorin 2-oxide) or fortified with the oxidase cofactor (3 μmol of NADPH). All incubation mixtures were lyophilized prior to extraction with methanol and analysis by TLC (BAW). The identity of **2A** was confirmed by two-dimensional TLC (MTBW × BAW) comparison with the unlabeled standard.

Effect of Tetrahydrophthalimide and NEM on Mouse Liver Thiol Levels. Male albino mice (~20 g) were treated intraperitoneally (ip) with **1B** or NEM (1.3 mmol kg^{-1}) (8–12 mice/compound) by using methoxy triglycol (MTG) as the carrier vehicle (50 μL) or with MTG alone (50 μL). (Tetramethrin was not examined because at this dose it is lethal within 5 min.) After 1 h the livers were removed and homogenized individually in 5% trichloroacetic acid (4× fresh weight of liver by volume). An aliquot (0.1 mL) of the 10000*g* supernatant fraction was diluted to 3 mL with phosphate buffer (pH 8; 0.1 M) containing DTNB (1.5 μmol) for colorimetric analysis at 412 nm (Lay and Casida, 1976).

Fate of [*acid*-^{14}C]- and [*alcohol*-^{14}C]Tetramethrin and [^{14}C]Chrysanthemic Acid in Rats and Mice. The labeled compounds (Yamamoto and Casida, 1968) were administered ip (30 μmol kg^{-1}) in MTG (150 μL for 200-g male albino rates, 30 μL for 20-g male albino mice), and the urine was collected for 48 h. In separate experiments bile was obtained from cannulated rats. Urine and bile were analyzed by direct spotting for two-dimensional TLC cochromatography (MTBW × BAW; Table I), detecting metabolites by radioautography and standards by iodine vapor.

RESULTS AND DISCUSSION

Reaction of Tetramethrin and Related Compounds with GSH and *N*-Acetylcysteine. Adducts **2A**, **3A**, and **3B** are readily formed from the appropriate tetrahydrophthalimides and thiols in methanol containing triethylamine. GSH in aqueous methanol at pH 8 reacts at virtually identical initial rates with **1A** and **1B** ($k_2 = 8 \times 10^{-2}$ M^{-1} s^{-1}; $t_{1/2} \sim 10^4$ s) but much faster with NEM ($t_{1/2} <$ 5 s). This rate difference between NEM and the tetrahydrophthalimides is in accord with the observation that 2,3 disubstitution at the double bond of NEM vastly retards its reaction rate. Thus, GSH reacts 4×10^5 times faster with NEM than with 2,3-Me_2-NEM (Miyadera et al., 1971). The tetramethrin–GSH conjugate (**2A**) is less stable than the mercapturic acids, undergoing partial reversion to starting material on storage several days even at 0 °C.

Reaction of Tetramethrin with GSH in the Presence of Mouse Liver and Housefly Abdomen Homogenate Fractions. Tetramethrin is converted to **2A** in 15–30% yield on incubation with 10 mM GSH at pH 7.4 for 1 h at 30 °C or 0.5 h at 37 °C. **2A** is not detected on incubation of tetramethrin with microsomal or soluble fractions of mouse liver or housefly abdomen homogenates, including fresh and heat-denatured preparations. The 15–30% yield of **2A** in the 10 mM GSH incubation mixture is not altered by any of the following additions: soluble fraction of mouse liver or housefly abdomen homogenate; heat-denatured soluble or microsomal fraction of mouse liver or housefly abdomen homogenate. These observations indicate that GSH conjugation with tetramethrin is not an enzymatically mediated reaction. The yield of **2A** from tetramethrin in 10 mM GSH is lowered under conditions favoring competitive oxidase reactions (i.e., adding NADPH to housefly and liver microsomes) and is elevated on inhibiting competitive esterase reactions (i.e., adding an organophosphorus esterase inhibitor to mouse microsomes). Thus, it appears that under some in vitro reaction conditions the direct chemical conjugation of tetramethrin and GSH is competitive with oxidase and esterase metabolism.

Action of Tetrahydrophthalimide and NEM in Vivo on Mouse Liver Thiols. Soluble thiols (mostly GSH) in mouse liver are decreased by 15 and 22% 1 h after 1.3 mmol kg^{-1} ip doses of **1B** and NEM, respectively. These findings suggest but do not in themselves establish in vivo formation of GSH conjugates of these imides.

Fate of Tetramethrin in Rats and Mice. The acid-^{14}C and alcohol-^{14}C preparations give no common metabolite in rat urine or bile or mouse urine indicating complete hydrolysis of the ester bond. Thus, tetramethrin conju-

gates **2A** and **3A** are not present in the bile or urine of ip-treated rats and mice.

The metabolites in bile from [*acid*-^{14}C]tetramethrin are identical with those from [^{14}C]chrysanthemic acid (i.e., three polar compounds that are probably conjugates: R_f 0.33, 0.37, and 0.43; BAW), despite large differences in the rates of biliary excretion (71% in 2 h for chrysanthemic acid and 6% in 2 h and 51% in 24 h with **1A**). A portion of these biliary conjugates probably undergoes enterohepatic circulation and cleavage prior to urinary excretion since oral (Elliott et al., 1972) and ip (current study) administration of [^{14}C]chrysanthemic acid yields 50–66% urinary radiocarbon in 24 h.

Speculation on Possible Neuropharmacological Significance of Thiol Adducts of Tetramethrin. Tetramethrin differs from other commercial pyrethroids in its uniquely high potency (3×10^{-13} M) and transient action on cockroach cercal sensory nerves (Gammon et al., 1981) and in readily adding sulfur nucleophiles. These phenomena may be related if tetramethrin undergoes reversible coupling with a critical thiol in the pyrethroid receptor site.

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Analysis of Formaldehyde in Shrimp by High-Pressure Liquid Chromatography

A method is described for the analysis of formaldehyde in shrimp. The procedure involves converting formaldehyde to its 2,4-dinitrophenylhydrazone which is analyzed by high-pressure liquid chromatography. Characteristics of the method include an estimated detection limit of 0.05 mg of formaldehyde/kg of shrimp, an average recovery of 72.3% at the 10 mg/kg level, and a total analysis time of 2 h. The results of formaldehyde analyses of fresh shrimp obtained from typical commercial outlets, and also of shrimp maintained live in a laboratory aquarium, are reported.

Recent reports of the health hazards of formaldehyde (Loomis, 1979) have stimulated an interest in methods for its analysis both in biological systems and in the environment (Janos et al., 1980; Jordan, 1980). It has long been accepted that formaldehyde develops post-mortem in marine fish and crustaceae (Sundsvold et al., 1969), probably from enzymatic reduction of trimethylamine oxide (Amano and Yamada, 1964). Thus, the analysis of seafood for formaldehyde is of particular importance.

Shrimp represent a major seafood resource. Many of the methods previously employed for the determination of formaldehyde in shrimp involve the use of specific reagents to generate colored complexes which are analyzed photometrically (Hansel and Wurziger, 1968; Sundsvold et al., 1971a; Flores and Crawford, 1973). The direct application of colorimetric methods to analyze formaldehyde in shrimp homogenates may be limited by interferences from other organic compounds. In addition, the drastic conditions used to form the complexes may result in the production of formaldehyde from trimethylamine oxide or from other amino compounds which occur naturally in shrimp (Sundsvold et al., 1971b). Isolation of formaldehyde from shrimp homogenates by distillation effectively eliminates these drawbacks, but obtaining reproducible results using this procedure appears to require considerable technique (Sundsvold et al., 1971a).

A traditional approach to the determination of low molecular weight aldehydes and ketones in foods has been to isolate them as 2,4-dinitrophenylhydrazones [for example, see Halvarson (1972)]. The mixture of 2,4-dinitrophenylhydrazones is either analyzed directly by thin-layer chromatography or converted to a mixture of the free carbonyl compounds which is analyzed by gas chromatography. Recently the separation of low molecular weight 2,4-dinitrophenylhydrazones by high-pressure liquid chromatography (HPLC) was described (Selim, 1977).

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