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Abstract

BACKGROUND: Monoterpenoids are a large group of plant secondary metabolites. Many of these naturally occurring compounds have shown good insecticidal potency on pest insects. Previous studies in this laboratory have indicated that some monoterpenoids have positive modulatory effects on insect GABA receptors. In this study, the key properties of monoterpenoids involved in monoterpenoid binding activity at the housefly GABA receptor were determined by developing quantitative structure-activity relationship (QSAR) models, and the relationship between the toxicities of these monoterpenoids and their GABA receptor binding activities was evaluated.

RESULTS: Two QSAR models were determined for nine monoterpenoids showing significant effects on [^{3}H]-TBOB binding and for nine *p*-menthane analogs with at least one oxygen atom attached to the ring. The Mulliken charges on certain carbon atoms, the log *P* value and the total energy showed significant relationships with binding activities to the housefly GABA receptor in these two QSAR models.

CONCLUSIONS: From the QSAR models, some chemical and structural parameters, including the electronic properties, hydrophobicity and stability of monoterpenoid molecules, were suggested to be strongly involved in binding activities to the housefly GABA receptor. These findings will help to understand the mode of action of these natural insecticides, and provide guidance to predict more monoterpenoid insecticides. Copyright © 2012 Society of Chemical Industry

Keywords

monoterpenoid, insecticide, quantitative structure-activity relationship (QSAR), GABA receptor, [3H]-TBOB

Disciplines

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Quantitative structure-activity relationships of monoterpenoid binding activities to house fly GABA receptor

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ABSTRACT:

BACKGROUND: Monoterpenoids are a large group of plant secondary metabolites. Many of these naturally occurring compounds showed good insecticidal potency on pest insects. Previous studies in this laboratory have indicated that some monoterpenoids have positive modulatory effects on insect GABA receptors. In this study, we determined the key properties of monoterpenoids involved in the monoterpenoids' binding activity at house fly GABA receptor by developing quantitative structure-activity relationship (QSAR) models, and evaluated the relationship between the toxicities of these monoterpenoids and their GABA receptor binding activities.

RESULTS: Two QSAR models were determined for nine monoterpenoids that showed significant effects on [^{3}H]-TBOB binding, and nine p-menthane analogs with at least one oxygen atom attached to the ring. Mulliken charges on certain carbon atoms, log P value, and total energy showed significant relationships with binding activities to the house fly GABA receptor in these two QSAR models.

CONCLUSIONS: From the QSAR models, some chemical and structural parameters, including electronic properties, hydrophobicity, and stability of monoterpenoid molecules were suggested to be strongly involved in the binding activities with the house fly GABA receptor. These findings will help to understand the mode of action of these natural insecticides, and to provide guidance to predict more monoterpenoids' insecticides.

KEYWORDS: Monoterpenoid; insecticide; quantitative structure-activity relationship (QSAR); GABA receptor; [^{3}H]-TBOB

1. INTRODUCTION:

Monoterpenoids are derived from or structurally related to monoterpenes, which are terpenes containing two isoprene units. Monoterpenoids are mostly found in plant essential oils. These natural products are secondary metabolites in higher-order plants. Unlike primary metabolites of plants, which are necessary in growth, development, and reproduction of plants, monoterpenoids are often involved in plant defense against herbivores and pathogens [1-3].

For hundreds of years, monoterpenoids have been used in the production of food additives, cosmetics, perfumes, shampoos and other personal care products, due to their pleasant, natural flavors and fragrances, and/or their antimicrobial properties. In the past 20 years, some monoterpenoids have shown very good insecticidal or insect repellent activities [1, 4-12]. These compounds have been considered as good alternatives for conventional synthetic insecticides, based on their wide-spectrum insecticidal activities, their low toxicities to mammals and other non-target organisms, and their biodegradability in the environment [3, 13-15].

Although some monoterpenoid insecticides are used commercially, the mechanisms of action of these botanical insecticides have not been fully elucidated. Previous studies on modes of action of some monoterpenoids revealed several possible protein targets in the insect nervous system, including ionotropic γ-aminobutyric acid (GABA) receptors [16-18], octopamine receptors [19, 20], tyramine receptors [21, 22], acetylcholinesterase (AChE) [23]and nicotinic acetylcholine receptors (nAChR) [24, 25]. Among these targets, the ionotropic GABA receptor may be involved

in the fast response to monoterpenoids in both central and peripheral nervous system in insects. From earlier studies of monoterpenoids' effects on insect GABA receptors, some monoterpenoids were indicated to bind to the insect GABA receptor, and interfere with the chloride movement mediated by GABA. Thymol was reported to be a positive allosteric modulator of a homo-oligomeric GABA receptor from *Drosophila melanogaster* [16]. Thymol, carvacrol, and pulegone were also indicated to increase the binding of [^{3}H]-TBOB, which is a non-competitive antagonist for insect GABA receptors, in house fly head membrane preparations, and potentiate $^{36}Cl^{-}$ uptake induced by GABA in ventral nerve cords of American cockroach [17]. However, the quantitative-structure activity relationship (QSAR) between monoterpenoid molecules and their binding activities to insect GABA receptor has not been evaluated yet, so the specific physicochemical properties of monoterpenoids that determine the binding of a monoterpenoid to the GABA receptor are unknown.

In this paper, the TBOB-binding activities of 22 monoterpenoids to house fly GABA receptors were determined using radioligand binding assays, and the binding data were used to build QSARs with a variety of descriptors, which can describe physical, chemical, structural, and electronic properties of monoterpenoids tested in this binding assay; they also help to explain ligand-receptor relationships. The QSAR models will be helpful to illustrate and predict the interactions between monoterpenoids and insect GABA receptors and provide guidance for searches for more potent analogs.

2. MATERIALS AND METHODS:

2.1 Chemicals. Monoterpenoids (eugenol, thymol, carvacrol, linalool, alpha-terpineol, menthol, vanillin, citronellal, citronellic acid, cinnamic acid, 1,8-cineole, 1,4-cineole, limonene epoxide, limonene, p-cymene, methyl salicylate, phenethyl propionate (PEP), piperonal, safrole, camphor, menthol, pulegone) and the GABA receptor antagonist convulsant picrotoxin (PTX) were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO. The [^{3}H]- *t*-butylbicycloorthobenzoate (TBOB) were purchased from GE Healthcare Life Sciences, Piscataway, NJ.

2.2 [^{3}H]-TBOB Binding Assay. House fly heads (0.8g) were homogenized in 10 mM tris-HCL buffer (pH 7.5) containing 0.25M sucrose (buffer A) with a glass homogenizer. The homogenate was centrifuged at 1,000*xg* for 5 minutes. The supernatant was filtered through four layers of cheesecloth and centrifuged at 25,000*xg*, and 4 ^{o}C for 40 minutes. The supernatant was discarded, and the pellet was homogenized and resuspended in ice cold buffer A for 30 minutes. The suspension was centrifuged at 25,000*xg*, and 4 ^{o}C for 40 minutes. The final pellet was suspended in 2 mL of 10 mM phosphate buffer (pH 7.5) containing 300 mM NaCl (buffer B) and used directly for the assays. Lowry protein assay was used to determine a final concentration of protein [26].

Membrane preparation containing 20 µg of protein was incubated for 90 minutes at room temperature (20 ^{o}C) with 4 nM [^{3}H]-TBOB (specific activity 22 Ci mmol^{-1}), 500 µM of candidate monoterpenoids and buffer B. The total assay buffer volume was 200 µL. After incubation, samples were filtered on glass fiber filter papers (Whatman

GF/B) and washed with 10 mL ice-cold buffer B three times. Radioactivity was measured by a Beckman liquid scintillation counter LS5000 CE. Specific binding was used to estimate the binding activities of candidate chemicals and was calculated as the difference between the total ^{3}H-bound and nonspecific ^{3}H-bound with 100 μM PTX. The specific binding was 60-70% of total binding at 4 nM [^{3}H]-TBOB. Each experiment was repeated at least three times using different membrane homogenates.[17, 27, 28]

The specific [^{3}H]-TBOB binding value in the absence of the any candidate chemicals was expressed as 100%. The percentage of a monoterpenoid's effect at a concentration of 500 μM on the [^{3}H]-TBOB binding to house fly head membrane preparations was calculated using the following formula:

percentage monoterpenoid's effect = (specific [^{3}H]-TBOB binding with 500 μM monoterpenoid/ specific [^{3}H]-TBOB binding w/o monoterpenoid) * 100

The difference between the effect of a monoterpenoid and 100 was used to develop QSAR models in the next step. These data described the efficiency of monoterpenoids' binding activities to the house fly GABA receptor at a concentration of 500 μM.

2.3 QSAR Analysis. Descriptors related to receptor-ligand interactions, including log P (octanol-water partition coefficient), Mulliken charge, dipole moment, total energy, highest occupied molecular orbital (HOMO), lowest unoccupied molecular

orbital (LUMO), and electrotopological state (E-state), were selected to describe chemical, physical, molecular, and topological properties of the monoterpenoids. Mulliken charge, dipole moment, total energy, HOMO, and LUMO were calculated in GAMESS, using an interface with ChemBio3D Ultra 12.0 (CambridgeSoft Corp., Cambridge, MA). The energy and geometry of all candidate monoterpenoids were analyzed with a split valence basis set and a polarization function (6-21G) calculation using GAMESS. Log P values were calculated in a free on-line cheminformatics services provided by www.molinspiration.com. Electrotopological state descriptors (E-state) were calculated in E-Calc (SciVision, Inc., Burlington, MA).

Descriptors and the [^{3}H]-TBOB binding data were analyzed by simple linear and multiple linear regressions for evidence of correlation. The [^{3}H]-TBOB binding data were shown as log (TB), which expresses the log value of the difference between the percentage of effect of a monoterpenoid on [^{3}H]-TBOB binding and 100. The square of the correlation coefficient (R^2) and cross-validation (Q^2) were used to evaluate the fitness of regression models. All linear and multiple regressions were analyzed using SAS 9.1. Regression models with $R^2 > 0.8$ were selected first, and then validation of these models were examined by using the leave-one-out method using the following equations:

$$\text{Cross-validation } Q^2 = 1 - (\text{PRESS/SSTO})$$

where

$$\text{PRESS} = \sum_y (Y_{\text{predicted}} - Y_{\text{actual}})^2$$

and SSTO is the sum of squares total. Any models with cross validation (Q^2) values >0.6 were suggested to be a nonrandom relationship [29].

2.4 Acute Toxicity Correlation with [^{3}H]-TBOB Binding Data. 24-hour topical lethal dose 50% (LD_{50}) values were determined for 22 monoterpenoids. Adult house flies (a mix of males and females) were subdued with CO_2 and placed on a piece of aluminum foil sitting on ice. 1 μL of various concentrations of monoterpenoids was applied to the pronotum of house flies using a microsyringe and repeating dispenser (Hamilton Company USA, Reno, NV). Treated house flies were placed in a mason jar with wire mesh lid, and maintained on a saturated sucrose solution. 10 to 20 house flies were treated for each concentration. For each concentration, three replications were used. Six to ten treatment concentrations (diluted in acetone) were tested for each monoterpenoid used in this assay to produce mortalities from 0% to 100%. Controls were examined for each of the treatments by applying 1 μL of acetone to the pronotum of the house flies. After 24-hour exposure, mortalities of the house flies were recorded. LD_{50} values of all the monoterpenoids were calculated by using SAS software (PROC PROBIT, SAS 9.1). LD_{50} values were converted to be expressed as μg /fly.

Log (LD_{50}) values of monoterpenoids from the two subsets and log (TB) values, which expresses the log values of the difference between the percentage of effect of a monoterpenoid on [^{3}H]-TBOB binding and 100, were analyzed by simple linear regressions for evidence of correlation. The square of the correlation coefficient (R^2) values were calculated by using SAS 9.1 to evaluate the fitness of regression.

3. RESULTS:

3.1 Effects of Monoterpenoids on [^{3}H]-TBOB Binding to House Ffly GABA Receptors.

The 22 monoterpenoids (Fig. 1) were selected to test their efficacies to modulate the [^{3}H]-TBOB binding in house fly head membrane preparations at a concentration of 500 μM. Among these candidates, only nine of them resulted in significant changes of [^{3}H]-TBOB binding in the house fly head membrane homogenates. In these nine compounds, 1,8-cineole, carvacrol, citronellic acid, pulegone, and thymol potentiated the [^{3}H]-TBOB binding in house fly head membrane preparations, which indicated that these monoterpenoids could bind to the house fly GABA receptor at a different binding site from the TBOB binding site; the others, including camphor, menthol, safrole, and vanillin, inhibited the [^{3}H]-TBOB binding significantly, suggesting that these compounds could also bind to house fly GABA receptor, either at the TBOB binding site to inhibit the TBOB binding competitively, or at an allosteric binding site to inhibit TBOB binding non-competitively (Table 1). The binding data for these nine monoterpenoids were used to develop the QSAR model in the following step. Furthermore, we found that six monoterpenoids out of these nine compounds showed structural similarity to p-menthane, so we also selected a subset of nine p-menthane analogs, each with at least one oxygen atom bonded to the ring from the 22-monoterpenoid list to develop another QSAR model.

3.2 Numbering of Carbon Atoms for Monoterpenoids. The structures of the selected 22 monoterpenoids show some similarities. Most of them are cyclic monoterpenoids (hexane or aromatic ring), except for citronellic acid, which can exist in a conformation that can resemble an aliphatic ring, and all of them have at least one carbon atom in the ring bonded with an oxygen atom. Based on these structural details, we numbered the carbon connected to an oxygen atom on the ring as carbon 1. (Fig. 2, and Fig. 3)

3.3 QSAR Models. The log values of differences between the percentage effects of monoterpenoids on TBOB binding and 100 were used to develop QSAR models (log (TB)). For the nine monoterpenoids which showed significant differences for [^{3}H]-TBOB binding, we found an excellent multiple regression model which contained log P, and Mulliken Charge on carbon atom 4 (MULC-C4): log (TB) = 2.02 (±0.27) + 0.13 (±0.05) [log P] + 2.81(±0.69) [MULC-C4]. The fitness of the model and the cross-validation provided good evidence of this multiple regression model (N = 9, F = 13.73, R^2 = 0.82, Q^2 = 0.78). Observed and calculated log (TB) values are compared in Table 2, and a good correlation between them is shown in Fig. 4. This model indicates the monoterpenoid's binding efficiency to house fly GABA receptor is positively related to the partition coefficient of this molecule as well as the charge on the carbon atom 4.

The second QSAR model was developed from nine monoterpenoids which are analogs of p-menthane with at least one oxygen atom connected to the ring. The structures and the numbering of carbon atoms are shown in Fig. 3. The Mulliken

charges on the carbon atom 6 (MULC-C6) and total energy (TE) were illustrated to be involved in this model, which is described: log (TB) = 67.26 (±21.3) + 226.82*10^{-6} (±74*10^{-6}) [TE] + 0.65 (±0.15) [MULC-C6]. Strong evidence based on the fitness of the model and the cross-validation showed a multiple linear relationship of this model (N = 9, F = 11.34, R^2 = 0.80, Q^2 = 0.74). Observed and calculated log (TB) values are compared in Table 3, and a good correlation between them is shown in Fig. 5. This relationship suggests that as the charge on the carbon atom 6 or the total energy increases, the monoterpenoid's binding efficiency also increases.

3.4 Relationship between Monoterpenoid Toxicity and GABA Receptor Binding. In insects, monoterpenoids may have various targets. In order to determine how much the binding of monoterpenoids to the GABA receptor contributes their toxicities to house fly, simple linear regression models between monoterpenoids' LD_{50} values (Table 4) and [^{3}H]-TBOB binding data (Table 1) of two different sets of monoterpenoids were calculated. The log values of monoterpenoids' LD_{50} values and [^{3}H]-TBOB binding data (log (TB)) were used in these two models.

The first set of monoterpenoids, which consisted of those with significant differences for [^{3}H]-TBOB binding, showed a linear relationship with log values of monoterpenoids' LD_{50} (R^2 = 0.60), which suggested that 60% of the variation in toxicities can be explained by the receptor binding of this set of monoterpenoids.

In the second set of monoterpenoids, which are p-menthane analogs, linear correlation was also found between the receptor binding and toxicity to the house fly with R^2 = 0.58. This relationship indicated that 58% of the variation in their toxicities

can be explained by the binding of the monoterpenoids to the house fly's GABA receptor for that set of p-menthane analogs.

In this study, GABA receptor binding data shows modest correlation with monoterpenoids' toxicities to house fly. The 0.58 to 0.60 correlation may indicate partial importance of GABA binding, or importance for only certain monoterpenoids. Firstly, these monoterpenoids kill insects by acting on multiple targets in insects besides the GABA receptor, including the nicotinic acetylcholine receptor and the octopamine receptor. Secondly, toxicokinetic factors influence the toxicities of these monoterpenoids, including volatility of these chemicals off the house fly cuticle, penetration of these insecticides into the cuticle, binding of monoterpenoids with proteins in insect hemolymph, detoxification of monoterpenoids by the insect enzyme system.

4. DISCUSSION

Of the 22 monoterpenoids tested in this study, two subsets of them were selected to develop QSAR models, due to their significant effects on the [^{3}H]-TBOB binding in house fly head membrane preparations, to evaluate the relationships between chemical properties of these monoterpenoids and their binding activities at insect GABA receptors. We found that moieties apparently needed to contain an oxygen atom for these monoterpenoids to interact strongly with the house fly GABA receptor, because all nine compounds with strong effects on the house fly GABA receptor have at least one carbon in the ring bonded to oxygen to form an alcohol, phenol, ketone, ether, or carboxyl group. Moreover, six of these monoterpenoids are analogs of

p-menthane, indicating that this type of monoterpenoids skeleton may also play an important role in the binding between monoterpenoids and insect GABA receptors. Based on these structural features, two QSAR models were developed from two subsets of nine compounds to illustrate relationships between monoterpenoid structures and their GABA receptor binding activities.

For both models, the electronic properties of the monoterpenoids were important to the binding of these compounds to the GABA receptor. As the charge on certain carbon atoms increased, the binding efficiency of monoterpenoids to the receptor also increased. This relationship indicated that the carbon atom 4 (for significant effects on TBOB binding model) and 6 (for p-menthane analogs model) might be directly involved in the binding of these compounds to the house fly GABA receptor. The log P is also crucial factor in the first QSAR model (significant effects on TBOB binding model). The enhanced log P value improved the binding efficiency of these monoterpenoids to house fly GABA receptors, which suggested the lipophilic interaction might play a key role in the binding between this set of monoterpenoids and the GABA receptor, and lipophilic moieties might be included in the binding site on the GABA receptor. Total energy is another key parameter in the p-menthane analogs model. Total energy has been used in other QSAR studies to show stability of a molecule [30]. The relationship with total energy indicated that the binding efficiency is related to the stability of monoterpenoids with p-menthane skeleton.

Although some of monoterpenoids showed significant effects on the binding of [^{3}H]-TBOB, which is a non-competitive inhibitor binding to the picrotoxin binding

site at insect GABA receptors, the binding sites of these compounds may not be the same due to their different patterns of [^{3}H]-TBOB binding, as well as their variation in structural features. 1,8-Cineole, carvacrol, citronellic acid, pulegone, and thymol enhanced the [^{3}H]-TBOB binding to house fly head membrane preparations. Among these five chemicals, carvacrol and thymol, which are phenols, may bind at the same site on the house fly GABA receptor, based on their similar structures and similar modulatory effects on insect GABA receptor from our previous study [17]. 1,8-Cineole, citronellic acid, and pulegone, which are a bicyclic ether, acyclic acid, and cyclic ketone, respectively, may not share the same binding site with carvacrol and thymol due to the lack of electron donors, which is important for the binding of phenols to the GABA receptor [31], although the previous study from our laboratory demonstrated that pulegone had effects similar to thymol and carvacrol on [^{3}H]-TBOB binding and $^{36}Cl^-$ uptake in insect nervous system [17]. The other four monoterpenoids, camphor, menthol, safrole, and vanillin significantly inhibited the [^{3}H]-TBOB binding to house fly head membrane preparations, but we could not determine if this inhibition was competitive (binding to the same site as GABA receptor inhibitors TBOB and picrotoxin to inhibit GABA responses) or non-competitive (binding to an allosteric site, not the TBOB binding site) from this assay. According to A.C. Hall *et al.*[32], camphor and menthol, analogs of p-menthane, showed positive modulatory effects on recombinant human $GABA_A$ receptor expressed in *Xenopus* oocytes, which indicated they did not bind to the non-competitive antagonist (TBOB/picrotoxin) binding site on human $GABA_A$ receptor. Aoshima *et al.*[33] found that vanillin inhibited GABA-induced Cl^- current

non-competitively in rat $GABA_A$ receptor expressed in *Xenopus* oocytes at a high concentration (10mM), which illustrated that vanillin might also bind to the non-competitive antagonist binding site on mammalian GABA receptors. However, in insects, we have not found any evidence for the binding sites or modulation of insect GABA receptors for these four monoterpenoids yet.

Mechanism-of-action research of monoterpenoids on insects faces numerous challenges. Monoterpenoids are a large group of compounds with diverse structural skeletons and functional groups. They may have multiple targets in insects and mammals. Many researchers have shown mechanisms of action of monoterpenoids on target other than GABA in either insects or mammals, such as octopamine receptors [19, 20], tyramine receptors [21, 22], nicotinic acetylcholine receptors [24, 25], thermo-transient receptors [34, 35] and acetylcholinesterase [23]. Furthermore, even targeting the same protein (receptors or enzymes), different categories of monoterpenoids may have different binding sites and different effects on the functions of these targets. Octopamine receptors in insects, as an example, were shown as targets for many monoterpenoids, however, one aromatic monoterpenoid (carvacrol) enhanced the binding of octopamine to the receptor, while others diminished the octopamine binding to the receptor; those included eugenol, vanillin, pulegone, and camphor, [19, 20]. On the other hand, a single monoterpenoid may interact with various targets contributing to the toxicity to insects. For example, carvacrol and pulegone were illustrated to have effects on both octopamine and GABA receptors in insects [17, 19, 20].

In this study, we focused on explaining the interaction between the chemical and structural properties of monoterpenoids and their binding activities at the house fly GABA receptor. Our two QSAR models provided evidence that (i) functional groups with oxygen atom(s) may be necessary for the binding, (ii) electronic properties on carbons 4 and 6 affect the binding activities, and (iii) the hydrophobicity and total energy level of monoterpenoid molecules play key roles for the binding to the house fly GABA receptor.

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TABLES

Table 1. Effects of monoterpenoids on [^{3}H]-TBOB-binding in house fly head membrane preparations

monoterpenoids	% of [^{3}H]-TBOB-binding (mean ± SEM)	Difference from 100%
1,4-cineole	117 ± 20	17
1,8-cineole	122 ± 8*	22
α-terpineol	95 ± 10	5
camphor	70 ± 7*	30
carvacrol	156 ± 2*	56
cinnamic acid	101 ± 5	1
citronellal	92 ± 7	8
citronellic acid	138 ± 12*	38
eugenol	88 ± 7	12
limonene	86 ± 7	14
limonene oxide	141 ± 20	41
linalool	124 ± 22	24
menthol	80 ± 10*	20
menthone	86 ± 13	14
methyl salicylate	103 ± 8	3
p-cymene	90 ± 8	10
phenethyl propionate	95 ± 10	5
piperonal	93 ± 8	7
pulegone	132 ± 4*	32
safrole	43 ± 3*	57
thymol	180 ± 12*	80
vanillin	82 ± 5*	18

* indicats significant difference from 100.

Table 2. [^{3}H]-TBOB binding data, calculated values, and residual values for 9 monoterpenoids with significant effects

monoterpenoids	observed TB[a]	observed log (TB)	calculated log (TB)	residual
1,8-cineole	22	1.33	1.37	0.04
camphor	30	1.47	1.51	0.04
carvacrol	56	1.75	1.85	0.1
citronellic acid	38	1.58	1.57	-0.01
menthol	20	1.31	1.4	0.09
pulegone	32	1.51	1.32	-0.19
safrole	57	1.75	1.72	-0.03
thymol	80	1.9	1.79	-0.11
vanillin	18	1.25	1.32	0.07

[a] TB means the difference between the percentage effect of a monoterpenoid on [^{3}H]-TBOB binding and 100.

Table 3. [^{3}H]-TBOB binding data, calculated values, and residual values for 9 p-menthane analogs

monoterpenoids	observed TB[a]	observed log (TB)	calculated log (TB)	residual
1,4-cineole	17	1.24	1.37	0.13
1,8-cineole	22	1.33	1.33	0
camphor	30	1.47	1.56	0.09
carvacrol	56	1.75	1.84	0.09
limonene oxide	41	1.61	1.5	-0.11
menthol	20	1.31	1.16	-0.15
menthone	14	1.13	1.26	0.13
pulegone	32	1.51	1.46	-0.05
thymol	80	1.9	1.76	-0.14

[a] TB means the difference between the percentage effect of a monoterpenoid on [^{3}H]-TBOB binding and 100.

Table 4. Monoterpenoids' LD_{50} values to house flies

monoterpenoids	LD_{50} (μg/fly)	95% confidence interval	number of insects
1,4-cineole	151.7	97.7-265.1	480
1,8-cineole	63.2	49.2-81.2	480
α-terpineol	137.5	122.5-153.9	480
camphor	149.5	135.4-164.2	480
carvacrol	60.1	36.4 - 86.2	180
cinnamic acid	>500		240
citronellal	46.8	40.1-54.4	600
citronellic acid	25.2	14.4-45.2	600
eugenol	46.4	41.7-51	480
limonene	89.4	72.3-114.5	480
limonene oxide	67.8	62.8 - 73.7	300
linalool	72.3	65-80.5	480
menthol	128.9	111.9 - 146.5	360
menthone	96.5	62.0 - 133.6	360
methyl salicylate	41.2	24.3-58	480
p-cymene	94.7	88.1-101.5	480
phenethyl propionate	66.6	36.1-108.7	480
piperonal	36.1	30.5-41.8	600
pulegone	92.2	78.5 - 106.2	180
safrole	18.3	13.2-28.4	600
thymol	26.3	23.6-29.6	390
vanillin	>500		240

FIGURE LEGENDS

Figure 1. Structures of 22 monoterpenoids tested in this study.

Fig. 1

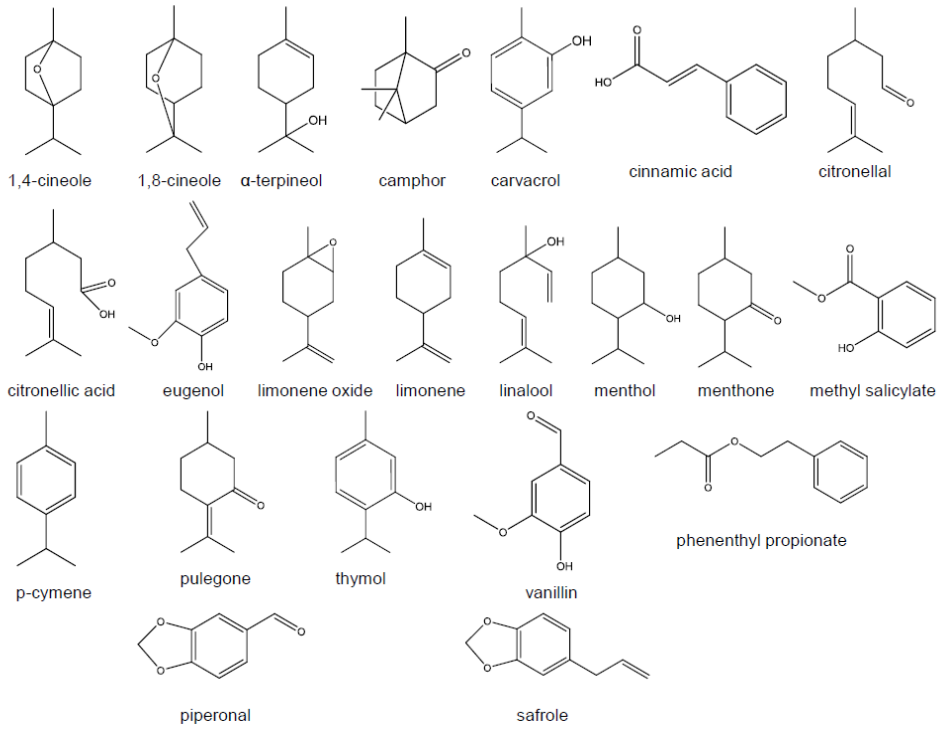

Figure 2. Numbering of the carbon atoms for monoterpenoids with significant effects on [^{3}H]-TBOB binding to house fly head membrane preparations.

Fig. 2

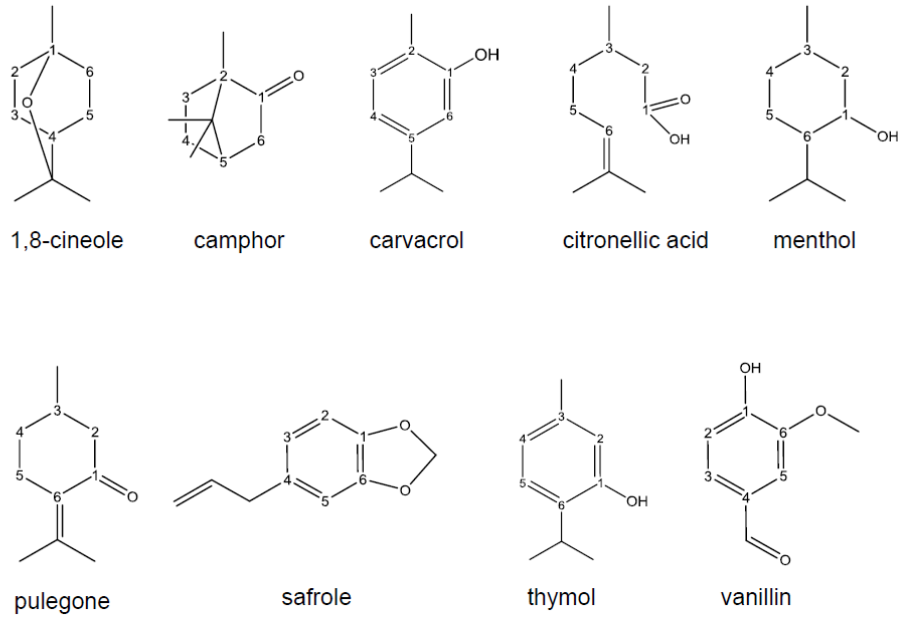

Figure 3. Structures of p-menthane and numbering of the carbon atoms for nine analogs with at least one oxygen atom connected with the ring.

Fig. 3

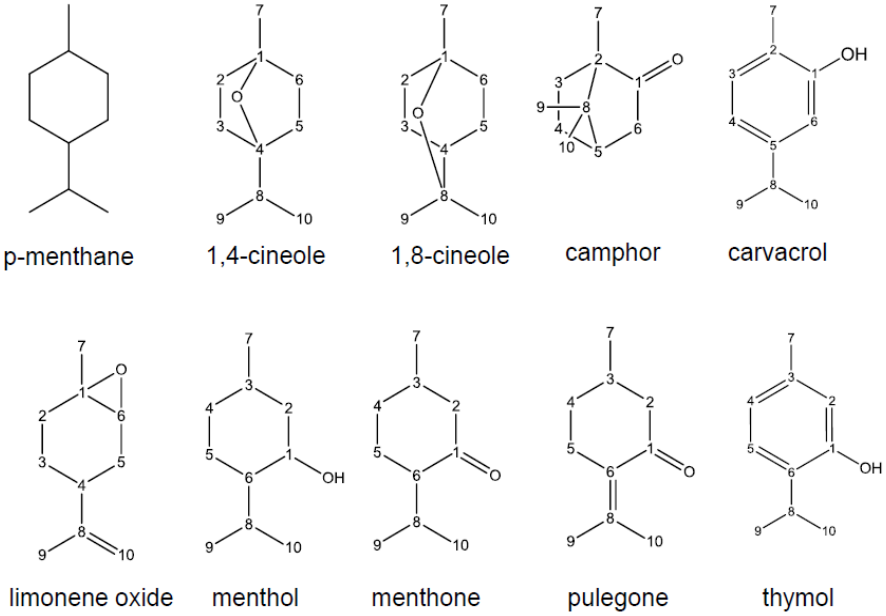

Figure 4. Plot of observed versus calculated [^{3}H]-TBOB binding activities for nine monoterpenoids with significant effects on [^{3}H]-TBOB binding. TB means the difference between the percentage effect of a monoterpenoid on [^{3}H]-TBOB binding and 100.

Fig. 4

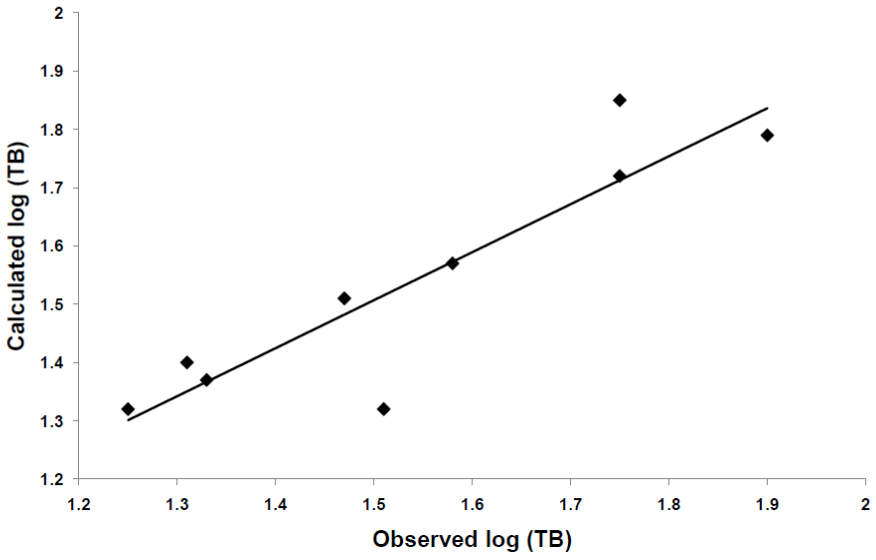

Figure 5. Plot of observed versus calculated [^{3}H]-TBOB binding activities for nine analogs of p-menthane. TB means the difference between the percentage effect of a monoterpenoid on [^{3}H]-TBOB binding and 100.

Fig. 5