Oxime Ether Pyrethroids and Hydroxylamine Ether Propyrethroids: Photochemistry, Biological Activity, and Metabolism

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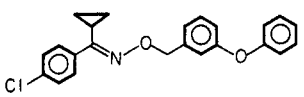
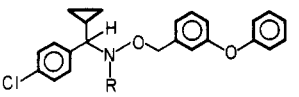
The potent insecticide 4-chlorophenyl cyclopropyl ketoxime (3-phenoxy)benzyl *O*-ether (**1**) is more resistant to photodegradation at 300 nm in hexane than the corresponding ester and current commercial pyrethroids. The predominant photochemical process is isomerization ($\phi_{E\rightarrow Z}$ = 0.26) resulting in photoactivation of the non-insecticidal (*Z*)-**1** to (*E*)-**1**. Hydroxylamine ethers of moderate insecticidal activity are obtained on reduction of **1** (for **2-H**) and N-derivatization of **2-H** (for *N*-methyl and *N*-formyl derivatives, **2-Me** and **2-CHO**). Hypochlorite oxidizes **2-H** to **1**. In contrast, *m*-chloroperoxybenzoic acid cleaves the ether linkages of **2-H** and **2-Me** but does not react with **2-CHO**. Houseflies and cabbage loopers oxidize and conjugate **2-Me**, probably to an *N*-hydroxymethyl glucoside, and hydrolyze **2-CHO** to **2-H**. These insects and mouse liver microsomes also oxidize **2-H** to **1**. Piperonyl butoxide retards housefly and cockroach knockdown by **2-Me**, and *S,S,S*-tributyl phosphorotrithioate delays housefly knockdown by **2-CHO**. **2-Me** and **2-CHO** are inactive on the cockroach cercal sensory nerve at 10^{-5} M whereas **1** is active at 5×10^{-8} M. Thus, hydroxylamine ethers **2-H**, **2-Me**, and **2-CHO** appear to be propyrethroids and oxime ether **1** is the actual toxicant.

All commercial pyrethroids are esters and alternative linkages usually yield compounds of diminished insecticidal activity (Elliott and Janes, 1978). An exception is a recent class of pyrethroids in which the ester group is replaced with an oxime ether linkage where only the *E* isomer is insecticidal (Bull et al., 1980; Nanjo et al., 1980). The nature of the neurophysiological activity of one of the most potent oxime ethers (**1**) (Bull et al., 1980) is indistinguishable from that of the equivalent ester (Bull et al., 1980) and related pyrethoids (Gammon et al., 1981). The reduced oxime ether, i.e., the hydroxylamine ether, and several of its N derivatives are claimed as insecticides without quantitative data on their activity (Henry, 1978); although not specifically described (Henry, 1978), compounds **2-H**, **2-Me**, and **2-CHO** are of this type.



1 (*E* isomer)



2-H, **2-Me**, **2-CHO**

The carboxylate linkage limits the persistence and photostability of pyrethroids (Ruzo, 1982). Photochemical reactions of the oxime ether pyrethroids such as **1** should be similar to those of the *O*-methyl oxime ethers that undergo some photocleavage of the N–O bond but react predominantly by photoisomerization (Padwa and Albrecht, 1974; Sato et al., 1972). Hydroxylamine ethers are candidate propyrethoids; they might be oxidized back to their insecticidal progenitors, e.g., **2-H** giving **1**. Thus, N-monosubstituted hydroxylamines undergo chemical and metabolic oxidation to oximes (Beckett and Jones, 1977; Beckett and Purkaystha, 1975). Propyrethroids with additional substituents as in **2-Me** and **2-CHO** would require further metabolic steps for reversion to the oxime ether.

This study examines the photochemistry and metabolism of oxime ether **1** and uses a variety of approaches to evaluate the possibility that hydroxylamine ethers **2-H**, **2-Me**, and **2-CHO** are propyrethroids.

MATERIALS AND METHODS

Chromatography and Spectroscopy. Thin-layer chromatography (TLC) utilized silica gel 60 F-254 chromatoplates (0.25-mm thickness) developed with solvents specified in Table I. Labeled compounds were detected by autoradiography and unlabeled compounds by their quenching of gel fluorescence under 254-nm light. Cochromatography with authentic standards constituted one method of compound identification. Reversed-phase TLC was used to determine the relative lipophilicities of different pyrethroids (Briggs et al., 1974, 1976). Gas–liquid chromatography (GLC) utilized the following conditions: 1.7 m × 3 mm i.d. glass column with 3% OV-101 on Chromosorb W (80–100 mesh); 100–245 °C, 6 °C/min; N_2 as the carrier gas at 20 mL/min; flame ionization detector; authentic standards to quantitate individual peak areas (Table I).

Nuclear magnetic resonance (NMR) spectra were determined at 90 MHz for samples in chloroform-*d* with tetramethylsilane as the internal standard. Ultraviolet (UV) spectra were taken for samples in various spectrograde solvents. Chemical ionization mass spectrometry (CI-MS) and GLC–CI-MS used the Finnigan Model 3200 spectrometer and Model 9500 GLC with a column of 5% OV-101 on Chromosorb W (80–100 mesh) operated at 100–245 °C (8 °C/min) with methane as the carrier gas and ionizing reagent (20 mL/min, 0.8 torr, 70 eV).

Chemicals. The chemicals are designated as indicated above or in Figure 1. In addition, PBalc, PBald, and PBacid refer to 3-phenoxybenzyl alcohol and the corresponding aldehyde and acid. All compounds were purified by TLC and gave appropriate NMR and CI-MS data. Oxime ether **1** (*Z*/*E* ratio of 1/2.2) was prepared via ketone **3** and oxime **5** (Bull et al., 1980). The *E* and *Z* isomers of **1** were separated by TLC with toluene using silica gel activated at 200 °C for 24 h (the lower band is (*Z*)-**1** and the upper band is (*E*)-**1**) (Bull et al., 1980; Brown, 1983). Oxime ether **1** was reduced to **2-H** with sodium cyanoborohydride in methanol at room temperature, with addition of HCl to maintain an acidic solution (Bernhart and Wermuth, 1974; Henry, 1978). Alternatively, **5** was reduced by this method and the hydroxylamine O anion, prepared with NaH, was coupled with 3-phenoxybenzyl

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Table I. Chromatographic and NMR Spectral Characteristics of Key Compounds

compound	GLC, R_t, min[a]	TLC, R_f[b] HE	HM	^{1}H NMR, δ[c]
(*E*)-1	22.0	0.62	0.68	7.4-6.9 (m, Ph), 5.18 (s, Bzl), 2.15 (m, cp), 1.0-0.4 (m, cp)
(*Z*)-1	21.1	0.54	0.58	7.4-6.8 (m, Ph), 5.00 (s, Bzl), 1.65 (m, cp), 0.9-0.6 (m, cp)
2-H		0.32	0.42	7.6-6.7 (m, Ph), 4.49 (s, Bzl), 3.14 (d, Bzl), 1.3-0.8 (m, cp), 0.8-0.0 (m, cp)
2-Me		0.47	0.52	7.6-6.7 (m, Ph), 4.51 (AB q, Bzl), 2.8-2.6 (m, Bzl + Me), 1.4-0.8 (m, cp), 0.8-0.1 (m, cp)
2-CHO		0.18	0.14	8.29 (s, CHO), 7.6-6.8 (m, Ph), 4.70 (s, Bzl), 4.5-4.2 (m, Bzl), 1.4-0.8 (m, cp), 0.8-0.1 (m, cp)
3	7.2	0.50	0.68	7.69 (AB q, Ph), 2.61 (m, cp), 1.4-1.0 (m, cp)
4-Ac$_2$[d]	18.5	0.09	0.04	7.5-6.9 (m, Ph), 5.07 (s, Bzl), 2.49 (s, Me)
5		0.05	0.06	7.32 (s, Ph), 2.2 (m, cp), 1.1-0.1 (m, cp)
6				7.25 (m, Ph), 2.68 (m, Bzl + Me), 1.3-0.8 (m, cp), 0.8-0.2 (m, cp)
PBalc	13.7	0.05	0.06	
PBalc-Ac[e]	13.0	0.34	0.24	
PBald	9.6	0.44	0.50	
PBacid		0.04	0.00	

[a] See Materials and Methods for conditions. R_t values for the two possible photo-Beckmann rearrangement products of 1 are 15.1 min for *N*-cyclopropyl-4-chlorobenzamide and 16.3 min for *N*-(4-chlorophenyl)cyclopropanecarboxamide. [b] TLC solvent systems: HE, hexane/ether (4/1); HM, hexane/methylene chloride (1/1). See Materials and Methods for other conditions. [c] NMR abbreviations: AB q, AB quartet; Bzl, benzyl; cp, cyclopropyl; d, doublet; m, multiplet; Me, methyl; Ph, phenyl; s, singlet. [d] Diacetate derivative. [e] Acetate derivative.

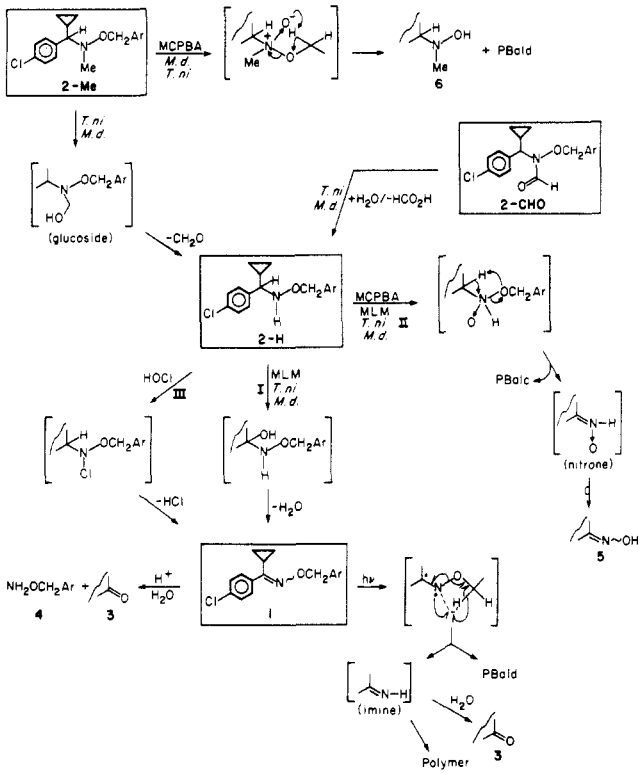

Figure 1. Chemical, photochemical, and metabolic reactions of oxime ether 1 and hydroxylamine ethers 2-H, 2-Me, and 2-CHO. Abbreviations: Ar, 3-phenoxyphenyl; *hν*, irradiation at 300 nm; MCPBA, *m*-chloroperoxybenzoic acid; *M. d.*, *Musca domestica* adults; MLM, mouse liver microsomes; *T. ni*, *Trichoplusia ni* larvae. Oxime ether 1 was used as a *Z*/*E* mixture (~2/1) except for some photochemical studies where individual isomers were required.

bromide to give 2-H. Hydroxylamine ether 2-H was derivatized by treating with an appropriate electrophile, i.e., dimethyl sulfate to prepare 2-Me, the mixed anhydride of formic and acetic acids to prepare 2-CHO, or others as appropriate (Brown, 1983). Analogous procedures were used for radiosynthesis of benzylic methylene ^{14}C preparations of 1 (*Z*/*E* = ~2/1), 2-H, 2-Me, and 2-CHO (each at 5.7 mCi/mmol) (Brown, 1983). [^{14}C]-2-H in ethanol slowly oxidizes to 1, even at −10 °C, requiring repurification. The other labeled compounds (1, 2-Me, and 2-CHO) were stable in ethanol or acetone even at 25 °C.

Chemical and Photochemical Reactions. The reactions of 1, 2-H, 2-Me, and 2-CHO were examined under various oxidative and hydrolytic conditions. Oxime ether 1 is stable to treatment with 1.0 M KOH in 20% aqueous methanol (4-h reflux) but completely hydrolyzes in 1.0 M HCl in 12% aqueous methanol (1 h, 55 °C). The HCl hydrolysate of 1 was extracted with chloroform, the organosoluble portion was examined by NMR, and the product in the aqueous phase was acetylated (acetyl chloride/pyridine in methylene chloride) for analysis by NMR and TLC. Hydroxylamine ether 2-H (25 mg) in water (1 mL) was treated with excess aqueous NaOCl (5.25%, 0.2 mL) and aqueous HCl (1 M, 0.2 mL) and shaken for 30 s. Extraction with chloroform gave a single product (18 mg). Treatment of 2-H (20 mg) in chloroform-*d* (0.3 mL) with equimolar *m*-chloroperoxybenzoic acid (MCPBA) at 25 °C resulted in essentially complete loss of the starting material and a simple product mixture (NMR) within 1 min. After filtration to remove *m*-chlorobenzoic acid, the products were further characterized by TLC. The same procedure was used to react 2-Me with MCPBA and to analyze the products. Treatment of 1 and 2-CHO with MCPBA under these conditions gave no products within 18 h.

Photodegradation studies involved irradiation of samples in spectrograde solvents (3 mL) (degassed when specified by repeated freeze–pump–thaw cycles) for 10 min–24 h in Pyrex tubes held in a merry-go-round arrangement in a Rayonette reactor (the Southern N. E. Ultraviolet Co., Middletown, CT) using two RPR 3000 lamps to examine photoisomerization or 14 RPR 3000 or 3500A lamps to study triplet sensitization or other photoreactions. Samples were adjusted in concentration to absorb 37–99% of the incident light. Actinometry was based on the Norrish type II reaction of 2-hexanone (ϕ = 0.252 at 310 nm) to give acetone quantitated by GLC using a Carbowax column as described by Murov (1973). Treatment with singlet oxygen involved photolysis of an oxygenated solution of 1% Rose Bengal in methanol. Photolysis of thin films (10–25 μg of pyrethroid in a 10 cm diameter Petri dish) utilized direct exposure to sunlight. The starting material and the photoproducts 3, PBalc, and PBald were identified by GLC, TLC, and GLC–CI-MS. Polar products such as 4 were examined by GLC and TLC following methylation (diazomethane in diethyl ether) or acetylation (as above).

Insecticidal, Knockdown, and Neurophysiological Activity. The insects utilized were adult female houseflies (*Musca domestica* L., SCR strain, 3–5 days after emergence, ~20 mg), third instar cabbage looper larvae (*Trichoplusia ni* Hübner, reared on an artificial diet, ~50 mg), and adult male cockroaches (*Periplaneta americana* L., reared on dry dog food, ~0.8 g). In topical tests the compound(s) was (were) applied in acetone to the ventrum of the abdomen (1 μL for houseflies and 2 μL for cockroaches) or to the mid-dorsal region (1 μL for cabbage loopers). The treated flies were provided sugar and water and the loopers lettuce leaves. Mortality at 24 h is reported as LD_{50} values with 95% confidence limits (Litchfield and Wilcoxon, 1949).

The effect of esterase and oxidase inhibitors on housefly knockdown (KD) was evaluated by topical application of *S,S,S*-tributyl phosphorotrithioate (DEF), phenyl saligenin cyclic phosphonate (PSCP), or piperonyl butoxide (Pip) at 250 μg/g followed after 3 h by the pyrethroids at LD_{85} doses. The time for 50% KD (KT_{50}) was determined from log time–probit KD plots. Pip was also evaluated under identical conditions as a synergist for 1 and deltamethrin. In synergism studies using Pip with cockroaches the KT_{50} was determined for 4–12 insects as the mean time to incoordination [see Gammon et al., (1981)]. Repetitive firing following electrical stimulation in cockroach cercal sensory nerve axons was used to evaluate pyrethroid activity in vitro (Gammon et al., 1981); when used, Pip and DEF were applied 30 min before the pyrethroid.

Metabolism. The metabolism of ^{14}C preparations of **1**, **2-H**, **2-Me**, and **2-CHO** was examined in housefly adults, cabbage looper larvae, and a mouse liver microsomal (MLM) system.

Housefly adults or heat-inactivated flies (80 °C, 4 min; controls) were treated topically (1 μL of acetone carrier) or injected (0.1 μL of ethanol carrier) with the labeled pyrethroid and held for 15 min to 4 h before analysis. The doses used gave no poisoning signs (**2-Me** topical), KD (**2-H** and **2-CHO** topical) or intense poisoning signs (**2-H** injected). The flies were extracted with acetone (**2-H** and **2-Me** topical) or methanol (**2-H** injected and **2-CHO** topical). Cleanup of the acetone extracts involved evaporation, lyophilization, and passage of the methylene chloride soluble portion through a short silica gel column. The methanol extracts were cleaned up by evaporation, partitioning between hexane and acetonitrile, evaporation of the acetonitrile layer, and dissolving the residue in methylene chloride. TLC of the cleaned up acetone and methanol extracts involved two-dimensional TLC (HE and then HM, Table I). Labeled material at the origin with **2-Me** extracts was rechromatographed, resolving two spots (R_f 0.6 and 0.8) with methanol/toluene/1-butanol/water (10/5/5/4). The combined material was incubated with β-glucosidase (6 mg, Calbiochem-Behring, La Jolla, CA) in pH 4.8, acetate buffer (2.5 mL) for 24 h at 37 °C (Gaughan and Casida, 1978). Addition of $(NH_4)_2SO_4$ (500 mg) and HCl (to pH 1), and extraction with chloroform gave 87% radiocarbon recovery in the chloroform, which was subjected to TLC.

Cabbage looper larvae (held without food for 24 h) were treated topically with **2-H** (dose producing intense poisoning signs, 1 μL of ethanol carrier, fourth instar) or **2-Me** (nontoxic dose, 1 μL of acetone carrier, third instar). After 30–90 min the loopers were extracted with methanol (for **2-H**) or acetonitrile (for **2-Me**), and the extracts were cleaned up and chromatographed as with houseflies. Labeled material at the origin with the **2-Me** extracts was rechromatographed as above, and 97% of the radiolabel was extracted into chloroform after treatment with β-glucosidase. A separate experiment used a brief hexane rinse to differentiate surface and penetrated material 6 h after application of **2-H**.

Table II. Effect of Solvent on Extent of Photodegradation and Photoproduct Distribution of Oxime Ether 1 Irradiated at 300 nM for 24 h

	recovery, % equiv[b]		
		cleavage products	
solvent[a]	1	3	PBald
methanol	50 ± 1	5.5 ± 0.5	12 ± 1.0
ethanol	62 ± 5	4.1 ± 1.0	1.6 ± 1.0
1-butanol	54 ± 5	4.5 ± 0.5	2.0 ± 0.5
hexane	76 ± 2	10.2 ± 1.0	2.0 ± 1.0

[a] 1 at 3.2 mM in methanol and 1-butanol and 6.4 mM in ethanol and hexane. [b] Other products were insoluble or remained at the origin on TLC.

Mouse liver microsomes (100 mg fresh weight equivalent, washed with buffer) were incubated for 30 min at 37 °C with [^{14}C]-**2-H** (0.5 μmol added to 10 μL of ethanol) and NADPH (0 or 2 μmol) in 1.0 mL of 50 mM, pH 7.4, phosphate buffer. Controls involved microsomes held for 5 min at 90 °C prior to use. Following lyophilization, the methylene chloride soluble portion was subjected to TLC (HE).

RESULTS

Figure 1 includes the chemical, photochemical, and metabolic reactions considered below.

Oxidation and Hydrolysis. Oxime ether **1** is stable to treatment with MCPBA in chloroform, or KOH in aqueous methanol, but is quantitatively cleaved by HCl in aqueous methanol to ketone **3** and *O*-hydroxylamine ether **4**.

Hydroxylamine ether **2-H** undergoes almost quantitative reaction on treatment with HOCl in water or MCPBA in chloroform; hypochlorite yields oxime ether **1** whereas MCPBA gives oxime **5** and PBalc (itself stable to oxidation under these conditions) plus a trace of PBald. **2-Me** is also readily oxidized by MCPBA, cleaving quantitatively to hydroxylamine **6** and PBald. In contrast, **2-CHO** does not react with MCPBA.

Photochemistry. *Photoreactivity of Oxime Ether 1.* Oxime ether **1** shows λ_{max} 210 nm (ϵ = 30 000) for the individual *Z* and *E* isomers, with a shoulder at 258 nm (ϵ = 7300 for (*Z*)-**1** and 10 500 for (*E*)-**1**). Its extinction coefficient in cyclohexane at 300 nm (ϵ = 309) is 10 times greater than that of its ester analogue (Brown, 1983), indicating that the oxime ether linkage is a relatively efficient chromophore at longer wavelengths. Oxime ether **1** is also somewhat more stable than *cis*-permethrin (ϵ_{300} in 1-propanol = 5) when irradiated at 300 nm for 1.5 h in hexane at concentrations absorbing 99% of the incident light, i.e., 94, 44, and 88% recoveries for **1**, the corresponding ester, and permethrin, respectively. The quantum yield for photodecomposition of **1** in alcohol solvents is approximately 0.003 and is slightly lower in hexane (Table II).

In each solvent the only major photoproducts are ketone **3** and PBald with their ratio dependent on the solvent; in methanol and hexane PBalc and *O*-hydroxylamine ether **4** are also detected (<1%). The unidentified photoproducts (12–39% of the starting material) are not detected on GLC and remain at the origin on TLC even when subjected to acetylation and methylation; they therefore do not include the two amides from photo-Beckmann rearrangements or PBacid (Table I). Further, photolysis gives appreciable amounts of material deposited on the

Table III. Effect of Triplet Sensitizer Energy on the Z/E Photoequilibrium of Oxime Ether 1 at 0.53 mM in Degassed Benzene Irradiated at 360 nM

sensitizer			oxime ether,
compound	mM[a]	E_T	$[Z]/[E]$[b]
none			0.75
anthracene	0.59	42.0	0.87
pyrene	10	48.7	0.69
benzil	14	53.7	1.3
benzophenone	14	68.5	4.6
xanthone	5	74.2	3.2

[a] Absorbance = 1.0 at 366 nm. [b] The Z/E ratio prior to irradiation was 0.45. Samples were irradiated until photoequilibrium was achieved (5–40 min).

glass, presumably polymeric photoproducts. The rate of photodegradation of 1 is the same in hexane, cyclohexane, and benzene and is unaffected by dissolved oxygen. Oxime ether 1 does not react with singlet oxygen.

The quantum yield for Z/E isomerization in methanol at 300 nm is 0.26 ± 0.015. Photoequilibrium in methanol or hexane at 300 nm is 1.88 ± 0.03 $[Z]/[E]$; oxygen does not affect this photoequilibrium or the photoisomerization rate in cyclohexane. Isomerization at 360 nm is sensitized by triplet sensitizers of appropriate energy (Table III) and is not quenched by 1,3-cyclohexadiene except at very high concentrations, i.e., 0, 22, and 85% reduction at 10^{-3}, 10^{-2}, and 10^{-1} M, respectively.

Photoisomerization is the only reaction observed on exposing 1 as a thin film to sunlight, i.e., 15% conversion of 25 μg of (*Z*)-1 to (*E*)-1 in 4 h, with quantitative recovery of a (*Z*/*E*)-1 mixture. The insecticidally inactive (*Z*)-1 can therefore serve as a photoactivatable pyrethroid. Thus, 10 μg of (*Z*)-1 introduced into a Petri dish and exposed to sunlight for 1 h causes 100% mortality with houseflies whereas the dark control shows no mortality within 24 h. On a comparable basis, isomerization of (*E*)-1 on exposure to sunlight would reduce its insecticidal activity.

Photolysis of Other Compounds. Ketone 3 is stable in methanol but undergoes very slow photolysis in hexane at 300 nm giving small amounts of 4-chloroacetophenone (identified by GLC–CI-MS and NMR) and a second product with NMR and GLC–CI-MS characteristics consistent with 4-chloro-*n*-butyrophenone. Photolysis of 2-H, 2-Me, 2-CHO, and the corresponding *N*-$C(O)CH_3$, *N*-$C(O)OCH_3$, and *N*-$C(O)NHCH_3$ analogues in methanol at 300 nm never yields any 1, and these compounds appear to be much more photolabile than 1.

Lipophilicities. The reversed-phase TLC method revealed relative lipophilicities as follows: 2-CHO < tetramethrin < hydroxylamine ether 2-H = the ester analogue of 1 < oxime ether 1 = permethrin (Brown, 1983). Of these compounds, only 2-CHO falls outside of the normal range for pyrethroids with KD and insecticidal properties (Briggs et al., 1974).

Comparative Activities of Oxime Ether 1 and Its Hydroxylamine Ether Derivatives. The topical toxicity to both housefly adults and cabbage looper larvae is reduced upon converting 1 to 2-H and is further reduced by derivatization of this hydroxylamine ether, i.e., 2-Me and 2-CHO are only moderately toxic (Table IV). Other N substituents examined essentially destroy all activity (Table IV). The –CDNHO– analogue of 2-H is identical in activity on houseflies with 2-H itself based on their 24-h LD_{50} values and the time to onset of poisoning signs.

Oxime ether 1 induces repetitive firing in the cockroach cercal sensory nerve at 5×10^{-8} M within 2 min but is the least active of the insecticidal "type I" pyrethroids examined (Gammon et al., 1981). 2-H undergoes air oxidation to 1 under the assay conditions. 2-Me is inactive in this preparation at 10^{-6} M but induces repetitive firing at 10^{-5} M after a latency period of 40–120 min. Pip pretreatment at 10^{-5} M has no effect on the response of this preparation to 2-Me at 10^{-5} M, producing a latency of 70 min. At 10^{-4} M, however, Pip completely blocks any activity of 2-Me at 10^{-5} M for up to 2 h after treatment but has no effect on the activity of 1 (i.e., 10^{-7} M causes repetitive firing in 30 s). 2-CHO is also inactive at 10^{-6} M but at 2×10^{-5} M it induces repetitive firing in 9.0 ± 1.5 min. DEF pretreatment at 2.5×10^{-4} M either blocks or greatly retards the onset of repetitive firing with 2×10^{-5} M 2-CHO, i.e., 26 min in one experiment and greater than 1 h in two other experiments.

Table IV. Comparative Topical Toxicity to Housefly Adults and Cabbage Looper Larvae of Oxime Ether 1 and Its Hydroxylamine Ether Derivatives

	LD_{50}, μg/g (95% confidence)	
compound	housefly[a]	cabbage looper
oxime ether 1[b]	1.2 (1.0-1.5)	1.0 (0.7-1.4)
hydroxylamine ethers[c]		
2-H	14 (11-18)	4.0 (3.0-5.0)
2-Me	25 (21-30)	8.0 (6.0-11)
2-CHO	40 (29-56)	8.0 (5.0-13)

[a] Pip synergizes the 24-h LD_{50} of 1 by 10-fold, the same factor found for deltamethrin. The toxicity of 2-CHO is also synergized by Pip, giving 100% mortality with a non-synergized LD_{30} dose. [b] Z/E ratio = 2.2/1. [c] Analogues of 2 with *N*-$C(O)CH_3$, *N*-$C(O)OCH_3$, *N*-$C(O)NHCH_3$, and *N*-$C(O)(CH_2)_2C(O)OCH_3$ substituents give $LD_{50} > 100$ μg/g for both housefly and cabbage looper.

Table V. Effect of Esterase and Oxidase Inhibitors on the Knockdown Time (KT_{50}) of Housefly Adults with Oxime Ether 1 and Hydroxylamine Ethers 2-H, 2-Me, and 2-CHO

	KT_{50}, min, ± SE		ratio[c]
compound[a]	control	treatment[b]	(significance)
	DEF		
1	21 ± 1	19 ± 1	0.90 (NS)
2-CHO	31 ± 2	53 ± 3	1.71 (S)
	PSCP		
2-CHO	38 ± 4	64 ± 7	1.68 (S)
	Pip		
1	16 ± 1	14 ± 1	0.88 (NS)
2-H	14 ± 1	12 ± 1	0.86 (NS)
2-Me	37 ± 3	>100[d]	>2.7[d] (S)

[a] Approximate LD_{85} doses, i.e., 5, 30, 75, and 100 μg/g for 1, 2-H, 2-Me, and 2-CHO, respectively. [b] 250 μg/g 3 h before the pyrethroid. [c] NS = ratio not significantly different from 1.0; S = ratio significantly different from 1.0 at $p < 0.01$ by the Student's *t* test. [d] 30% KD at 100 min, and thus significance is calculated based on KT_{50} as 100 min.

Interactions with Esterase and Oxidase Inhibitors in Adult Houseflies and Cockroaches. DEF significantly delays housefly KD from topically applied 2-CHO but not 1 (Table V) although it does not appreciably alter their 24-h LD_{50} values. PSCP also delays KD by 2-CHO (Table V) without affecting the 24-h LD_{50}. Pip greatly delays the mean time to KD with 2-Me (Table V) although it only slightly synergizes its 24-h LD_{50}. The KD with 1 and 2-H is not significantly affected by Pip (Table V).

Treatment of cockroaches with Pip followed after 3 h with the pyrethroid has no significant effect on the KD of 1 but greatly delays the effect of 2-Me (Table VI). Moreover, at the mean time to incoordination from 2-Me for control insects there are no signs of incoordination in

Table VI. Effect of an Oxidase Inhibitor on the Knockdown Time (KT_{50}) of Cockroaches with Oxime Ether 1 and Hydroxylamine Ether 2-Me

compound[a]	KT_{50}, min, ± SE		ratio[b] (significance)
	control	Pip[a]	
1	134 ± 30	200 ± 38	1.5 (NS)
2-Me	214 ± 19[c]	700–1000[d]	3.3–4.7 (S)

[a] Pip applied at 250 μg/g 3 h before 1 or 2-Me at 1 or 10 μg/g, respectively. [b] NS = ratio not significantly different from 1.0; S = ratio significantly different from 1.0 at $p < 0.01$ by the Student's t test. [c] Significantly longer than the value of 1 without Pip at $p < 0.05$. [d] KD 0% at 214 min, <50% at 700 min, and 100% at 1000 min.

Table VII. Metabolism of Oxime Ether 1 and Hydroxylamine Ethers 2-H, 2-Me, and 2-CHO by Housefly Adults, Cabbage Looper Larvae, and the Mouse Liver Microsome/NADPH System

pyrethroid	recovery, % equiv[a]					
	1	2-H	2-Me	2-CHO	PBalc	other
Housefly Topical, 5 μg/g,[b] 15–60 min						
2-H	3.7	92.6			3.7	
2-Me		<0.1[c]	70		2[c]	28 PBald
2-CHO		2.4		93.8		3.8[d]
Housefly Injected, 1.2 μg/g, 4 h						
2-H	6.4	86.2			7.4	
Cabbage Looper Topical, 1.3 μg/g, 1.5 h						
2-H[e]	3.5	92.4				4.1
2-Me		0.4[c]	63.7		13.5[c]	22.4 PBald
Mouse Liver Microsome/NADPH[f]						
1	94.9	0.0			1.9	3.2[g]
2-H	10.3	82.4			7.3	

[a] Normalized to 100%. [b] 2-H at 15 μg/g. [c] Quantitated after β-glucosidase hydrolysis of a conjugate. [d] TLC R_f 0.0 with methylene chloride/hexane (2/1). [e] In a separate 6-h experiment, a hexane rinse contained 70.6% 2-H and 7.4% 1 but no polar metabolites. The penetrated material consisted of 9.5% 2-H, 1.0% 1 and 11.5% polar metabolites. [f] Recovery values without NADPH were 99.1% 1 and 0.9% PBalc and 99.0% 2-H and 1.0% 1. [g] TLC R_f 0.5 with HM.

any Pip-pretreated cockroaches.

Metabolism by Housefly Adults, Cabbage Looper Larvae, and the Mouse Liver Microsomal System. The sequential conversion of 2-CHO → 2-H → 1 involving hydrolysis and then oxidation is established in several systems (Table VII). Topically treated housefly adults convert 2.4% of 2-CHO to 2-H and 3.7% of 2-H to 1, as compared to conversions in heat-inactivated controls of 0.9 and 1.3%, respectively. Cabbage looper larvae give 1.0–3.5% of 1 from 2-H. Housefly adults convert 6.4% of injected 2-H to 1, vs. 4.3% in heat-inactivated controls. The mouse microsomal system requires NADPH in converting 2-H to 1 and PBalc in 10 and 7% yields, respectively. Under these conditions oxime ether 1 itself is relatively stable, yielding PBalc and a product (3.2%) that chromatographs as anticipated for 4′-hydroxy-1. Other polar unknowns are formed in trace amounts both in vivo and in vitro from all radiolabeled compounds tested.

2-Me is slowly metabolized in topically treated housefly adults and cabbage looper larvae, resulting in recovery of mostly starting material (64–70%) along with PBald (22–28%). Both species give polar metabolites cleaved by β-glucosidase to two products identified by two-dimensional TLC cochromatography as PBalc and 2-H (Table VII). The higher apparent conversion to these products with cabbage looper larvae than with houseflies may be due to differences in technique; i.e., acetonitrile was used to extract the loopers and acetone the flies, the former solvent probably being more effective for dissolving and extracting glucosides.

DISCUSSION

Oxidation of hydroxylamine ether 2-H appears to involve three pathways (I, II, and III of Figure 1). The bioactivation pathway (I) forming oxime ether 1 may proceed via benzylic hydroxylation and dehydration (analogous to oxime ether formation from a ketone and NH_2OH), but hydroxylation is probably not the rate-limiting step since the –CDNHO– analogue of 2-H is indistinguishable in insecticidal activity from 2-H; i.e., there is no isotope effect. Pathway II is important on MCPBA oxidation of 2-H, and possibly in biological systems, and leads exclusively to cleavage products. The initial intermediate is probably the hydroxylamine N-oxide, which cleaves to PBalc and the nitrone, the latter tautomerizing to the free oxime. Analogous reactions include metabolic oxidation of N,N-disubstituted hydroxylamines ultimately to disubstituted nitrones (Poulsen et al., 1974) and oxidation with molecular oxygen of N-monosubstituted hydroxylamines to oximes (Beckett and Purkaystha, 1975). Additional polar metabolites of 2-H in insects and the MLM system include PBalc and conjugates possibly formed via N-oxidation and ether cleavage. HOCl cleavage of 2-H probably involves an initial N-chloro derivative that rapidly dehydrochlorinates to the corresponding oxime (pathway III) by analogy with N-chloroaniline proposed as an intermediate in the reaction of hypochlorite with aniline (Haberfield and Paul, 1965).

Oxidation of 2-Me probably also involves two pathways, the first N-demethylation to 2-H and the second cleavage to PBald and hydroxylamine 6 (Figure 1). The first pathway presumably gives the N-hydroxymethyl intermediate via either N-oxidation or direct methyl hydroxylation, with subsequent cleavage to the amine and formaldehyde [for analogous reactions with dimethylaniline, see Willi and Bickel (1973)]. MCPBA oxidation of 2-Me proceeds only via the N-oxidation pathway. PBald is formed directly, since PBalc is not oxidized to PBald with MCPBA. The presumed N-oxide intermediate undergoes either inter- or intramolecular hydride shift from the benzylic position of the alcohol moiety. N-Oxidation may be the principal pathway for metabolism of 2-H and 2-Me in insects and the MLM system since PBalc and PBald are major metabolites.

The photostability is greatly increased on replacement of the ester with the oxime ether linkage, despite only small differences in their UV spectra (λ_{max} and ϵ) (Holmstead et al., 1978). Thus, the quantum yield for photodecomposition of 1 in methanol is at least 6-fold lower than that of any commercial pyrethroid (Ruzo and Casida, 1980) or the ester analogue of 1. This is at least partially because the energy absorbed by the oxime ether is so efficiently dissipated by Z/E interconversions. Isomerization on direct irradiation probably involves a singlet excited state or perhaps a short-lived triplet, since the rate of isomerization is not affected by dissolved oxygen or 1,3-cyclohexadiene, except at very high concentrations. With triplet sensitizers the Z/E ratio depends on the triplet sensitizer energy. These phenomena of direct singlet excitation with high quantum yield and indirect triplet excitation involving non-vertical transfer of energy are consistent with photoisomerization processes reported for O-methyl oxime ethers (Padwa and Albrecht, 1974). Fragmentation of oxime ether 1 is probably due to N–O bond cleavage to form a biradical, followed by hydrogen abstraction to form the corresponding imine and PBald (Figure 1) [analogous

processes are proposed for other oxime ethers: Ito and Matsuura (1975); Sato et al. (1972)]; the imine would rapidly hydrolyze or polymerize. Thermally, oxime ethers are very stable relative to their imine counterparts, showing little tendency even to isomerize (Curtin et al., 1966). Photochemically, PBald is probably formed directly rather than via PBalc since the alcohol is present in <1% amount and is not photooxidized to PBald.

The present studies show pyrethroid-like activity with relatively non-lipophilic propyrethroids that undergo metabolic conversion to neuroactive products; i.e., 2-H, 2-Me, and 2-CHO appear to be propyrethroids acting only after conversion to 1. Their toxicity to insects decreases in the sequence 1 > 2-H > 2-Me or 2-CHO and on the cockroach cercal sensory nerve in the order 1 > 2-Me or 2-CHO. The use of DEF with 2-CHO and Pip with 2-Me has little or no effect on their 24-h LD_{50} values but clearly increases the time for KD_{50}, presumably via inhibition of activation (Sun and Johnson, 1960). Although 1 is active at 5×10^{-8} M in the nerve assay, 2-Me and 2-CHO are inactive at 10^{-6} M and their activity at 10^{-5} M is attenuated or blocked by Pip and DEF, respectively. There is thus in vivo and in vitro evidence that the cercal nerve and/or other insect tissues hydrolyze 2-CHO and oxidize 2-Me to 2-H, which in turn is oxidized to 1, the active pyrethroid. The principles of derivatization used in the present study may be applicable in other pyrethroid series to obtain propyrethroids of modified and possibly improved physical or toxicological properties.

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Registry No. (*E*)-1, 67038-97-5; (*Z*)-1, 67038-98-6; 2-H, 86374-22-3; 2-Me, 86374-23-4; 2-CHO, 86374-24-5.

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Direct Synthesis of Dipteran Sex Pheromones by the Wittig Reaction in a Heterogeneous Medium

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The Wittig reaction carried out in a solid–liquid medium under mild experimental conditions led to the single-step synthesis of various Dipteran sex pheromones from nonanal and tetradecanal. This method of synthesis was characterized by its excellent yield.

The synthesis of pheromones specific to some insect species has proved to be liable to improve the efficiency of pest control in agriculture (Morgan et al., 1974; Jacobson, 1975; Rossi, 1977; Campion et al., 1978). The use of the attractant power of these derivatives, coupled with that of an insecticide (Boyd et al., 1974; Carlson and Beroza, 1973; Carlson et al., 1971; Kinzer and Mc Daniel, 1978), affords a more efficient action on a particular insect species; there is no harmful ecological interaction with the environment, contrary to what is often observed when high rates of insecticides are sprayed.

The most active *Musca domestica* pheromones are (*Z*)-9-tricosene (Carlson et al., 1971, 1974; Richter et al., 1976) and (*Z*)-9-heneicosene (Richter, 1974). Their mixture in the 7:3 ratio is known to be particularly efficient (Bestmann et al., 1974). In *Musca autumnalis*, (*Z*)-14-nonacosene has been used as a mating stimulant (Uebel et al., 1975).

The potential interest of the housefly pheromones (Julia, 1976; Kuepper and Streck, 1976) led to the investigation of various methods of synthesis. Kuepper and Streck

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