1-(4-Ethynylphenyl)-4-substituted-2,6,7-trioxabicyclo[2.2.2]octanes: Effect of 4-Substituent on Toxicity to Houseflies and Mice and Potency at the GABA-Gated Chloride Channel†

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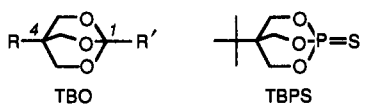
1-(4-Ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes with appropriate 4-substituents are among the most potent insecticides and $GABA_A$ receptor antagonists. Thirty-five analogues were prepared with varying 4-substituents comprising six *n*-alkyl, seven cycloalkyl, six branched alkyl, five alkenyl, eight aryl, and three others. About half of these compounds are highly potent insecticides with topical LD_{50}s for adult houseflies of 0.1–1 μg/g and with 2- to >50-fold synergism by piperonyl butoxide. Most of the same compounds are also very active in mammalian systems with mouse intraperitoneal LD_{50}s of 0.1–1 mg/kg and IC_{50}s of 0.4–5 nM for inhibiting the GABA-gated chloride channel (*tert*-butylbicyclophosphorothionate or TBPS assay). Potency in the TBPS receptor assay generally correlates with the toxicity to mice ($r = 0.76$; $n = 30$). Particularly effective 4-substituents in these assays are C_{3-4} *n*-alkyl, C_{3-6} cycloalkyl, *tert*-butyl (near optimal) and several other C_{3-5} branched alkyls, and phenyl or fluorophenyl. Some selective toxicity to mice is conferred by dimethylamino and to houseflies by *n*-butyl and 4-fluorophenyl substituents, indicating possible differences in receptor specificity.

INTRODUCTION

2,6,7-Trioxabicyclo[2.2.2]octanes (TBOs) with suitable substituents in the 1- and 4-positions are potent insecticides (Palmer and Casida, 1985, 1987, 1989) and inhibitors of the GABA-gated chloride channel (Casida and Palmer, 1988; Casida et al., 1985). An interesting level of insecticidal activity was first recognized with the 1-(4-chlorophenyl)-TBOs in a study with 11 examples of 4-substituents assayed for toxicity to houseflies or houseflies pretreated with piperonyl butoxide (PB) to inhibit their oxidative detoxification; preferred 4-substituents (LD_{50}s 0.5–8 μg/g with PB) were *n*- and *i*-Pr, *n*-, *s*-, and *t*-Bu, *c*-Pen, *c*-Hex, and Ph (Palmer and Casida, 1985).



TBO TBPS

The structurally related bicyclophosphorus esters (phosphites, phosphates, and phosphorothionates) have little or no topical insecticidal activity yet are highly toxic to mammals by virtue of their action as noncompetitive GABA antagonists (Bellet and Casida, 1973; Eto et al., 1976; Milbrath et al., 1979; Ozoe et al., 1983; Wu et al., 1989). Their optimal 4-substituent is *t*-Bu (Eto et al., 1976) leading to the development of *tert*-butylbicyclophosphorothionate (TBPS) (Milbrath et al., 1979) as a radioligand for the GABA-gated chloride channel (Squires et al., 1983; Obata et al., 1988). The toxicity to mice of the bicyclophosphorus esters with varying 4-substituents correlates well with their potency for inhibiting [^{35}S]-TBPS binding to brain membranes (Casida et al., 1985) in a manner characteristic of small or compact cage convulsants leading to their designation as type B action (Palmer and Casida, 1988). The (4-chlorophenyl)-TBOs as larger or extended cage convulsants are much more potent inhibitors of TBPS binding relative to their toxicity than the bicyclophosphorus esters, leading to their designation as type A action (Palmer and Casida, 1988). The optimal 4-substituents are generally the same for types A and B action (Milbrath et al., 1979; Palmer and Casida, 1988).

Structure–activity correlations for any particular substituent are most meaningful when the rest of the molecule is at or near the optimum, which is not the case for the chlorophenyl-TBOs and bicyclophosphorus esters when assayed as insecticides. Fortunately, the 1-(4-ethynylphenyl)-TBOs are outstanding in potency to houseflies both with and without PB, achieving a level of activity comparable to that of the most effective established insecticides acting at other target sites (Palmer and Casida, 1989). Further structure optimization therefore focused on the effect of the 4-substituent of the 1-(4-ethynylphenyl)-TBOs relative to their toxicity to houseflies, their ease of oxidative detoxification (assayed as PB synergism), their toxicity to mice, and their potency at the TBPS receptor.

MATERIALS AND METHODS

Abbreviations. Substituents are designated as Me, methyl; Et, ethyl; Pr, propyl; Bu, butyl; Pen, pentyl; Hex, hexyl; Hept, heptyl; Ph, phenyl; *n*, normal; *i*, iso; *s*, secondary; *t*, tertiary; and *c*, cyclo.

Bioassays. LD_{50} values were determined for adult female houseflies (*Musca domestica* L., SCR strain, ~20 mg each) held 24 h at 25 °C after application of the test compound in acetone solution to the ventrum of the abdomen (Palmer and Casida, 1985). Synergized toxicity was evaluated by using flies pretreated topically with PB at 250 μg/g 2 h before the toxicant was administered. Each experiment was repeated on at least three separate days with 10 or 20 flies per group and a dose differential of 2-fold. Male albino Swiss-Webster mice (17–20 g) were employed for determining LD_{50}s 24 h after intraperitoneal (ip) treatments with the test compounds in methoxytriglycol as the carrier vehicle. Five to nine mice were used at each dose. LD_{50}

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Table I. Characterization of 4-Substituted-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes

no.	R[a]	mp, °C	CI-MS $[M+1]^+$	NMR ($CDCl_3$),[b] ppm: $C(CH_2O)_3$ (6 H, s)	NMR ($CDCl_3$),[b] ppm: other
				n-Alkyl	
1	Me	118–120	231	4.08	0.88 (3 H, s, CH_3)
2	Et	73–75	245	4.08	0.88 (3 H, t, CH_3CH_2), 1.32 (2 H, q, CH_2CH_3)
5	*n*-Pen	92–93	287	4.10	0.90 (3 H, t, CH_3), 1.25 (8 H, m, $(CH_2)_4CH_3$)
6	*n*-Hex	92–93	301	4.10	0.88 (3 H, t, CH_3CH_2), 1.25 (10 H, m, $(CH_2)_5$)
				Cycloalkyl	
7	*c*-Pr	180–182	257	4.02	0.27 (2 H, m, CHCH), 0.45 (2 H, m, CHCH), 0.55 (1 H, m, $CH(CH_2)_2$)
8	1-Me-*c*-Pr	139–142	271	4.07	0.22 (2 H, m, CHCH), 0.48 (2 H, m, CHCH), 1.00 (3 H, s, CH_3)
9	*trans*-2-Me-*c*-Pr	128–131	271	4.02	0.20 (2 H, m, CH_2CH), 0.42 (1 H, m, $CHCH_2$), 0.67 (1 H, m, $CHCH_3$), 1.02 (3 H, d, CH_3CH)
10	*c*-Bu	162–163	271	4.08	1.70–1.95 (6 H, m, $(CH_2)_3CH$), 2.23 (1 H, m, $CHCH_2$)
11	*c*-Pen	154–156	285	4.12	1.25 and 1.60 (9 H, m, $(CH_2)_4CH$)
13	*c*-Hept	199–200	313	4.15	1.15–1.70 (13 H, m, $(CH_2)_6CH$)
				Branched Alkyl	
14	*i*-Pr	127–128	259	4.12	0.90 (6 H, d, $(CH_3)_2CH$), 1.60 (1 H, m, $CH(CH_3)_2$)
16	*s*-Bu	109–111	273	4.15	0.88 (3 H, d, CH_3CH), 0.91 (3 H, t, CH_3CH_2), 1.00 (1 H, m, $CHCH_2$), 1.28 and 1.50 (each 1 H, m, CH_2CH)
17	*i*-Bu	122–123	273	4.15	0.95 (6 H, d, $(CH_3)_2CH$), 1.16 (2 H, d, CH_2CH), 1.68 (1 H, m, CH_2CH)
18	*t*-$BuCH_2$	152–153	287	4.20	1.02 (9 H, s, $(CH_3)_3C$), 1.25 (2 H, s, CH_2C)
19	3-Me-Bu	101–102	287	4.10	0.80 (6 H, d, $(CH_3)_2CH$), 1.00–1.40 (5 H, m, $CHCH_2CH_2$)
				Alkenyl	
20	*i*-propenyl	127–129	257	4.25	1.72 (3 H, br s, CH_3C), 4.72 (1 H, s, CH=C), 5.00 (1 H, m, CH=C)
21	prop-2-enyl	93–95	257	4.10	2.02 (2 H, d, CH_2CH), 5.12 (2 H, m, CH_2=CH), 5.65 (1 H, m, CH=CH_2)
22	1-Me-prop-2-enyl	98–99	271	4.10	1.00 (3 H, d, CH_3CH), 2.12 (1 H, m, $CHCH_3$), 5.00–5.10 (2 H, m, CH_2=CH), 5.60 (1 H, m, CH_2=CH)
23	2-Me-prop-2-enyl	122	271	4.15	1.76 (3 H, s, CH_3C), 2.00 (2 H, s, CH_2C), 4.72 (1 H, m, CH=C), 4.95 (1 H, m, CH=C)
24	*c*-hex-2-enyl	151–152	297	4.18	1.25–1.85 (4 H, m, CH_2CH_2), 2.00 (2 H, m, CH_2CH), 2.15 (1 H, m, CHCH), 5.50 (1 H, m, CH=CH), 5.85 (1 H, m, CH=CH)
				Aryl	
25	Ph	196–197	293	4.50	7.20 (2 H, m, aromatic), 7.40 (3 H, m, aromatic)
26	2-F-Ph	163–165	311	4.57	7.00–7.20 (3 H, m, aromatic), 7.32 (1 H, m, aromatic)
27	3-F-Ph	193–194	311	4.47	6.90 (1 H, dt, aromatic), 6.98 (1 H, dt, aromatic), 7.05 (1 H, dt, aromatic), 7.05 (1 H, dt, aromatic), 7.38 (1 H, dt, aromatic)
28	4-F-Ph	239–241	311	4.45	7.05–7.20 (4 H, m, aromatic)
29	4-Cl-Ph	236–238	327	4.45	7.12 and 7.38 (each 2 H, AA'BB', aromatic)
30	3,4-F_2-Ph	204–205	329	4.42	6.90 (1 H, m, aromatic), 7.00 (1 H, m, aromatic), 7.18 (1 H, m, aromatic)
31	4-Me-Ph	243–245	307	4.45	2.50 (3 H, s, $ArCH_3$), 7.08 and 7.20 (each 2 H, AA'BB', aromatic)
32	4-MeO-Ph	219–221	323	4.45	3.80 (3 H, s, CH_3O), 6.90 and 7.10 (each 2 H, AA'BB', aromatic)
				Other	
33	$ClCH_2$	130–132	265	4.20	3.38 (2 H, s, CH_2Cl)
34	Me_2N	152–153	260	4.20	2.30 (6 H, s, $(CH_3)_2N$)
35	Et_2N	113–114	288	4.20	1.02 (6 H, t, $(CH_3CH_2)_2N$), 2.65 (4 H, q, $(CH_3CH_2)_2N$)

[a] $RC(CH_2O)_3CPh$-4-C≡CH. **3**, **4**, **12**, and **15** with *n*-Pr, *n*-Bu, *c*-Hex, and *t*-Bu substituents, respectively, are reported by Palmer and Casida (1989) and Palmer et al. (1990). [b] Additional signals in common for all compounds are 3.07 (1 H, s, C≡CH) and 7.47 and 7.57 (each 2 H, AA', BB', aromatic).

values for the houseflies and mice were based on log dose–probit mortality plots and were reproducible within 1.5-fold. The [^{35}S]-TBPS assay was used for IC_{50} determinations with mouse brain P_2 membranes (Cole et al., 1984). The 95% confidence limits had an average range of ~1.6-fold relative to the IC_{50} value (Casida et al., 1985).

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).

SYNTHESES

TBOs **3**, **4**, **12**, and **15** were available from earlier studies (Palmer and Casida, 1989; Palmer et al., 1990). Characterization data for the other TBOs synthesized for this investigation are given in Table I. Compounds **1**, **2**, **13**, **17**, **20**, **22**, **24**, and **30–35** were prepared by direct condensation of the appropriate 2-substituted-2-(hydroxymethyl)propane-1,3-diol (triol) described later with methyl 4-ethynylorthobenzoate (Figure 1) using *p*-toluenesulfonic acid (*p*-TSA) by a similar procedure to that described earlier (Palmer and Casida, 1985). To prepare methyl 4-ethynylorthobenzoate, a mixture of methyl 4-bromoorthobenzoate (Palmer and Casida, 1985) (6.5 g, 25 mmol), (trimethylsilyl)-acetylene (5 g, 50 mmol), triphenylphosphine (300 mg), and palladium(II) acetate (150 mg) in dry triethylamine (25 mL) was heated to reflux overnight under a nitrogen atmosphere. Evaporation of the solvent left methyl 4-[(trimethylsilyl)ethynyl]-orthobenzoate as an oil [NMR δ 0.25 (9 H, s, Me_3Si), 3.10 (9 H, s, $(MeO)_3C$), 7.48 (4 H, AA'BB', aromatic)], which was not purified further. To this ortho ester in dry tetrahydrofuran (THF) (100 mL) at 0 °C under nitrogen atmosphere was added a 1 M solution of tetrabutylammonium fluoride in THF (30 mL, 30 mmol). The mixture was stirred for 2 h and then evaporated. The residue

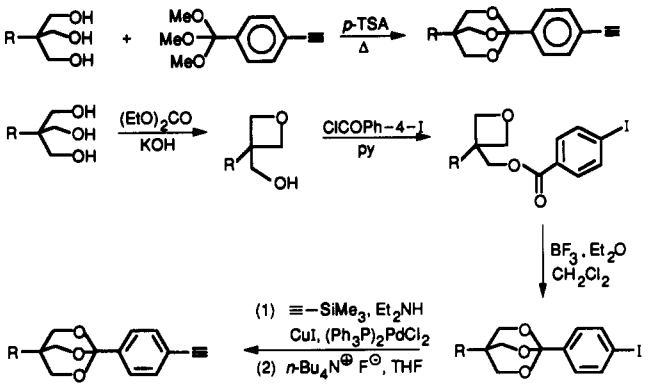

Figure 1. Two methods of synthesis of 1-(4-ethynylphenyl)-trioxabicyclooctanes.

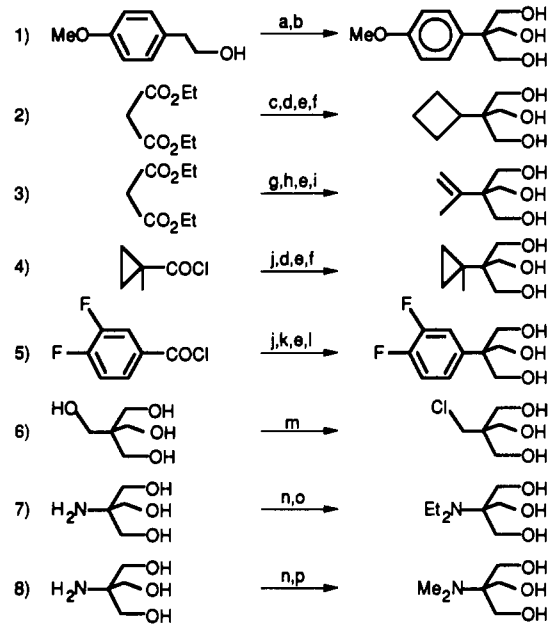

Figure 2. Synthesis of triol intermediates. (a) PCC/CH_2Cl_2; (b) $(CH_2O)_n/NaOH$ (aq); (c) (1) NaOEt/EtOH, (2) R-X; (d) (1) NaH/THF, (2) $PhCH_2OCH_2Cl$; (e) $LiAlH_4$; (f) Na/liquid NH_3; (g) $(CH_3)_2CO$, Ac_2O, $ZnCl_2$; (h) (1) LDA, THF/HMPA, (2) $PhCH_2OCH_2Cl$; (i) Ca/liquid NH_3; (j) (1) N_2CHCO_2Et, (2) Ag_2O, (3) EtOH; (k) (1) NaH/THF, (2) MeO-4-$PhCH_2OCH_2Cl$; (l) (1) $(CH_3)_2CO$, H^+, (2) DDQ, (3) H^+, H_2O; (m) $SOCl_2$, py; (n) $(CH_2O)_n$, benzene, p-TSA; (o) MeMgBr, Et_2O; (p) (1) HCO_2H, (2) HCl, (3) NaOH.

was partitioned between methylene chloride and water. The organic layer was separated, dried (K_2CO_3), evaporated, and purified on a basic alumina column. Elution with hexane gave methyl 4-ethynylorthobenzoate (2.8 g, 54%) as a yellow solid: mp 62–64 °C; MS $[M + 1]^+$ 207; NMR δ 3.08 (1 H, s, C≡CH), 3.10 (9 H, s, $(MeO)_3C$), 7.50 (4 H, AA'BB', aromatic).

The remaining TBOs used in this study were prepared from the appropriate triols via the corresponding (iodobenzoyl)oxymethyloxetane (Figure 1) according to the procedures described earlier (Palmer and Casida, 1989).

Eight procedures were developed for preparation of the various triols, with examples shown in Figure 2. Method 1 involved oxidation with pyridinium chlorochromate (PCC) (Corey and Suggs, 1975) of a 2-substituted ethanol to form the corresponding acetaldehyde which with formaldehyde underwent hydroxymethylation and a subsequent crossed-Cannizzaro reaction to give the triol (Dermer and Solomon, 1954; Ketslakh et al., 1963) (*n*-Pr, *n*-Bu, *n*-Pen, *c*-Pen, *c*-Hex, *c*-Hept, *i*-Pr, *t*-Bu, *s*-Bu, *i*-Bu, prop-2-enyl, 1-Me-prop-2-enyl, Ph, 2-F-Ph, 3-F-Ph, 4-F-Ph, 4-Cl-Ph, 4-Me-Ph, and 4-MeO-Ph). Method 2 involved alkylation of diethyl malonate with sodium ethoxide and an alkyl bromide (Marvel, 1955) followed by a second alkylation with sodium hydride and chloromethyl benzyl ether (Cooper et al., 1978). Lithium aluminum hydride reduction of the resultant malonate to the diol followed by removal of the benzyl group with sodium in liquid ammonia gave the triol (Cooper et al., 1978) (*n*-Hex, *c*-Bu, *c*-hex-2-enyl, *t*-$BuCH_2$, 3-Me-Bu, 2-Me-prop-2-enyl). Method 3 also started from diethyl malonate, condensing with acetone to form the isopropylidene malonate (Eliel et al., 1970). On treatment with lithium diisopropylamide (LDA) in hexamethylphosphoramide (HMPA) followed by chloromethyl benzyl ether the isopropylidene malonate underwent deconjugative alkylation to give the isopropenyl malonate (Tsuboi et al., 1986). After reduction of the malonate to the diol as above, calcium in liquid ammonia (Hwu et al., 1986) selectively cleaved the benzyl group without alkene reduction to give the isopropenyl triol. Method 4 involved conversion of an acid chloride, by reaction with ethyl diazoacetate, into the acyl diazo ester. This diazo ester, when heated to reflux with silver oxide in toluene, rearranged to the ketene, which upon reaction with ethanol formed the substituted malonate (Smith and McKenzie, 1950). This alkylated malonate underwent conversion to the triol via the steps described in method 2 (*c*-Pr, 1-Me-*c*-Pr, *trans*-2-Me-*c*-Pr). Method 5 involved formation of the difluorophenyl malonate using the acid chloride/diazo ester route described in method 4 followed by alkylation with chloromethyl *p*-methoxybenzyl ether (Benneche et al., 1983). Reduction of this malonate with lithium aluminum hydride to the diol, followed by hydroxyl protection as the acetal, permitted *p*-methoxybenzyl cleavage by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Kozikowski and Wu, 1987); subsequent acid cleavage of the acetal (Ho, 1978) gave the difluorophenyl triol. Method 6 utilized thionyl chloride in pyridine (py) to convert pentaerythritol to the chloromethyl triol (Mooradian and Cloke, 1945). Method 7 involved condensing tris(hydroxymethyl)aminomethane with paraformaldehyde to form a substituted 1-aza-3,7-dioxabicyclo-[3.3.0]octane, which upon treatment with methylmagnesium bromide afforded the diethylamino triol (Senkus, 1945). Method 8 proceeded by reduction of the 1-aza-3,7-dioxabicyclo[3.3.0]-octane from method 7 with formic acid to form the dimethylamino triol (Wawzonek, 1981). The methyl and ethyl triols were commercially available.

RESULTS

Insecticidal Activity (Table II). *General.* A remarkable number of the 35 4-substituted-1-(4-ethynylphenyl)-TBOs examined fall in the same potency range to houseflies (alone and with PB) as (1*R*,*cis*)-permethrin and parathion. They are discussed below on the basis of six series of 4-substituents.

n-Alkyl Series (Compounds 1-6). Insecticidal activity is highly dependent on the chain length, increasing progressively from Me to Et to Pr and reaching an optimum at Pr or Bu followed by a progressive decline with Pen and Hex. The factor of synergism by PB decreases progressively with *n*-alkyl chain length.

Cycloalkyl Series (Compounds 7–13). High activity is conferred by C_{3-6} cycloalkyl substituents, with surprisingly little potency difference with ring size, although there is a marked decline for *c*-Hept. Substituting a methyl group at either the 1- or 2-position of the *c*-Pr ring has relatively little effect on potency. The synergism factor is low with *c*-Pr relative to that with the other cycloalkyl substituents.

Branched Alkyl Series (Compounds 14–19). The position and degree of branching greatly affect the activity. The *t*-Bu compound is the most potent in this study. Relative to the *n*-Pr and *n*-Bu compounds, there appears to be no real advantage for other branched C_{3-5} alkyl groups, with *i*-Pr, *s*-Bu, and *i*-Bu conferring similar synergized levels of activity but only *i*-Bu exhibiting a comparable unsynergized level of activity. Branching at C_3 results in a large decrease in potency.

Alkenyl Series (Compounds 20–24). The C_{3-4} alkenyl compounds are less active, both synergized and unsynergized, than their saturated counterparts. Thus, unsat-

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

		housefly			mouse	
		LD_{50},[b] μg/g				
no.	R[a]	alone	with PB	factor of synergism	TBPS receptor IC_{50}, nM	LD_{50}, mg/kg
			n-Alkyl			
1	Me	>500	13	>38	200	12.5
2	Et	15	0.48	31	32	0.75
3	*n*-Pr	0.68	0.023	30	2	0.92
4	*n*-Bu	0.24	0.054	4.4	5	1.1
5	*n*-Pen	2.2	0.50	4.4	34	4.7
6	*n*-Hex	85	37	2.3	1290	>250
			Cycloalkyl			
7	*c*-Pr	0.48	0.11	4.4	1	0.44
8	1-Me-*c*-Pr	0.63	0.029	22	3	0.35
9	*trans*-2-Me-*c*-Pr	0.63	0.063	10	2	1.0
10	*c*-Bu	0.56	0.015	37	0.4	0.50
11	*c*-Pen	1.0	0.036	28	2	0.23
12	*c*-Hex	0.53	0.030	18	1	0.17
13	*c*-Hept	13	0.26	50	8	3.0
			Branched Alkyl			
14	*i*-Pr	0.95	0.027	35	1	0.25
15	*t*-Bu	0.090	0.011	8	1	0.11
16	*s*-Bu	1.6	0.027	59	1	0.35
17	*i*-Bu	0.50	0.035	14	1	0.17
18	*t*-$BuCH_2$	0.78	0.060	13	1	0.23
19	3-Me-Bu	3.0	0.21	14	20	6.0
			Alkenyl			
20	isopropenyl	13	0.35	37	1	0.75
21	prop-2-enyl	3.2	0.15	21	10	0.55
22	1-Me-Prop-2-enyl	10	0.45	22	1	0.55
23	2-Me-prop-2-enyl	1.8	0.21	8.6	4	2.3
24	*c*-hex-2-enyl	0.88	0.046	19	2	0.60
			Aryl			
25	Ph	0.30	0.028	11	2	0.53
26	2-F-Ph	2.0	0.35	5.7	8	1.6
27	3-F-Ph	0.15	0.036	4.2	3	0.60
28	4-F-Ph	0.098	0.032	3.1	1	1.1
29	4-Cl-Ph	87	22	4	15	>50
30	3,4-F_2-Ph	0.21	0.095	2.2	2	NT[c]
31	4-Me-Ph	>500	10	>50	130	>200
32	4-MeO-Ph	>500	>500		760	>300
			Other			
33	$ClCH_2$	170	3.4	50	86	2.9
34	Me_2N	130	6.0	22	24	0.75
35	Et_2N	11	1.3	8.5	8	1.4

[a] $RC(CH_2O)_3CPh$-4-C≡CH. [b] LD_{50} values for established insecticides (alone and with PB) are 0.21 and 0.012 μg/g for (1*R*,*cis*)-permethrin and 1.3 and 0.43 μg/g for parathion (Palmer and Casida, 1989), respectively. [c] Not tested.

uration between either C_1 and C_2 or C_2 and C_3 of the alkyl group reduces activity relative to the saturated compound. Only the *c*-hex-2-enyl compound exhibits a level of activity approaching that of its saturated analogue.

Aryl Series (*Compounds 25–32*). The nature and position of the substituent on the aromatic ring have a profound influence on activity. Of the substituents examined, only fluorine is tolerated, and it results in compounds with very low factors of synergism. Thus, introduction of fluorine into the 2-position reduces activity 7–13-fold, whereas its introduction into the 3- or 4-position improves the unsynergized activity 2–3-fold without changing the synergized activity. Introduction of two fluorine atoms into the 3- and 4-positions has little effect on unsynergized activity and apparently reduces synergized activity. Substituents examined other than fluorine drastically reduce the potency, i.e., chloro, methyl, and methoxy.

Other Substituents (*Compounds 33–35*). There is only weak activity for compounds with chloromethyl and dialkylamino substituents, the best being the diethylamino compound.

Activity in Mammalian Systems (Table II). *TPBS Receptor Potency.* Twenty-one of the compounds are highly potent with IC_{50} values of 0.4–5 nM. Compounds of particularly high potency in each series are *n*-Pr of the *n*-alkyls, *c*-Bu of the cycloalkyls, a variety of branched alkyls of similar potency, isopropenyl and 1-Me-prop-2-enyl in the alkenyls, 4-F-Ph in the aryls, and (although much less active) Et_2N in the other substituents.

Mouse Toxicity. More than half of the 1-(4-ethynylphenyl)-TBOs have mouse ip LD_{50}s of 0.1–1.1 mg/kg. This toxicity range includes compounds with the following 4-substituents: C_{2-4} *n*-alkyls; C_{3-6} cycloalkyls or Me-substituted *c*-Pr; C_{3-5} branched alkyls except 3-Me-Bu; C_{3-6} alkenyls except 2-Me-prop-2-enyl; Ph and 3- and 4-F-Ph; Me_2N. Other substituents generally reduce the toxicity to mice.

DISCUSSION

A surprisingly large number of the 35 4-substituents examined in the 1-(4-ethynylphenyl)-TBOs confer very high toxicity to houseflies and mice and potency at the

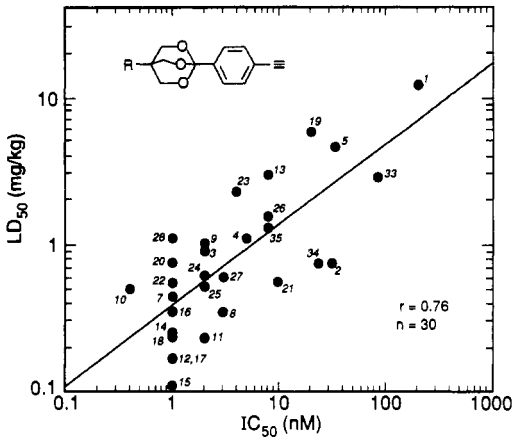

Figure 3. Relation of potency in inhibiting [^{35}S]TBPS binding to mouse brain membranes and ip toxicity to mice for 1-(4-ethynylphenyl)trioxabicyclooctanes. The compounds are identified by numbers and 4-substituents in Table II. All compounds with discrete IC_{50} and LD_{50} values are used in this correlation.

TBPS receptor. The optimal substituent in this series is almost always *t*-Bu as is also the case for more limited studies with 1-alkyl-TBOs, 1-phenyl-TBOs, 1-(4-chlorophenyl)-TBOs, and bicyclic phosphorus esters (Eto et al., 1976; Milbrath et al., 1979; Palmer and Casida, 1985; Casida et al., 1985). It is important to note that other apolar, moderate-sized 4-substituents approach the effectiveness of the *t*-Bu moiety in conferring potency in the 1-(4-ethynylphenyl)-TBOs, i.e., C_{3-4} *n*-alkyls, C_{3-6} cycloalkyls, several C_{3-5} branched alkyls, and phenyl or fluorophenyl.

Oxidative metabolism limits the toxicity of the 1-(4-ethynylphenyl)-TBOs. PB synergism factors of <6-fold suggest that the highly effective compounds with *n*-Bu, *c*-Pr, and fluorophenyl substituents are not readily oxidized in houseflies. In contrast, synergism factors of >35-fold are found for the high potency *c*-Bu, *c*-Hept, *i*-Pr, *s*-Bu, and isopropenyl derivatives, indicating that these substituents confer facile oxidative detoxification. The large factor of synergism of the *s*-Bu compound is probably associated with rapid hydroxylation at this moiety (Deng et al., 1990). The effect of the 4-substituent in conferring biodegradability is considerably different for the 1-(ethynylphenyl)-TBOs in houseflies on the basis of PB synergism (this study) than for the 1-(iodophenyl)-TBOs in mouse microsomes on the basis of GC analysis (Cole et al., 1991). Although not particularly labile, the ethynyl substituent itself is also oxidized in the mouse microsomal mixed-function oxidase system (Cole et al., 1991).

The potency of a 1-(4-ethynylphenyl)-TBO at the TBPS receptor is a moderately good predictor of its mouse toxicity (r = 0.76 for the 30 compounds with discrete IC_{50} and LD_{50} values) (Figure 3). All of the compounds fall within the normal range for type A inhibitors of the GABA-gated chloride channel (Palmer and Casida, 1988), providing one type of evidence that they all act at the same receptor in the same way.

The TBOs, picrotoxinin, and the cyclodiene insecticides act at the same or closely coupled sites in the GABA receptor/chloride ionophore (Squires et al., 1983; Lawrence and Casida, 1983, 1984; Casida and Lawrence, 1985). In molecular modeling, the 4-substitutent of the TBOs may be equivalent to the isopropenyl group of picrotoxinin and the *gem*-dichloro moiety of cyclodiene insecticides (Ozoe and Matsumura, 1986; Ozoe et al., 1990). The most appropriate data set for deducing receptor conformation is that for the TBOs because of the variety of compounds examined and their generally high potency. The 4-substituent of the TBOs appears to fit in an apolar pocket of the mammalian $GABA_A$ receptor that accepts almost equally well a broad array of moderate-sized alkyl, cycloalkyl, alkenyl, and aryl moieties.

The 1-(4-ethynylphenyl)-TBOs with moderate to high insecticidal activity are also quite toxic to mice; i.e., there is relatively little selective toxicity with mouse/unsynergized housefly LD_{50} ratios of, for example, 1 for *t*-Bu and 2 for *n*-Pen. In the few cases where some selectivity is evident (LD_{50} ratios of <0.006 for Me_2N, 0.06 for isopropenyl, 4 for *n*-Bu and 3-F-Ph, and 11 for 4-F-Ph), the species differences may be due in part to variations in the receptor specificity of houseflies and mice. The design of proinsecticides provides another approach to improved selectivity (Palmer et al., 1990, 1991).

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