Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1873–1881

BIOORGANIC & MEDICINAL CHEMISTRY

Structure–Activity Relationships of *seco*-Prezizaane Terpenoids in γ-Aminobutyric Acid Receptors of Houseflies and Rats

Tadahiko Kuriyama,[a] Thomas J. Schmidt,[b] Emi Okuyama[c] and Yoshihisa Ozoe[a,*]

[a]*Department of Life Science and Biotechnology, Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane 690-8504, Japan*

[b]*Institut für Pharmazeutische Biologie der Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany*

[c]*Faculty of Pharmaceutical Sciences, Chiba University, Inage-Ku, Chiba 263-8522, Japan*

Received 12 November 2001; accepted 20 December 2001

Abstract—Thirteen *seco*-prezizaane terpenoids isolated from star anise species (*Illcium floridanum, Illcium parviflorum,* and *Illcium verum*) were investigated for their ability to inhibit the specific binding of [^{3}H]4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB), a non-competitive antagonist of γ-aminobutyric acid (GABA) receptors, to housefly-head and rat-brain membranes. Veranisatin A was found to be the most potent inhibitor in both membranes, with an IC_{50}^{fly} of 78.5 nM and an IC_{50}^{rat} of 271 nM, followed by anisatin (IC_{50}^{fly} = 123 nM; IC_{50}^{rat} = 282 nM). Six of the other 11 tested compounds were effective only in housefly-head membranes. Pseudoanisatin proved to display a high (>26-fold) selectivity for housefly versus rat GABA receptors (IC_{50}^{fly} = 376 nM; IC_{50}^{rat} >10,000 nM). Although pseudoanisatin does not structurally resemble EBOB, Scatchard plots indicated that the two compounds bind to the same site in housefly receptors. Anisatin and pseudoanisatin exhibited moderate insecticidal activity against German cockroaches. Comparative molecular field analysis (CoMFA), a method of three-dimensional quantitative structure–activity relationship (3D-QSAR) analysis, demonstrated that *seco*-prezizaane terpenoids can bind to the same site as do picrotoxane terpenoids such as picrotoxinin and picrodendrins, and the CoMFA maps allowed us to identify the parts of the molecules essential to high activity in housefly GABA receptors.

Introduction

γ-Aminobutyric acid (GABA) plays an important role as a major inhibitory neurotransmitter in both vertebrates and invertebrates. In vertebrates, GABA binds to both ionotropic and G protein-coupled (GPCR) receptors, two major receptor types, to regulate the central nervous system.[1] The former receptors are hetero-pentameric ligand-gated ion channels, where GABA induces the opening of the channels and increases the membrane conductance to chloride ions, making the generation of an action potential more difficult.[2] Insects have similar but pharmacologically distinct GABA receptors in not only the central but also the peripheral nervous system,[3,4] so that their GABA receptors have been regarded as a promising target for insecticides. Actually, fipronil, a novel insecticide, has been found to act as a non-competitive antagonist selectively on insect ionotropic GABA receptors.[5]

To date, a variety of terpenoids of plant origin such as picrotoxinin, picrodendrins, and anisatin have been shown to be non-competitive antagonists of ionotropic GABA receptors. Picrotoxinin is a long-established picrotoxane-type sesquiterpene antagonist isolated from Menispermaceae plants.[6] Picrodendrins are another group of picrotoxane terpenoids that have been isolated from a Euphorbiaceae plant *Picrodendron baccatum* (L.) Krug and Urban.[7,8] Anisatin is a poisonous sesquiterpene isolated from Japanese star anise (*Illcium anisatum* L.).[9–11] Recently, anisatin and several other *seco*-prezizaane-type terpenoids bearing an anisatin-like skeleton have been isolated from American (*Illcium floridanum* Ellis and *Illcium parviflorum* Michaux ex Ventenat)[12–14] and Chinese (*Illcium verum* Hook. fil.)[15] star anise species and studied with regard to their pharmacological and toxicological properties.[15,16] These compounds include analogues that differ from anisatin in the modes of cyclization and substitution. These novel compounds, along with other known terpenoids, might be useful as probes for GABA receptors or as lead compounds for insecticides.

*Corresponding author. Tel.: +81-852-32-6573; fax: +81-852-32-6092; e-mail: ozoe-y@life.shimane-u.ac.jp

0968-0896/02/$ - see front matter
PII: S0968-0896(02)00011-1

In the present study, we investigated the action of anisatin and 12 analogues (Fig. 1) on GABA receptors by determining their ability to inhibit the specific binding of the non-competitive GABA antagonist [^{3}H]4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB, Fig. 1) to housefly-head and rat-brain membranes. We also performed three-dimensional quantitative structure–activity relationship (3D-QSAR) analysis of *seco*-prezizaane-type and picrotoxane-type terpenoid GABA antagonists on housefly GABA receptors, and report here the results.

Results

Inhibition of [^{3}H]EBOB binding to housefly-head membranes

Eight of 13 *seco*-prezizaane sesquiterpenes exhibited a concentration-dependent inhibition of specific [^{3}H]EBOB binding to housefly-head membranes (Fig. 2), with IC_{50}s of less than 10 μM (Table 1). Veranisatin A (**2**) was the most potent inhibitor in housefly receptors, with an IC_{50} of 78.5 nM, which is approximately one-third the magnitude of its IC_{50} in rat receptors. Anisatin (**1**, IC_{50} = 123 nM) was the second most potent inhibitor. Quite noteworthy, the third most potent inhibitor was pseudoanisatin (**10**, IC_{50} = 376 nM), which has previously been demonstrated to be non-toxic to mice[16] and has proved to be almost inactive in rat-brain membranes as described below. Scatchard analysis was performed to investigate whether **10** is a competitive inhibitor, like anisatin,[11] even though the modes of cyclization and substitution differ significantly between the two terpenoids. The results indicated that **10** and EBOB, which do not structurally resemble each other, bind to the same site; that is, **10** is a competitive inhibitor of [^{3}H]EBOB binding and a non-competitive antagonist of GABA receptors (Fig. 3). The shift of a hydroxyl group of anisatin to produce

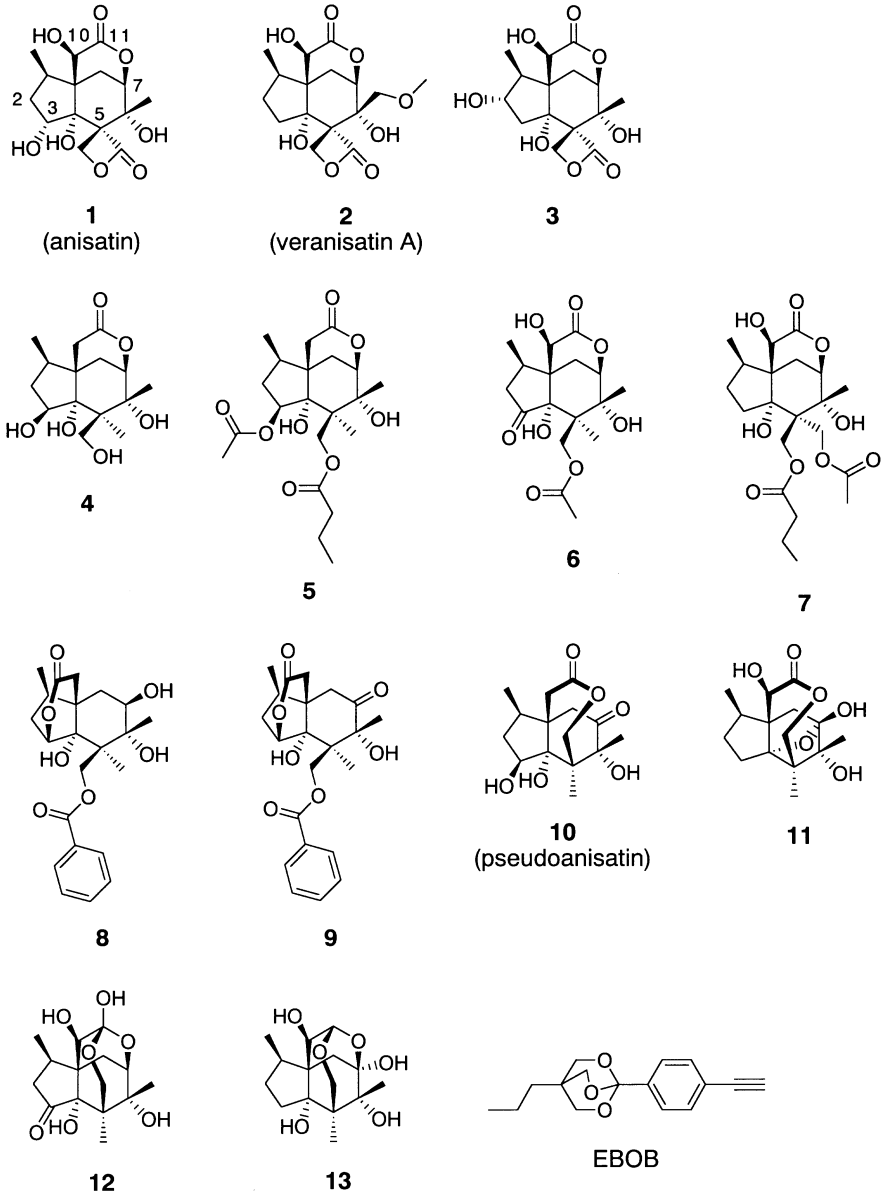


Figure 1. Structures of *seco*-prezizaane terpenoids and EBOB.

2α-hydroxyneoanisatin (**3**) resulted in a 53-fold reduction in potency, although **3** still retained activity. The opening of the β-lactone ring (as seen in **4**) and the substitution of bulky groups at the C5 position (as seen in **6** and **7**) were tolerable to some extent, while the acetylation of the C3 hydroxyl group (as seen in **5**) was detrimental to the activity (for position number, see anisatin's structure in Fig. 1). In the case of analogues with a different cyclization mode (i.e., lacking the lactone ring closed between C11 and C7), **8**, **9**, **12**, and **13** were almost inactive, while **10** and **11** were active.

Inhibition of [^{3}H]EBOB binding to rat-brain membranes

Anisatin and veranisatin A, both known to be strong convulsants in mice,[15] were equipotent in [^{3}H]EBOB-binding assays using rat-brain membranes, with IC_{50}s of 282 nM and 271 nM (Table 1), respectively; their concentration–inhibition curves are shown in Figure 4. The other compounds tested were almost inactive, unlike the case of housefly-head membranes. It is worth noting that only the shift of a hydroxyl group from the 3-position to the 2-position led to a loss of activity, as seen in the comparison of **3** with anisatin (**1**) (for position number, see anisatin's structure in Fig. 1). This finding is in agreement with the results of toxicity tests and shows that unfavorable interaction with the receptor site and not a lack of absorption due to higher hydrophilicity is the reason for the absence of toxicity of **3**.[16]

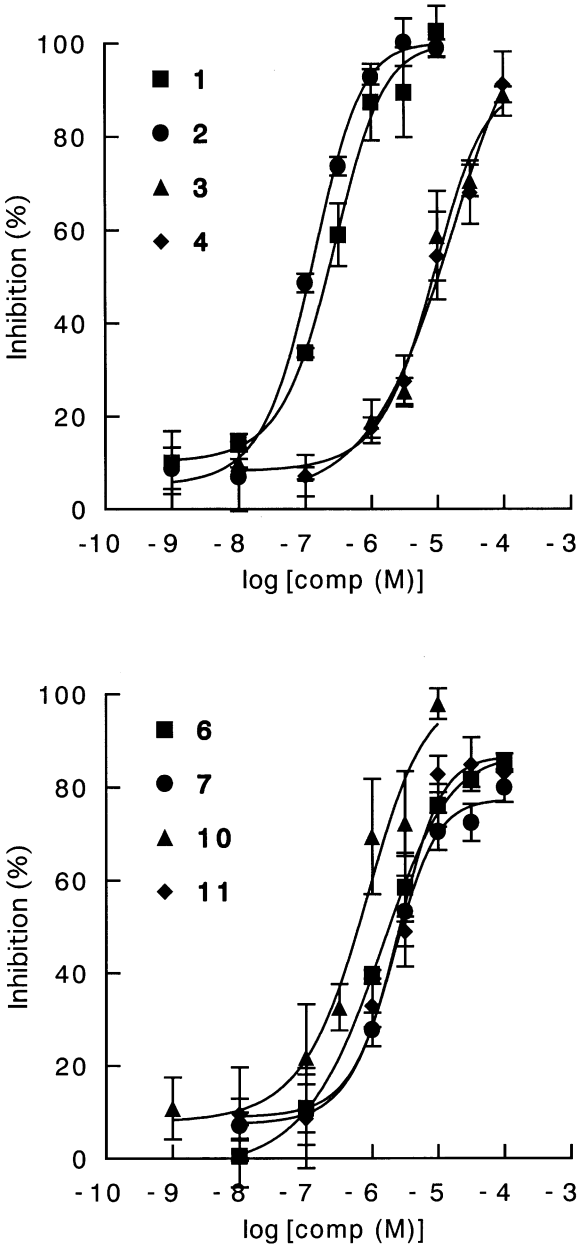


Figure 2. Concentration–inhibition curves of *seco*-prezizaane terpenoids in the inhibition of [^{3}H]EBOB binding to housefly-head membranes. Data are mean ±standard deviation ($n=3$).

Insecticidal activity against German cockroaches

Generally, non-competitive GABA receptor antagonists are toxic to animals. In the present study, the insecticidal activities of anisatin and pseudoanisatin were investigated by injecting them into German cockroaches (*Blattella germanica* L.) that had been topically pretreated with the cytochrome P450 inhibitor piperonyl butoxide to depress the oxidative metabolism of the compounds. Pseudoanisatin exhibited a similar level of insecticidal activity to that of anisatin, with LD_{50}s of 26 and 70 μg/g, respectively; their dose–mortality curves are shown in Figure 5.

3D-QSAR analysis of terpenoid antagonists in housefly GABA receptors

The procedure of comparative molecular field analysis (CoMFA)[17] was employed to investigate the 3D-QSAR of terpenoid GABA antagonists in housefly GABA receptors. The compounds used for analysis included not only the eight *seco*-prezizaanolides assayed here but also 17 picrotoxane-type terpenoids reported previously[7] (Fig. 6). The compounds were superposed in the manner proposed by Schmidt et al.[16] and as described in the experimental section, as three regions of the compounds, designated as a, b, and c (Fig. 7), appeared

Table 1. Potency of *seco*-prezizaanolides in inhibiting [^{3}H]EBOB binding to housefly-head and rat-brain membranes

Compd	IC_{50} (μM)	
	Housefly	Rat
1 (anisatin)	0.123 (0.089–0.170[a])	0.282 (0.213–0.375[a])
2 (veranisatin A)	0.0785 (0.0588–0.1046[a])	0.271 (0.211–0.348[a])
3	6.52 (4.61–9.25[a])	>10 (10.7[b])
4	7.84 (5.95–10.33[a])	>10 (0.8[b])
5	>10 (23.1[b])	>10 (3.3[b])
6	2.31 (1.67–3.19[a])	>10 (8.8[b])
7	3.55 (2.48–5.08[a])	>10 (2.8[b])
8	>10 (21.9[b])	>10 (8.5[b])
9	>10 (42.7[b])	>10 (15.6[b])
10 (pseudoanisatin)[c]	0.376 (0.271–0.522[a])	>10 (10.1[b])
11[d]	2.19 (1.57–3.06[a])	>10 (11.9[b])
12	>10 (32.9[b])	>10 (8.0[b])
13	>10 (36.0[b])	>10 (9.1[b])

[a]95% confidence limits.
[b]Inhibition% at 10μM.
[c]A mixture of the 7-keto (90%) and the 4,7-hemiketal (10%) forms.
[d]A mixture of the 7-keto (20%) and the 4,7-hemiketal (80%) forms.

to correspond well with each other in the three-dimensional structures (Fig. 8). The CoMFA results are summarized in Table 2. Although **3**, picrodendrin C, and picrodendrin O were not included in this analysis, the derived CoMFA equation gave a cross-validated r^2 (q^2) of 0.446, indicating that the quality of the model is not sufficiently high, but is significant (Table 2). Other modes of superposition tested did not give good results. These findings indicate that *seco*-prezizaane and picrotoxane terpenoids share a common binding site in housefly GABA receptors.

The CoMFA field maps display some of the structural portions of terpenoid antagonists required for them to exhibit high activity (Fig. 9). The red contours indicate regions where the more electronegative electrostatic interaction with the binding site increases the activity, whereas the blue contours show regions where the more electropositive electrostatic interaction increases the activity. The green contours indicate regions where the submolecular bulk is well accommodated with an increase in activity, whereas the yellow contours indicate regions where the submolecular bulk is unfavorable to the activity. The red contours surrounding regions a and b in Figure 9 indicate the importance of the electronegativity of these regions for high activity. The 1-methoxyethylidene moiety of the spiro γ-lactone ring of picrodendrin Q is sterically favorable, as shown by the green contour (Fig. 9, C and D). The methoxymethyl group of veranisatin A is facing the same green contour (Fig. 9, A and B), indicating that this substituent might contribute to a slight increase in the potency of this compound over anisatin.

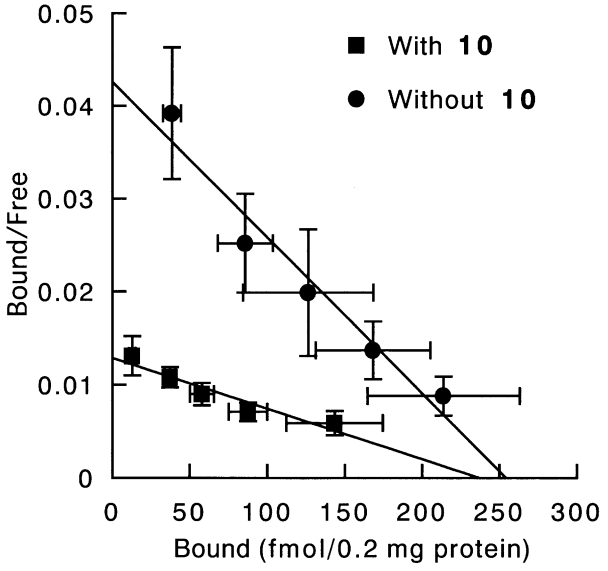


Figure 3. Scatchard plots of [^{3}H]EBOB binding to housefly-head membranes in the presence and absence of 3 μM pseudoanisatin (**10**). In the absence of **10**: $K_d = 5.97$ nM; $B_{max} = 254$ fmol/0.2 mg protein. In the presence of **10**: $K_d = 18.4$ nM; $B_{max} = 237$ fmol/0.2 mg protein. Data are mean ±standard deviation ($n = 3$).

Discussion

Picrotoxinin, picrodendrins, and anisatin are terpenoids that have been shown to be non-competitive antagonists of ionotropic GABA receptors.[6–11] In the current study, we examined the interaction of anisatin and of 12 anisatin analogues with GABA receptors by determining their abilities to inhibit the specific binding of [^{3}H]EBOB, a non-competitive GABA receptor antagonist, to membranes prepared from rat brains and housefly heads. Consequently, anisatin and veranisatin A were found to bind to housefly as well as rat GABA receptors with high-nanomolar affinity (Table 1). Six of the other 11 compounds tested were active in receptor assays using housefly-head membranes, whereas none was active in assays using rat-brain membranes. Thus, six *seco*-prezizaane terpenoids proved to be selective for housefly versus rat GABA receptors. Judging from the

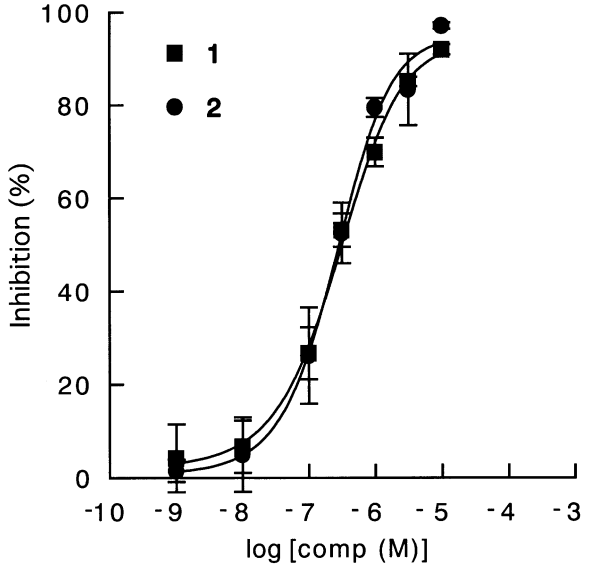


Figure 4. Concentration–inhibition curves of anisatin (**1**) and veranisatin A (**2**) in the inhibition of [^{3}H]EBOB binding to rat-brain membranes. Data are mean ±standard deviation ($n = 3$).

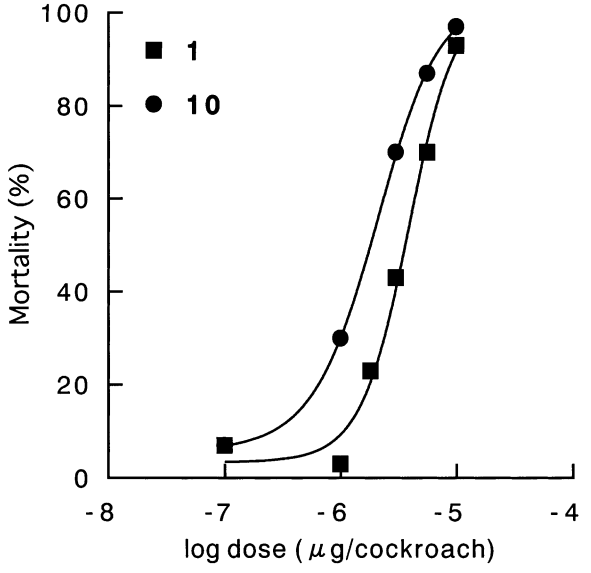
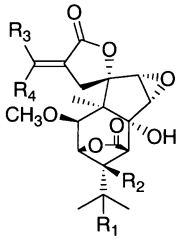


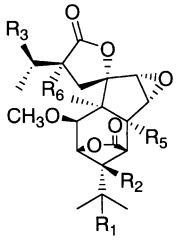
Figure 5. Dose–mortality curves of anisatin (**1**) and pseudoanisatin (**10**) in insecticidal assays using German cockroaches. Mortality was determined using 30 cockroaches for each dose.

$IC_{50}^{rat}/IC_{50}^{fly}$ ratio, pseudoanisatin displayed the highest (>26-fold) selectivity among the compounds tested (Table 1). Pseudoanisatin (**10**) was the third most potent inhibitor in housefly receptors and showed moderate insecticidal activity. It is important to note here that **10** and **11** exist in equilibria between the 7-keto and the 4,7-hemiketal forms. In the case of **10**, the keto form is predominant (about 90%), and the hemiketal form is minor (about 10%). In the case of **11**, approximately 80% are in the hemiketal form and only 20% are in the keto form.[14] It is not clear whether only one or both forms contribute to the activity. However, it does not appear reasonable that the keto form of **10** and the hemiketal form of **11** would be responsible for the activity, as both compounds are very similar to each other in their respective isomeric forms. It was therefore assumed that only the keto form is active in both **10** and **11**. Based on the ratio of the keto and the hemiketal forms, the IC_{50} of the keto form of **11** was calculated to be 438 nM, which is at the same level as the value calculated for the keto form of **10** (338 nM). If the hemiketal forms were the bioactive ones, this calculation would not lead to a reasonable result. As a result of 3D-QSAR analysis, a significant CoMFA equation including data for **10** and **11** could be obtained only when the keto forms and the converted values were used. This result indicates that the C3 and the C10 hydroxyl groups of **10** and **11** are not essential to activity in housefly receptors. The structural distortion might render the hemiketal forms inactive.

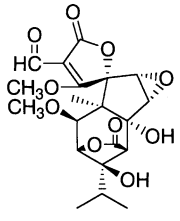
The skeleton of anisatin differs from that of the representative non-competitive GABA antagonist picrotoxinin, and the conformation change of rat GABA receptors allosterically induced by competitive antagonists affected the binding of anisatin to the receptors differently from that of picrotoxinin.[18] Therefore, it was assumed that the mode of binding of anisatin must be different from that of picrotoxinin, although there is evidence showing that both sesquiterpenes bind to the same site. On the other hand, the comparison of the three-dimensional structures and the electrostatic properties of *seco*-prezizaane terpenoids with those of picrotoxane terpenoids led to an identification of a possible common pharmacophore for the two different classes of terpenoid antagonists.[16] To examine the way in which



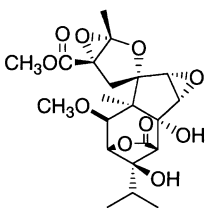
	R_1	R_2	R_3	R_4
PD-A:	H	OH	CH_2OH	OCH_3
PD-M:	OH	H	CH_3	OCH_3
PD-Q:	H	H	CH_3	OCH_3
PD-T:	H	OH	OCH_3	CH_2OH



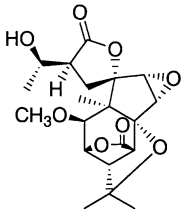
	R_1	R_2	R_3	R_5	R_6
PD-E:	OH	H	OH	OH	H
PD-E-18-Ac:	OH	H	$OCOCH_3$	OH	H
PD-F:	H	OH	OH	OH	OH
PD-L:	H	OH	OH	H	OH
PD-O:	H	H	OH	OH	H



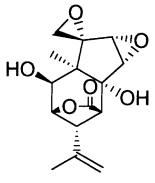
PD-B



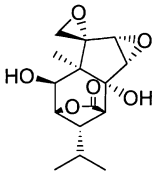
PD-G



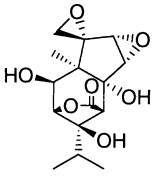
PD-P



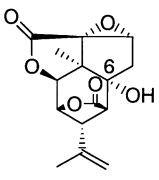
Tutin



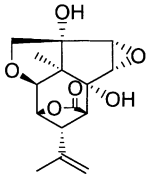
Dihydrotutin



PD-C



Picrotoxinin



Corianin

Figure 6. Structures of picrotoxane terpenoids used in 3D-QSAR analysis. PD, picrodendrin.

the two classes of terpenoids share a common binding site, we performed 3D-QSAR analysis of *seco*-prezizaane terpenoids along with picrotoxane terpenoids, using data obtained from assays using housefly-head membranes. In the CoMFA analysis, the compounds were superposed in the manner proposed by Schmidt et al. (1999).[16] This resulted in a model with significant reliability, validating that the two classes of terpenoid antagonists bind to the same site at least in housefly GABA receptors. Ozoe and colleagues[19,20] have proposed that there are four important subsites in the noncompetitive antagonist-binding site of GABA receptors and that the interaction of compounds with at least two of the four subsites leads to high antagonism. Three of the four subsites might interact with a, b, and c in Figure 7. Schmidt et al.[16] have also pointed out that the interaction of a, b, c, and the C10 hydroxyl group of anisatin (the C6 hydroxyl group of picrotoxinin) with receptors contributes to high toxicity to mice (for position number, see anisatin's and picrotoxinin's structures in Figs 1 and 6, respectively). The CoMFA maps presented here emphasize the importance of the electronegativity of a and b, although the significance of the other parts is not clearly displayed (Fig. 9), probably because of the lack of variability of compounds with respect to the structural features of these parts.

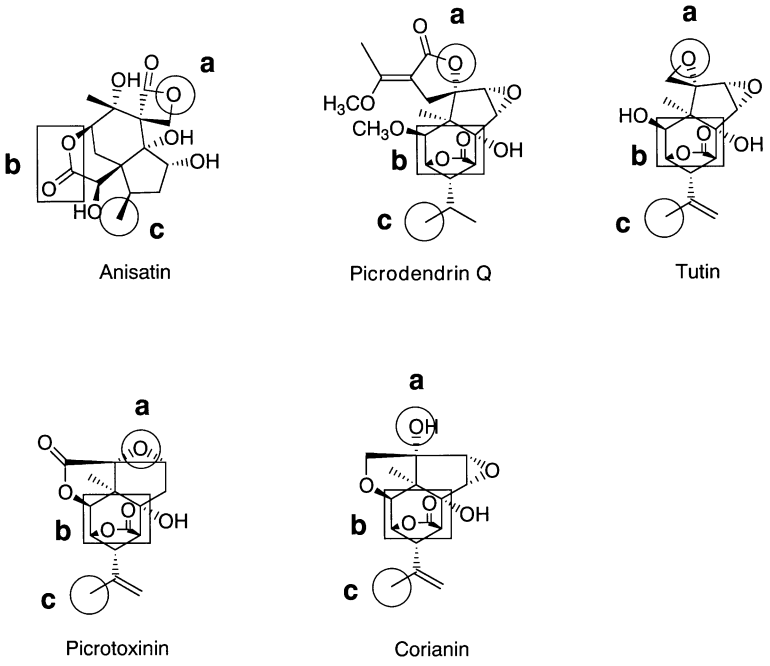


Figure 7. Comparison of the structures of anisatin and picrotoxane terpenoids. Terpenoids were superposed at a, b, and c for CoMFA analysis; that is, with the oxygen atom of the β-lactone (a), the oxygen atoms of the δ-lactone (b), and the carbon atom of the methyl group (c) of anisatin versus the oxygen atom of the spiro γ-lactone, the epoxide, or the hydroxyl group (a), the oxygen atoms of the bridged γ-lactone (b), and the methyl carbon atom of the isopropenyl (isopropyl) group (c) of picrotoxane terpenoids, respectively.

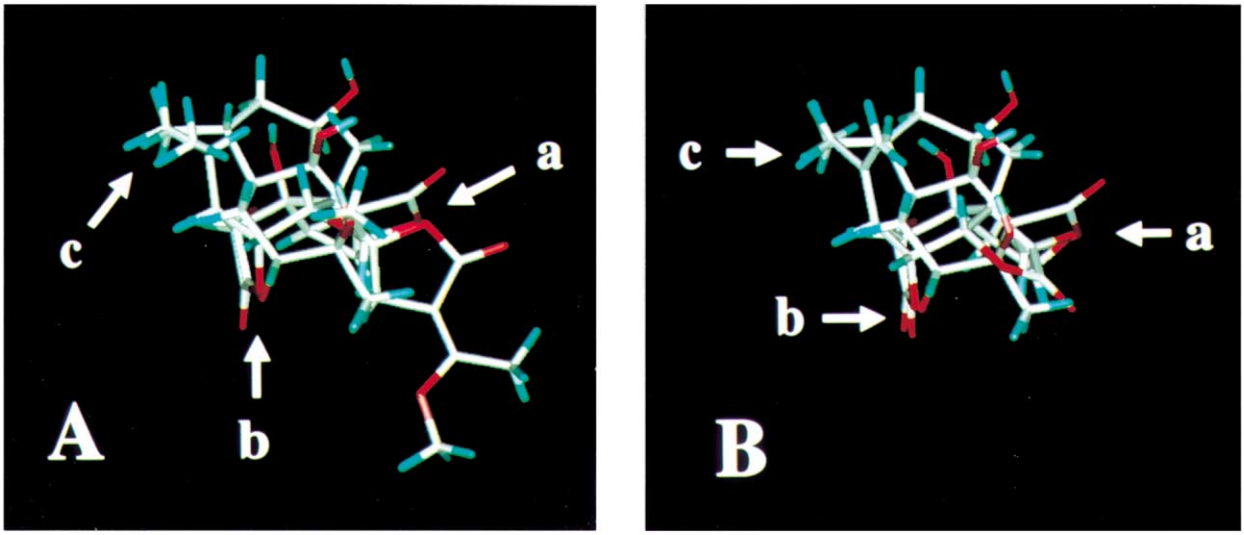


Figure 8. Alignment of terpenoids. Anisatin is aligned with picrodendrin Q (in A) and picrotoxinin (in B) by the fit-atoms procedure of SYBYL, as shown in Figure 7.

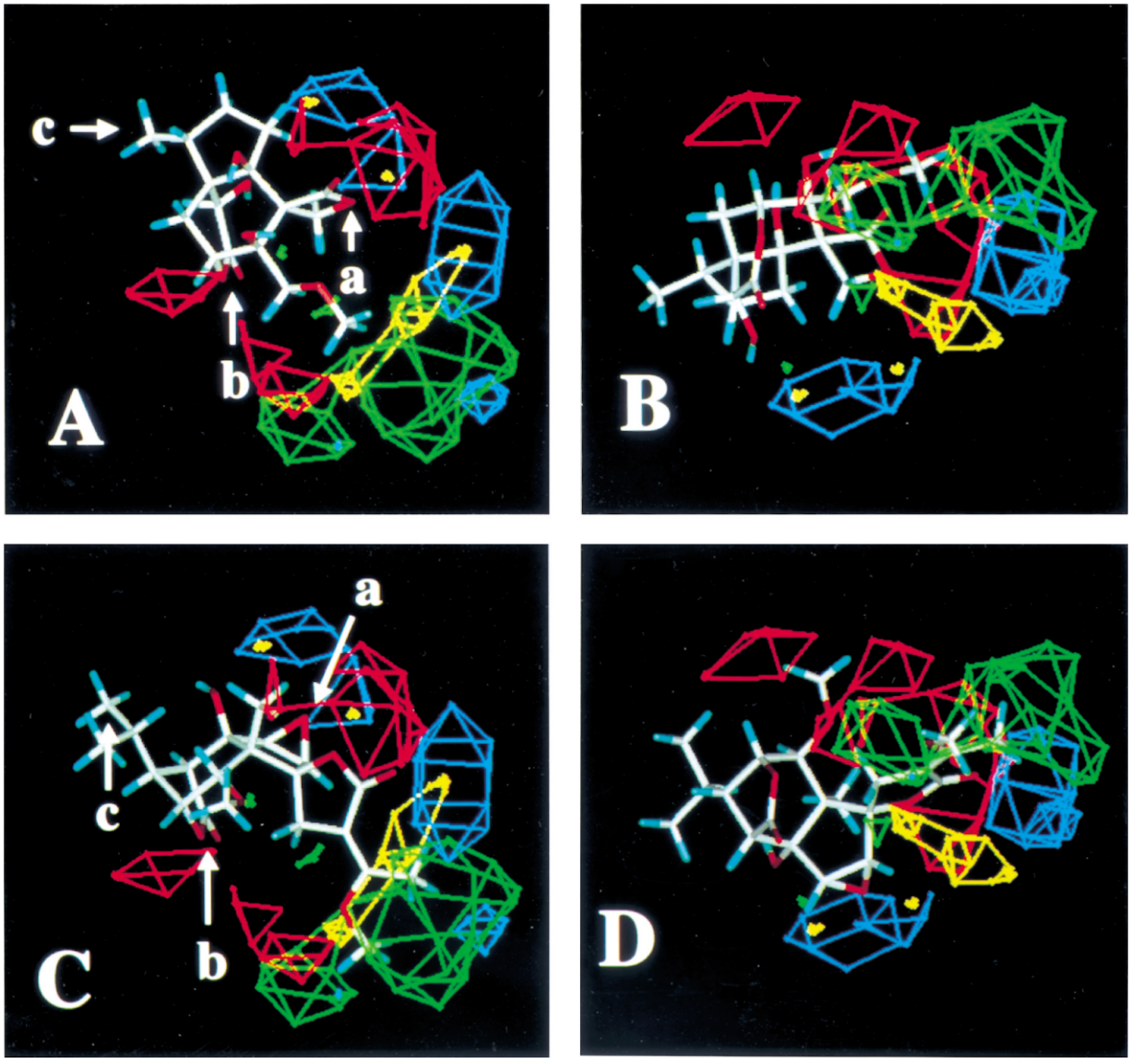


Figure 9. CoMFA field maps. The red contours indicate regions where the more electronegative electrostatic interaction with the binding site increases the activity, whereas the blue contours show regions where the more electropositive electrostatic interaction increases the activity. The green contours indicate regions where the submolecular bulk is well accommodated with an increase in activity, whereas the yellow contours indicate regions where the submolecular bulk is unfavorable to the activity. Veranisatin A is displayed in A and B. Picrodendrin Q is displayed in C and D.

Table 2. Summary of CoMFA results

a	Optimum no. of components	*n*	*s*	r^2	Cross-validated		Relative contribution	
					s_{press}	q^2	Steric	Electrostatic
6.185	3	22	0.243	0.903	0.582	0.446	0.396	0.604

$pIC_{50} = a + [CoMFA\ field\ terms]$

In conclusion, the present study generated a model confirming the hypothesis that *seco*-prezizaane and picrotoxane terpenoids, having different skeletons, interact with the same binding site in GABA receptors. Several *seco*-prezizaane terpenoids are selective antagonists for housefly versus rat GABA receptors, although the mechanisms underlying the selectivity remain to be elucidated. Information from the present 3D-QSAR analysis might be utilized for the design of safer insecticides. Further work in this direction is well under way.

Experimental

Chemicals

Thirteen *seco*-prezizaane-type sesquiterpenes used in the present study were isolated from *I. floridanum* Ellis, *I. parviflorum* Michaux ex Ventenat (**1**, **3**–**13**),[12–14] and *I.*

verum Hook. fil. (**2**).[15] 4′-Ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB) was prepared according to the method of Palmer et al.[21] [^{3}H]EBOB (1650.2 GBq/mmol) was purchased from NEN Life Science Products, Inc.

[^{3}H]EBOB binding assays

The procedure of Deng et al.[22] was modified to prepare P_2 membranes from the heads of adult houseflies (*Musca domestica* L.). The heads were homogenized in ice-cold 10 mM Tris–HCl buffer (pH 7.5) containing 0.25 M sucrose (buffer A) with a Teflon-glass homogenizer, and the homogenate was centrifuged at 500*g* for 5 min after filtration through four layers of a 64-μm mesh screen. The supernatant was centrifuged at 25,000*g* for 30 min after filtration through the screen. The resulting pellets were resuspended in buffer A and allowed to stand on ice for 30 min. The suspension was recentrifuged at 25,000 *g* for 30 min, and the resulting pellets were finally suspended in ice-cold 10 mM phosphate buffer (pH 7.5) containing 300 mM NaCl (buffer B) and used directly for the binding assays. Rat brain P_2 membranes were prepared from 5-week-old male Wistar rats by a modification of the method of Squires et al.[23] Whole rat brains stored at −80 °C were thawed and homogenized in ice-cold 1 mM EDTA using a Teflon-glass homogenizer. The homogenate was centrifuged at 1000*g* for 10 min, and the supernatant was then centrifuged at 25,000*g* for 30 min. The resulting pellets were suspended in 1 mM EDTA, packed into cellophane tubing, and dialyzed against distilled/deionized water in an ice-bath (1–2 L, 2 h × 3). The inner suspension was then centrifuged at 25,000*g* for 30 min, and the pellets were stored at −80 °C. On the day of the binding experiments, the pellets were suspended in ice-cold buffer B for the binding assays. [^{3}H]EBOB binding assays were carried out according to Cole and Casida[24] and Deng et al.[22] Briefly, terpenoids were incubated with rat-brain membranes (125 μg protein) or housefly-head membranes (200 μg protein) and 0.5 nM [^{3}H]EBOB in 1.0 mL of buffer B at 37 °C for 90 min (rat) or at 22 °C for 70 min (housefly). Terpenoids in DMSO (4 μL) were added to the reaction mixtures so as to give the desired final concentrations. After incubation, the mixtures were filtered through GF/B filters and rapidly rinsed twice with 5 mL of cold buffer B using a Brandel M-24 cell harvester. The radioactivity of [^{3}H]EBOB that specifically bound to membranes on the filters was measured with a Beckman LS 6000SE liquid scintillation spectrometer. Nonspecific binding was determined in the presence of 5 μM unlabeled EBOB. Each experiment was performed in triplicate and repeated three times.

Insecticidal assays

Acetone solutions (1 μL) of piperonyl butoxide (30 μg) were topically applied to the ventral side of the thorax of male adult German cockroaches (*Blattella germanica* L.). After 1 h, 0.25 μL DMSO solutions containing anisatin and pseudoanisatin were injected into the ventral portion of the abdomen with a Kloehn microsyringe. After injection, 10 cockroaches were placed in each glass vial with water and sugar and held at 25 °C. Thirty cockroaches were used for each dose. Mortality was recorded 24 h after the injection of compounds.

Molecular modeling and 3D-QSAR analysis

All computations were performed using the molecular modeling software SYBYL, version 6.5.[25] To determine the initial conformations of terpenoids, the X-ray crystallographic coordinates of anisatin (**1**),[26] **3**, pseudoanisatin (**10**),[27] **6**,[12] **9**,[13] **4**,[12] picrotoxinin,[28] picrodendrin A,[29] and picrodendrin F-18-*p*-bromobenzoate[30] were obtained from the Cambridge Crystallographic Database. The structures of the other terpenoids were modeled on the basis of these structures. The coordinates of the modified portions were calculated using the SYBYL standard values for bond lengths and angles. The initial coordinates thus obtained were fully optimized by the semi-empirical molecular orbital method PM3.[31–33] For the conformation with the optimized coordinates fixed, the atomic charges were calculated using MNDO.[31,34,35]*seco*-Prezizaanolides were aligned by the fit atoms procedure of SYBYL with all nine atoms of the perhydroindane ring. Picrotoxane terpenoids and anisatin were similarly superposed at the a, b, and c parts, as shown in Figure 7; that is, with the oxygen atom of the β-lactone (a), the oxygen atoms of the δ-lactone (b), and the carbon atom of the methyl group (c) of anisatin versus the oxygen of the spiro γ-lactone, the epoxide, or the hydroxyl group (a), the oxygen atoms of the bridged γ-lactone (b), and the methyl carbon atom of the isopropenyl (isopropyl) group (c) of picrotoxane terpenoids, respectively. The pIC_{50}s [$\log(1/IC_{50}$ (M))] of a superposed set of terpenoid GABA antagonists in the inhibition of [^{3}H]EBOB binding to housefly-head membranes were analyzed by the CoMFA/QSAR module of SYBYL as described previously.[19] Unless otherwise stated, default settings were used throughout. The IC_{50}s of picrotoxane terpenoids were available from an earlier study.[7] In the case of pseudoanisatin, which contained the 7-keto (90%) and the 4,7-hemiketal (10%) forms, the more stable keto form was assumed to be an active form. Also in the case of **11**, which contained the keto (20%) and the hemiketal (80%) forms, the keto form was presumed to primarily contribute to the activity. The structures of the keto forms and their IC_{50}s (pseudoanisatin, 338 nM; **11**, 438 nM) converted on this assumption were used in CoMFA.

Data analysis

Linear regression analysis with DeltaGraph (Polaroid) was used to determine the dissociation constant (K_d) and the maximal binding capacity (B_{max}). IC_{50}s and LD_{50}s were calculated using mean values by the Probit method. Dose–response data were fitted to the logistic equation using DeltaGraph.

Acknowledgements

This work was supported in part by Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

1. Bormann, J. *Trends Pharmacol. Sci.* **2000**, *21*, 16.
2. Rabow, L. E.; Russek, S. J.; Farb, D. H. *Synapse* **1995**, *21*, 189.
3. Hosie, A. M.; Aronstein, K.; Sattelle, D. B.; ffrench-Constant, R. H. *Trends Neurosci.* **1997**, *20*, 578.
4. Mezler, M.; Müller, T.; Raming, K. *Eur. J. Neurosci.* **2001**, *13*, 477.
5. Gant, D. B.; Chalmers, A. E.; Wolff, M. A.; Hoffman, H. B.; Bushey, D. F. *Rev. Toxicol.* **1998**, *2*, 147.
6. Takeuchi, A.; Takeuchi, N. *J. Physiol.* **1969**, *205*, 377.
7. Ozoe, Y.; Akamatsu, M.; Higata, T.; Ikeda, I.; Mochida, K.; Koike, K.; Ohmoto, T.; Nikaido, T. *Bioorg. Med. Chem.* **1998**, *6*, 481.
8. Hosie, A. M.; Ozoe, Y.; Koike, K.; Ohmoto, T.; Nikaido, T.; Sattelle, D. B. *Br. J. Pharmacol.* **1996**, *119*, 1569.
9. Shinozaki, H.; Ishida, M.; Kudo, Y. *Brain Res.* **1981**, *222*, 401.
10. Kudo, Y.; Oka, J.; Yamada, K. *Neurosci. Lett.* **1981**, *25*, 83.
11. Ikeda, T.; Ozoe, Y.; Okuyama, E.; Nagata, K.; Honda, H.; Shono, T.; Narahashi, T. *Br. J. Pharmacol.* **1999**, *127*, 1567.
12. Schmidt, T. J.; Schmidt, H. M.; Müller, E.; Peters, W.; Fronczek, F. R.; Truesdale, A.; Fischer, N. H. *J. Nat. Prod.* **1998**, *61*, 230.
13. Schmidt, T. J.; Peters, W.; Fronczek, F. R.; Fischer, N. H. *J. Nat. Prod.* **1997**, *60*, 783.
14. Schmidt, T. J. *J. Nat. Prod.* **1999**, *62*, 687.
15. Nakamura, T.; Okuyama, E.; Yamazaki, M. *Chem. Pharm. Bull.* **1996**, *44*, 1908.
16. Schmidt, T. J.; Okuyama, E.; Fronczek, F. R. *Bioorg. Med. Chem.* **1999**, *7*, 2857.
17. Cramer, R. D., III; Patterson, D. E.; Bunce, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 5959.
18. Kakemoto, E.; Okuyama, E.; Nagata, K.; Ozoe, Y. *Biochem. Pharmacol.* **1999**, *58*, 617.
19. Akamatsu, M.; Ozoe, Y.; Ueno, T.; Fujita, T.; Mochida, K.; Nakamura, T.; Matsumura, F. *Pestic. Sci.* **1997**, *49*, 319.
20. Ozoe, Y.; Akamatsu, M. *Pest Manag. Sci.* **2001**, *57*, 923.
21. Palmer, C. J.; Cole, L. M.; Larkin, J. P.; Smith, I. H.; Casida, J. E. *J. Agric. Food Chem.* **1991**, *39*, 1329.
22. Deng, Y.; Palmer, C. J.; Casida, J. E. *Pestic. Biochem. Physiol.* **1993**, *47*, 98.
23. Squires, R. F.; Casida, J. E.; Richardson, M.; Saederup, E. *Mol. Pharmacol.* **1983**, *23*, 326.
24. Cole, L. M.; Casida, J. E. *Pestic. Biochem. Physiol.* **1992**, *44*, 1.
25. *SYBYL Molecular Modeling Software*; Tripos Associates: St. Louis, MO.
26. Wong, M. G.; Gulbis, J. M.; Mackay, M. F.; Craik, D. J.; Andrews, P. R. *Aust. J. Chem.* **1988**, *41*, 1071.
27. Kouno, I.; Irie, H.; Kawano, N. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2511.
28. Mackay, M.; Sadek, M. *Aust. J. Chem.* **1983**, *36*, 2111.
29. Ohmoto, T.; Koike, K.; Fukuda, H.; Mitsunaga, K.; Kagei, K.; Kawai, T.; Sato, T. *Chem. Pharm. Bull.* **1989**, *37*, 1805.
30. Koike, K.; Ohmoto, T.; Kawai, T.; Sato, T. *Phytochemistry* **1991**, *30*, 3353.
31. Stewart, J.J.P. *MOPAC Ver.5, Program No. 455*; Quantum Chemistry Program Exchange: Indiana University, Bloomington, IN.
32. Stewart, J. J. P. *J. Compt. Chem.* **1989**, *10*, 209.
33. Stewart, J. J. P. *J. Compt. Chem.* **1989**, *10*, 221.
34. Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899.
35. Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4907.