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Structural Requirements for Bridged Bicyclic Compounds Acting on Picrotoxinin Receptor

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To study essential requirements of cyclodiene type chemicals to interact with the picrotoxinin receptor, several cyclic hydrocarbons were synthesized. 8-Isopropylidenebicyclo[3.2.1]oct-6-en-3-one derivatives with equatorial *cis*-2,4-dimethyl substituents were found to be toxic to the German cockroach. By contrast, the axial cis and trans isomers were nontoxic. Some derivatives of bicyclo[2.2.1]heptene were moderately toxic. The endo cyclic sulfite of 5,6-bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene was most toxic. The cyclodiene-resistant strain of the German cockroach exhibited cross-resistance to representative bridged bicyclic compounds. These compounds also inhibited specific [³H]-α-dihydropicrotoxinin binding to the American cockroach brain membrane components. The potency of *endo*-5,6-bis(chloromethyl)-7-isopropenylbicyclo[2.2.1]heptan-2-one was comparable to those of cyclodiene insecticides. These observations suggest that these insecticidal bridged bicyclic compounds act at the picrotoxinin binding site. There appears to be a minimum requirement for an active ligand to possess at least two of the three active sites: two electronegative and one steric bulkiness (hydrophobicity) centers.

INTRODUCTION

γ-Aminobutyric acid (GABA) is regarded as an inhibitory neurotransmitter in a variety of animals. The mechanism of GABAergic synaptic transmission is rapidly being elucidated at subcellular levels. The GABA system in the central nervous system (CNS) of mammalian species consists of at least three closely coupled components, i.e., the GABA receptor, the chloride ionophore, and the benzodiazepine recognition site (Ticku, 1983; Bowery, 1984). γ-Aminobutyric acid released from the nerve terminal binds to the GABA receptor on the postsynaptic membrane. The binding causes an increase in the membrane permeability to chloride. A naturally occurring convulsant, picrotoxinin, is known to antagonize the action of GABA by blocking the ionophore (Takeuchi and Takeuchi, 1969; Ticku et al., 1978).

Recently, Kadous et al. (1983), Matsumura and Ghiasuddin (1983), and Tanaka et al. (1984) have presented the evidence that cyclodiene insecticides and γ-BHC compete with picrotoxinin at a common binding site in the cockroach brain. As a result they have concluded that their interaction with the picrotoxinin receptor plays an important role in their convulsive action in insect CNS. This conclusion is based on the following evidence. The cyclodiene-resistant strains of the German cockroach show cross-resistance to picrotoxinin. Cyclodiene-type insecticides, including γ-BHC, inhibit the specific binding of [³H]-α-dihydropicrotoxinin (an active analogue of picrotoxinin) at the picrotoxinin receptor. The neurophysiological effect of picrotoxinin was similar to that of cyclodiene-type insecticides.

In addition, Matsumura and Ghiasuddin (1983) noticed structural similarity among picrotoxinin, heptachlor epoxide, and γ-BHC (Figure 1). The first two compounds have bridged bicyclic structure: 6-oxabicyclo[3.2.1]octan-

7-one for picrotoxinin; bicyclo[2.2.1]heptene for heptachlor epoxide. Two rings at the 1-, 2-, and 6-positions of picrotoxinin may correspond to the epoxycyclohexane ring of heptachlor epoxide and three equatorial chlorines of γ-BHC. γ-BHC does not have any bridged bicyclic structures but two axial chlorines that may correspond to the γ-lactone ring. Also the central axial chlorine of γ-BHC has the same orientation as the isopropenyl group of picrotoxinin.

Structure–insecticidal activity relationship of picrotoxinin analogues and related compounds has been studied by several researchers (Miller et al., 1979; Kuwano et al., 1980), who noticed that the bridged bicyclic lactone skelton and the *trans*-isopropenyl or isopropyl group are essential for insecticidal activity. Structure–activity relationship of cyclodiene insecticides and BHC has also been thoroughly discussed (Soloway, 1965; Brooks, 1973, 1974). However, these discussions were held before the realization of the nature of their biological target site(s).

In view of the recent discovery of similarities of action patterns between picrotoxinin and cyclodiene-type insecticides as described above (cf., Miller et al., 1979; Matsumura and Tanaka, 1984), it appears worthwhile to reexamine structural requirement of picrotoxinin-type convulsants for interaction with the specific picrotoxinin binding site. Another objective of this study is to obtain supporting evidence for the role of the picrotoxinin receptor in the mode of action of cyclodiene-type insecticides by synthesizing compounds that structurally bridge the gap between cyclodiene compounds and picrotoxinin.

MATERIALS AND METHODS

Chemicals. Picrotoxinin and picrotin were separated from commercially available picrotoxin with silica gel column. Camphor, norcamphor, and norbornylene were provided by Aldrich Chemical Co. α- and β-endosulfan were supplied by the Environmental Protection Agency. Alodan was synthesized by lithium aluminum hydride reduction of the Diels–Alder adduct between penta-

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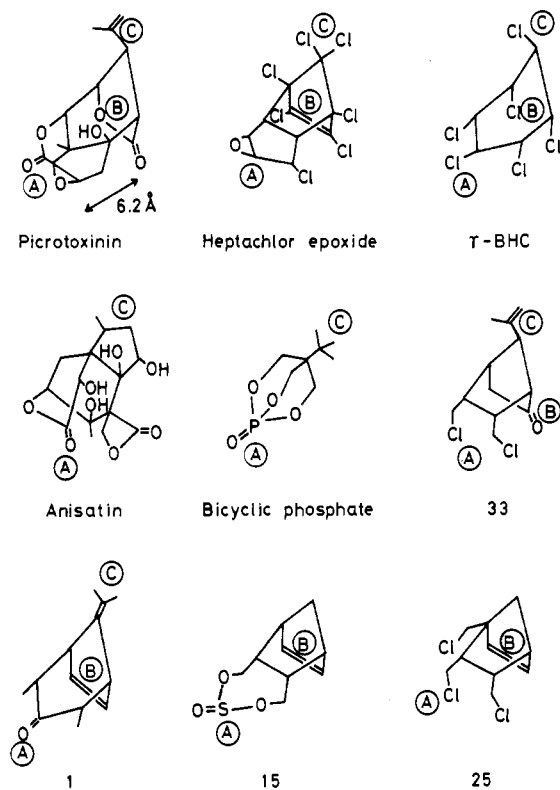
A and B: Electronegative centers; C: Hydrophobic mass.

Figure 1. Electronegative and sterical bulkiness sites of compounds acting on picrotoxinin receptor.

chlorocyclopentadiene and maleic anhydride followed by tosylation and chlorination with lithium chloride in dimethylformamide. The product was purified twice by recrystallization from ethyl acetate: mp 103 °C; MS, *m/z* 394 (M$^+$). The M$^+$ + 2, M$^+$ + 4, and M$^+$ + 6 peaks were observed at correct intensity corresponding to eight chlorines. $\beta,\beta,\gamma,\gamma$-Tetramethyl-$\gamma$-butyrolactone was synthesized by intramolecular cyclization of ethyl 3-hydroxy-3,4,4-trimethylpentanoate (Burgstahler and Wetmore, 1961); mp (sublimation) 85 °C; NMR δ 1.07 (6 H, s, CH$_3$), 1.30 (6 H, s, CH$_3$), 2.38 (2 H, s, CH$_2$). α- and β-dihydropicrotoxinin were prepared by hydrogenation of picrotoxinin with 5% Pt/C in acetic acid and Pd/C in ethyl acetate, respectively (Mercer and Robertson, 1936; O'-Donnell et al., 1939). α-Dihydropicrotoxinin: mp 256 °C; MS, *m/z* 294 (M$^+$, 3), 109 (32), 40 (100). β-Dihydropicrotoxinin: mp 267 °C; MS, *m/z* 294 (M$^+$, 15), 109 (86), 44 (99), 43 (100). Shift positions (NMR) of two methyl groups of the isopropyl group of the former were different whereas those of the latter were the same. α-[8,10-^{3}H]-Dihydropicrotoxinin (30.0 Ci/mmol) was purchased from New England Nuclear.

2,4-Dimethyl-8-isopropylidenebicyclo[3.2.1]oct-6-en-3-one was prepared from 2,4-dibromo-3-pentanone and isopropylidenecyclopentadiene by the modification of the method of Hoffmann and Igbal (1975). The reaction products were purified by removal of the precipitates caused by addition of petroleum ether and ethanol, followed by silica gel column chromatography (with chloroform). The products mainly consisted of the equatorial *cis*- and axial *cis*-2,4-dimethyl isomers (**1** and **8**). The former was carefully distilled at 101–102 °C (1 mmHg) (17 % yield), and the latter was recrystallized from pentane (21% yield). **1**: mp 34 °C; NMR δ 1.10 (6 H, d, *J* = 7 Hz, CH$_3$), 1.78 (6 H, s, =C(CH$_3$)$_2$), 2.48 (2 H, dq, *J* = 3, 7 Hz, CH-2,4), 3.31 (2 H, brs, CH-1,5), 6.15 (2 H, t, *J* = 1 Hz,

CH-6,7). **8**: mp 57 °C; NMR δ 1.20 (6 H, d, *J* = 7 Hz, CH$_3$), 1.83 (6 H, s, =C(CH$_3$)$_2$), 2.30 (2 H, q, *J* = 7 Hz, CH-2,4), 3.20 (2 H, brs, CH-1,5), 6.10 (2 H, s, CH-6,7). The *trans*-2,4-dimethyl isomer (**12**) was sometimes produced in a few percent yield through isomerization of cis isomers during column chromatography. **12**: bp 82 °C (2 mmHg); ^{1}H NMR δ 1.00 (3 H, d, *J* = 4 Hz, CH$_3$), 1.12 (3 H, d, *J* = 6 Hz, CH$_3$), 1.70 (3 H, s, =C(CH$_3$)$_2$), 1.73 (3 H, s, =C-(CH$_3$)$_2$), 2.2–2.8 (2 H, CH-2,4), 3.18 (2 H, q, *J* = 1 Hz, CH-1,5), 6.00 (2 H, t, *J* = 1 Hz, CH-6,7). Purity was checked by gas chromatography (silicon OV-17 or SE-30). The crude product of usual lithium aluminum hydride reduction of **1** was distilled at 95 °C (1 mmHg) to give a mixture of alcohols **2** and **3** in 55% yield. Axial 3-OH isomer **3** was obtained by recrystallization from pentane. **3**: mp 105–108 °C; NMR δ 1.02 (6 H, d, *J* = 6 Hz, CH$_3$), 1.1–1.7 (2 H, m, CH-2,4), 1.50 (1 H, s, OH), 1.63 (6 H, s, =C(CH$_3$)$_2$), 2.82 (1 H, t, *J* = 8 Hz, CH-3), 3.04 (2 H, brs, CH-1,5), 6.02 (2 H, t, *J* = 1 Hz, CH-6,7). Equatorial 3-OH isomer **2** remained in the solution: mp 31–33 °C; NMR δ 1.08 (6 H, d, *J* = 8 Hz, CH$_3$), 1.62 (6 H, s, =C(CH$_3$)$_2$), 1.80 (1 H, s, OH), 1.8–2.2 (2 H, m, CH-2,4), 2.97 (2 H, brs, CH-1,5), 3.55 (1 H, t, *J* = 5 Hz, CH-3), 6.32 (2 H, t, *J* = 1 Hz, CH-6,7). Oxidation of **1**, **8**, and **12** with 1 equiv of *m*-chloroperbenzoic acid (MCPBA) in chloroform (room temperature, 1–5 h) gave epoxides **4**, **9**, and **13** in 40–70% yield, respectively. **4**: mp 108 °C; MS, *m/z* 206 (M$^+$); NMR δ 1.05 (6 H, d, *J* = 6 Hz, CH$_3$), 1.45 (6 H, s, Epo-(CH$_3$)$_2$ (Epo = epoxy)), 2.4–2.7 (4 H, CH-1,2,4,5), 6.07 (2 H, t, *J* = 1 Hz, CH-6,7). **9**: mp 42 °C; MS, *m/z* 206 (M$^+$); NMR δ 1.27 (6 H, d, *J* = 7 Hz, CH$_3$), 1.49 (6 H, s, Epo-(CH$_3$)$_2$), 2.46 (2 H, brs, CH-1,5), 2.53 (2 H, q, *J* = 7 Hz, CH-2,4), 6.21 (2 H, brs, CH-6,7). **13**: mp 60 °C; NMR δ 1.02 (3 H, d, *J* = 7 Hz, CH$_3$), 1.13 (3 H, d, *J* = 8 Hz, CH$_3$), 1.41, 1.43 (6 H, s, Epo(CH$_3$)$_2$), 2.0–2.8 (4 H, CH-1,2,4,5), 6.06 (2 H, t, *J* = 1 Hz, CH-6,7). The carbonyl group of **4** was reduced with lithium aluminum hydride in ether in 58% yield. **5**: mp 74 °C; NMR δ 1.12 (6 H, d, *J* = 8 Hz, CH$_3$), 1.37 (6 H, s, =C(CH$_3$)$_2$), 1.90 (1 H, s, OH), 1.7–2.1 (2 H, m, CH-2,4), 2.16 (2 H, brs, CH-1,5), 3.56 (1 H, t, *J* = 5 Hz, CH-3), 5.98 and 6.30 (eq:ax = ca. 9:1, 2 H, t, *J* = 1 Hz, CH-6,7). Diepoxy analogues **6**, **10**, and **14** were produced by reaction of **1**, **8**, and **12**, respectively, with 2–5 molar excess of MCPBA for 24 h in about 40% yield. **6**: mp (sublimation) 140 °C; MS, *m/z* 222 (M$^+$); NMR δ 1.20 (6 H, d, *J* = 7 Hz, CH$_3$), 1.46 (6 H, s, Epo(CH$_3$)$_2$), 2.21 (2 H, d, *J* = 3 Hz, CH-1,5), 2.62 (2 H, dq, *J* = 3, 7 Hz, CH-2,4), 3.47 (2 H, s, CH-6,7). **10**: mp 115 °C; MS, *m/z* 222 (M$^+$); NMR δ 1.27 (6 H, d, *J* = 8 Hz, CH$_3$), 1.40 (6 H, s, Epo(CH$_3$)$_2$), 2.25 (2 H, s, CH-1,5), 2.50 (2 H, q, *J* = 8 Hz, CH-2,4), 3.61 (2 H, s, CH-6,7). **14**: mp 105–106 °C; MS, *m/z* 222 (M$^+$); NMR δ 1.14 (3 H, d, *J* = 7 Hz, CH$_3$), 1.19 (3 H, d, *J* = 7 Hz, CH$_3$), 1.39 (6 H, s, Epo(CH$_3$)$_2$), 2.23 (2 H, brs, CH-1,5), 2.3–2.9 (2 H, CH-2,4), 3.41 (2 H, brs, CH-6,7).

2,4-Dibromo-8-isopropylidenebicyclo[3.2.1]oct-6-en-3-one was prepared by the same procedure as that for the 2,4-dimethyl analogue except that 1,1,3,3-tetrabromoacetone was used instead of 2,4-dibromo-3-pentanone. The axial and equatorial dibromo isomers were purified by silica gel column chromatography (with chloroform). The eluted product was recrystallized from ether to produce plates and needles. The plates and needles were separated from each other. Each form of crystals was repeatedly recrystallized. The plates (1.3% yield) and needles (0.4% yield) proved to be equatorial and axial isomers, respectively, by NMR spectrometry. **7**: mp 154 °C; NMR δ 1.89 (6 H, s, =C(CH$_3$)$_2$), 3.76 (2 H, dd, *J* = 1, 3 Hz, CH-1,5),

4.22 (2 H, d, J = 3 Hz, CH-2,4), 6.20 (2 H, t, J = 1 Hz, CH-6,7). 11: mp (sublimation) 150 °C; NMR δ 1.80 (6 H, s, =C(CH₃)₂), 3.84 (2 H, dd, J = 1, 4 Hz, CH-1,5), 4.63 (2 H, d, J = 4 Hz, CH-2,4), 6.34 (2 H, t, J = 1 Hz, CH-6,7). The ether solution contained a mixture of both isomers (eq:ax = 4:1; 1.4% yield).

7-Isopropylidenebicyclo[2.2.1]hept-2-ene-5,6-dicarboxylic anhydride was prepared by the Diels–Alder reaction between isopropylidenecyclopentadiene and maleic anhydride in ether (Alder and Rühmann, 1950). The exo isomer (mp 136–138 °C) precipitated as crystals, and the ether solution contained the endo isomer (mp 112 °C). The latter was purified by repeated recrystallization from ether. Purity was checked by NMR spectrometry. The shift position of the protons attached to C-5 and C-6 of the endo isomer was clearly different from that of the exo isomer (endo, a doublet of doublet at δ 3.47; exo, a singlet at δ 3.03). Both isomers were reduced with lithium aluminum hydride to the corresponding two isomers of 7-isopropylidene-5,6-bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene (endo diol, mp 113 °C; exo diol, mp 113 °C). The diols were allowed to react with an equivalent amount of thionyl chloride in chloroform at room temperature for 4 h to yield the cyclic sulfites of the diols in about 70% yield. The cyclic sulfites were recrystallized from hexane. Endo isomer 18: mp 104 °C; NMR δ 1.56 (6 H, s, =C(CH₃)₂), 2.73 (2 H, dm, J = 12 Hz, CH-5,6), 3.27 (2 H, m, CH-1,4), 3.70 (2 H, dd, J = 4, 12 Hz, CH₂), 4.56 (2 H, t, J = 2 Hz, CH-2,3), 6.26 (2 H, t, J = 2 Hz, CH-2,3). Exo isomer 20: mp 93 °C; NMR δ 1.61 (6 H, s, =C(CH₃)₂), 1.9–2.3 (2 H, m, CH-5,6), 2.96 (2 H, t, J = 2 Hz, CH-1,4), 3.73 (2 H, dd, J = 4, 12 Hz, CH₂), 4.70 (2 H, t, J = 12 Hz, CH₂), 6.33 (2 H, t, J = 2 Hz, CH-2,3). When the exo cyclic sulfite was allowed to react with an equimolar amount of MCPBA in chloroform at room temperature for 1 h, the sulfite was converted to the mixture of epoxides 21 and 22. Epoxide 21 was obtained by recrystallization of the crude product from acetone in 21% yield: mp 179 °C; MS, m/z 256 (M⁺); NMR δ 1.67 (6 H, s, =C(CH₃)₂), 2.1–2.5 (2 H, m, CH-5,6), 2.82 (2 H, s, CH-1,4), 3.92 (2 H, s, CH-2,3), 3.63 (2 H, dd, J = 4 Hz, CH₂), 4.67 (2 H, t, J = 12 Hz, CH₂). Epoxide 22 obtained from the mother liquor was recrystallized from ether (12% yield): mp 112–113 °C; NMR δ 1.41 (6 H, s, Epo(CH₃)₂), 2.0–2.6 (2 H, m, CH-5,6), 2.31 (2 H, t, J = 2 Hz, CH-1,4), 3.67 (2 H, dd, J = 4, 12 Hz, CH₂), 4.90 (2 H, t, J = 12 Hz, CH₂), 6.33 (2 H, t, J = 2 Hz, CH-2,3). Reaction of the cyclic sulfite with double the molar quantity of MCPBA afforded diepoxide 23, which was recrystallized from acetone (40% yield): mp 201–203 °C; NMR δ 1.38 (6 H, s, Epo(CH₃)₂), 2.12 (2 H, s, CH-1,4), 2.40 (2 H, m, CH-5,6), 3.17 (2 H, s, CH-2,3), 3.58 (2 H, dd, J = 4, 12 Hz, CH₂), 4.88 (2 H, t, J = 12 Hz, CH₂). Anal. Calcd for C₁₂H₁₆O₅S: C, 52.94; H, 5.88. Found: C, 52.64; H, 5.90. Two isomeric compounds (19) epoxidized at the isopropylidene moiety of endo cyclic sulfite 18 were formed by the reaction of 18 with 1 equiv of MCPBA (room temperature, 24 h). Mixture 19I (mp 107–117 °C) was obtained as crystals by recrystallization of the product from acetone (56% yield), and mixture 19II (mp 64-9 °C) was from the mother liquor (28% yield). The ratios of the isomer epoxidized at the side of the cyclic sulfite moiety against that epoxidized at the side of the C(2)–C(3) double bond were ca. 2:8 and 5:4 in mixtures 19I and 19II, respectively (estimated from the ratio of triplets at δ 6.15 and 6.26 assigned to C(2)-H and C(3)-H).

On the other hand, the endo and exo diols were tosylated in pyridine in a usual manner (the endo tosylate, mp 119 °C; the exo tosylate, mp 144 °C). The yield was low

(30–40%) because cyclic ethers (30–60% yield) were formed as a side reaction product through dehydration between the two hydroxyl groups. The endo and exo tosylates were heated at 70 °C with a twofold molar excess of lithium chloride in dimethylformamide for 24 h to give 5,6-bis(chloromethyl) analogues (40–80% yield). endo-5,6-Bis(chloromethyl) isomer 30: mp 82 °C; MS, m/z 232 (M⁺ + 2, 4.9), 230 (M⁺, 7.3); NMR δ 1.58 (6 H, s, CH₃), 2.3–2.8 (2 H, m, CH-5,6), 2.9–3.6 (6 H, CH-1,4, CH₂), 6.32 (2 H, t, J = 2 Hz, CH-2,3). exo-5,6-Bis(chloromethyl) isomer 34: mp 69 °C; NMR δ 1.64 (6 H, s, CH₃), 1.8–2.3 (2 H, m, CH-5,6), 3.20 (2 H, t, J = 10 Hz, CH₂), 3.70 (2 H, dd, J = 5 Hz, CH₂), 6.27 (2 H, t, J = 2 Hz, CH-2,3). The exo-5,6-bis(chloromethyl) isomer was epoxidized with MCPBA to give the 2,3-epoxy and 7,8-epoxy analogues. The 2,3-epoxy analogue was purified by silica gel column chromatography (15% yield). 35: mp 140 °C; NMR δ 1.68 (6 H, s, CH₃), 2.0–2.4 (2 H, m, CH-5,6), 3.14 (2 H, t, J = 10 Hz, CH₂), 3.20 (2 H, s, CH-1,4), 3.25 (2 H, s, CH-2,3), 3.54 (2 H, dd, J = 5, 10 Hz, CH₂). The endo-5,6-bis(chloromethyl) isomer was allowed to react with 1 equiv of 1 M diborane–THF solution at room temperature for 24 h and then with alkaline hydrogen peroxide at 40–50 °C for 2 h. The crude products were purified by silica gel column chromatography followed by recrystallization from ether to give diol 31 (24% yield): mp 154 °C; NMR δ 1.33 (6 H, s, CH₃), 1.5 (1 H), 1.8 (1 H, OH), 1.9 (2 H), 2.4 (1 h), 2.6 (3 H), 3.5 (4 H), 3.8 (1 H, OH), 3.9 (1 H). The secondary hydroxyl group of the diol was oxidized with chromium trioxide–pyridine complex (Ratcliffe and Rodehorst, 1970). The product (32) was recrystallized from dichloromethane (60% yield): mp 146 °C; NMR δ 1.27 (3 H, s, CH₃), 1.5 (1 H), 1.7 (1 H), 1.9 (2 H), 2.5–3.0 (4 H), 3.1–4.0 (4 H). To compound 32 in pyridine cooled at 0 °C was added in drops a sixfold molar excess of thionyl chloride. The mixture was left at 0 °C for 1 h to give dehydrated compound 33 (40% yield): mp 65 °C; MS, m/z 248 (M⁺ + 2, 3.2), 246 (M⁺, 4.8), 197 (89), 155 (100), 93 (86), 91 (93); NMR δ 1.8 (3 H), 2.0 (1 H), 2.5–3.0 (6 H), 3.0–4.0 (4 H), 4.67 (1 H), 4.87 (1 H). Hydroboration–oxidation was similarly applied to the exo-5,6-bis(chloromethyl) isomer. However, a monohydroxy derivative was produced instead of a diol. 36: mp 120–121 °C; NMR δ 1.3–1.6 (2 H), 1.76 (6 H, s, CH₃), 1.7–2.2 (3 H), 2.6–4.0 (7 H). Ketone 37 was prepared by oxidation of the compound. 37: mp 94 °C; NMR δ 1.70 (3 H, s, CH₃), 1.77 (3 H, s, CH₃), 2.2–2.6 (4 H), 3.2–3.9 (6 H).

The Diels–Alder adduct (sublimation, 130 °C) between cyclopentadiene and maleic anhydride was reduced with lithium aluminum hydride in ether–tetrahydrofuran (3:1) to give an endo diol (mp 67 °C). Cyclic sulfite 17 was prepared by hydrogenation of the diol with Pt/C in acetic acid (75% yield) followed by reaction with thionyl chloride (66% yield). 17: bp 113 °C (2 mmHg); mp 44 °C; NMR δ 1.43 (6 H), 2.1–2.6 (4 H), 3.50 (2 H, dd, J = 3, 12 Hz, CH₂), 4.97 (2 H, t, J = 12 Hz, CH₂). Reaction of the diol with thionyl chloride gave cyclic sulfite 15 in 55% yield. 15: mp 48 °C; MS, m/z 200 (M⁺, 16), 91 (92), 67 (100), 39 (87); NMR δ 1.60 (2 H, CH₂), 2.8 (4 H, CH-1,4,5,6,), 3.63 (2 H, dd, J = 8, 12 Hz, CH₂), 6.10 (2 H, CH-2,3). The sulfite was epoxidized with 1.3-fold molar excess of MCPBA in chloroform at room temperature (10 h) in 65% yield. 16: mp 111 °C; NMR δ 1.0 (2 H), 1.6 (2 H), 2.4–2.9 (4 H), 3.3 (2 H, s), 3.7 (2 H, dd, J = 3, 12 Hz, CH₂), 5.1 (2 H, t, J = 12 Hz, CH₂).

On the other hand, the diol was tosylated in a usual manner. The tosylate was converted to chlorinated compound 24 with lithium chloride as described above. 24:

bp 93–94 °C (2 mmHg); 35% yield; MS, m/z 192 (M^+ + 2), 190 (M^+); NMR δ 1.2–1.7 (2 H), 2.4–2.8 (2 H), 2.7–3.7 (6 H), 6.23 (2 H, t, J = 2 Hz). Alcohol **26** was prepared by hydroboration–oxidation of **24** and purified by distillation at 136–139 °C (1 mmHg) and recrystallization from ether–hexane (50–70% yield). **26**: mp 58 °C; NMR δ 1.0–1.6 (4 H), 1.6–2.0 (2 H), 3.0–3.9 (4 H), 3.8–4.1 (1 H). Oxidation of **26** with chromium trioxide–pyridine gave **27** in 72% yield. **27**: mp 72 °C; MS, m/z 208 (M^+ + 2), 206 (M^+); NMR δ 1.80 (2 H), 2.10 (2 H), 2.4–3.0 (4 H), 3.0–4.0 (4 H).

1-(Methoxycarbonyl)bicyclo[2.2.1]hept-2-ene-*endo*-5,6-dicarboxylic anhydride (Grunewald and Davis, 1978) (mp 150 °C) was reduced with lithium aluminum hydride in tetrahydrofuran to give a triol, 1,5,6-tris(hydroxymethyl)bicyclo[2.2.1]hept-2-ene. The tosylate of the triol was allowed to react with lithium chloride to give trichloro analogue **25**, which was distilled twice at 90–130 °C (2 mmHg). **25**: NMR δ 1.3–1.9 (2 H), 2.6–4.5 (9 H), 5.9–6.5 (2 H). Hydroboration–oxidation of **25** gave an alcohol, 1,5,6-tris(chloromethyl)bicyclo[2.2.1]heptan-2-ol (bp 130–60 °C (2 mmHg)) in 63% yield. Ketone **28** was scraped from the silica gel TLC plate on which the chromium trioxide oxidation product of the above alcohol was developed and was recrystallized from ether–hexane (11% yield). **28**: mp 85–86 °C; MS, m/z 258 (M^+ + 4, 0.8), 256 (M^+ + 2, 3.9), 254 (M^+, 4.4), 178 (61), 176 (100), 143 (18), 141 (59), 105 (56), 91 (55); NMR δ 1.93 (2 H), 2.28 (2 H), 2.7–3.1 (3 H), 3.1–3.6 (3 H), 3.77 (2 H), 3.8–4.2 (1 H). Lactone **29** was prepared by oxidation of ketone **27** with sixfold molar excess of MCPBA in chloroform at room temperature (56% yield). **29**: mp 108–110 °C; NMR δ 1.8–2.1 (2 H), 2.5–3.0 (5 H), 3.3–4.0 (4 H), 4.7–5.0 (1 H).

Bioassay. Insecticidal activity of synthesized compounds was tested by injection of a dimethyl sulfoxide solution (0.25 μL) to the ventral portion of abdomen of the adult male German cockroach, *Blattella germanica* (L.), 1 h after topical application of piperonyl butoxide (30 μg/μL) in acetone (1 μL). Mortality was determined 24 h after injection.

[³H]-α-Dihydropicrotoxinin Binding Assay. Twenty-five heads of the male American cockroach, *Periplaneta americana* (L.), were homogenized in ice-cooled 0.25 M sucrose (25 mL) with a glass homogenizer. The homogenate was centrifuged at 1000g for 10 min. The supernatant was filtered through a glass wool plug. The filtrate was centrifuged at 20000g for 45 min. The precipitate was suspended in 13.5 mL of ice-cooled 0.2 M NaCl–5 mM sodium phosphate buffer (pH 7.0). To centrifuge tubes kept in an ice bath were added 0.7-mL aliquots of the suspension in triplicate and the test chemicals dissolved in acetone (1 μL). After the tubes were kept in an ice bath for 10 min, with occasional shaking, 0.3-mL aliquots of [³H]-α-dihydropicrotoxinin (final concentration 9–16 nM) solution in 0.2 M NaCl–5 mM sodium phosphate buffer (pH 7.0) were added. The solution was incubated in an ice bath for 15 min with occasional shaking. The reaction was terminated by centrifugation of the solution at 20000g for 20 min. The supernatant was pipetted out, and the precipitate was quickly rinsed once with 4 mL of ice-cooled 0.2 M NaCl–5 mM sodium phosphate buffer (pH 7.0). The inside of the centrifuge tube was carefully wiped with tissue paper, 0.5-mL aliquots of 0.2 M sodium hydroxide solution were added, and the tubes were incubated at 50 °C to dissolve the pellet. Bound radioactivity in the pellet was assayed by liquid scintillation spectrometry. Specific α-dihydropicrotoxinin binding was defined as the difference between binding in the absence and the presence of

0.1 mM unlabeled α-dihydropicrotoxinin (Kadous et al., 1983).

RESULTS

Chemistry. On the basis of the discussion by Matsumura and Ghiasuddin (1983), several bridged bicyclic compounds were synthesized. Cycloaddition of allyl cations to isopropylidenecyclopentadiene yielded three epimers of 2,4-dimethyl-8-isopropylidenebicyclo[3.2.1]oct-6-en-3-one, i.e., equatorial *cis*-2,4-dimethyl (**1**), axial *cis* 2,4-dimethyl (**8**), and *trans*-2,4-dimethyl (**12**) analogues, and two epimers of 2,4-dibromo analogues (**7** and **11**). Several epoxy and hydroxy analogues were prepared by epoxidation of double bonds and reduction of carbonyl groups, respectively.

The Diels–Alder reaction was utilized to construct bicyclo[2.2.1]heptane or bicyclo[2.2.1]heptene structures. Reaction of isopropylidenecyclopentadiene with maleic anhydride produced the endo and exo configurational isomers of 8-isopropylidenebicyclo[2.2.1]hept-2-ene-5,6-dicarboxylic anhydride. Similar reactions were applied to unsubstituted and 1-(methoxycarbonyl)cyclopentadienes to give the corresponding anhydrides. The anhydrides were reduced to alcohols, which were then allowed to react with thionyl chloride or tosyl chloride to give cyclic sulfites or tosylates. Reaction of the tosylates with lithium chloride gave chlorinated compounds. Hydration of the double bonds of the chlorinated compounds was accomplished by hydroboration–oxidation. Diol **31** thus obtained was converted to 5,6-bis(chloromethyl)-7-isopropylidenebicyclo[2.2.1]heptan-2-one (**33**) by chromium trioxide oxidation of the secondary hydroxyl group followed by dehydration at the C-7 substituent.

Insecticidal Activity. Generally, the insecticidal activity of synthesized compounds was not so high. However, they were active enough to serve as a tool for the current toxicological study. Generally speaking, active compounds (e.g., **1** and **15**) caused convulsion, and some of them (e.g., **33**) caused incoordinated walking in the German cockroach. The onset of sign of poisoning was early, as compared with that caused by cyclodiene insecticides such as dieldrin. Table I shows the insecticidal activities of 2,4-dimethyl-8-isopropylidenebicyclo[3.2.1]oct-6-en-3-one and related compounds by injection. The equatorial *cis*-2,4-dimethyl epimer **1** was active among three epimers of 2,4-dimethyl-8-isopropylidenebicyclo[3.2.1]oct-6-en-3-one. The trans isomer **12** was ca. 10 times less active than the equatorial cis isomer. The epoxide derivatives (**4** and **6**) had almost the same activity as the parent compound. Reduction of the carbonyl group (**2** and **3**) lowered the activity. the 2,4-dibromo derivatives **7** and **11** were inactive.

Table II lists the insecticidal activity of cyclic sulfites. In this case, the exo cyclic sulfites of 5,6-bis(hydroxymethyl)-7-isopropylidenebicyclo[2.2.1]hept-2-ene **20** was active whereas the corresponding endo isomer **18** was inactive. Monoepoxidation (**21** and **22**) and diepoxidation (**23**) of the exo cyclic sulfite resulted in slight increase in activity. Compound **15** was the most active among this series of compounds synthesized. The LD$_{50}$ value of this compound was estimated to be about 1 μg/fly when topically applied to houseflies without any synergists (data not shown). The C-7 isopropylidene group proved to be unnecessary for high activity by comparison with compound **18**. The activity decreased by epoxidation (**16**) or hydrogenation (**17**) of the endocyclic double bond. The cyclodiene insecticide endosulfan was about 10 times as active as **15**.

Another series of compounds are 5,6-bis(chloro-

Table I. Insecticidal Activity of Bicyclo[3.2.1]oct-6-en-3-one Analogues

compd no.	X	Y	Z	R	dose, μg/roach	mortality
		2,4-Diequatorial Cis Isomers				
1	HC=CH	>C=C(CH₃)₂	C=O	CH₃	10 / 3	6/11 / 3/10
2	HC=CH	>C=C(CH₃)₂	CH(OH) (eq)	CH₃	10	0/10
3	HC=CH	>C=C(CH₃)₂	CH(OH) (ax)	CH₃	10	0/10
4	HC=CH	>C—C(CH₃)₂ (epoxide)	C=O	CH₃	10 / 3	8/10 / 2/10
5	HC=CH	>C—C(CH₃)₂ (epoxide)	CH(OH)	CH₃	10	1/10
6	HC—CH (epoxide)	>C—C(CH₃)₂ (epoxide)	C=O	CH₃	10 / 3	7/10 / 0/10
7	HC=CH	>C=C(CH₃)₂	C=O	Br	10	0/10
		2,4-Diaxial Cis Isomers				
8	HC=CH	>C=C(CH₃)₂	C=O	CH₃	10	1/10
9	HC=CH	>C—C(CH₃)₂ (epoxide)	C=O	CH₃	100 / 10	6/10 / 0/10
10	HC—CH (epoxide)	>C—C(CH₃)₂ (epoxide)	C=O	CH₃	10	0/10
11	HC=CH	>C=C(CH₃)₂	C=O	Br	10	0/10
		2,4-Trans Isomers				
12	HC=CH	>C=C(CH₃)₂	C=O	CH₃	10	0/10
13	HC=CH	>C—C(CH₃)₂ (epoxide)	C=O	CH₃	100 / 10	9/10 / 0/10
14	HC—CH (epoxide)	>C—C(CH₃)₂ (epoxide)	C=O	CH₃	10	1/10

methyl)bicyclo[2.2.1]hept-2-ene and related compounds (Table III). Compound **24** was active. The activity was slightly raised by the introduction of a chloromethyl group to the bridgehead (**25**). Ketone **27** prepared by hydroboration–oxidation of **24** was active. Introduction of a chloromethyl group to the bridgehead of **27** resulted in a decrease in activity (**28**). Compound **30** was inactive, indicating that the isopropylidene group at the 7-position is not desirable for insecticidal activity. Ketone **33**, which has an isopropenyl group at the 7-position, had activity.

Table IV shows the insecticidal activity of commercially available bicyclic terpenes. Camphor and norcamphor were not toxic at 10 μg/roach. However, the knockdown effect of norcamphor was remarkable.

Table V is comparison of insecticidal activity of picrotoxinin and three derivatives. Picrotin was about 30 times less toxic than picrotoxinin. α- and β-dihydropicrotoxinin were approximately 3 and 100 times less toxic than picrotoxinin, respectively. The *trans*-isopropyl group proved to be favorable for high activity by comparison of α- and β-dihydropicrotoxinin. This finding is consistent with that of Kuwano et al. (1980), who synthesized the cis and trans isomers of 8-isopropyl-6-oxabicyclo[3.2.1]octan-7-one.

If the insecticidal activity of the synthesized bridged bicyclic compounds is due to the interaction with the picrotoxinin binding site, cyclodiene-resistant German cockroaches must show cross-resistance to these compounds, since it has been clearly shown by Kadous et al. (1983) and Tanaka et al. (1984) that the resistance phenomenon is due to the alteration of the picrotoxinin site.
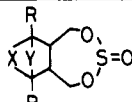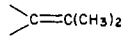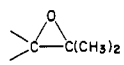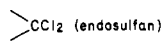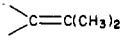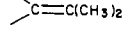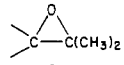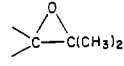
Table VI shows the insecticidal activity of selected compounds against the cyclodiene-susceptible strain (Columbus) and the resistant strain (LPP) of the German cockroach. The LPP strain showed about 30-fold resistance to endosulfan, alodan, and picrotoxinin, compared with the Columbus strain. The cyclodiene-resistant strain was about 3–10 times resistant to three selected bicyclic compounds. However, the resistance to a simple γ-butyrolactone was not significant.

Inhibition of Specific [³H]-α-Dihydropicrotoxinin Binding. Specific binding of [³H]-α-dihydropicrotoxinin was inhibited by some bridged bicyclic compounds (Table VII). Compound **33** was a potent inhibitor, which was comparable to endosulfan, although the insecticidal activity of the former compound was somewhat lower than that of the latter. Cyclic sulfite **15** was also active in displacing [³H]-α-dihydropicrotoxinin. Noninsecticidal analogue **10** was not quite active in this regard, but nor is the insecticidal equatorial cis isomer **6**. A simple lactone and norcamphor were also weak inhibitors. Cyclohexane had almost no activity. Judging by the fact that the degree of resistance shown by LPP to the lactone compound and to compound **6** was very modest, it appears that the extent of interaction of these compounds with the picrotoxinin receptor of the cockroach is less significant than that of **15** and **33**.

DISCUSSION

Molecular topography of cyclodiene insecticides was extensively studied by Soloway (1965), who suggested the

Picrotoxinin Receptor Binding

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Table II. Insecticidal Activity of the Cyclic Sulfites of 5,6-Bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene Analogues

compd no.	X	Y	R		dose, μg/roach	mortality
			Endo Cyclic Sulfites			
15	HC=CH	CH_2	H		10	10/10
					3	9/10
					1	3/10
					0.3	0/10
16	HC—CH (epoxide)	CH_2	H		10	10/10
					3	4/10
					1	0/10
17	H_2CCH_2	CH_2	H		10	8/10
					3	0/10
18	HC=CH	C=C(CH3)2	H		10	0/10
19	HC=CH	C—C(CH3)2 (epoxide)	H	I[a]	30	1/10
					10	0/10
				II[a]	30	6/10
					10	0/10
	ClC=CCl	CCl2 (endosulfan)	Cl	α	0.3	10/10
					0.1	8/10
				β	0.3	8/10
					0.1	0/10
			Exo Cyclic Sulfites			
20	HC=CH	C=C(CH3)2	H		10	5/10
					3	1/10
21	HC—CH (epoxide)	C=C(CH3)2	H		10	8/10
					3	0/10
22	HC=CH	C—C(CH3)2 (epoxide)	H		10	9/10
					3	1/10
23	HC—CH (epoxide)	C—C(CH3)2 (epoxide)	H		10	10/10
					3	0/10

[a] See Materials and Methods for differences.

significance of the presence of two electronegative centers of biologically active cyclodiene compounds. According to his hypothesis, one center is composed of chlorines of hexachloronorbornene nucleus and the other is the double bond or oxygen atom of the second ring system. In the case of γ-BHC, one includes three equatorial chlorines and the two axial chlorines on the same side of the ring as the equatorial chlorines, and the other is the center axial chlorine. The two electronegative centers were supposed to interact with an unknown receptor.

We provide another possibility of the molecular topography of picrotoxinin-type convulsants in this paper. The similarity is that two electronegative centers are provided by both the olefinic chlorines of the hexachloronorbornene nucleus and the double bond or oxygen atom of the second ring system in the case of cyclodiene compounds such as aldrin, dieldrin and heptachlor epoxide (A and B in Figure 1). The differences are that we propose that there is one additional hydrophobic center that provides steric bulkiness (C in Figure 1) and that to act on the receptor the compound must at least possess two of these three characteristics. γ-BHC has three equatorial chlorines and two axial chlorines as the electronegative center. However, the isopropenyl group of picrotoxinin, the center axial chlorine of γ-BHC, and one chlorine of dichloromethylene bridge of cyclodiene insecticides appear to work as steric bulkiness or a hydrophobic center rather than an electronegative center. Another type of convulsant, bicyclic phosphates (Ticku and Olsen, 1979; Ozoe et al., 1982; Squires et al., 1983), which act at the picrotoxinin binding site also in

cockroaches (Tanaka et al., 1984), has a phosphoryl moiety as an electronegative center but does not have another one. Rather, the bulkiness or hydrophobicity of the bridgehead substituent plays an important role as another center for high affinity to the binding site (Eto et al., 1976). Another example is a picrotoxinin-like convulsant anisatin which is isolated from the seed of a toxic plant (Kudo et al., 1981). This compound does not seem to have the second electronegative center (Figure 1).

Insecticidal compounds synthesized for the present study satisfy structural requirement as described above although they are not complete. Actually, they inhibited [3H]-α-dihydropicrotoxinin binding and were less toxic to the cyclodiene-resistant strain of the German cockroach than the susceptible strain, indicating that they act at the picrotoxinin site. In the case of bicyclo[3.2.1]oct-6-en-3-one analogues, all insecticidal (below 10 μg) compounds have equatorial methyl groups at the 2- and 4-positions. The equatorial cis-2,4-dimethyl isomers are in the chair conformation whereas axial cis isomers are in the boat form, in which 1,3-diaxial nonbonded interactions are minimized (A of Figure 2). On the other hand, trans isomers are supposed to interconvert between the chair and boat conformations (Kashman and Rudi, 1974). Chair conformation and substituents in equatorial position seem to be favorable for the interaction with the picrotoxinin binding site. This is also the case for picrotoxinin, γ-BHC, and cyclodiene insecticides that have equatorial or endo substituents at the corresponding positions. The carbonyl oxygen of boat conformers possibly cannot constitute the

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Table III. Insecticidal Activity of 5,6-Bis(chloromethyl)bicyclo[2.2.1]hept-2-ene Analogues
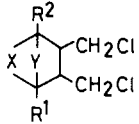
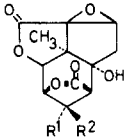| compd no.	X	Y	R^1	R^2	dose, μg/roach	mortality
		endo-Bis(chloromethyl) Analogues				
24	HC=CH	CH$_2$	H	H	10	6/10
					3	1/10
25	HC=CH	CH$_2$	CH$_2$Cl	H	10	10/10
					3	0/10
26	CH$_2$CH(OH)	CH$_2$	H	H	10	0/10
27	CH$_2$CO	CH$_2$	H	H	10	5/10
					3	0/10
28	CH$_2$CO	CH$_2$	CH$_2$Cl	H	10	2/10
29	CH$_2$COO	CH$_2$	H	H	10	2/10
30	HC=CH	C=C(CH$_3$)$_2$	H	H	10	0/10
31	CH$_2$CH(OH)	CHC(CH$_3$)$_2$(OH)	H	H	10	0/10
32	CH$_2$CO	CHC(CH$_3$)$_2$(OH)	H	H	10	0/10
33	CH$_2$CO	CHC(CH$_3$)=CH$_2$	H	H	10	5/10
					3	0/10
	ClC=CCl	CCl$_2$ (alodan)	Cl	Cl	0.1	5/10
					0.03	0/10
		exo-Bis(chloromethyl) Analogues				
34	HC=CH	C=C(CH$_3$)$_2$	H	H	10	0/10
35	HC—CH (epoxide)	C=C(CH$_3$)$_2$	H	H	10	0/10
36	CH$_2$CH(OH)	C=C(CH$_3$)$_2$	H	H	10	0/10
37	CH$_2$CO	C=C(CH$_3$)$_2$	H	H	10	0/10

Table IV. Insecticidal Activity of Bridged Bicyclic Terpenes

X	Y	R	dose, μg/roach	kd^a	mortality
CH$_2$CO	C(CH$_3$)$_2$ (camphor)	CH$_3$ (+)	100	10/10	10/10
			10	4/10	1/10
		(−)	100	9/10	10/10
			10	1/10	1/10
CH$_2$CO	CH$_2$ (norcamphor)	H	100	10/10	10/10
			30	11/11	5/11
			10	10/10	0/10
HC=CH	CH$_2$ (norbornylene)	H	30	0/10	0/10

^{a} The number of roaches knocked down 2 h after injection.

Table V. Insecticidal Activity of Picrotoxinin Derivatives

R^1	R^2	dose, μg/roach	mortality
C(CH$_3$)=CH$_2$ (picrotoxinin)	H	0.3	8/10
		0.1	4/10
(CH$_3$)$_2$(OH) (picrotin)	H	10	9/10
		3	0/10
CH(CH$_3$)$_2$ (α-dihydropicrotoxinin)	H	1	7/10
		0.3	1/10
H (β-dihydropicrotoxinin)	CH(CH$_3$)$_2$	30	5/10
		10	1/10

electronegative center with the double bond or epoxy group at the 6- and 7-positions whereas that of chair conformers can. This may be proven by ligand–receptor binding assay. However, there was no great difference in specific [^{3}H]-α-dihydropicrotoxinin binding inhibitory activity between 6 and 10. The binding assay seems to be less sensitive in estimating biological activities of compounds than bioassay with the German cockroach. Although the binding inhibitory activity of 6 was not so high, the cross-resistance shown by cyclodiene-resistant roaches clearly indicated that this compound acted on the picrotoxinin receptor. On the other hand, epoxidation of the double bonds of 1 did not improve the insecticidal activity. This finding indicates that compound 1 with double bonds is possibly converted to the compounds with epoxy groups (4 and 6) in the insect body to exert an activity similar to the latter compounds or that both the epoxy group and double bond work almost equally as an electronegative center as for the 6- and 7-positions, i.e., B of 1 in Figure 1. As to the 8-position (C of 1 in Figure 1), the epoxy group as well as the isopropylidene group may not be adequate for the interaction with the binding site.

Compound 15 was the most highly insecticidal analogue. This compound can be regarded as a dechlorinated analogue of endosulfan. The difference in insecticidal activity between the two compounds was only 10 times, which could be explained by expected metabolic differences. Chlorine atoms of cyclodiene insecticides are not necessarily essential for insecticidal activity. Soloway (1965) found the very high toxicity of 1,4,10,10-tetrachloro analogue of aldrin, indicating that the double bond of hexachloronorbornene nucleus can replace the olefinic chlorines as a part of the electronegative center. It is also worth

Table VI. Insecticidal Activity of Bridged Bicyclic Compounds against Cyclodiene Insecticide Susceptible and Resistant Strains of the German Cockroach

compd	dose, μg/ roach	mortality susceptible	mortality resistant
6	25		5/10
	10	7/10	0/10
	3	0/10	0/10
15	100		10/10
	30		5/10
	10	10/10	0/10
	3	9/10	0/10
	1	3/10	
	0.3	0/10	
33	100		10/10
	30		5/10
	10	5/10	0/10
	3	0/10	
β,β,γ,γ-tetramethyl-	30		10/10
γ-butyrolactone	10	10/10	5/10
	3	0/10	0/10
endosulfan (α)	10		10/10
	3		1/10
	1		1/10
	0.3	10/10	0/10
	0.1	8/10	
alodan	10		4/10
	3		4/10
	0.3	10/10	
	0.1	5/10	
	0.03	0/10	
picrotoxinin	10		10/10
	3		1/10
	1		2/10
	0.3	8/10	
	0.1	4/10	

Table VII. Specific [³H]-α-Dihydropicrotoxinin Binding Inhibitory Activity of Bridged Bicyclic Compounds and Related Compounds

compd	concn, μM	inhibn,[a] %
cyclohexane	30	10 ± 19
β,β,γ,γ-tetramethyl-	10	20 ± 8
γ-butyrolactone	30	17 ± 3
norcamphor	10	23 ± 2
	30	22 ± 10
6	10	38 ± 21
	30	16 ± 9
10	10	21 ± 12
	30	27 ± 30
15	10	28 ± 8
	30	58 ± 18
33	10	60 ± 21
	30.	102 ± 33
endosulfan (α)	10	63 ± 3
alodan	10	78 ± 18

[a] Each value is the mean ± SD of three or four experiments, each performed in triplicate.

noting the difference in activity between 15 and 18 (B of Figure 2). The difference in structure is just an iso-propylidene group at the 7-position. The isopropylidene group unlike the isopropenyl moiety of picrotoxinin has a projection angle away from the electronegative center A. This makes the distance between A and C too great for 18, indicating that the isopropylidene group is unfavorable for the interaction with the picrotoxinin binding site. The fact that compound 20 is active despite having unfavorable exo configuration supports the above conclusion (B of Figure 2). A similar argument may be made for compound 24, for 33 vs. 30, and for α- vs. β-endosulfan (C and D of Figure 2). The activity of epoxidized and hydrogenated analogues (16 and 17) of 15 decreased in that order due to reduced electronegativity. This may mean
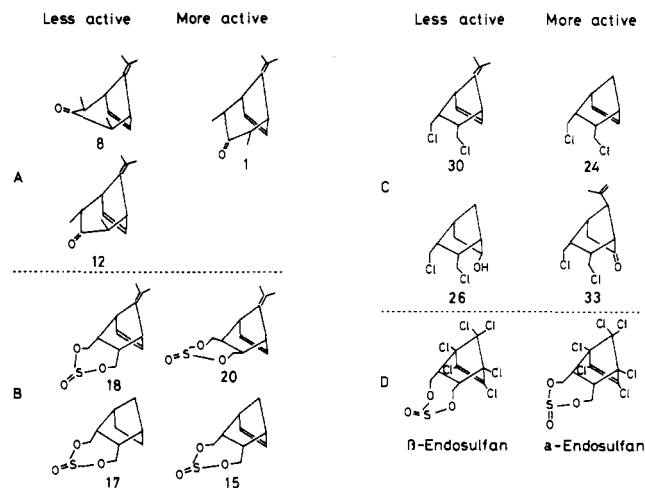
Figure 2. Structure–activity relationships of bridged bicyclic compounds.

the significance of electronegativity of the B site of 15 (Figure 1), with which cyclic sulfite moiety A works as electronegative centers.

Several endo-5,6-bis(chloromethyl)bicyclo[2.2.1]heptenes and heptanones had insecticidal activity. The lactone moiety of picrotoxinin is a metabolically vulunerable point, and the insecticidal activity may increase by replacement of the lactone by a carbonyl group. This is the reason why some ketones were synthesized. Compound 33 has an isopropenyl group like picrotoxinin and two endo chloromethyl groups like the cyclodiene insecticide alodan. In this case, two chloromethyl groups and the carbonyl group probably constitute two electronegative centers (Figure 1). The isopropenyl group may play an important role as steric bulkiness in interacting the critical binding site. This compound was intrinsically the most active, although the insecticidal activity was weaker than expected. The inhibitory activity of specific [³H]-α-dihydropicrotoxinin binding was comparable to those of cyclodiene insecticides, indicating that 33 satisfies structural requirement as a ligand to the picrotoxinin site to a great extent. However, high affinity of a compound to the site of action does not necessarily mean high insecticidal activity of the compound. Hydrophobicity of the whole molecule and metabolic vulnerability should be considered to obtain highly insecticidal compounds.

In contrast to the above groups of compound there is some evidence to show that γ-butyrolactones do not act on the picrotoxinin receptor, though they have a partial structure of picrotoxinin. Eto (1983) described the insecticidal activity of β-isopropyl-γ-butyrolactone. Klunk et al. (1983) provided a hypothesis that alkyl-γ-butyrolactones act at the same site as picrotoxinin, based on structural and pharmacological studies. If simple alkyl-γ-butyrolactones act at the picrotoxinin binding site, cyclodiene-resistant German cockroaches must show cross-resistance to the compounds and specific [³H]-α-dihydropicrotoxinin binding must be inhibited by them. Tables VI and VII show the results of bioassay and ligand–receptor binding assay of β,β,γ,γ-tetramethyl-γ-butyrolactone. The lactone interacted with the picrotoxinin receptor to some extent in vitro, but judging from its very low resistance ratio the interaction is not so significant. These findings suggest other site(s) of action for these compounds rather than the picrotoxinin binding site.

In conclusion, the picrotoxinin binding site can accept a variety of compounds as well as cyclodiene insecticides, γ-BHC, toxaphen, bicyclic phosphates, and picrotoxinin

(Tanaka et al., 1984). While the mode of interaction seems to be somewhat flexible, there appears to be a minimum requirement to possess at least two of the three active sites: two electronegative and one steric bulkiness (or hydrophobicity) sites within a definite range of distance. Bridged bicyclic structures are suited for preparation of compounds which satisfy such structural requirements.

Registry No. 1, 53033-91-3; 2, 99618-55-0; 3, 99664-04-7; 4, 99618-56-1; 5, 99618-57-2; 6, 99618-58-3; 7, 99618-59-4; 8, 53033-90-2; 11, 99618-60-7; 12, 99664-05-8; 15, 99664-06-9; 16, 99618-61-8; 17, 99618-62-9; 18, 99618-63-0; 19, 99618-64-1; 20, 99664-07-0; 21, 99618-65-2; 23, 99618-66-3; 24, 70096-05-8; 25, 99618-67-4; 26, 99618-68-5; 27, 99618-69-6; 28, 99618-70-9; 29, 99618-71-0; 30, 99618-72-1; 31, 99618-73-2; 32, 99618-74-3; 33, 99618-75-4; 34, 99618-76-5; 35, 99618-77-6; 36, 99618-78-7; 37, 99618-79-8; camphor, 76-22-2; norcamphor, 497-38-1; norbornylene, 498-66-8; picrotoxinin, 17617-45-7; picrotin, 21416-53-5; α-hydropicrotoxinin, 17617-46-8; β-dihydropicrotoxinin, 62697-27-2.

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Purification of a Proteolytic Enzyme from *Adenopus breviflorus* Fruits

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A neutral protease with some similar physicochemical properties to papain was extracted and purified from the fruit of *Adenopus breviflorus*. The pH range of optimum activity in Tris-HCl buffer was between 7.0 and 9.0, and activity was enhanced by the addition of sodium cyanide, cysteine hydrochloride, 2-mercaptoethanol, poly(ethylene glycol)-6000, or polyclar AT, while some inhibition was noticed by the addition of common biocides. Degree of purification achieved was 100.4-fold while overall yield was 15.75%. Its molecular weight was estimated to be 69 500, and it was found to be highly thermostable and significantly stable toward acids and alkalies, organic solvents, freezing, and thawing. Its maximal UV absorption was at 275 nm.

The fruit of *Adenopus breviflorus* is a small green melon with irregular yellow markings and a bitter astringent taste. It belongs to the Curcurbitaceae family and its main distribution is in the tropical regions of Africa and Central America (Irvine, 1961). It is empirically used in Nigeria by rural tanners for the depilation of raw hides and skins, a process suspected to be enzymatic in nature. The objective of this paper is to report on systematic steps taken to extract and purify a proteolytic enzyme from the fruits in order to enhance its economic importance, particularly with respect to the replacement of toxic sodium sulfide in unhairing operations and the reduction of both the pollutional effects of tannery effluents and the number of unit operations required for modern leather manufacture, as earlier proposed by Adewoye and Bangaruswamy (1984).

MATERIALS AND METHODS

Materials. Fresh fruits of *A. breviflorus* were collected from forest clearings around Oyo City, Oyo State, Nigeria. Polyclar AT powder came from GAF Corp. NY, poly(ethylene glycol)-6000 from J. T. Baker Chemical Co., Phillipsburg, NJ, and the SDS-acrylamide kit from BIO-

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