Silylated 1-(4-Ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes: Structural Features and Mechanisms of Proinsecticidal Action and Selective Toxicity†

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Proinsecticidal action and selective toxicity to houseflies vs mice are characteristic features for most of the 18 4-substituted 1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octanes and 13 silylated analogues examined. Optimal silylated derivatives are equal in insecticidal activity to the corresponding highly potent ethynyl compounds, and piperonyl butoxide (PB) strongly antagonizes their action. The carbon isostere (Me_3C vs Me_3Si) is inactive. Studies with a ^3H-labeled (trimethylsilyl)-ethynyl proinsecticide establish that it undergoes PB-sensitive desilylation and that the activation product is the ethynyl compound in houseflies and mice and their microsomal mixed-function oxidase systems. Desilylation is probably initiated by oxidation at the silicon atom rather than any carbon substituent. Receptor assays for the GABA-gated chloride channels of housefly head and mouse brain support other evidence that the selective toxicity of the (trimethylsilyl)ethynyl compounds is attributable to the much greater importance of their oxidative bioactivation in houseflies than in mice.

INTRODUCTION

2,6,7-Trioxabicyclo[2.2.2]octanes (TBOs) with appropriate 1- and 4-substituents are potent insecticides (Palmer and Casida, 1985). They are also quite toxic to mammals by virtue of their action as $GABA_A$ receptor antagonists (Casida et al., 1985) to block the GABA-gated chloride channel (Obata et al., 1988). The 1-(4-ethynylphenyl) substituent confers particularly high toxicity to insects and mammals and potency at the $GABA_A$ receptor (Palmer and Casida, 1989; Palmer et al., 1988). Improved selectivity for insects vs mammals might be achieved by target site or metabolic specificity. Structure–activity studies varying the 4-substituent in the 1-(4-ethynylphenyl) series indicate that perhaps only a moderate level of specificity may be involved at the receptor level (Palmer et al., 1991). An alternative approach to improved selectivity is the design and use of proinsecticides which undergo metabolic activation in insects more efficiently than in mammals. This proved to be successful with the 1-[4-[(trimethylsilyl)-ethynyl]phenyl]-TBOs, which have three interesting properties: their toxicity to houseflies may approach that of the corresponding ethynylphenyl analogues; the microsomal mixed-function oxidase (MFO) inhibitor piperonyl butoxide (PB) greatly reduces their toxicity to houseflies; they have decreased toxicity to mice (Palmer et al., 1990).

The 1-[4-[(trimethylsilyl)ethynyl]phenyl]-TBOs appear to be proinsecticides which undergo MFO-catalyzed metabolic activation in houseflies possibly by removing the trimethylsilyl group to give the corresponding ethynylphenyl compounds. If so, more rapid desilylation in houseflies than in mice might account for the improved selectivity (Palmer et al., 1990) (Figure 1). The present study uses four approaches to (a) further investigate the structural features and mechanisms of proinsecticidal action and selective toxicity and (b) identify the activation product. First, the ethynylphenyl compounds are compared with their trimethylsilyl derivatives for a series with 18 different 4-substituents. Second, other silyl protective groups and similar moieties are examined. Third, metabolism of a representative [(trimethylsilyl)ethynyl]phenyl compound, radiolabeled with tritium, is studied in houseflies and mice and their microsomal MFO systems, and the metabolites are identified. Fourth, receptor assays involving radioligands for the GABA-gated chloride channels of housefly head and mouse brain are used in conjunction with toxicity studies to differentiate direct-acting compounds from those undergoing bioactivation.

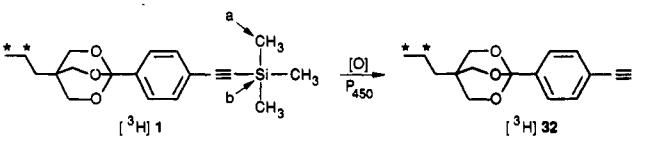

Figure 1. Metabolic conversion of the proinsecticide 4-[^3H]-*n*-propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-TBO ([^3H]-1) to its more potent ethynyl analogue ([^3H]-32). Desilylation involves oxidation at carbon (a) or silicon (b) probably catalyzed by cytochrome P450.

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 deuterochloroform. Mass spectrometry (MS) utilized the Hewlett-Packard 5985 system with chemical ionization (CI) (230 eV with methane at 0.8 Torr).

Chemicals. Compounds **1**, **2**, **10**, **12**, and **32** have been described previously (Palmer and Casida, 1989; Palmer et al., 1990) as has [^3H]-1 (58.4 Ci/mmol) (Palmer and Casida, 1991). The new silylated 1-(4-ethynylphenyl)-TBOs and related compounds (Table I) were prepared by two general methods (Figure 2). Method A, involving palladium-catalyzed coupling of the appropriate 1-(4-iodophenyl)-TBO with (trimethylsilyl)acetylene as described by Palmer and Casida (1989), was used to synthesize **3**–**9**, **11**, and **13**–**18**. An analogous procedure gave **33** from 3,3-dimethylbut-1-yne. Compounds **19**–**31** were prepared by using method B, involving silylation of **32** (Palmer and Casida,

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Table I. Characterization of 4-(Alkyl or Aryl)-1-[(4-silylated-ethynyl)phenyl]-TBOs and Related Compounds

no.	R or R′ substituent[a]	mp, °C	CI-MS $[M+1]^+$	$C(CH_2O)_3$ (6 H, s)	NMR ($CDCl_3$),[b] ppm R or R′ substituent
	$RC(CH_2O)_3CC_6H_4$-4-C≡$CSiMe_3$				
3	*n*-Pen	172–173	359	4.10	0.90 (3 H, t, CH_3CH_2), 1.25 (8 H, m, $(CH_2)_4$)
4	*n*-Hex	152–153	373	4.08	0.88 (3 H, t, CH_3), 1.20–1.30 (10 H, m, $(CH_2)_5$)
5	*c*-Pr	189–191	329	4.02	0.25 (2 H, m, *CHCH*), 0.42 (2 H, m, *CHCH*), 0.55 (1 H, m, $CH(CH_2)_2$)
6	1-Me-*c*-Pr	235–237	343	4.07	0.20 (2 H, m, *CHCH*), 0.48 (2 H, m, *CHCH*), 1.00 (3 H, s, CH_3C)
7	*trans*-2-Me-*c*-Pr	139–141	343	4.00	0.20 (2 H, m, CH_2CH), 0.42 (1 H, m, $CHCHCH_3$), 0.68 (1 H, m, CH_3CH), 1.02 (3 H, d, CH_3CH)
8	*c*-Bu	174–176	343	4.08	1.70–1.90 (6 H, m, $(CH_2)_3$), 2.25 (1 H, m, $CHCH_2$)
9	*c*-Pen	188–190	357	4.10	1.25–1.60 (9 H, m, $(CH_2)_4CH$)
11	*i*-Pr	204–205	331	4.10	0.90 (6 H, d, $(CH_3)_2CH$), 1.60 (1 H, m, $CH(CH_3)_2$)
13	s-Bu	157–159	345	4.12	0.87 (3 H, d, CH_3CH), 0.90 (3 H, t, CH_3CH_2), 1.00 (1 H, m, $CHCH_3$), 1.25 and 1.50 (each 1 H, m, CH_2CH)
14	3-Me-Bu		359	4.08	0.88 (6 H, d, $(CH_3)_2CH$), 1.00–1.30 (4 H, m, CH_2CH_2), 1.40–1.50 (1 H, m, $(CH_3)_2CH$)
15	prop-2-enyl	163–164	329	4.10	2.00 (2 H, d, CH_2CH), 5.12 (2 H, m, CH_2=CH), 5.65 (1 H, m, CH_2=CH)
16	Ph	220–222	365	4.48	7.20 (2 H, m, aromatic), 7.35 (3 H, m, aromatic)
17	3-F-Ph	232–234	383	4.45	6.88 (1 H, dt, aromatic), 6.98 (1 H, d, aromatic), 7.02 (1 H, dt, aromatic), 7.38 (1 H, dt, aromatic)
18	4-F-Ph	266–268	383	4.45	7.05–7.20 (4 H, m, aromatic)
	n-$PrC(CH_2O)_3CC_6H_4$-4-C≡CR′ R′ = $SiR_1R_2R_3$				
19	$SiMe_2H$	114–116	317	4.10	0.30 (6 H, d, $(CH_3)_2SiH$), 4.25 (1 H, m, Si*H*)
20	$SiMe_2Et$	121–122	345	4.10	0.20 (6 H, s, $(CH_3)_2Si$), 0.65 (2 H, q, CH_2Si), 1.00 (3 H, t, CH_3CH_2Si)
21	$SiMe_2$(*i*-Pr)	106–107	359	4.10	0.18 (6 H, s, $(CH_3)_2Si$), 0.88 (1 H, m, $SiCHCH_3$), 1.05 (6 H, d, $(CH_3)_2CH$)
22	$SiMe_2$(*t*-Bu)	140	373	4.10	0.17 (6 H, s, $(CH_3)_2Si$), 1.00 (9 H, s, $(CH_3)_3C$)
23	$SiMe_2$(3-Me-*n*-Bu)	86	387	4.08	0.20 (6 H, s, $(CH_3)_2Si$), 0.67 (2 H, m, CH_2Si), 0.89 (6 H, d, $(CH_3)_2CH$), 1.20–1.30 (2 H, m, $SiCH_2CH_2$), 1.50 (1 H, m, $(CH_3)_2CH$)
24	$SiMe_2$(prop-2-enyl)	100–101	357	4.10	0.21 (6 H, s, $(CH_3)_2Si$), 1.70 (2 H, m, $SiCH_2CH$), 4.90 (2 H, m, CH_2=CH), 5.87 (1 H, m, CH_2=CH)
25	$SiMe_2[(CH_2)_3CN]$	118–119	384	4.10	0.22 (6 H, s, $(CH_3)_2Si$), 0.82 (2 H, m, CH_2Si), 1.75–1.85 (2 H, m, CH_2CH_2CN), 2.41 (2 H, t, CH_2CN)
26	$SiMe_2(CH_2Cl)$	138	365	4.10	0.35 (6 H, s, $(CH_3)_2Si$), 2.93 (2 H, s, CH_2Cl)
27	$SiMe_2(CH_2Ph)$	112–113	407	4.10	0.20 (6 H, s, $(CH_3)_2Si$), 2.27 (2 H, s, $PhCH_2Si$), 7.05–7.25 (5 H, m, aromatic)
28	$SiMe_2Ph$	113–114	373	4.10	0.48 (6 H, s, $(CH_3)_2Si$), 7.35 (3 H, m, aromatic), 7.65 (2 H, m, aromatic)
29	$SiMePh_2$	123–124	455	4.10	0.77 (3 H, s, CH_3Si), 7.35–7.72 (10 H, m, aromatic)
30	$SiEt_3$	98–99	373	4.10	0.65 (6 H, q, $(CH_2)_3Si$), 1.02 (9 H, t, $(CH_3CH_2)_3Si$)
31	$Si(n\text{-}Pr)_3$	96	415	4.10	0.60–0.70 (6 H, m, $(CH_2)_3Si$), 1.00 (9 H, t, $Si(CH_2CH_2CH_3)_3$), 1.40–1.50 (6 H, m, $(CH_2CH_2CH_3)_3$
	R′ = other substituents				
33	CMe_3	163–164	315	4.10	1.30 (9 H, s, $(CH_3)_3C$)
34	$GeMe_3$	174–176	373	4.10	0.40 (9 H, s, $(CH_3)_3Ge$)
35	$SnMe_3$	88–90	419	4.10	0.35 (9 H, s, $(CH_3)_3Sn$)
36	$Si(CD_3)_3$	174–176		4.08	

[a] Compounds 1, 2, 10, and 12 with *n*-Pr, *n*-Bu, *c*-Hex, and *t*-Bu substituents, respectively, and compound 32 are reported by Palmer and Casida (1989) and Palmer et al. (1990). [b] Additional signals in common for compounds 3–9, 11, and 13–18 are 0.22 (9 H, s, $(CH_3)_3Si$) and 7.43 and 7.55 (each 2 H, AA′BB′, aromatic) and for compounds 19–36 are 0.90 (3 H, t, CH_3CH_2) and 1.25 (4 H, m, CH_2CH_2) and 7.43 and 7.55 (each 2 H, AA′BB′, aromatic).

1989) with *n*-butyllithium and the appropriate silyl chloride. For example, to a stirred solution of **32** (1 mmol) in dry tetrahydrofuran (25 mL) at −78 °C under nitrogen atmosphere was added *n*-butyllithium (1.2 mmol of 2 M solution in hexane). After the solution was stirred for 30 min, the appropriate silyl chloride (1.2 mmol) was added. The solution was allowed to warm to room temperature and then evaporated, and the residue was partitioned between ether and water. The organic layer was dried (magnesium sulfate) and evaporated to obtain the silylated derivatives of **32**. Compounds **34–36** were obtained in an analogous manner from chlorotrimethylgermane, chlorotrimethyltin, and *N*-(trimethyl-d_9-silyl)imidazole, respectively.

To prepare candidate metabolites, **1** and **32** were converted to their corresponding ring-opened derivatives by dissolving the TBO (10 mg) in methylene chloride (5 mL) to which was added 6 N aqueous hydrochloric acid (10 μL). After the solution was stirred for 2 h at 25 °C, partial evaporation and cooling afforded the white crystalline esters in ~90% yield. 4-*n*-$PrC(CH_2OH)_2CH_2OCOPh$-4-C≡$CSiMe_3$ (ring-opened **1**): mp 80–81 °C; $[M+1]^+$ 349; NMR δ 0.25 [9 H, s, $(CH_3)_3Si$], 0.92 (3 H, t, CH_3CH_2), 1.25–1.40 (4 H, m, $CH_3CH_2CH_2$), 2.70–2.85 (2 H, br s, OH), 3.65 (4 H, AB, 2 × CH_2OH), 4.55 (2 H, s, CH_2OCO), 7.52 and 7.95 (each 2 H, AA′BB′, aromatic). 4-*n*-$PrC(CH_2OH)_2CH_2OCOPh$-4-C≡CH (ring-opened **32**): mp 76–77 °C; $[M+1]^+$ 277; NMR δ 0.92 (3 H, t, CH_3CH_2), 1.25–1.40 (4 H, m, $CH_3CH_2CH_2$), 2.75–2.90 (2 H, br s, OH), 3.25 (1 H, s, C≡CH), 3.65 (4 H, AB, 2 × CH_2OH), 4.55 (2 H, s, CH_2OCO), 7.55 and 7.95 (each 2 H, AA′BB′, aromatic).

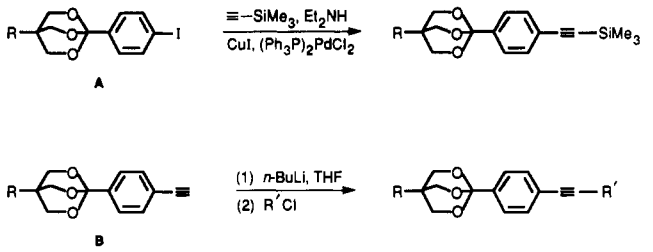

Figure 2. Two methods of synthesis for 1-[4-[(trimethylsilyl)-ethynyl]phenyl]-TBOs and related compounds. Detailed syntheses are given in the text for methods A and B.

Bioassays. The procedures of Palmer et al. (1991) were used for LD_{50} determinations. Adult female houseflies (*Musca domestica* L.) were held 24 h at 25 °C after application of the test compound to the ventrum of the abdomen. Synergism or antagonism was evaluated for flies pretreated with PB at 250 μg/g 2 h before the toxicant was administered. Mouse LD_{50}s were determined 24 h after intraperitoneal (ip) administration of the TBOs to albino Swiss-Webster males (18–22 g) with methoxytriglycol (MTG) (50 μL) as the carrier vehicle. In some cases the mice were pretreated ip with PB at 150 mg/kg (administered in 25 μL of MTG) 1 h before the toxicant.

Receptor and Enzyme Preparations. Receptor assays used EDTA/water-dialyzed mouse brain P_2 membranes (Squires et al., 1983). Mouse liver microsomes were prepared and washed in 100 mM sodium phosphate, pH 7.4, buffer and stored frozen (Cole et al., 1991). Housefly microsomes were obtained from thoraces plus abdomens by homogenization and centrifugation as with the liver microsomes, but they were always used freshly prepared. Protein was determined according to the method of Bradford (1976).

Determination of Receptor Potency. *tert*-Butylbicyclophosphorothionate (TBPS) assays for the GABA-gated chloride channel (Squires et al., 1983) followed the procedure of Cole and Casida (1986). The test compounds at various concentrations added in dimethyl sulfoxide (5 μL) and [^{35}S]TBPS at 2 nM were incubated with the receptor preparation (200 μg of protein) in 200 mM sodium chloride/50 mM sodium phosphate, pH 7.4, buffer (1 mL) for 30 min at 37 °C. Nonspecific binding was determined by addition of 2 μM unlabeled TBPS.

Recognition of Metabolic Activation by Coupled Microsomal MFO/Brain Membrane TBPS Binding Site Assay. The effect of oxidative metabolism on inhibitor potency was determined as described by Casida et al. (1985), involving fortification of the mouse brain receptor [^{35}S]TBPS binding assay with mouse liver or housefly microsomes (25 and 200 μg of protein, respectively) and NADPH (2 μmol for the oxidase system and 0 μmol for the control). TBO analogues were used at 1–100 nM in comparison with aldrin (100 nM) as a standard. Controls were included to correct for ~11% higher background binding with NADPH, attributable to phosphorothionate oxidation and binding of the ^{35}S liberated from [^{35}S]TBPS.

Metabolites of 4-[^{3}H]-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-TBO. Metabolites from MFO systems were generated in standard incubation mixtures consisting of the microsomal protein (500 μg for mouse, 1000 μg for housefly) and NADPH in 100 mM sodium phosphate, pH 7.4, buffer (1.2 mL) to which was added the substrate in acetone (2 μL) to give final concentrations of 0 or 1 mM NADPH and 22 μM [^{3}H]-1. Following incubation with shaking in air for 60 min at 37 °C and cooling on ice, the mixtures were saturated with NaCl (~0.4 g) and extracted with diethyl ether (1 mL × 2) (at ~5 °C in the case of housefly microsomal mixtures to minimize emulsion formation). For in vivo studies houseflies were treated topically as above with acetone or acetone containing PB (5 μg/fly), and after 75 min, they received [^{3}H]-1 (0.1 μg/fly). At various periods (0, 0.5, 1, and 3 h) these flies in groups of 10 were rinsed with acetone (2 mL × 2) (to remove unpenetrated material) and then homogenized in acetone [2 mL containing 0.1% triethylamine (TEA) to prevent acid-catalyzed ring opening]. On a similar basis, mice were pretreated ip with PB (136 mg/kg) in MTG (25 μL) or with MTG only. One hour later they received [^{3}H]-1 (1 mg/kg), and after an additional 1 h, the liver was removed and homogenized in acetone (5 mL containing 0.1% TEA). Following quantitation by liquid scintillation counting, the ether and acetone extracts were evaporated under nitrogen for chromatography.

Quantitation and identification of individual metabolites involved thin-layer chromatography (TLC) and cochromatography using UV visualization of the authentic standards and radioautography to detect the ^{3}H-labeled metabolites. Silica gel 60 F_{254} chromatoplates (0.25 mm gel thickness) were pretreated by first developing with hexane/acetone (7:2 + 0.07% TEA), and then the origin areas of the plates were prespotted with 1% aqueous sodium hydroxide and allowed to dry prior to spotting the silylated compounds to minimize their decomposition on the chromatoplate. The solvent systems of hexane/acetone (7:2 + 0.07% TEA) and toluene/ethyl acetate/methanol (15:5:1 + 0.07% TEA) provided separation of the relevant compounds, i.e., 1 R_f 0.47 and 0.71, 32 R_f 0.34 and 0.66, ring-opened 1 R_f 0.14 and 0.30, and ring-opened 32 R_f 0.09 and 0.26, respectively.

Analysis of Formaldehyde as a Metabolite. Eight substrates, each at 78 nmol, were examined for possible formaldehyde liberation on incubation with mouse liver microsomes (0 or 250 μg of protein) and NADPH (0 or 1 μmol) in 100 mM sodium phosphate, pH 7.4, buffer (1.2 mL) for 1 h at 37 °C. Formaldehyde was analyzed by gas chromatography with a flame ionization detector after conversion to the 2,4-dinitrophenylhydrazone (Jacobsen et al., 1991). MFO-dependent formaldehyde liberation was considered to be that amount formed in the presence of both microsomes and NADPH but not in the absence of either component.

Table II. Biological Activity of 4-Substituted-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octanes

		housefly			mouse	
		LD_{50}, μg/g (R′ = $SiMe_3$)[a]		LD_{50} ratio (alone) (R′ = $SiMe_3$/R′ = H)[c]		
no.	R[a]	alone	with PB[b]		TBPS receptor IC_{50}, nM	LD_{50}, mg/kg
1	*n*-Pr	0.53	102	0.8	65	7[d]
2	*n*-Bu	0.43	124	1.8	1260	>400
3	*n*-Pen	14	>500	6.4	>10000	>75
4	*n*-Hex	>500	>500	>5.9	>10000	>250
5	*c*-Pr	0.63	420	1.3	47	16
6	1-Me-*c*-Pr	1.1	>500	1.8	117	43
7	*trans*-2-Me-*c*-Pr	0.53	>500	0.8	92	29
8	*c*-Bu	0.63	125	1.1	85	16
9	*c*-Pen	2.6	>500	2.6	311	5
10	*c*-Hex	68	>500	128	3300	125
11	*i*-Pr	5.0	63	5.3	34	5.5
12	*t*-Bu	0.7	>500	7.8	223	3.2
13	*s*-Bu	1.7	43	1.1	116	2.5
14	3-Me-Bu	3.1	370	1.0	5000	NT[e]
15	prop-2-enyl	5.0	105	1.6	538	>250
16	Ph	0.37	>500	1.2	2600	18
17	3-F-Ph	0.22	>500	1.5	74	25
18	4-F-Ph	0.58	>500	5.9	163	12.5

[a] $RC(CH_2O)_3CPh$-4-C≡CR′. [b] Preliminary observations indicate that with some compounds the LD_{50} decreases between 24 and 48 h. [c] Data for R′ = H from Palmer et al. (1991). [d] 3 mg/kg with PB. [e] Not tested.

RESULTS

Effect of 4-Substituent on the Insecticidal Activity of 1-[4-[(Trimethylsilyl)ethynyl]phenyl]-TBOs Relative to Their 1-(4-Ethynylphenyl) Analogues (Table II). Housefly LD_{50}s are below 1 μg/g for 9 of the 18 C≡C$SiMe_3$ compounds examined. In most cases the C≡C$SiMe_3$ derivative is almost equitoxic with the corresponding C≡CH analogue (LD_{50} ratios R′ = $SiMe_3$/R′ = H of 0.8–1.8). Only the *c*-Hex compound shows >8-fold lower toxicity for the $SiMe_3$ derivative. PB almost completely blocks the activity of the $SiMe_3$-derivatized compounds at 24 h.

Table III. Biological Activity of 4-*n*-Propyl-1-[4-[(substituted)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octanes

		housefly			mouse	
		LD_{50}, μg/g				
no.	R′[a]	alone	with PB	factor of antagonism	TBPS receptor IC_{50}, nM	LD_{50}, mg/kg
	R′ = $SiR_1R_2R_3$					
19	$SiMe_2H$	0.63	0.55	0.87	4	1.8
1	$SiMe_3$	0.53	102	192	65	7
20	$SiMe_2Et$	0.65	4.6	7.1	369	20
21	$SiMe_2$(*i*-Pr)	1.25	13	10	7400	12
22	$SiMe_2$(*t*-Bu)	3.2	23	7.2	625	11
23	$SiMe_2$(3-Me-*n*-Bu)	8	29	3.6	>10000	NT[d]
24	$SiMe_2$(prop-2-enyl)	1.3	7	5.4	90	12
25	$SiMe_2[(CH_2)_3CN]$	1.7	20	12	43	1.7
26	$SiMe_2(CH_2Cl)$	4.0	0.9	0.2	10	50
27	$SiMe_2(CH_2Ph)$	3.0	17	6	>10000	225
28	$SiMe_2Ph$	360	90	0.25	104	18
29	$SiMePh_2$	>500	63	<0.13	600	100
30	$SiEt_3$	1.3	10	7.7	4200	25
31	Si(*n*-Pr)$_3$	>500	>500		>10000	>33
	R′ = other substituents					
32	H	0.68	0.023	0.03	2	0.92
33	CMe_3	>500	>500		>10000	>250
34	$GeMe_3$	2.5	32	13	>10000	80
35	$SnMe_3$[b]	1.8	0.06	0.03	3	NT
36	$Si(CD_3)_3$	1.0[c]	5.5[c]	5.5	NT	NT

[a] 4-*n*-PrC$(CH_2O)_3$CPh-4-C≡CR′. [b] Chemically unstable, slowly reverting to **32**. [c] Comparable values for 1 (R′ = $SiMe_3$) as a control in the same experiment were 0.95 and 85 alone and with PB, respectively. [d] Not tested.

Effect of Various Silyl and Other Groups on the Insecticidal Activity of 4-*n*-Propyl-1-[4-[(substituted)ethynyl]phenyl]-TBOs (Table III). The unsynergized toxicity to houseflies decreases with various silyl derivatives as follows (R′ = $SiR_1R_2R_3$ or R_3 substituent of $SiMe_2R_3$): H, Me, Et > *i*-Pr, prop-2-enyl, $(CH_2)_3CN$, $SiEt_3$ > *t*-Bu, CH_2Cl, CH_2Ph > 3-Me-*n*-Bu ≫ Ph, $SiMePh_2$, Si(*n*-Pr)$_3$. PB reduces the toxicity of all the silylated ethynylphenyl derivatives except the $SiMe_2H$, $SiMe_2(CH_2Cl)$, $SiMe_2Ph$, and $SiMePh_2$ derivatives, which are increased somewhat in potency by PB but much less so than the ethynylphenyl compound.

Replacement of $SiMe_3$ with CMe_3 destroys activity both with and without PB. The $GeMe_3$ compound is a proinsecticide but less effective than its $SiMe_3$ counterpart. The substituent $SnMe_3$ does not appear to confer proinsecticidal activity, and this compound is similar to the ethynylphenyl in potency and PB effect. The $Si(CD_3)_3$ compound is essentially identical with the $SiMe_3$ compound in unsynergized toxicity, although it is reduced 15-fold in PB-synergized toxicity.

Effect of 4-Substituent on the Toxicity to Mice of 1-[4-[(Trimethylsilyl)ethynyl]phenyl]-TBOs Relative to Their Ethynylphenyl Analogues (Table II). All of the compounds are of reduced mammalian toxicity relative to their ethynylphenyl analogues [see Palmer et al. (1991)]. PB increases the toxicity to mice in the one case examined in contrast to always reducing it to houseflies. While several compounds are of quite low toxicity to mice, three in particular show very good selectivity ratios, i.e., >930 for *n*-Bu, 114 for 3-F-Ph, and 55 for *trans*-2-Me-*c*-Pr. This is in contrast to *n*-Pr and *t*-Bu which have very little selectivity.

Effect of Various Silyl and Other Groups on the Toxicity to Mice of 4-*n*-Propyl-1-[4-[(substituted)-ethynyl]phenyl-TBOs (Table III). Each of the substituents examined reduces the toxicity of **32** to mice by 1.9-fold for $SiMe_2H$ and $SiMe_2[(CH_2)_3CN]$ to 245-fold for $SiMe_2(CH_2Ph)$. Of the potent insecticides, selective toxicity to houseflies vs mice of 19–75-fold is conferred by $SiMe_2Et$, $SiMe_2(CH_2Ph)$, $SiEt_3$, and $GeMe_3$ substituents vs 13-fold for $SiMe_3$.

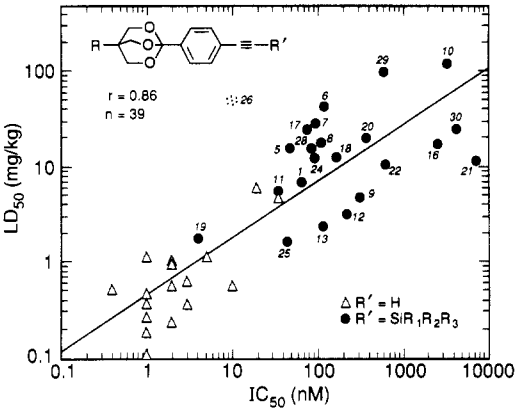

Figure 3. Relationship between potency for 1-(4-ethynylphenyl)-TBOs (R′ = H) and their silylated derivatives (R′ = $SiR_1R_2R_3$) as inhibitors of the TBPS binding site and as toxicants to mice. Compound **26** is not included in the correlation coefficient.

Table IV. Metabolic Activation of 4-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane in the Mouse Liver and Housefly Microsomal Mixed-Function Oxidase Systems

4-*n*-PrC$(CH_2O)_3$-CPh-4-C≡CR′ R′ substituent	microsome source	substrate, nM	inhibition of [^{35}S]TBPS binding,[a,b] % control	% oxidase
$SiMe_3$ (1)	mouse	30	13 ± 4	39 ■ 7
	housefly	30	23 ■ 5	38 ■ 5
$SiMe_2H$ (19)	mouse	3	32 ± 9	28 ± 4
	housefly	3	38 ± 7	42 ± 4
H (32)	mouse	3	79 ± 3	74 ± 3
	housefly	3	64 ± 6	60 ± 7
H (32)	mouse	1	34 ± 9	35 ■ 4
	housefly	1	31 ± 6	38 ± 6

[a] Aldrin examined as a standard at 100 nM showed the expected activation (Casida et al., 1985), i.e., 5 ■ 2 and 4 ± 2% inhibition for the control with mouse and housefly microsomes, respectively; 40 ± 8 and 34 ■ 2% for the oxidase with mouse and housefly microsomes, respectively. [b] Comparable values for 4-PhC$(CH_2O)_3$CPh-4-C≡CSiMe$_3$ as a substrate at 100 nM with mouse and housefly microsomes (200 μg of protein) are 0 and 15% inhibition for controls vs 33 and 25% inhibition for the oxidase, respectively.

Relation between Potency at the TBPS Receptor and Toxicity to Mice (Tables II and III, Figure 3). Potency in the TBPS receptor assay is a good predictor of toxicity to mice (r = 0.86, n = 39) for both the ethynylphenyl compounds and their silylated derivatives. One compound that deviates from the correlation line is the $SiMe_2(CH_2Cl)$ derivative.

Metabolic Activation of 4-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-TBO in the Mouse Liver and Housefly Microsomal MFO Systems (Table IV). The MFO systems of mouse liver and houseflies strongly activate **1** relative to its inhibition of [^{35}S]TBPS binding. These oxidases have little or no effect on the potency of **19** or **32**, which were tested at lower concentrations because of their higher innate potency. These findings suggest an MFO-dependent activation of the C≡CSiMe$_3$ compound **19** in forming the C≡CH compound **32**, and similar activation was observed for 4-PhC$(CH_2O)_3$CPh-4-

Table V. Metabolic Desilylation of 4-[^{3}H]-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane in the Mouse and Housefly Microsomal Mixed-Function Oxidase Systems

	substrate and products,[a] %					
	mouse			housefly		
compound or fraction	buffer + NADPH	microsome	microsome + NADPH	buffer + NADPH	microsome[b]	microsome[b] + NADPH
(trimethylsilyl)ethynylphenyl						
1	20	18, 23	20, 20	46	39, 33	21, 23
ring-opened 1[c]	47	58, 54	30, 34	28	25, 27	16, 14
ethynylphenyl						
32	3	1, 1	7, 9	2	2, 3	13, 14
ring-opened **32**[d]	7	3, 3	9, 7	7	5, 7	21, 23
others						
unknowns[e]	17	9, 5	12, 8	11	17, 16	11, 11
origins	6	11, 14	22, 22	6	12, 14	18, 15

[a] Each value is from a separate experiment. Similar relationships were observed in each of two other experiments. [b] Corrected for incomplete recoveries (31% with microsomes and 46% with microsomes and NADPH) which are probably attributable to inefficient extraction at ~5 °C to minimize emulsion formation. [c] 4-*n*-PrC(CH_2OH)$_2$$CH_2$OCOPh-4-C≡$CSiMe_3$. [d] 4-*n*-PrC($CH_2OH$)$_2$$CH_2$OCOPh-4-C≡CH. [e] Intermediate R_f values not cochromatographing with standards.

Table VI. Metabolic Desilylation of 4-[^{3}H]-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-2,6,7-trioxabicyclo[2.2.2]octane in Houseflies and Mice and Effect of Piperonyl Butoxide

			substrate and products,[a] %				
			(trimethylsilyl)ethynylphenyl		ethynylphenyl		
species	time, h	PB	1	ring-opened 1[b]	**32**	ring-opened **32**[b]	other
fly[c]	0	−	2.1, 1.7	0.2, 0.4	0.1, 0.1	0.0, 0.0	0.3, 0.0
		+	1.8, 1.4	0.4, 0.1	0.1, 0.1	0.0, 0.0	0.2, 0.2
	0.5	−	4.6, 6.5	1.0, 1.0	0.8, 1.2	0.3, 0.1	1.4, 1.4
		+	6.0, 7.6	1.1, 0.9	0.4, 0.2	0.1, 0.1	1.1, 1.3
	1	−	6.0, 6.3	1.1, 1.2	1.1, 1.5	0.3, 0.2	2.8, 3.2
		+	6.8, 8.7	1.1, 1.4	0.1, 0.4	0.1, 0.1	0.1, 0.1
	3	−	8.8, 8.7	1.5, 2.3	4.2, 5.6	1.1, 2.1	8.5, 7.4
		+	21.9, 22.8	3.2, 3.5	1.0, 0.8	0.3, 0.3	2.9, 2.0
mouse liver	1	−	0.5, 0.7	0.3, 0.3	0.3, 0.4	0.1, 0.4	1.7, 1.3
		+	0.9, 1.1	0.2, 0.2	0.2, 0.2	0.1, 0.0	0.9, 0.8

[a] Each value is from a separate experiment. [b] See Table V. [c] Penetrated substrate and products as percent of applied dose. The remaining material was removed in a surface rinse of the houseflies.

C≡$CSiMe_3$. The findings are inconclusive on whether the C≡$CSiMe_2H$ compound **19** acts directly or after desilylation.

Metabolic Desilylation of 4-[^{3}H]-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-TBO in the Mouse Liver and Housefly Microsomal MFO Systems (Table V). [^{3}H]-1 yields three primary metabolites and degradation products, each identified by TLC cochromatography in two different solvent systems, i.e., **32** and the ring-opened derivatives of 1 and **32**. Fortification of the mouse or housefly microsomes with NADPH increases the loss of [(trimethylsilyl)ethynyl]phenyl derivatives and the recovery of ethynylphenyl compounds. The highest yields of **32** and its ring-opened derivative appear in the housefly MFO system; i.e., they are formed in larger amounts when the microsomes are fortified with NADPH (37 and 34% in two separate experiments) than without NADPH (7 and 10%). This relationship is also clear for the NADPH-dependent formation of **32** and ring-opened **32** by mouse microsomes, i.e., 16 and 16% yields in two experiments with NADPH vs 4 and 4% for controls.

Metabolic Desilylation of 4-[^{3}H]-*n*-Propyl-1-[4-[(trimethylsilyl)ethynyl]phenyl]-TBO in Houseflies and Mice and Effect of Piperonyl Butoxide (Table VI). Houseflies metabolize topically applied [^{3}H]-1 to a single major metabolite recovered on extraction which cochromatographs with [^{3}H]-**32**. About one-fourth of the desilylated compound is detected as ring-opened **32** as is also the case for [^{3}H]-1 itself. The amount of ring-opened product is not time dependent and is not altered by PB and is therefore considered to be an artifact of analysis rather than a metabolite. PB decreases the amount of desilylation to 25 ± 4% (SE, n = 6) of that without PB, and the penetrated and desilylated materials in two experiments without PB account for 5.3 and 7.7% of the dose after 3 h.

The liver of mice treated ip with [^{3}H]-1 contains the administered compound and [^{3}H]-**32** with almost equal amounts of their ring-opened derivatives. When expressed relative to the administered dose, the products in the liver at 1 h are 0.6% 1, 0.3% ring-opened 1, 0.4% **32**, 0.3% ring-opened **32**, and 1.5% other metabolites. With PB, the comparable values are 1.0, 0.2, 0.2, 0.1, and 1.0%, respectively. The desilylation reaction is definitely inhibited by PB, and the ring opening also appears to be PB sensitive [see also Deng et al. (1990b)].

Formaldehyde as a Possible Metabolite of [(Trimethylsilyl)ethynyl]phenyl Compounds. No formaldehyde is detected (i.e., <1% yield) as a mouse liver microsome- and NADPH-dependent metabolite of any one of 1, 19, **32**, $PhSiMe_3$, or $PhSiMe_2H$. These compounds therefore are not or are poor formaldehyde progenitors relative to others examined in the same system (>10% conversion to formaldehyde), i.e., N-demethylation of diuron and O-demethylation of *p*-nitroanisole and alachlor (Jacobson et al., 1991).

DISCUSSION

Silylated 1-(4-ethynylphenyl)-TBOs are a novel type of proinsecticide (Palmer et al., 1990). The $SiMe_3$ derivatives are similar in toxicity to houseflies to their ethynylphenyl

analogues in the majority of 18 comparisons made by varying the 4-substituent. PB partially or completely blocks the insecticidal activity of essentially all of the $SiMe_3$ derivatives, indicating that they undergo P450-mediated oxidative bioactivation. The 4-*c*-Hex compound is the principal exception with low activity as the $SiMe_3$ derivative possibly due to more rapid detoxification at the metabolically sensitive *c*-Hex substituent (Cole et al., 1991) than activation by oxidative desilylation. Other trialkylsilyl substituents (e.g., those of compounds **20**, **21**, **24**, **25**, and **30**) approach the effectiveness of $SiMe_3$ in conferring proinsecticidal activity which is presumably associated with good chemical stability and yet facile metabolic desilylation. The high potency of the $SiMe_2H$ and $SiMe_2(CH_2Cl)$ derivatives is apparently not blocked by PB; their toxicity is probably not dependent on oxidative bioactivation. The moderate activity of **20–25**, **27**, **30**, **34**, and **36** with PB may be due to only partial inhibition of their oxidative bioactivation or to trace levels (<0.5%) of the very potent **32** as an impurity. In contrast, substituents such as $SiMe_2Ph$ and $SiMePh_2$ practically destroy the insecticidal activity and therefore these analogues do not undergo oxidative activation in houseflies. The insecticidal activity ultimately results from the ethynyl compounds as established by studies with [^{3}H]-**1** showing PB-sensitive conversion to [^{3}H]-**32** by the housefly MFO system and by houseflies in vivo.

Replacement of silicon by other members of the group IV element series establishes the importance of the atom attached directly to the ethynyl function in conferring proinsecticidal activity. $GeMe_3$ instead of $SiMe_3$ only slightly reduces the proinsecticidal activity, suggesting similar efficient biooxidative cleavage. The $SnMe_3$ compound does not appear to be a proinsecticide, probably due to rapid chemical hydrolysis to the ethynyl compound. The $C{\equiv}CCMe_3$ analogue is totally inactive either alone or with PB, establishing that it is not metabolized to the corresponding C≡CH compound and supporting the view that the isosteric $C{\equiv}CSiMe_3$ derivative is intrinsically inactive.

The $SiMe_3$ derivatives are proantagonists for the GABA receptor/ionophore of insects and mammals. They have greatly reduced potency relative to the ethynylphenyl-TBOs in the [^{3}H]-**32** binding assay with housefly head (IC_{50} 2.6 nM for **32** and 125 nM for **1**) (Deng et al., 1990a) and in the TBPS binding assay with mouse brain. They are activated in the coupled housefly or mouse liver MFO/mouse brain TBPS receptor assay. PB greatly antagonizes the toxicity of **1** in houseflies but slightly synergizes it in mice. In contrast to houseflies, in mice the silylated ethynylphenyl compounds appear to act directly rather than after desilylation. Thus, the potency of both the silylated ethynylphenyl and the ethynylphenyl compounds in the TBPS receptor assay is a good predictor of their toxicity to mice ($r = 0.86$, $n = 39$) (Figure 3), and the compounds fit on the same correlation line indicating that the silylated derivatives are not bioactivated in mice to increase their toxicity. However, the bioactivation process appears to be toxicologically significant in houseflies. An apparent exception is the $SiMe_2(CH_2Cl)$ compound, which does not fit the correlation line and is not a proinsecticide in houseflies. It is not clear whether the activity of the $SiMe_2H$ derivative in houseflies and mice is dependent on desilylation or if it acts directly.

The $SiMe_3$ substituent provides a means to achieve selective toxicity for ethynylphenyl-TBOs relative to houseflies and mice. The maximum effect is for the 4-*n*-Bu analogue, which is >400-fold more toxic to houseflies than to mice, a remarkable achievement considering that the corresponding ethynylphenyl-TBO is equitoxic to houseflies and mice. Enzyme studies establish that both species carry out the oxidative-desilylation reaction, whereas PB synergism establishes a greater toxicological significance of this process in houseflies than in mice. The 4-*n*-butyl substituent confers facile metabolism of 4-iodophenyl-TBOs in the mouse MFO system (Cole et al., 1991). The magnitude of selectivity is also dependent on the ethynyl protecting group, suggesting that there is a species-dependent balance with oxidative activation favored in houseflies and detoxification in mice.

The overall bioactivation mechanism involves cytochrome P450 catalyzed oxidative cleavage of the C–$SiMe_3$ bond (Figure 1). The initial step is probably not oxidation at a methyl group (a) on the basis of several observations: unsynergized fly toxicity is essentially the same for the $SiMe_3$ and $Si(CD_3)_3$ derivatives, whereas a significant isotope effect is expected for hydroxylation of a methyl vs a deuteriomethyl substituent (Mitoma et al., 1967; Tanaka et al., 1976) leading in this case to reduced insecticidal potency; the methyl groups can be replaced with ethyl and even bulkier alkyl groups without greatly affecting the proinsecticidal activity; formaldehyde is not an intermediate, and the $SiMe_2H$ compound is not detected. Initial oxidation does not occur at the ethynyl substituent because this would modify and probably detoxify this important moiety. The alternative is oxidation at silicon (b), which is a reasonable possibility as all members of the group IV elements except carbon (which as the Me_3C group does not confer proinsecticidal activity) have empty d orbitals, allowing for pentacoordination (e.g., the N → Si bond of the silatranes; Voronkov, 1966). Cytochrome P450 catalyzed formation of an oxygenated silicon intermediate (or transition state) may therefore facilitate or lead to cleavage of the ethynyl carbon–silicon bond. This is also consistent with the similar insecticidal potency when the methyls of the silyl moiety are replaced with other alkyl groups and with the loss of proinsecticidal activity when the nature of the substituents directly bonded to silicon is more dramatically changed, e.g. H or CH_2Cl as in $SiMe_2H$ or $SiMe_2(CH_2Cl)$ or Ph as in $SiMe_2Ph$ or $SiMePh_2$. Although no metabolism studies are reported on silylated ethynylphenyl compounds, the metabolism of $PhSiMe_3$ in rats gives $PhSiMe_2(CH_2OH)$ in the urine, free and as a conjugate, and no silicon oxygenation is observed (Fessenden and Hartman, 1970), consistent with the SiPh compounds in the present study. It is possible that the d orbitals of silicon interact with the π orbitals of the phenyl group in a manner unfavorable to silicon oxygenation. Although the precise mechanism of oxidative desilylation remains to be defined, it appears to involve direct oxidative attack at the silicon. The extent to which this particular bioactivation phenomenon is applicable to other types of compounds remains to be established.

ABBREVIATIONS USED

CI, chemical ionization; $GABA_A$ receptor, sensitive to muscimol, bicuculline, and picrotoxinin but insensitive to baclofen; ip, intraperitoneal; MFO, mixed-function oxidase; MS, mass spectrometry; MTG, methoxytriglycol; NMR, nuclear magnetic resonance; PB, piperonyl butoxide; TBO, 2,6,7-trioxabicyclo[2.2.2]octane; TBPS, *tert*-butylbicyclophosphorothionate; TEA, triethylamine; TLC, thin-layer chromatography. Substituents: Me, methyl; Et, ethyl; Pr, propyl; Bu, butyl; Pen, pentyl; Hex, hexyl; Ph, phenyl; *n*, normal; *i*, iso; *s*, secondary; *t*, tertiary; *c*, cyclo.

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