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1-[4-[(Trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octanes: A Novel Type of Selective Proinsecticide

Christopher J. Palmer,*,† Ian H. Smith,‡ Matthew D. V. Moss,‡ and John E. Casida†

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720, and Wellcome Research Laboratories, Berkhamsted, Hertfordshire, United Kingdom

4-Alkyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octanes are potent insecticides with 24-h housefly topical LD_{50}s depending on the 4-substituent, i.e., 0.4–0.7 μg/g for *n*-propyl, *n*-butyl, and *tert*-butyl and 68 μg/g for cyclohexyl. The 4-*n*-propyl- and 4-*n*-butyl-1-[4-[(trimethylsilyl)ethynyl]phenyl] compounds have almost the same potency to houseflies as their 1-(4-ethynylphenyl) analogues, suggesting that the trimethylsilyl compounds may be proinsecticides. The microsomal cytochrome P_{450} inhibitor piperonyl butoxide antagonizes the toxicity of the [(trimethylsilyl)ethynyl]phenyl compounds by up to >714-fold and synergizes that of the ethynylphenyl compounds by 4- to 30-fold, indicating oxidative activation and detoxification, respectively. Mouse intraperitoneal LD_{50} values are 0.1–1.1 mg/kg for the ethynylphenyl compounds versus 3–>400 mg/kg for the [(trimethylsilyl)ethynyl]phenyl compounds. The 4-*n*-butyl-1-[4-[(trimethylsilyl)ethynyl]-phenyl] analogue is >930-fold more toxic to houseflies than to mice, establishing a remarkable level of selective toxicity among the trioxabicyclooctanes probably attributable to oxidative metabolic activation in houseflies but not in mice.

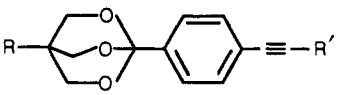
1,4-Disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes are potent insecticides acting as $GABA_A$ receptor antagonists to inhibit GABAergic synaptic transmission (Palmer and Casida, 1985, 1987; Casida et al., 1988). 4-Alkyl-1-(4-ethynylphenyl)trioxabicyclooctanes are highly toxic to houseflies both with and without the synergist piperonyl butoxide (PB), achieving a level of potency comparable to that of the most effective established insecticides acting at other targets (Palmer and Casida, 1989). As with many other trioxabicyclooctanes (Casida et al., 1985), the 4-alkyl-1-(4-ethynylphenyl) analogues typically exhibit intraperitoneal LD_{50} values below 1 mg/kg to mice. Structural modification for selective toxicity therefore warrants special consideration.

This study focuses on possible proinsecticides that might more effectively undergo metabolic activation in insects than in mammals. Among the substituents examined, the [(trimethylsilyl)ethynyl]phenyl group proved to be of particular interest.

MATERIALS AND METHODS

Spectroscopy. Proton nuclear magnetic resonance (NMR) spectra were obtained at 300 MHz with a Bruker WM-300 spectrometer for samples dissolved in deuteriochloroform. Mass spectrometry (MS) utilized the Hewlett-Packard 5985 system with chemical ionization (230 eV with methane at 0.8 Torr).

Syntheses. The compounds examined are shown below:



	R			
R′	*n*-Pr	*n*-Bu	*t*-Bu	c-Hx
$SiMe_3$	1	2	3	4
H	5	6	7	8

Syntheses of **3**, **5**, and **7** have been described earlier (Palmer and Casida, 1989). Compounds **1**, **2**, **4**, **6**, and **8** were prepared by a similar route.

4-*n*-Butyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane (**2**) was obtained as light brown needles: mp 124–126 °C: MS, $[M + 1]^+$ 345; NMR δ 7.55 and 7.40 (each 2 H, AA′BB′, aromatic), 4.10 (6 H, s, $CH_2O \times 3$), 1.35–1.15 (6 H, m, $(CH_2)_3$), 0.90 (3 H, t, CH_3CH_2), 0.20 (9 H, s, $(CH_3)_3Si$).

4-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane (**1**) was obtained as light tan flakes: mp 174–176 °C; MS, $[m + 1]^+$ 331; NMR δ 7.52 and 7.41 (each 2 H, AA′BB′, aromatic), 4.08 (6 H, s, $CH_2O \times 3$), 1.29–1.17 (4 H, m, CH_2CH_2), 0.91 (3 H, t, CH_3CH_2).

4-*n*-Butyl-1-(4-ethynylphenyl)-2, 6, 7-trioxabicyclo[2.2.2]octane (**6**) was obtained as pale yellow crystals: mp 85–87 °C; MS,

† University of California.
‡ Wellcome Research Laboratories.

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Table I. Housefly Topical LD_{50} Alone and with Piperonyl Butoxide of 4-Alkyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]- and 4-Alkyl-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]-octanes $RC(CH_2O)_3CC_6H_4$-4-C≡CR′

	housefly LD_{50}				
	μg/g		ratio	housefly + PB LD_{50}, μg/g	
R	R′ = $SiMe_3$	R′ = H	$SiMe_3$/H	R′ = $SiMe_3$	R′ = H
n-Pr	0.53	0.68	0.8	102	0.023
n-Bu	0.43	0.24	1.8	124	0.054
t-Bu	0.70	0.09	7.8	>500	0.011
c-Hx	68	0.58	116	>500	0.049

$[M + 1]^+$ 273; NMR δ 7.55 and 7.45 (each 2 H, AA′BB′, aromatic), 4.10 (6 H, s, $CH_2O \times 3$), 3.05 (1 H, s, C≡C*H*), 1.35–1.15 (6 H, m, $(CH_2)_3$), 0.90 (3 H, *t*, CH_3CH_2).

4-Cyclohexyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane (4) was obtained as brown crystals: mp 223–226 °C; MS, $[M + 1]^+$ 371; NMR δ 7.50 and 7.40 (each 2 H, AA′BB′, aromatic), 4.10 (6 H, s, $CH_2O \times 3$), 1.85–1.55 and 1.30–0.92 (11 H, m, $(CH_2)_5CH$), 0.20 (9 H, s, $(CH_3)_3Si$).

4-Cyclohexyl-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]-octane (8) was obtained as pale yellow crystals: mp 190–192 °C; MS, $[M + 1]^+$ 299; NMR δ 7.55 and 7.45 (each 2 H, AA′BB′, aromatic), 4.10 (6 H, s, $CH_2O \times 3$), 3.05 (1 H, s, C≡C*H*), 1.85–1.55 and 1.30–0.90 (11 H, m, $(CH_2)_5CH$).

The (*RS*)-α-(trimethylsilyl)ethynyl analogue of deltamethrin was prepared by sequentially adding *n*-butyllithium (2.5 M solution in hexane) (1.25 equiv) followed by 3-phenoxybenzaldehyde (1 equiv) to (trimethylsilyl)acetylene (1.25 equiv) in dry tetrahydrofuran at −78 °C under an N_2 atmosphere. The resultant benzyl alcohol was esterified with (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid chloride (1 equiv) in dry dichloromethane/pyridine to give (*RS*)-α-[(trimethylsilyl)ethynyl]-3-phenoxybenzyl (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate: colorless oil; MS, $[M + 1]^+$ 575; NMR (mixture of diastereomers) δ 7.40–6.95 (9 H, m, aromatic), 6.75 (1 H, d, Br_2C=C*H*), 6.40 and 6.35 (1 H, 2 s, OC*H*), 2.00–1.85 (2 H, m, C*H*CHCO), 1.25 and 1.20 (3 H, 2 d, $(CH_3)_2C$), 0.15 (9 H, 2 s, $(CH_3)_3Si$).

Bioassays. Housefly LD_{50} values were determined for adult female *Musca domestica* L. (SCR strain, ~20 mg each) held 24 h at 25 °C after application of the test compound to the ventrum of the abdomen (Palmer and Casida, 1985). Synergized toxicity was evaluated for flies pretreated topically with PB at 250 μg/g 2 h before the toxicant was administered. Mouse intraperitoneal LD_{50}s were determined 24 h after male albino Swiss–Webster mice (18–22 g) were treated with methoxytriglycol (50 μL) as the carrier vehicle. In one case the mice were pretreated intraperitoneally with PB at 150 mg/kg (administered in 25 μL of methoxytriglycol) 1 h before the toxicant.

RESULTS AND DISCUSSION

Housefly Toxicity (Table I). The trimethylsilyl derivatives and particularly the *n*-Pr and *n*-Bu compounds approach the toxicity of their corresponding ethynyl analogues. On the other hand, the toxicity of the cyclohexyl [(trimethylsilyl)ethynyl]phenyl compound is exceptionally low relative to its ethynylphenyl analogue.

Piperonyl butoxide antagonizes the toxicity of the [(trimethylsilyl)ethynyl]phenyl compounds so that with this oxidase inhibitor they are virtually inactive with determinations at 24 h, and although not tabulated, they are also of low activity in longer term assays. In contrast, their ethynylphenyl analogues are synergized about 10-fold by PB. These findings are consistent with the [(trimethylsilyl)ethynyl]phenyl compounds undergoing metabolic oxidative activation and the ethynylphenyl compounds undergoing oxidative metabolic detoxification. On this basis, the most effective [(trimethylsilyl)ethynyl]phenyl analogues as proinsecticides appear to be the *n*-propyl, *n*-butyl, and *tert*-butyl analogues, with the less efficient activation of the cyclohexyl analogue possibly due to unusually rapid detoxification as noted earlier with its analogue wherein the (trimethylsilyl)ethynyl group is replaced by a chloro substituent (Casida et al., 1985).

Table II. Mouse Intraperitoneal LD_{50} and Mouse/Housefly LD_{50} Ratios of 4-Alkyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]- and 4-Alkyl-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes $RC(CH_2O)_3CC_6H_4$-4-C≡CR′

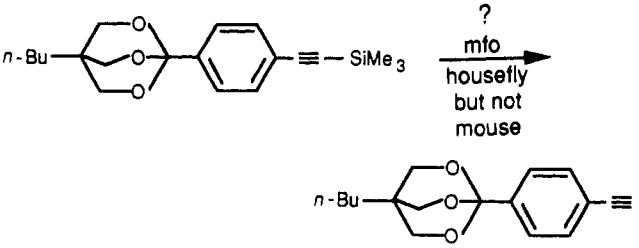
	mouse LD_{50}, mg/kg		LD_{50} ratio, mouse/housefly	
R	R′ = $SiMe_3$	R′ = H	R′ = $SiMe_3$	R′ = H
n-Pr	7[a]	0.92	13	1.3
n-Bu	>400	1.1	>930	4.6
t-Bu	3.2	0.11	4.6	1.2
c-Hx	125	0.17	1.8	0.29

[a] LD_{50} with PB is 3 mg/kg.

In contrast to the trioxabicyclooctanes considered above, the α-(trimethylsilyl)ethynyl analogue of deltamethrin (replacement of the α-cyano group with the (trimethylsilyl)ethynyl substituent) does not appear to be a proinsecticide, giving housefly LD_{50}s of 10 μg/g alone and 1 μg/g with PB.

Mouse Toxicity (Table II). The ethynylphenyl compounds are highly toxic to mice with LD_{50}s of 0.11–1.1 mg/kg relative to the [(trimethylsilyl)ethynyl]phenyl analogues with LD_{50}s of 3–>400 mg/kg. The toxicity of the *n*-propyl [(trimethylsilyl)ethynyl]phenyl compound is increased by about 2-fold on pretreatment of the mice with PB (a typical level of PB synergism in mice with compounds of this type; Casida et al., 1985), indicating that this derivative does not undergo oxidative activation as in houseflies but instead undergoes oxidative detoxification.

Selective Toxicity. The 4-*n*-butyl 1-[4-[(trimethylsilyl)ethynyl]phenyl] compound is the most selective trioxabicyclooctane reported. This selectivity may be rationalized on the basis of the following reaction:



This is the first example of the trimethylsilyl substituent in a proinsecticide and as the moiety conferring selective toxicity.

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Antimicrobial *Piper* Metabolite and Related Compounds

Muraleedharan G. Nair*,† and Basil A. Burke

Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568

4,5-Dimethoxy-2,3-(methylenedioxy)-1-allylbenzene, a natural isolate of *Piper hispidum* and *Piper aduncum*, is found to have strong antimicrobial activity. This natural product and three other related compounds, 4-(5′-hydroxy-5′-nonanyl)-1,2-(methylenedioxy)benzene, 4-(5′-non-4′-enyl)-1,2-(methylenedioxy)benzene, and 6-methoxy-2,3-(methylenedioxy)-4-allylphenol, were synthesized from piperonal and screened for their biological activity. All four compounds showed high levels of antifungal and antibacterial activity on various fungi and bacteria. In vivo experiments with wheat powdery mildew pathogen *Erysiphe graminis* gave excellent disease control at 100 ppm for most of the test compounds.

Extracts of *Piper* species (Piperaceae) have reportedly found widespread application in medicinal practices and have also been used against insects (Atal et al., 1967; Asprey and Thornton, 1976; Escobar, 1972) in several parts of the world, although the active compounds have not been specifically defined. We have previously reported the isolation and characterization of 4,5-dimethoxy-2,3-(methylenedioxy)allylbenzene, pseudodillapiole, from the Jamaican *Piper aduncum* and *Piper hispidum* (Burke and Nair, 1986). Tumor inhibitory compounds and lignins with (methylenedioxy)phenyl substituents were isolated from *Piper novae-hollandiae* and *Piper cubeba*, respectively (Loden et al., 1969; Prabhu and Mulchandani, 1985). Synergistic activity of several (methylenedioxy)phenyl compounds with pyrethrum insecticides is also known (Devakumar et al., 1985; Indian Patent, 1969). The reported use of *Piper* plants as insect repellants (Escobar, 1972) and isolation of (methylenedioxy)phenyl propenoids from the Jamaican species of *Piper* prompted us to investigate the biological activity of compounds 4–7. The natural product and its chemically related compounds, 4–7, were prepared and investigated for antifungal, antibacterial, insecticidal, and herbicidal activities.

EXPERIMENTAL SECTION

Instrumentation. UV–visible and IR absorption spectra were recorded on Perkin-Elmer Lambda 5 and Perkin-Elmer 1420 spectrophotometers, respectively. NMR spectra were obtained on a Varian XL-300 (300 MHz for ^1H and 75 MHz for ^{13}C). Mass spectrometry was carried out by a VG analytical 7070E spectrometer. Melting points were determined on a Kofler hot stage and are uncorrected.

Materials Used. For *Xanthomonas campestris* bacteria, the enriched nutrient medium (ENM) used was made by mixing 0.5% glucose and 1.5% commercial nutrient agar. The Wantanabe broth used for bacterial suspension was made up with 0.1% L-glutamic acid, 0.5% L-methionine, 0.3% NH_4HPO_4, 0.2% KH_2PO_4, 0.1% $MgCl_2 \cdot 6H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.000 075% $MnSO_4 \cdot H_2O$, and 0.5% sucrose, and the pH was adjusted to 6.5–7.0. The *Agrobacterium tumefaciens* was assayed in Luria–Bertani (LB) medium containing 1% trylone, 0.5% yeast extract, 1% NaCl, and 1% NaOH. *Rhizobium japonicum* was assayed in yeast–mannitol medium containing 0.05% NaCl, 0.01% yeast extract, 0.02% K_2HPO_4, 1% mannitol, and 0.2% concentrated salt solution (the concentrated salts were 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $FeCl_3$, 0.04 g of $CaCl_2$, 0.83 mL of HCl, and 99.0 mL of H_2O), and the pH was adjusted to 7.2. The medium for yeast contained 1% yeast extract, 2% bactopeptone, 25% adenine (1 mg/mL) by volume, 2% uracil (1 mg/mL) by volume, 2% agar, and 4% glucose (50%) by volume. The V-8 medium for fungi consisted of V-8 juice (200 mL), $CaCO_3$ (3.0 g), and agar (15.0 g) per 1000 mL of medium, and the pH was adjusted to 7.2. All the cultures were incubated at 27 °C for 3–7 days.

Determination of ED_{50} on Fungi. ED_{50} values for the fungi were calculated by determining inhibition of mycelial growth on solid nutrient medium (V-8 juice agar). A small plug of the desired fungus on solid nutrient agar was placed on solid nutrient agar previously incorporated with the compounds under investigation. The concentrations used were from 5 to 100 ppm. The inhibition of the mycelial growth was recorded at the end of 72 h.

Determination of ED_{50} on Bacteria and Yeast. Bacterial and yeast bioassay were carried out in their respective liquid nutrient mediums. The compound to be assayed was incor-

† Present address: 420 Plant and Soil Sciences Building, Michigan State University, East Lansing, MI 48824.

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