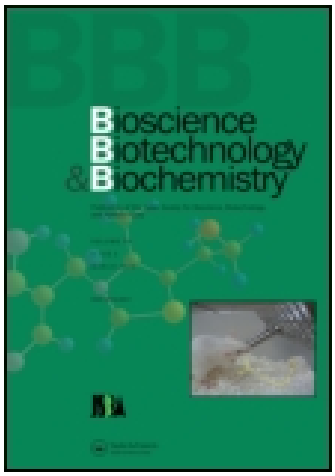

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Six-membered Cyclic Phosphonate GABA Antagonists, 2, 5-Disubstituted 1,3,2-Dioxaphosphorinanes

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Note

Six-membered Cyclic Phosphonate GABA Antagonists, 2,5-Disubstituted 1,3,2-Dioxaphosphorinanes

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Received May 1, 1995

The *trans* isomer of 2-(4-bromophenyl)-5-*tert*-butyl-2-thiono-1,3,2-dioxaphosphorinane competitively inhibited the specific binding of ^{35}S-*tert*-butylbicyclophosphorothionate to rat brain membranes with an IC_{50} value of 0.52 μM, and showed insecticidal activity against houseflies with an LD_{50} value of 2.4 μg/fly. This compound and its analogues acted as noncompetitive $GABA_A$ receptor antagonists (NGRAs), and phosphorus-containing cyclohexane skeletons may prove useful for the design of novel NGRAs.

Organophosphorus (OP) compounds play important roles not only as biomolecules but also as agrochemicals, *etc.* Most OP agrochemicals are insecticides, the primary action site of which is acetylcholinesterase, a key enzyme in the nervous system, although they also include herbicides, fungicides, and other bioregulators.[1,2] It has been shown that certain insecticidal bicyclophosphorus esters (BPs, 4-substituted 2,6,7-trioxa-1-phosphabicyclo[2.2.2]octanes) did not act as anticholinesterase agents, unlike conventional OP insecticides, but as noncompetitive GABA (γ-aminobutyric acid)$_A$ receptor antagonists (NGRAs).[3] These BPs noncompetitively antagonized the action of the neurotransmitter GABA by binding to a site in the GABA-gated Cl^- channel, and were the first and only known group of OP NGRAs. However, we have recently discovered a novel tricyclophosphonate NGRA, 5-oxo-5-phenyl-2,3:8,7-*endo*-4,6-dioxa-5-phosphatricyclo[7.2.1.0^{2,8}]dodec-10-ene (DPTD), during the structural modification of known NGRAs.[4] We report here the synthesis of several DPTD-related compounds, 2,5-disubstituted 1,3,2-dioxaphosphorinanes, and their activities as NGRAs and insecticides.

The reaction of 2-alkyl-1,3-propanediols with phenylphosphonic dichloride or its derivatives afforded mixtures of the respective *trans* and *cis* isomers of 2,5-disubstituted 1,3,2-dioxaphosphorinanes **1**–**5**. Compounds **1**–**4** were used for subsequent bioassays without separating the isomers, although **5** was separated into the *trans* (**5t**) and *cis* (**5c**) isomers. The *trans*/*cis* ratios of **1**–**4** were determined by comparing their ^{1}H-NMR spectra with those of **5t** and **5c** (Table).

The 400 MHz ^{1}H-NMR spectra of **5t** and **5c** exhibited patterns of AA'BB'MX that approximated to A_2B_2MX (X = ^{31}P). The large $J_{H4,6ax\text{-}H5ax}$ (11.5, 9.7 Hz) and small $J_{H4,6eq\text{-}H5ax}$ (4.3, 4.5 Hz) values for both isomers indicate the preference of a chair conformer with the *tert*-butyl group equatorial. This conclusion is supported by an analysis of the coupling constants between the 4,6-protons and the phosphorus nucleus [large $J_{P2\text{-}H4,6eq}$ (23.7, 18.6 Hz) and small $J_{P2\text{-}H4,6ax}$ (4.7, 8.0 Hz) values]. The downfield shift of the axial 4,6-protons of **5t** may be explained by the deshielding effect of the axial P=S group.

The potency of dioxaphosphorinanes as NGRAs was examined by binding assays, using ^{35}S-*tert*-butylbicyclophosphorothionate (TBPS), a radioactive NGRA. Dioxaphosphorinanes with a *tert*-butyl group at the 5-position (**3**–**5**) inhibited the specific binding of ^{35}S-TBPS to rat brain membranes, while those with *n*-propyl and *n*-butyl groups (**1** and **2**) were inactive at 10 μM (Table).

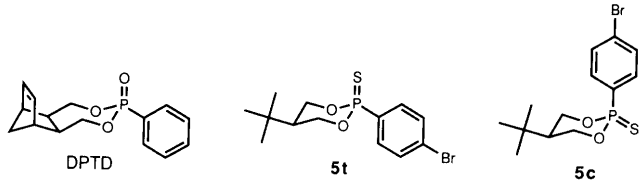
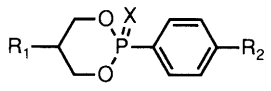


Table Potency in Inhibiting ^{35}S-TBPS Binding to Rat Brain Membranes and Synergized Insecticidal Activity against Houseflies



No.	R_1	R_2	X	*trans*/*cis*	^{35}S-TBPS binding assay		Insecticidal assay LD_{50} (μg/fly)
					Inhibition (%) at 10 μM	IC_{50} (μM)	
1	*n*-Pr	H	O	54/46	3.9±4.1[a]	—	>10
2	*n*-Bu	H	O	55/45	1.6±1.1[a]	—	>10
3	*t*-Bu	H	O	42/58	20.9±6.2[a]	—	>10
4	*t*-Bu	H	S	22/78	44.2±3.0[a]	—	5.8 (5.5–6.0)[b]
5t	*t*-Bu	Br	S	100/0	—	0.52±0.15[a]	2.4 (2.3–2.6)[b]
5c	*t*-Bu	Br	S	0/100	—	14.9 ±1.9[a]	>10

[a] Mean±SD (n=3).
[b] Numbers in parenthesis represent the 95% confidence limit.

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Abbreviations: ax, axial; BP, bicyclophosphate (4-substituted 2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane); DPTD, 5-oxo-5-phenyl-2,3:8,7-*endo*-4,6-dioxa-5-phosphatricyclo[7.2.1.0^{2,8}]dodec-10-ene; eq, equatorial; GABA, γ-aminobutyric acid; NGRA, noncompetitive $GABA_A$ receptor antagonist; OP, organophosphorus; TBPS, *tert*-butylbicyclophosphorothionate.

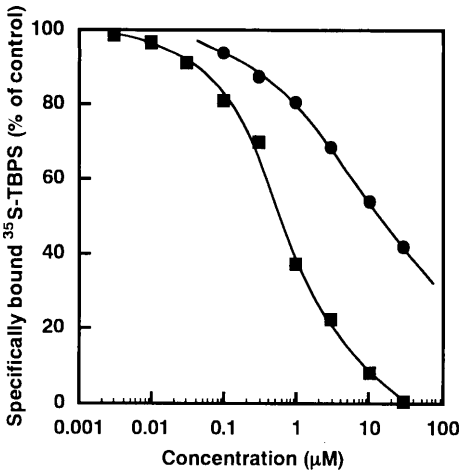

Fig. Inhibition of Specific ^{35}S-TBPS Binding to Rat Brain Membranes by **5t** and **5c**.

Rat brain membranes were incubated with 1.0 nM ^{35}S-TBPS and the indicated concentrations of **5t** (■) and **5c** (●). Experimental procedures are described in the text. Each plot represents the mean of three experiments with similar results, each done in duplicate.

Some bulkiness of the equatorial 5-substituent would possibly be required for high inhibitory activity, as in the case of 1,3-dithianes.[8] Inhibition of ^{35}S-TBPS binding by a thiono analogue (**4**) was higher than that by the corresponding oxon analogue (**3**), although **4** contained predominantly the *cis* isomer, which was presumed to be less active than the *trans* isomer from the analogy with **5t** and **5c**. The introduction of a bromine atom into the *p*-position of the benzene ring of **4** to produce **5** resulted in increased inhibitory activity. The *trans* isomer (**5t**) was a relatively potent inhibitor of ^{35}S-TBPS binding with an IC_{50} value of 0.52 μM (Fig.). A Scatchard analysis of the effect of **5t** revealed that the inhibition was competitive (data not shown), indicating that **5t** acted as an NGRA. The *cis* isomer (**5c**) was about 29-fold less potent than **5t**.

Compounds **4** and **5t** showed moderate insecticidal activity against houseflies by topical application (Table). The oxon analogues of these compounds at 10 μM did not inhibit acetylcholinesterase in housefly heads (data not shown), so the inhibition of acetylcholinesterase was not involved in the insecticidal action of **4** and **5t**. We have reported a relationship between $\log LD_{50}$ (housefly) and $\log IC_{50}$ (rat) for 29 various NGRAs ($\log 1/LD_{50} = 3.956 + 0.743 \log 1/IC_{50}$).[6] The activities of **5t** as an NGRA and insecticide do not greatly deviate from the reported relationship, indicating that this novel NGRA would not be a selective one to mammals or insects. Reconfirmation of this finding is currently in progress, using another radioligand suitable for both mammalian and insect GABA receptors.

We have demonstrated here that phosphorus-containing cyclohexane skeletons may prove useful for the design of novel NGRAs. The potency of the most potent analogue (**5t**) as an inhibitor of ^{35}S-TBPS binding (rat) and as a toxicant to houseflies does not exceed that of its prototype compound, DPTD ($IC_{50} = 0.65$ μM; $LD_{50} = 1.9$ μg/fly).[9] As shown with 1,3-dithianes and 2,6,7-trioxabicyclo[2.2.2]octanes,[10,11] however, replacement of the bromine atom of **5t** by an ethynyl group or a substituted ethynyl group can be expected to increase both activities.

Experimental

General Methods. Mass spectra were obtained with a Hitachi M-80 B/M-0101 mass spectrometer, and nuclear magnetic resonance (NMR) spectra were measured in $CDCl_3$ at 270 MHz with a JEOL JNM-GX 270 spectrometer. NMR spectra of **5t** and **5c** were analyzed in further detail at 400 MHz with a JEOL JNM-A400 spectrometer. Melting point (mp) data were determined with Yanako MP-500D apparatus and are uncorrected.

2-Oxo-2-phenyl-5-n-propyl-1,3,2-dioxaphosphorinane (**1**). Phenylphosphonic dichloride (160 mg, 0.85 mmol) in dry ether (2 ml) was added dropwise to a stirred, ice-cold solution of 2-*n*-propyl-1,3-propanediol (100 mg, 0.85 mmol) and pyridine (130 mg, 1.7 mmol) in dry ether (1 ml). After the addition, the mixture was stirred at room temperature for 1 h. The ethereal solution was washed with water, dried (Na_2SO_4) and concentrated, and the residue was purified by silica-gel column chromatography (chloroform : acetone = 1 : 1) to give 101 mg (50% yield) of **1** (*trans* : *cis* = 27 : 23) as an oil. ^{1}H-NMR δ: 0.92 (*cis* isomer, t, J = 7.32 Hz, CH_3), 0.95 (*trans* isomer, t, J = 7.32 Hz, CH_3), 1.24–1.40 (*trans* & *cis* isomers, m, $(CH_2)_2$), 2.26 (*trans* & *cis* isomers, m, H-5), 3.91 (*cis* isomer, m, H_{ax}-4 & H_{ax}-6), 4.19–4.41 (*trans* & *cis* isomers, m, H_{eq}-4 & H_{eq}-6), 4.51 (*trans* isomer, m, H_{ax}-4 & H_{ax}-6), 7.43–7.61, 7.73–7.89 (*trans* & *cis* isomers, m, Ar). HRMS *m/z* (M^+): calcd. for $C_{12}H_{17}O_3P$, 240.0915; found, 240.0927.

5-n-Butyl-2-oxo-2-phenyl-1,3,2-dioxaphosphorinane (**2**). Compound **2** (*trans* : *cis* = 11 : 9) was obtained by using 2-*n*-butyl-1,3-propanediol in a 68% yield in the manner just described as an oil. ^{1}H-NMR δ: 0.89 (*cis* isomer, t, CH_3), 0.91 (*trans* isomer, t, CH_3), 1.30, 1.33 (*trans* & *cis* isomers, m, $(CH_2)_3$), 2.25 (*trans* & *cis* isomers, m, H-5), 3.92 (*cis* isomer, m, H_{ax}-4 & H_{ax}-6), 4.20–4.42 (*trans* & *cis* isomers, m, H_{eq}-4 & H_{eq}-6), 4.52 (*trans* isomer, m, H_{ax}-4 & H_{ax}-6), 7.44–7.61, 7.73–7.89 (*trans* & *cis* isomers, m, Ar). HRMS *m/z* (M^+): calcd. for $C_{13}H_{19}O_3P$, 254.1071; found, 254, 1075.

5-tert-Butyl-2-oxo-2-phenyl-1,3,2-dioxaphosphorinane (**3**). Compound **3** (*trans* : *cis* = 21 : 29) was obtained using 2-*tert*-butyl-1,3-propanediol in a 23% yield in the manner just described, mp 62.2°C, ^{1}H-NMR δ: 0.92 (*cis* isomer, s, *t*-Bu), 1.00 (*trans* isomer, s, *t*-Bu), 2.15 (*trans* isomer, m, H-5), 2.34 (*cis* isomer, m, H-5), 4.09 (*cis* isomer, m, H_{ax}-4 & H_{ax}-6), 4.30–4.63 (*trans* & *cis* isomers, m, H-4 & H-6), 7.43–7.60, 7.73–7.89 (*trans* & *cis* isomers, m, Ar). HRMS *m/z* (M^+): calcd. for $C_{13}H_{19}O_3P$, 254.1070; found, 254.1054.

5-tert-Butyl-2-phenyl-2-thiono-1,3,2-dioxaphosphorinane (**4**). Phenylthiophosphonic dichloride (240 mg, 1.14 mmol) in dry ether (5 ml) was added dropwise to a stirred, ice-cold solution of 2-*tert*-butyl-1,3-propanediol (150 mg, 1.14 mmol) and pyridine (180 mg, 2.27 mmol) in dry ether (5 ml). After this addition, the mixture was stirred at room temperature for 2 h. The ethereal solution was partitioned between chloroform and water, the chloroform layer being treated in the manner already described. Recrystallization from cyclohexane gave **4** (*trans* : *cis* = 11 : 39) in a 14% yield, mp 78.1°C. ^{1}H-NMR δ: 0.90 (*cis* isomer, s, *t*-Bu), 1.00 (*trans* isomer, s, *t*-Bu), 2.23 (*trans* & *cis* isomers, m, H-5), 4.09 (*cis* isomer, m, H_{ax}-4 & H_{ax}-6), 4.33 (*trans* isomer, m, H_{eq}-4 & H_{eq}-6), 4.55 (*cis* isomer, m, H_{eq}-4 & H_{eq}-6), 4.74 (*trans* isomer, m, H_{ax}-4 & H_{ax}-6), 7.51, 7.78, 8.01 (*trans* & *cis* isomers, m, Ar). HRMS *m/z* (M^+): calcd. for $C_{13}H_{19}O_2PS$, 270.0841; found, 270.0831.

2-(4-Bromophenyl)-5-tert-butyl-2-thiono-1,3,2-dioxaphosphorinane (**5**). 4-Bromophenylthiophosphonic dichloride[5] (327 mg, 1.13 mmol) in dry ether (5 ml) was added dropwise to a stirred, ice-cold solution of 2-*tert*-butyl-1,3-propanediol (150 mg, 1.13 mmol) and triethylamine (230 mg, 2.27 mmol) in dry ether (5 ml). After this addition, the mixture was stirred at room temperature for 21 h, before the reaction mixture was treated as described for **4**. The *trans* (**5t**) and *cis* (**5c**) isomers were separated by silica-gel column chromatography (benzene) in 3.4% and 1.7% yields, respectively. **5t**: mp 76.0°C; ^{1}H-NMR δ: 1.01 (9H, s, *t*-Bu), 2.19 (1H, tt, J = 11.5 & 4.3 Hz, H-5), 4.34 (2H, ddd, H_{eq}-4 & H_{eq}-6, J = 23.7, 11.4 & 4.3 Hz), 4.73 (2H, ddd, H_{ax}-4 & H_{ax}-6, J = 11.5, 11.4 & 4.7 Hz), 7.64 (2H, dd, J = 8.3 & 3.5 Hz, Ar), 7.88 (2H, dd, J = 14.4 & 8.3 Hz, Ar); EIMS *m/z* (rel. intensity): 350 ($M^+ + 2$) (32), 348 (M^+) (31), 293 (37), 291 (36), 259 (13), 257 (14), 237 (42), 235 (40), 221 (7), 219 (7), 41 (100). *Anal.* Calcd. for $C_{13}H_{18}BrO_2PS$: C, 44.71; H, 5.19%. Found: C, 44.81; H, 5.03%. **5c**: mp 114.4°C; ^{1}H-NMR δ: 0.91 (9H, s, *t*-Bu), 2.24 (1H, tt, J = 9.7 & 4.5 Hz, H-5), 4.07 (2H, ddd, H_{ax}-4 & H_{ax}-6, J = 11.4, 9.7 & 8.0 Hz), 4.55 (2H, ddd, H_{eq}-4 & H_{eq}-6, J = 18.6, 11.4 & 4.5 Hz), 7.62 (2H, s, Ar), 7.65 (2H, d, J = 2.7 Hz, Ar); EIMS *m/z* (rel. intensity): 350 ($M^+ + 2$) (18), 348 (M^+) (17), 293 (10), 291 (8), 255 (13), 253 (12), 237 (20), 235 (19), 221 (7), 219 (9), 41 (100). *Anal.* Calcd. for $C_{13}H_{18}BrO_2PS$: C, 44.71; H, 5.19%. Found: C, 45.16; H, 5.27%.

Radioreceptor assay. The potency of dioxaphosphorinanes as NGRAs

was measured by ^{35}S-TBPS binding assays, using EDTA/water-dialyzed membranes of rat brain, as described previously.[6] Briefly, a test compound (0.003–30 μM) was incubated with rat brain membranes (0.25 mg of protein) and 1.0 nM ^{35}S-TBPS in 1.0 ml of a 5 mM Tris–HCl buffer (pH 7.5) containing 0.2 M KBr and 1 mM EDTA at 25°C for 90 min. The membranes were collected on a Whatman GF/C filter by vacuum filtration and rapidly washed with the ice-cold buffer, before the radioactivity of ^{35}S-TBPS that specifically bound to the membranes was measured with a liquid scintillation spectrometer. Nonspecific binding was determined in the presence of 10 μM unlabeled TBPS. ^{35}S-TBPS (>2.22 TBq/mmol) was purchased from Du Pont/NEN Research Products (Boston, MA, U.S.A.), and unlabeled TBPS was available from our previous studies.[7]

Insecticidal assay. Insecticidal activity was measured by topical application of the dioxaphosphorinanes to piperonyl butoxide-treated adult female houseflies (WHO susceptible strain, *Musca domestica* L.) as described previously.[6]

Antiacetylcholinesterase assay. The 25,000 × *g* supernatant of the homogenate of housefly heads was used as an enzyme source. This assay was done by the method of Kojima and Ishizuka.[12,13]

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