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EPOXYCHRYSANTHEMIC ACID AS AN INTERMEDIATE IN METABOLIC DECARBOXYLATION OF CHRYSANTHEMATE INSECTICIDES

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Summary. Epoxychrysanthemic acid undergoes rapid decarboxylation in aqueous medium at physiological pH to form a ring-opened dienol. Epoxychrysanthemates degrade in dilute acid to cyclopropane ring-opened diols. Metabolic decarboxylation of tetramethrin (1) and related chrysanthemate insecticides involves sequential enzymatic isobutenyl epoxidation, hydrolysis of the epoxychrysanthemate, and a nonenzymatic concerted decarboxylation and oxirane-ring opening reaction.

Pyrethrins and several pyrethroids derived from chrysanthemic acid are potent insecticides[1] which undergo rapid metabolism in insects and mammals by processes that include ester hydrolysis and methyl, methylene, allyl and aryl oxidation[2-7]. Expired $^{14}CO_2$ accounts for 0.3 to 3.1% of the dose of [^{14}C=O]pyrethroids administered orally to rats and mice[4]. It appeared possible that the metabolic pathway for $^{14}CO_2$ evolution might be initiated by hydroxylation at cyclopropane C_1 with subsequent ring cleavage or by a reaction at the isobutenyl moiety. The latter hypothesis was favored since [^{14}C=O]pyrethroids with a dihalovinyl replacement for the isobutenyl group give a much lower yield of $^{14}CO_2$[8]. We therefore considered the possible role of epoxychrysanthemates in $^{14}CO_2$ production. The study focused on tetramethrin (1) because this was the compound giving the highest value for $^{14}CO_2$ evolution[4].

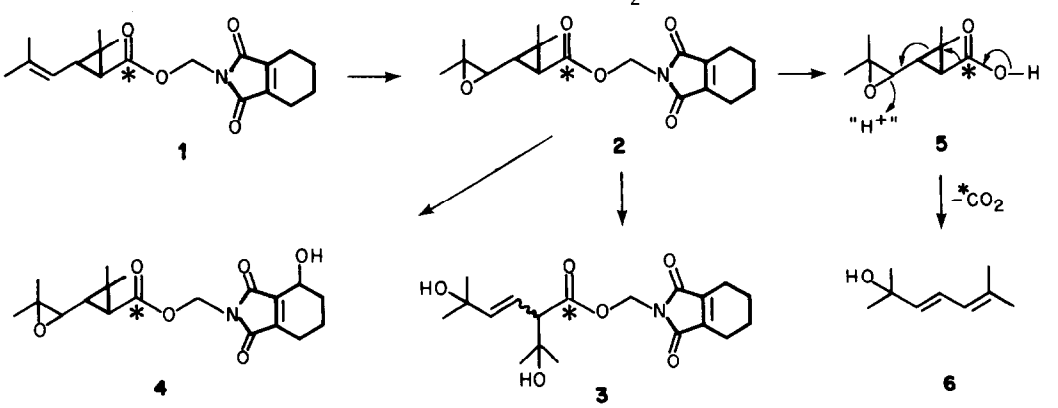
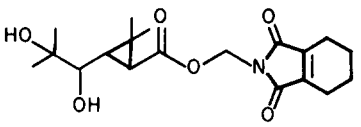


Figure. Metabolic formation and reactions of epoxytetramethrin (2) and epoxychrysanthemic acid (5).

Epoxidation was firmly established as a new[9] metabolic reaction of chrysanthemates by isolating epoxytetramethrin (2) in 6.4% yield from the reaction of 1 with mouse liver

microsomes. For maximum product isolation, the microsomes were treated with tetraethyl pyrophosphate (TEPP) or another esterase inhibitor and fortified with reduced nicotinamide-adenine dinucleotide phosphate (NADPH), the critical oxidase cofactor[10]. The compound obtained was identical in chemical ionization-mass spectral (CI-MS) fragmentation pattern with an authentic standard produced by reaction of 1 with m-chloroperoxybenzoic acid[11]. The metabolite co-chromatographed with only one of the two possible diastereoisomers of synthetic 2 in a variety of thin-layer chromatography (TLC) solvent systems[12] indicating metabolic stereospecificity.

Previous difficulties in defining epoxychrysanthemate metabolites probably resulted from their partial or complete destruction on acidifying the reaction mixtures prior to extraction for metabolite recovery. Dilute aqueous perchloric acid hydrolyzes 2 rapidly leading exclusively to the cyclopropane ring-opened diol (3) with no trace of the isomeric 1,2-diol (7A)[13] being obtained. This is in accord with an earlier observation[14] that p-toluenesulfonic acid treatment of 7B gave triene 8, which is equivalent to diol 3 under non-aqueous reaction conditions. Neither 3 nor 7A have, as yet, been identified as further metabolites of 1, although due to the aqueous stomach acid 3 undoubtedly forms in mammals on ingestion of 2.



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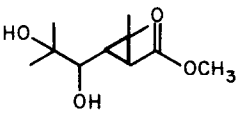
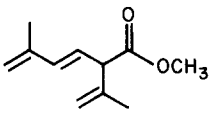


7B



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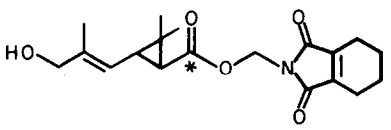

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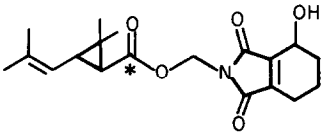

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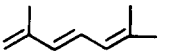

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Two of the tetramethrin metabolites (4 and 10) are formed by hydroxylation in the alcohol moiety. Thus 10 was identified (3.4%) from microsomal metabolism[10] of tetramethrin under conditions where other primary oxidative metabolites were the epoxide 2 (6.4%) and the usual[3,15] allylic alcohol 9 (5.8%). Epoxytetramethrin (2) is further metabolized to give one major NADPH-dependent product (12%), identified as 4 by CI-MS and TLC[12] comparison with an authentic sample from synthesis[16]. The oxirane ring of 2 apparently blocks further oxidation of the acid moiety side chain.

[^{14}C]Carbon dioxide is produced in in vitro experiments only under conditions where epoxychrysanthemic acid (5) can be formed. Thus, [^{14}C=O]1 plus mouse microsomes fortified with NADPH give 0.7% $^{14}CO_2$, an amount reduced to zero when the microsomal esterases are inhibited with TEPP. Similarly, [^{14}C=O]2 produces 7% $^{14}CO_2$ with mouse microsomes and 21% with rabbit microsomes, both reduced to zero by the addition of TEPP. Interestingly, the addition of NADPH to mouse microsomes also lowers the $^{14}CO_2$ recovery (3.0%) from 2 due to competitive oxidative metabolism. Preparation of 5 itself, by oxidation of chrysanthemic acid with

monoperphthalic acid[17], and examination of its stability reveal that the figure from rabbit microsomes is probably limiting under the reaction and CO_2 trapping conditions and that the decarboxylation is non-enzymatic in nature. Thus, [^{14}C=O]5 gives 26% $^{14}CO_2$ from aqueous buffer (pH 6.5) whether or not microsomes are present. In fact, 5 is inherently prone to decarboxylation; attempted purification by TLC or dissolution in aqueous buffer (pH 6.5 or 7.4) both lead only to the isolation of a new compound with no carbonyl group (IR). A Lanthanide shift reagent study (with $Eu(fod)_3$) of the NMR spectrum reveals this to be 6[18], produced presumably by a concerted decarboxylation and oxirane-ring opening reaction. Facile dehydration produces 11 with identical NMR data to the literature[19]. Corresponding *in vitro* studies with both [^{14}C=O]- and isobutenyl-methyl labeled epoxytetramethrin (2) and TLC cochromatography of the products have confirmed this sequence. The dienol (6) is only apparent as a mouse microsomal metabolite from isobutenyl-labeled 2 under conditions where the esterases are not blocked.

In vivo studies with male rats confirm the relevance of the *in vitro* metabolic reactions considered above. Under conditions where oral or intraperitoneal administration of [^{14}C=O]1 produces 0.6-2.7% $^{14}CO_2$, intraperitoneal administration of [^{14}C=O]2 gives 19% $^{14}CO_2$. Oral administration of 2, however, gives only 2.8% $^{14}CO_2$, presumably due to its instability in the stomach acid and hence, at least, partial conversion to 3. The diol 3 gives virtually no $^{14}CO_2$ upon intraperitoneal administration and the urinary and especially fecal metabolites show great similarities to those from 2 given orally.

Epoxytetramethrin (2) is detected as a metabolite in microsomal oxidase systems incubated with 1 as above and in the liver of mice treated intraperitoneally with 1. This is a metabolite and not an artifact formed on photooxidation, a point carefully examined since epoxychrysanthemates are also formed photochemically[11,20]. The metabolic and photochemical stabilities of chrysanthemates are limited by the reactivity of the isobutenyl substituent to both methyl oxidation and epoxidation. The latter process initiates a reaction sequence that ultimately degrades the cyclopropane ring with concomitant decarboxylation.

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9. Microsomal mixed-function oxidase formation of an epoxychrysanthemate was first suggested (Ref. 6) with cis-resmethrin, but the yield was only 0.6%.
10. Mouse liver microsomes (2.3% fresh liver wt equiv/vol) in phosphate buffer (pH 7.4; 50 mM; 2.2 ml) were pretreated with TEPP (87 μg) and incubated with tetramethrin or or epoxytetramethrin (100 μg) and NADPH (2.2 mg) for 0.5 hr at 37°C. With [^{14}C=O]-tetramethrin as the substrate, the recovered products in addition to epoxytetramethrin were unmetabolized tetramethrin (61.5%), 9 and 10 (9.2%), two apolar unknowns (7.2%) and polar products (15.7%).
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12. TLC cochromatography systems: chloroform/acetone (9:1) and hexane/toluene/ethyl acetate/methanol (9:6:4:1) for compounds 2, 6, 9 and 10; chloroform/methanol (99:1) for compounds 2 and 4; chloroform/acetone (4:1) for compound 4; hexane/ether (1:1) and chloroform/acetone (39:1) for compound 2.
13. The diol acid was prepared according to M. Matsui, M. Uchiyama and H. Yoshioka, Agr. Biol. Chem. 27, 554 (1963). Esterification of the potassium salt with the chloride of the alcohol, using 18-crown-6 as solubilizing agent, then gave 7A.
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16. Oxidation of N-(hydroxymethyl)-3,4,5,6-tetrahydrophthalimide (as the acetate) with selenium dioxide in glacial acetic acid produced the requisite diacetate. Acid catalyzed deprotection gave the diol which was monoesterified (chrysanthemoyl chloride) on the primary alcohol and epoxidized (m-chloroperoxybenzoic acid) to give synthetic 4. All spectral data were consistent.
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