Structure–Activity Relationships in a New Series of Insecticidally Active Dioxatricycloalkenes Derived by Structural Comparison of the GABA Antagonists Bicycloorthocarboxylates and Endosulfan

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To study structural requirements for picrotoxinin-type GABA (γ-aminobutyric acid) antagonists to interact with the receptor site, 5-substituted 4,6-dioxatricyclo[7.2.1.$0^{2,8}$]dodec-10-enes and related compounds were prepared and examined for their insecticidal activity and potency in displacing [^{35}S]-*tert*-butylbicyclophosphorothionate (TBPS) binding. Compounds with high insecticidal activity possessed a phenyl group with an electron-withdrawing para substituent, a cycloalkyl group, or a C_3–C_5 straight-chain alkyl group at the 5-position. The effect of the 5-substituents on insecticidal activity was very similar to that of the 1-substituents of the bicycloorthocarboxylate GABA antagonists. Representative dioxatricycloalkenes displaced the binding of the GABA antagonist [^{35}S]TBPS to housefly head membranes by 29–53% at 10 μM. X-ray crystal structure analysis demonstrated that this class of compounds had structures superimposable on those of 4-*tert*-butylbicycloorthocarboxylates. These findings indicate that the dioxatricycloalkenes and some other analogues occupy the picrotoxinin binding site in such a way that the fourth interacting subsite of the receptor site accommodates the 5-substituent.

Lindane and cyclodiene insecticides such as aldrin, dieldrin, and endosulfan mainly act as noncompetitive antagonists of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Their site of action is the binding site for picrotoxinin, a classical GABA antagonist, on the GABA receptor–chloride channel complex protein (Matsumura and Tanaka, 1984). Compounds thought to act on the same locus include cage convulsants such as bicyclo phosphorus esters and bicycloorthocarboxylates (Casida, 1987). It seems likely that these compounds modify the gating process of the chloride channel by binding to a site probably inside the channel, as demonstrated clearly with picrotoxinin (Inoue and Akaike, 1988). Under certain conditions these compounds could also directly block the channel (Ozoe, 1985; Havoundjian et al., 1986; Tehrani et al., 1986).

As compared with the progress made in the field of biochemical mode of action studies, the topographical studies of the actual binding site seem to be lagging behind. The classic structure–insecticidal activity study on cyclodienes by Soloway (1965), while very extensive, did not address the question on their relationship to the GABA receptor. Ticku and Maksay (1983) are the only scientists who presented a hypothetical model of the binding site, based on the structure and activity of various known convulsants. Structure–activity studies of a series of bicycloorthocarboxylates are also helpful in characterizing the binding site (Casida et al., 1985). To study structural requirements for the action, we have previously synthesized several new compounds that structurally bridge the gap between cyclodienes and picrotoxinin and have pointed out the requirement of two or three key moieties in their chemical structure for the compounds to react with the receptor site (Ozoe and Matsumura, 1986). As a continued part of the above research, we have prepared 5-substituted 4,6-dioxatricyclo[7.2.1.$0^{2,8}$]dodec-10-enes and related compounds based on structural comparison of bicycloorthocarboxylates and endosulfan (Figure 1), tested their insecticidal properties and interactions with the GABA-gated chloride channel, and now report the results.

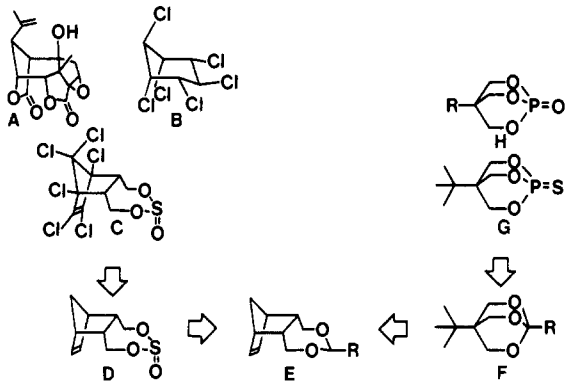

Figure 1. Structures of some of the known noncompetitive GABA antagonists and their modification: A, picrotoxinin; B, lindane; C, α-endosulfan; D, deschloroendosulfan; E, dioxatricycloalkenes; F, *tert*-butylbicycloorthocarboxylates; G, TBPS; H, bicyclophosphates.

MATERIALS AND METHODS

Deschloroendosulfan, *tert*-butylbicyclophosphorothionate (TBPS), and *tert*-butylbicycloorthobenzoate (TBOB) were available from earlier synthesis (Ozoe and Matsumura, 1986; Ozoe et al., 1988). Judging from comparison of ^{1}H NMR spectra measured at 270 MHz, deschloroendosulfan has most likely a conformation corresponding to α-endosulfan. Deschloroendosulfan: ^{1}H NMR ($CDCl_3$) δ 1.59 (1 H, d, J = 9 Hz, H-12), 1.63 (1 H, d, J = 9 Hz, H-12), 2.78 (2 H, dm, H-2, H-8), 2.80 (2 H, s, H-1, H-9), 3.70 (2 H, dd, J = 3, 12 Hz, H_e-3, H_e-7), 4.52 (2 H,

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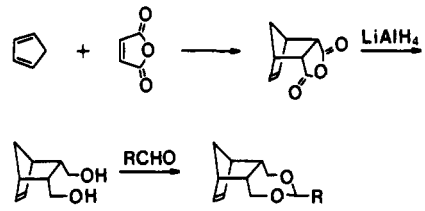
Scheme I



t, J = 12 Hz, H_a-3, H_a-7), 6.17 (2 H, s, H-10, H-11). α-Endosulfan was purchased from Wako Pure Chemical Industries, Ltd., Japan. Other compounds used in the present work were synthesized by the methods described below. The structures and purity were confirmed by ^{1}H NMR (JEOL JNM-GX 270) and mass (Hitachi M-80B/M-0101) spectra. Melting points (mp) were determined on a Yanaco MP-500D apparatus and are uncorrected.

General Procedure for Synthesis of 5-Substituted 2,3:8,7-*endo*-4,6-Dioxatricyclo[7.2.1.0^{2,8}]dodec-10-enes (enDTD's 1–12 and 15–32) (Scheme I). The endo Diels–Alder adduct between cyclopentadiene and maleic anhydride was reduced with lithium aluminum hydride to give 5,6-*endo*-bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene (endo BH diol). A mixture of endo BH diol (1.5 g, 10 mmol), an appropriate aldehyde (10 mmol), *p*-toluenesulfonic acid (0.3 g), and benzene (80 mL) was heated under reflux in a flask equipped with a Dean–Stark trap for separating codistilled water. After 15–240 min, the solution was cooled, washed with 5% sodium carbonate (70 mL × 3) and water (70 mL), and dried over anhydrous sodium sulfate. After evaporation of benzene, a crystalline mass or liquid obtained was purified by recrystallization (from ethanol, chloroform, benzene, hexane, or petroleum ether) or silica gel column chromatography with hexane–acetone eluants. The yields ranged from 2% to 90%. The melting points were as follows (°C): **1**, 126–129; **2**, 131–134; **3**, 141.5–143; **4**, 129–130; **5**, 104–107; **6**, 133–134; **7**, 112–114; **8**, 141–143; **9**, 114–115; **10**, 108–110; **11**, 118–121; **12**, 116–118; **15**, 122–123; **16**, 116–117; **17**, 122–124; **18**, 96–98; **19**, 127–128; **20**, 140–141.5; **21**, 82–84; **22**, 101–103; **23**, 36–38; **24**, 39–41, **25**, 89–91; **26**, 65–67; **32**, 75–77. Compounds **27–31** were liquids. All endo isomers thus prepared showed analogous ^{1}H NMR patterns. The molecular ion (M^+) was generally negligible in 70-eV electron impact mass spectra (EIMS) and sometimes the $M^+ + 1$ ion peak was also very small in chemical ionization mass spectra (CIMS) with isobutane. For example, 5-(4-CNPh)enDTD (**2**): ^{1}H NMR ($CDCl_3$) δ 1.53 (1 H, d, J = 8 Hz, *syn*-H-12), 1.61 (1 H, d, J = 8 Hz, *anti*-H-12), 2.78 (2 H, s, H-1, H-9), 2.80 (2 H, dd, H-2, H-8), 3.41 (2 H, t, J = 12 Hz, H_a-3, H_a-7), 4.22 (2 H, dd, J = 4, 12 Hz, H_e-3, H_e-7), 5.30 (1 H, s, H_a-5), 6.16 (2 H, s, H-10, H-11), 7.54–7.63 (4 H, m, aromatic); CIMS, m/z 268 ($M^+ + 1$).

Oxidation of 5-[4-(Methylthio)phenyl]-2,3:8,7-*endo*-4,6-dioxatricyclo[7.2.1.0^{2,8}]dodec-10-ene (12). A solution of 5-(4-MeSPh)enDTD (**12**) (1.0 g, 3.5 mmol) and 70% *m*-chloroperbenzoic acid (MCPBA) (3.5 mmol) in chloroform (20 mL) was stirred in an ice bath for 15 min. The solution was washed with ice-cold 5% sodium bicarbonate, dried, and concentrated, giving a solid. Purification of the solid by silica gel column chromatography (chloroform–acetone) and recrystallization (benzene–methanol) gave 5-(4-MeSOPh)enDTD (**13**): 0.30 g, 28%; mp 157–158 °C. Similar oxidation with 7.0 mmol of MCPBA afforded 5-(4-$MeSO_2$Ph)enDTD (**14**): 0.11 g, 10%; mp 191–192 °C.

5-(4-Cyanophenyl)-2,3:8,7-*exo*-4,6-dioxatricyclo-[7.2.1.0^{2,8}]dodec-10-ene (5-(4-CNPh)exDTD (33)). Compound **33** was prepared in 28% yield by the same method as that for the corresponding endo analogue **2**, using 5,6-*exo*-bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene (exo BH diol), which was obtained by $LiAlH_4$ reduction of the exo Diels–Alder adduct between cyclopentadiene and maleic anhydride (Craig, 1951). The purified material contained 2.5% of the endo analogue as an impurity. **33**: mp 118–122 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (1 H, d, J = 9 Hz, *syn*-H-12), 1.60 (1 H, d, J = 9 Hz, *anti*-H-12), 2.10 (2 H, dm, J = 12 Hz, H-2, H-8), 2.52 (2 H, s, H-1, H-9), 3.71 (2 H, t, J = 12 Hz, H_a-3, H_a-7), 4.31 (2 H, dd, J = 4, 12 Hz, H_e-3, H_e-7), 5.47 (1 H, s, H-5), 6.22 (2 H, s, H-10, H-11), 7.58–7.65 (4 H, m, aromatic); CIMS, m/z 268 ($M^+ + 1$).

5-(4-Cyanophenyl)-2,3:8,7-*endo*-4,6-dioxatricyclo-[7.2.1.0^{2,8}]dodecane (34). 10,11-Dihydro analogue **34** was prepared in 12% yield by the general procedure described above, using 2,3-bis(hydroxymethyl)bicyclo[2.2.1]heptane, which was obtained by hydrogenation of endo BH diol over 5% Pt/C in acetic acid. **34**: mp 120–121 °C; ^{1}H NMR ($CDCl_3$) δ 1.39–1.62 (6 H, H-10, H-11, H-12), 2.25 (2 H, s, H-1, H-9), 2.41–2.55 (2 H, m, H-2, H-8), 4.00 (2 H, t, J = 12 Hz, H_a-3, H_a-7), 4.13 (2 H, dd, J = 4, 12 Hz, H_e-3, H_e-7), 5.45 (1 H, s, H-5), 7.58–7.65 (4 H, m, aromatic); CIMS, m/z 270 ($M^+ + 1$).

Oxidation of 5-(4-Cyanophenyl)-2,3:8,7-*endo*-4,6-dioxatricyclo[7.2.1.0^{2,8}]dodec-10-ene (2). Oxidation of 5-(4-CNPh)enDTD (**2**) (0.16 g, 0.6 mmol) with 70% MCPBA (0.43 g, 1.7 mmol) gave 10,11-epoxy analogue **35**: 0.10 g, 59%; mp 191–193 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (1 H, d, J = 10 Hz, *syn*-H-12), 1.55 (1 H, d, J = 10 Hz, *anti*-H-12), 2.61 (2 H, s, H-1, H-9), 2.69 (2 H, dd, J = 4, 12 Hz, H-2, H-8), 3.31 (2 H, s, H-10, H-11), 4.00 (2 H, t, J = 12 Hz, H_a-3, H_a-7), 4.26 (2 H, dd, J = 4, 12 Hz, H_e-3, H_e-7), 5.43 (1 H, s, H-5), 7.57–7.65 (4 H, m, aromatic); CIMS, m/z 284 ($M^+ + 1$).

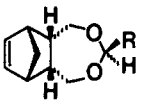
5-(4-Cyanophenyl)-2,3:8,7-*exo*-4,6,12-trioxatricyclo-[7.2.1.0^{2,8}]dodec-10-ene (36). Compound **36** was obtained in 25% yield by the general procedure described above, using 5,6-*exo*-bis(hydroxymethyl)-7-oxabicyclo[2.2.1]hept-2-ene, which was prepared by $LiAlH_4$ reduction of the exo Diels–Alder adduct between furan and maleic anhydride (Woodward and Baer, 1948). **36**: mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (2 H, dm, J = 12 Hz, H-2, H-8), 3.92 (2 H, t, J = 12 Hz, H_a-3, H_a-7), 4.42 (2 H, dd, J = 3, 12 Hz, H_e-3, H_e-7), 4.61 (2 H, s, H-1, H-9), 5.49 (1 H, s, H-5), 6.44 (2 H, s, H-10, H-11), 7.57–7.65 (4 H, m, aromatic); CIMS, m/z 270 ($M^+ + 1$).

1,9,10,11,12,12-Hexachloro-5-(4-cyanophenyl)-4,6-dioxatricyclo[7.2.1.0^{2,8}]dodec-10-ene (5-(4-CNPh)Cl$_6$DTD) (37, 38). The precursor 1,2,3,4,7,7-hexachloro-5,6-bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene was obtained by heating hexachlorocyclopentadiene, *cis*-2-butene-1,4-diol, and 1,4-dioxane in a sealed tube at 115–120 °C (Riemschneider et al., 1961). Endo (5-(4-CNPh)enCl$_6$DTD) (0.58 g, 37% yield) and exo (5-(4-CNPh)exCl$_6$DTD) (0.54 g, 35% yield) isomers were produced from the precursor diol (1.2 g, 3.3 mmol) and 4-cyanobenzaldehyde (0.43 g, 3.3 mmol) by the general procedure described above, followed by separation on a silica gel column (hexane:acetone = 3:1). 5-(4-CNPh)enCl$_6$DTD (**37**): mp 175–177 °C; ^{1}H NMR δ 3.50 (2 H, d, J = 12 Hz, H-2, H-8), 3.72 (2 H, t, J = 12 Hz, H_a-3, H_a-7), 4.46 (2 H, dd, J = 3, 12 Hz, H_e-3, H_e-7), 5.42 (1 H, s, H-5), 7.55 (2 H, d, J = 8 Hz, aromatic), 7.60 (2 H, d, J = 8 Hz, aromatic); CIMS, m/z 472 ($M^+ + 1$, 12.0%), 474 ($M^+ + 3$, 22.1%), 476 ($M^+ + 5$, 18.1%), 478 ($M^+ + 7$, 9.0%), 480 ($M^+ + 9$, 3.2%). 5-(4-CNPh)exCl$_6$DTD (**38**): mp 223–225 °C; ^{1}H NMR ($CDCl_3$) δ 3.09 (2 H, s, H-2, H-8), 4.04 (2 H, d, J = 14 Hz, H_a-3, H_a-7), 4.61 (2 H, d, J = 14 Hz, H_e-3, H_e-7), 5.19 (1 H, s, H-5), 7.56 (2 H, d, J = 8 Hz, aromatic), 7.64 (2 H, d, J = 8 Hz, aromatic); CIMS, m/z 472 ($M^+ + 1$, 3.2%), 474 ($M^+ + 3$, 4.7%), 476 ($M^+ + 5$, 4.4%), 478 ($M^+ + 7$, 2.3%), 480 ($M^+ + 9$, 0.7%).

5-Phenyl-2,3:8,7-*endo*-4,6-dioxa-5-phosphatricyclo-[7.2.1.0^{2,8}]dodec-10-ene 5-Oxide (39). A solution of endo BH diol (0.8 g, 5 mmol), phenylphosphonic dichloride (1.0 g, 5 mmol), and pyridine (0.8 g, 10 mmol) in acetonitrile (50 mL) was stirred overnight at room temperature. After the solvent was removed, the residue was partitioned between water and chloroform. The organic layer was dried and concentrated to give a solid, which on crystallization from ether afforded the desired phosphonate (**39**): 0.4 g, 29%; mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.54 (1 H, d, J = 8 Hz, H-12), 1.60 (1 H, d, J = 8 Hz, H-12), 2.87–2.90 (4 H, H-1, H-2, H-8, H-9), 4.05–4.34 (4 H, H-3, H-7), 6.16 (2 H, s, H-10, H-11), 7.41–7.89 (5 H, aromatic); CIMS, m/z 277 ($M^+ + 1$).

4-(4-Cyanophenyl)-3,5-dioxabicyclo[5.4.0]undec-9-ene (42). Reduction of *cis*-4-cyclohexene-1,2-dicarboxylic anhydride with $LiAlH_4$ gave 4,5-bis(hydroxymethyl)-1-cyclohexene. Compound **42** (0.83 g, 9%) was obtained from the crude diol (5.0 g) and 4-cyanobenzaldehyde (4.6 g, 35 mmol). **42**: mp 71–74 °C; ^{1}H NMR ($CDCl_3$) δ 2.17 (6 H, m, H-1, H-7, H_2-8, H_2-11), 3.71 (2 H, d, J = 13 Hz, H-2, H-6), 3.81 (2 H, d, J = 13 Hz,

Table I. Topical Insecticidal Activity of 5-Substituted 2,3:8,7-*endo*-4,6-Dioxatricyclo[7.2.1.$0^{2,8}$]dodec-10-enes to Houseflies



no.	R	LD_{50},[a] μg/fly	95% CL	no.	R	LD_{50},[a] μg/fly	95% CL
1	Ph	2.33	1.98–2.74	17	4-MePh	>10 (0)	
2	4-CNPh	0.11	0.10–0.12	18	4-MeOPh	>10 (44)	
3	4-BrPh	0.29	0.27–0.30	19	4-*i*-PrPh	>10 (0)	
4	4-ClPh	0.30	0.28–0.32	20	4-Me_2NPh	>10 (2)	
5	4-FPh	0.79	0.73–0.85	21	3-c-Hx[c]	1.19	1.11–1.27
6	4-NO_2Ph	1.07	1.01–1.13	22	c-Hx	0.24	0.23–0.26
7	3-ClPh	1.68	1.58–1.78	23	c-Oct	0.75	0.68–0.81
8	2-ClPh	>10 (0)[b]		24	$PhCH_2$	6.08	5.55–6.67
9	3,4-Cl_2Ph	0.48	0.44–0.51	25	Me	>10 (0)	
10	2,4-Cl_2Ph	>10 (13)		26	Et	>10 (1)	
11	4-CF_3Ph	>10 (40)		27	*n*-Pr	7.61	7.17–8.08
12	4-MeSPh	>10 (21)		28	*n*-Bu	3.30	3.04–3.59
13	4-MeSOPh	>10 (6)		29	*n*-Pen	2.35	2.19–2.51
14	4-$MeSO_2$Ph	>10 (2)		30	*n*-Hx	>10 (34)	
15	4-PhOPh	>10 (0)		31	*n*-Hep	>10 (4)	
16	3-PhOPh	>10 (0)		32	*t*-Bu	>10 (0)	

[a] Determined with PBO and corrected with 2 as standard. [b] The mortality (%) at a dosage of 10 μg/fly is given in parentheses in the case of LD_{50} > 10 μg/fly. [c] 3-Cyclohexenyl.

H-2, H-6), 5.58 (2 H, s, H-9, H-10), 5.75 (1 H, s, H-4), 7.58–7.66 (4 H, m, aromatic); CIMS, *m/z* 256 (M^+ + 1).

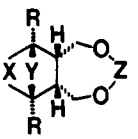
2-(4-Cyanophenyl)-1,3-dioxepane (43). Compound **43** (0.9 g, 44%) was obtained by the reaction between 1,4-butanediol (1.2 g, 13 mmol) and 4-cyanobenzaldehyde (1.3 g, 10 mmol). **43**: mp 84–85 °C; ^{1}H NMR ($CDCl_3$) δ 1.75–1.79 (4 H, m, H-5, H-6), 3.74–3.92 (4 H, H-4, H-7), 5.71 (1 H, s, H-2), 7.59–7.67 (4 H, m, aromatic); CIMS, *m/z* 204 (M^+ + 1).

Bioassays. Triplicate groups of 15 female adult houseflies (*Musca domestica* L., the WHO insecticide-susceptible strain, 3–5 days old) were used for each dosage at 25 °C. An acetone solution (1 μL/fly) containing a test compound was topically applied on the mesonotum 1 h after topical pretreatment with a 1-μL acetone solution of piperonyl butoxide (PBO) (10 μg/fly). The mortality was recorded 24 h after application of test compounds. LD_{50} values were calculated by the Probit method and corrected with the LD_{50} value of 5-(4-CNPh)enDTD (**2**) as a standard. Confidence limits (95%) were obtained by χ^2 tests and are given in Tables I and II.

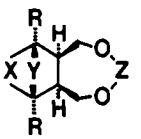
Membrane Preparation and Binding Assays. Heads of adult houseflies of the WHO susceptible strain were homogenized in 20 volumes of ice-cold 0.25 M sucrose containing 1 mM EDTA using a glass homogenizer and filtered through gauze. Pellets were removed twice by centrifugation of the filtrate at 1000*g* for 10 min. The supernatant was centrifuged at 25000*g* for 30 min. The pellet was superficially rinsed with 5 mM sodium phosphate buffer containing 0.2 M sodium chloride (assay buffer) (pH 7.0) and resuspended in assay buffer. The suspension was allowed to stand on ice for 30 min and then centrifuged at 25000*g* for 30 min. The resulting pellet was washed once more by repeating the above centrifugation procedure. The resulting pellet was finally suspended in assay buffer. Protein was determined by the Coomassie Brilliant Blue G method (Bradford, 1976) with bovine serum albumin as standard.

The membrane suspension (ca. 0.4 mg as protein) was incubated with 5.0 nM [^{35}S]*tert*-butylbicyclophosphorothionate (TBPS) (NEN, 73.7 Ci/mmol) and a test compound in assay buffer (1.0 mL) at 20 °C for 30 min. The incubation was terminated by filtration and washing with ice-cold assay buffer (5 mL × 2) under reduced pressure through prewetted glass fiber filters (Toyo GC-50). The filter was heated overnight in 0.2 M sodium hydroxide (0.5 mL) in a scintillation vial at 50 °C. Radioactivity was measured with an Aloka LSC-700 liquid scintillation spectrometer. Specific binding was defined as radioactivity displaced by 10 μM unlabeled TBPS. The potency of the test compounds to bind with the receptor was defined as their ability at 10 μM to displace 5 nM [^{35}S]TBPS. Test compounds and TBPS were added with 2 μL of acetone as a carrier. The control tubes received only the carrier.

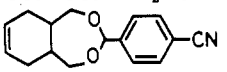
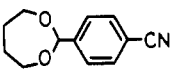
Table II. Topical Insecticidal Activity of Dioxatricycloalkenes and Related Compounds to Houseflies



Endo Type



Exo Type

no.	type	R	X	Y	Z	LD_{50},[a] μg/fly	95% CL
33	exo	H	CH=CH	CH_2	CHPhCN-4	0.32	0.31–0.34
34	endo	H	CH_2CH_2	CH_2	CHPhCN-4	0.25	0.23–0.27
35	endo	H		CH_2	CHPhCN-4	0.13	0.12–0.13
36	exo	H	CH=CH	O	CHPhCN-4	0.93	0.89–0.97
37	endo	Cl	CCl=CCl	CCl_2	CHPhCN-4	>10 (2)[b]	
38	exo	Cl	CCl=CCl	CCl_2	CHPhCN-4	>10 (2)	
39	endo	H	CH=CH	CH_2	P(O)Ph	1.89	1.78–2.01
40[c]	endo	H	CH=CH	CH_2	S=O	0.86	0.82–0.89
41[d]	endo	Cl	CCl=CCl	CCl_2	S=O	0.042	0.037–0.047
42						>10 (0)	
43						>10 (27)	

[a] Determined with PBO and corrected with 2 as standard. [b] The mortality (%) at a dosage of 10 μg/fly is given in parentheses in the case of LD_{50} > 10 μg/fly. [c] Deschloroendosulfan. [d] α-Endosulfan.

RESULTS

Structure–Insecticidal Activity Relationships. When assayed without synergist, a selected compound (**2**) was found to have only low activity (76% mortality at 10 μg). Therefore, the insecticidal activity of synthesized compounds was determined in the presence of a synergist, piperonyl butoxide (PBO). This was also due to the fact that synergized activity better reflects a molecular interaction between a compound and its site of action than unsynergized activity in most cases. Table I lists the topical synergized insecticidal activity of enDTD's against houseflies. The activity greatly depended on the substitution at the 5-position. 5-(4-CNPh)enDTD (**2**) was the most potent analogue among those tested (LD_{50} =

Table III. Effects of Dioxatricycloalkenes and Related Compounds on [^{35}S]TBPS Binding to Housefly Head Membranes

compound[a]	binding displacement,[b] %	LD_{50},[c] μg/fly
TBPS	100	0.032[d]
TBOB	85 ± 18	0.22
α-endosulfan (41)	76 ± 27	0.042
5-c-HxenDTD (22)	48 ± 24	0.24
5-(4-ClPh)enDTD (4)	53 ± 26	0.30
5-(3-ClPh)enDTD (7)	51 ± 24	1.68
5-(2-ClPh)enDTD (8)	48 ± 27	>10
5-MeenDTD (25)	29 ± 22	>10

[a] Tested at 10 μM. [b] Expressed as mean ± SD of three to five experiments. [c] Topical application, except TBPS. [d] Injection (Palmer and Casida, 1985).

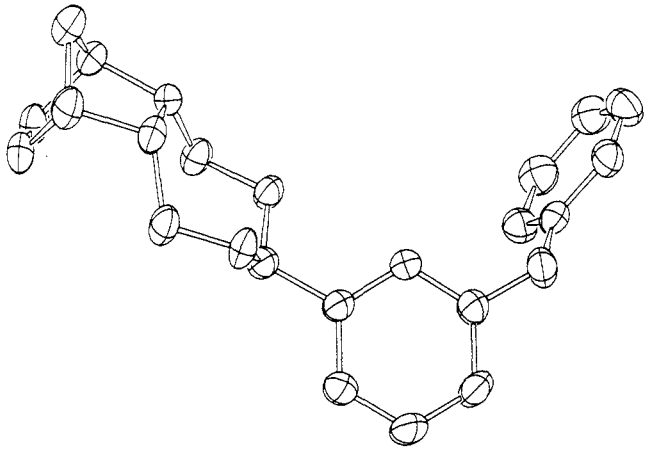


Figure 2. Structure of 5-(3-phenoxyphenyl)-2,3:8,7-*endo*-4,6-dioxatricyclo[7.2.1.$0^{2,8}$]dodec-10-ene (16) determined by X-ray crystallography.

0.11 μg/fly). Introduction of an electron-withdrawing group such as CN, NO_2, and halogens into the 4-position of the benzene ring of 5-PhenDTD increased the insecticidal activity. The effect of H, F, Cl, Br, and CN substituents can be explained by using the Hammet σ parameter (r^2 = 0.86). However, the relation did not apply to NO_2, CF_3, SOMe, and SO_2Me substituents. By contrast, analogues of 5-PhenDTD with an electron-donating group at the 4-position of the benzene ring were less active or inactive. By comparing 5-(3-ClPh)- and 5-(2-ClPh)enDTD's (7, 8) with 5-PhenDTD (1), the effect of the position of the same substituent became apparent: Cl at the 3-position had little influence but Cl at the 2-position reduced the activity. This relationship applies to 5-(4-ClPh)-, 5-(3,4-Cl_2Ph)-, and 5-(2,4-Cl_2Ph)-enDTD's (4, 9, 10). In a series of 5-alkyl-enDTD's, moderate activity was observed with C_3–C_5 straight-chain alkyl groups (27–29). Alkyl-enDTD's with a shorter or a longer chain at the 5-position were not active, and neither was 5-*t*-BuenDTD (32). 5-c-HxenDTD (22) showed high insecticidal activity, comparable to those shown by 5-(4-CNPh)-, 5-(4-BrPh)-, and 5-(4-ClPh)enDTD's (2–4). Replacement of the cyclohexyl group with a cyclooctyl or a 3-cyclohexenyl group resulted in a decreased activity.

5-(4-CNPh)exDTD (33), which had the exo configuration at the 2- and 8-positions, was 3 times less potent than the corresponding endo isomer 2 (Table II). Hydrogenation of the double bond of the norbornene moiety of 5-(4-CNPh)enDTD (2) reduced insecticidal activity to half the activity of 2. Epoxidation of the double bond had almost no influence on activity. Replacement of the C-12 methylene of 33 with an oxygen atom to give 36 resulted in a 3-fold reduction in its activity. The norbornene structure of DTD's was important for activity. Compound 42, a derivative having a cyclohexene ring instead of the norbornene moiety, was inactive, and dioxepane 43 was only weakly active. Phosphonate derivative 39 was comparable in activity to the corresponding enDTD 1, indicating that, without essential loss of activity, the acetal moiety of enDTD may be replaced by the phosphonate structure. Introduction of six chlorine atoms into the norbornene nucleus of 5-(4-CNPh)enDTD (2) and 5-(4-CNPh)exDTD (33), giving compounds 37 and 38, respectively, resulted in a complete loss in topical insecticidal activity.

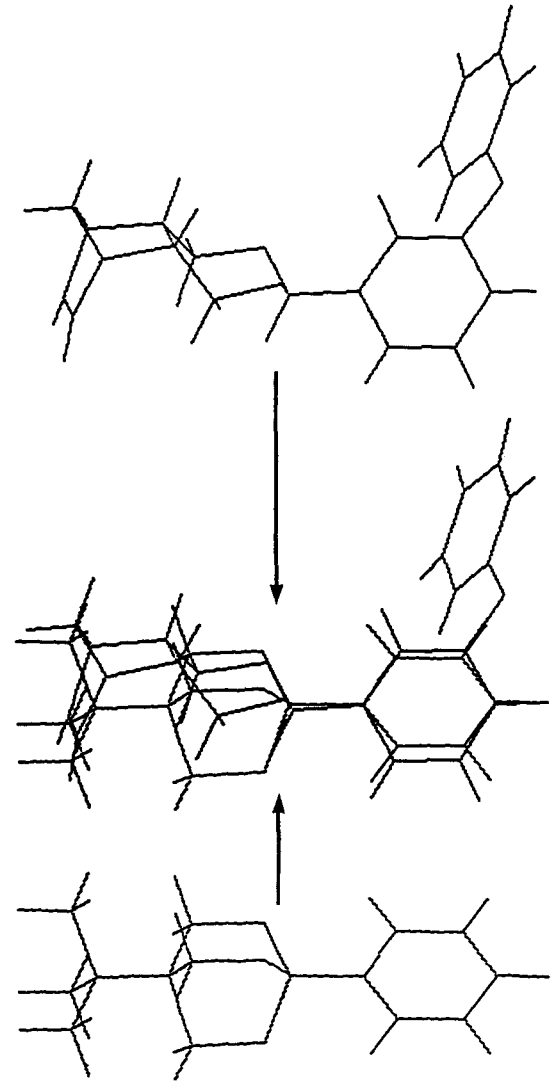


Figure 3. Structural overlap between dioxatricycloalkene 16 and *tert*-butylbicycloorthobenzoate.

Potency in Displacing [^{35}S]TBPS Binding. Table III shows the results of in vitro displacement studies using [^{35}S]TBPS, a radioligand for the GABA-gated chloride channel. In this experiment, a single concentration of 10 μM was utilized because compounds with no activity at 10 μM were considered to be inactive, judging from the activity of reported displacers. Thus, selected enDTD's at 10 μM displaced 29–53% of [^{35}S]TBPS binding to housefly head membranes, although detailed investigations could not be made because of great variability. They appeared 1.4–3.4 times less potent than TBPS, TBOB, and α-endosulfan. 5-(2-ClPh)- and 5-MeenDTD's (8, 25) seemed to interact with [^{35}S]TBPS binding sites to some extent but were insecticidally inactive.

DISCUSSION

The current study demonstrates that some analogues of dioxatricycloalkenes synthesized on the basis of structures of two different types of the noncompetitive GABA antagonists bicycloorthocarboxylates and endosulfan are insecticidally active against houseflies. The most potent compound was 5-(4-CNPh)enDTD (**2**). In comparison of synergized activity, this compound was 8 times more potent than deschloroendosulfan, and 3 and 23 times less potent than α-endosulfan and 4-*tert*-butyl-1-(4-cyanophenyl)bicycloorthocarboxylate (Palmer and Casida, 1985), respectively. Phenyl groups with an electron-withdrawing para substituent, cycloalkyl groups, and alkyl groups of intermediate size are favorable substituents at the 5-position for high activity. The norbornene or norbornane nucleus is important for activity. The acetal moiety is not essential as shown by the high activity of phosphonate derivative **39**, although the electronegativity of the portion is probably necessary. The endo configuration is somewhat preferable to the exo configuration at the 2- and 8-positions.

X-ray crystal structure analysis of 5-(3-PhOPh)enDTD (**16**) revealed that the seven-membered ring exists in the chair form and that the benzene ring at the 5-position is equatorial (Figure 2). As shown in Figure 3, the three-dimensional structure of the enDTD is superimposable on a structural model of TBOB constructed based on standard bond angles and lengths. The norbornene moiety of the former compound corresponds to the *tert*-butyl group of the latter compound. Each benzene ring and oxygen moiety coincide. These X-ray crystal data suggest that a series of DTD's shares a common site of action with bicycloorthocarboxylates, i.e., a site related to the GABA-gated chloride channel. This is supported by two findings: (1) The effect of various 5-substituents of enDTD's on insecticidal activity was very similar to that of the 1-substituents of bicycloorthocarboxylates described by Palmer and Casida (1985). (2) [^{35}S]TBPS binding to housefly head membranes was inhibited by several enDTD's. The relatively high insecticidal activity of deschloroendosulfan can be rationalized in light of its similar superimposition on *tert*-butyl bicyclophosphate.

In a previous paper (Ozoe and Matsumura, 1986), we proposed the hypothesis that there is a minimum requirement for a picrotoxinin-type convulsant to possess at least two of three active centers spaced approximately: two electronegative and one steric bulkiness (hydrophobic) centers. In other words, the receptor site consists of three subsites where critical interactions with compounds occur. The present work further suggests that the binding site might carry one more pocket to accommodate the 5-substituent of DTD's or the 1-substituent of bicycloorthocarboxylates and that substituents accommodated by the pocket must moreover possess appropriate properties to exert insecticidal activity. However, one must await further examination of the influence of chlorine atoms to determine whether the way in which the cyclodiene insecticides occupy the site is the same as that of DTD's, bicycloorthocarboxylates, etc., because chlorinated DTD's **37** and **38** had all four interacting centers but were insecticidally inactive.

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ABBREVIATIONS USED

GABA, γ-aminobutyric acid; TBPS, *tert*-butylbicyclophosphorothionate or 4-*tert*-butyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfide; TBOB, *tert*-butylbicycloorthobenzoate or 4-*tert*-butyl-1-phenyl-2,6,7-trioxabicyclo[2.2.2]octane; en, endo; ex, exo; DTD, 4,6-dioxatricyclo[7.2.1.0^{2,8}]dodec-10-ene; BH diol, 5,6-bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene; MCPBA, *m*-chloroperbenzoic acid; Cl_6DTD, 1,9,10,11,12,12-hexachloro-4,6-dioxatricyclo[7.2.1.0^{2,8}]dodec-10-ene; PBO, piperonyl butoxide.

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