Using Classic and Quantum Parameters to Determine Monoterpenoids' Insecticidal Quantitative Structure-Activity Relationships

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Abstract
Monoterpenoids are naturally occurring plant compounds that have been shown to have toxicity to insects. Quantitative structure-activity relationships (QSAR)s were developed for monoterpenoids and their derivatives. Monoterpenoid phenols and alcohols (thymol, carvacrol, carveol, and geraniol) and their ester derivatives were examined to determine the structural features of the molecules that are essential for their toxicity to house flies. Using a variety of classical and quantum parameters, we found that electronic properties within each monoterpenoid group showed a high correlation with house fly toxicity.

Disciplines
Animal Sciences | Entomology | Environmental Health | Toxicology

Comments
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Chapter 23

Using Classic and Quantum Parameters to Determine Monoterpenoids’ Insecticidal Quantitative Structure–Activity Relationships

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Monoterpenoids are naturally occurring plant compounds that have been shown to have toxicity to insects. Quantitative structure-activity relationships (QSARs) were developed for monoterpenoids and their derivatives. Monoterpenoid phenols and alcohols (thymol, carvacrol, carveol, and geraniol) and their ester derivatives were examined to determine the structural features of the molecules that are essential for their toxicity to house flies. Using a variety of classical and quantum parameters, we found that electronic properties within each monoterpenoid group showed a high correlation with house fly toxicity.

Monoterpenoids are naturally occurring plant compounds that are found in higher-order plants. These compounds are secondary metabolites; they are usually synthesized from two isoprene units, and are therefore 10-carbon molecules. Biosyntheses of monoterpenoids are accomplished via the mevalonic acid pathway. Monoterpenoids are further processed by the plant through various oxidation steps. These compounds seem to play no major role in the metabolic functioning of the plants, and their role is thought to be less critical (secondary). There are several functions for monoterpenoids in the plant. One
function is to aid in pollination of the plant by attracting certain insects to the plant. Another function of monoterpenoids is to defend against plant pathogens, herbivores, or competing plant species.

Plants and insects have co-evolved for millions of years. Plants have developed the capability to produce secondary metabolites in order to protect themselves against different types of pathogens and herbivores. The pathogens include fungi and bacteria, and the herbivores include insects, birds, mammals, etc. Secondary metabolites, such as monoterpenoids, are potentially good naturally occurring insecticides because of the co-evolution through which they were developed. Some monoterpenoids have shown insecticidal activity, and a few of these compounds are used as commercial pesticides (d-limonene, menthol, citronellal, and linalool) (1). Although, these monoterpenoids are being used commercially, the mode of action is still unknown. In addition, no quantitative structure-activity relationships (QSARs) have been determined up to this point.

We examined four monoterpenoids (phenols and alcohols) and their ester analogs. We tested linear monoterpenoids (geraniol), cyclic monoterpenoids (carveol), and aromatic monoterpenoids (carvacrol, thymol) to find a relationship between all the monoterpenoids and their toxicity. By using toxicity to house flies, we tried to correlate toxicity with various classical and quantum parameters. Specific parameters were chosen in order to help explain toxicity. These parameters were chosen to represent the features of molecules that are important in receptor-ligand interaction. Size and shape of a molecule is extremely important for receptor-ligand interactions. If the receptor does not accommodate the molecule because of its size or shape, then the molecule cannot generate its effect on the system. In our case, its effect would be toxicity to house flies. To discern if shape and size of the monoterpenoids are important for their toxicity, we examined several classical parameters. These independent variables are molecular connectivity indices (0,1,2), valence connectivity indices (0,1,2), shape indices (1,2,3), and molar refractivity.

The other important criterion that must be met for receptor-ligand interactions to occur is the adherence of the ligand to the receptor. Molecular interactions can be explained by affinity due to electrostatic interactions, London dispersion forces, and hydrophobic interactions. We examined classical and quantum parameters to help explain these interactions. Log P and molar refractivity are the classical parameters chosen to represent hydrophobic interactions and London dispersion forces (2). The quantum parameters were chosen to represent both electrostatic interactions and London dispersion forces. Highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), dipole moment, polarizability, and Mulliken population are the quantum parameters chosen to represent receptor-ligand interaction, which can ultimately cause mortality to house flies.
Synthesis of monoterpenoid esters

Monoterpenoid parent alcohols or phenols, carveol, geraniol, thymol, and carvacrol, (1 mole) were added to their corresponding anhydride or acid chloride (2 moles) to form ester derivatives in the presence of a catalytic amount of pyridine (2-5 drops). Methylene chloride was used as the solvent, and the reaction was allowed to stir for 24-48 hr at room temperature. The reactions were monitored by thin-layer chromatography using a 9:1 hexane:acetone mobile phase and developed by vanillin spray (8g vanillin, 1.25ml sulfuric acid brought up to 250ml with methanol). The reaction was worked up with four (NaHCO₃ and water) washes. Methylene chloride was removed using a rotary evaporator. Compounds were purified using silica gel-column clean up, using a 19:1 hexane:acetone solvent system. Identities of the esters were determined using TLC, comparing Rf values of the parent alcohols or phenols against reaction products. Identities were confirmed using ¹H-NMR 300 Mhz. A total of 25 monoterpenoids were used in this study, which includes the four parent molecules and 21 esters (Fig. 1) (Fig. 2). Four geranyl esters were made from geraniol, and five esters were made from each of the remaining monoterpenoids (thymol, carvacrol, and carveol).

House fly toxicity testing

LD₅₀ values were obtained for all 25 monoterpenoids. Topical application was used to apply 1 μL of various concentrations of monoterpenoid to the pronotum of Musca domestica (house fly). We placed 10 treated house flies in a jar and for each concentration, three replications of monoterpenoid were used. At the end of the 24-hr exposure, mortalities of the house flies were recorded. LD₅₀s of all the monoterpenoids were calculated using the Spearman-Karber method (3). LD₅₀ values are shown (Fig 1) (Fig 2). Some compounds’ LD₅₀ values were previously report from our lab (4). These showed a range of toxicity to house flies, ranging from LD₅₀ of 0.17 μmol/fly to 2.35 μmol/fly. The two monoterpenoids which have the greatest toxicity are geranyl chloroacetate with a LD₅₀ value of 0.17 μmol/fly and thymol with a LD₅₀ value of 0.22 μmol/fly. There is no obvious structural reason why these two compounds have the most insecticidal activity. Geranyl chloroacetate is a derivative of an acyclic monoterpenoid, and thymol is an aromatic monoterpenoid. In the thymol group, thymol was more toxic than its derivatives; however, in the geraniol group, all the derivatives were more toxic than geraniol. Also for the carveol group, carveol was one of the least toxic compounds within that group. Carvacrol, on the other hand, was one of the
Thymol Compounds:

- **Thymol**
  - LD$_{50}$ = 0.22 (0.20-0.24)

- **Thymyl pivalate**
  - LD$_{50}$ = 0.34 (0.22-0.42)

- **Thymyl propionate**
  - LD$_{50}$ = 0.49 (0.40-0.62)

- **Thymyl acetate**
  - LD$_{50}$ = 0.49 (0.44-0.54)

- **Thymyl trichloroacetate**
  - LD$_{50}$ = 0.62 (0.56-0.69)

- **Thymyl chloropivalate**
  - LD$_{50}$ = 1.12 (0.98-1.27)

Carvacrol Compounds:

- **Carvacryl dichloroacetate**
  - LD$_{50}$ = 0.39 (0.41-0.53)

- **Carvacrol**
  - LD$_{50}$ = 0.42 (0.40-0.43)

- **Carvacryl trifluoroacetate**
  - LD$_{50}$ = 0.46 (0.41-0.53)

- **Carvacryl trichloroacetate**
  - LD$_{50}$ = 0.47 (0.43-0.51)

- **Carvacryl acetate**
  - LD$_{50}$ = 0.55 (0.50-0.61)

- **Carvacryl propionate**
  - LD$_{50}$ = 0.65 (0.64-0.66)

*Figure 1. Structures and LD$_{50}$ (µmole/fly) of thymol and carvacrol compounds. 95% confidence intervals of LD50 values in parentheses.*
Geraniol Compounds:

Geranyl chloroacetate
LD<sub>50</sub>=0.17 (0.15-0.19)

Geranyl pivalate
LD<sub>50</sub>=0.39 (0.37-0.41)

Geraniol
LD<sub>50</sub>=0.84 (0.61-1.01)

Geranyl acetate
LD<sub>50</sub>=0.28 (0.25-0.30)

Geranyl trichloroacetate
LD<sub>50</sub>=0.45 (0.44-0.46)

Carveol Compounds:

Carvyl pivalate
LD<sub>50</sub>=0.37 (0.35-0.40)

Carvyl acetate
LD<sub>50</sub>=0.57 (0.54-0.61)

Carvyl chloropivalate
LD<sub>50</sub>=0.96 (0.85-1.09)

Carvyl propionate
LD<sub>50</sub>=0.99 (0.92-1.104)

Carveol
LD<sub>50</sub>=1.85 (1.64-2.09)

Carvyl trichloropivalate
LD<sub>50</sub>=2.35 (2.32-2.39)

Figure 2. Structures and LD<sub>50</sub> (μg/mole/fly) of geraniol and carveol compounds. 95% confidence intervals of LD<sub>50</sub> values in parentheses.
most toxic compounds within its group. There was no obvious trend in structure that helps explain toxicity of these compounds. To help clarify what moieties of the molecules are responsible for their toxicity, we examined classical and quantum parameters to try to explain their toxicity.

**Monoterpenoid QSAR analysis**

The classical parameters mentioned previously, molar refractivity, molecular connectivity indices (0,1,2), valance connectivity indices (0,1,2), shape indices (1,2,3), and Log P, were calculated by CAChe™ (Oxford Molecular). The quantum parameters, highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), dipole moment (magnitude and direction), Mulliken population, and polarizability, were calculated in GAMESS™. Geometry and energy of all the molecules were optimized using a split valance basis set and a polarization function (6-31*gd) calculation using GAMESS™. Hessian runs were performed using 6-31*gd calculations using GAMESS™ to show that all the molecules tested were at an energy-minimum conformation. Classical and quantum parameters were plotted against house fly LD$_{50}$S. All regression analyses were fitted using Microsoft Excel™.

A relationship was not found between all the monoterpenoids (and their derivatives) and their toxicity to house flies. We did find relationships within sub-groups such as, thymol and its derivatives. Thymol compounds, carveol compounds, and carvacrol compounds showed no correlation between classical parameters and toxicity. Log P is often used to explain chemical uptake and hydrophobic interactions between ligand and a receptor. The lack of correlation between Log P and the toxicity of monoterpenoids indicates that changing the ester group does not have a dramatic effect on uptake or hydrophobic interactions. No correlations were found for thymol compounds, carveol compounds, and carvacrol compounds, but there were correlations found between the toxicity of geraniol compounds and molar refractivity, molecular connectivity indices (0,1,2), valance connectivity (0,1,2), and shape indices (1,2,3) (Fig 3). However, the correlation between toxicity and the previously mentioned parameters was a parabolic relationship using only five data points. The parabolic relationships suggest that there is an optimal region for toxicity of that series of derivatives. More data points should be added to verify this relationship. No correlations were found between toxicity and molar refractivity, molecular connectivity indices (0,1,2), valance connectivity (0,1,2), or shape indices (1,2,3), for thymol, carveol, and carvacrol compounds, which indicates that modifying the esters at the –OH position of the monoterpenoids...
Figure 3. Relationships of geraniol compounds’ toxicity with connectivity index (0), molar refractivity valence connectivity index (0), and shape index (1).
does not seem to have a major effect on toxicity. The size or shape of the esters does not seem to be a major factor on toxicity to house flies.

Only one quantum parameter (Mulliken population) showed a correlation between toxicity of thymol, carveol, and carvaerol compounds. Geraniol compounds showed no correlation between their toxicity and any of the quantum parameters. We obtained a correlation between toxicity and Mulliken population within the thymol, carveol, and carvaerol groups. Our study revealed a linear trend of increasing toxicity within the various groups to Mulliken population of certain atoms within that group. Thymol and its derivatives showed a relationship between toxicity and the Mulliken population on three atoms. Thymol compounds revealed that as the Mulliken population around atom 13 increases, toxicity of the compound decreases. The numbers on the atoms for thymol, carvacryl, and carveol correspond to the order the atoms were added to the Z-matrix to construct the molecules (Fig. 4). Atom 12 of the thymol compounds showed that as Mulliken population decreased toxicity increased. Atom 11 of the thymol compounds revealed the inverse relationship of atoms 13 and 12. It showed that as Mulliken population increased, toxicity also increased. We obtained an $r^2=0.96$ for atom 13 with $n=6$ (Fig. 5). Atom 11 had an $r^2=0.83$ with $n=6$ (Fig. 6), and atom 12 had an $r^2=0.92$ with $n=6$ (Fig. 7). We also obtained a linear correlation with toxicity and Mulliken population within the carvacryl group. Two atoms within the carvacryl group (6 and 12) showed a relationship between Mulliken population and toxicity. As Mulliken population increases around these atoms, their toxicity also increases. We obtained an $r^2=0.78$ for atom 6 with an $n=6$ (Fig. 8), and for atom 12 we obtained an $r^2=0.86$ with $n=6$ (Fig. 9). The carvacryl group of compounds also had a relationship between toxicity and Mulliken population. As the Mulliken population around atom 6 increased, toxicity also increased ($r^2=0.86; n=6$) (Fig. 10). These correlations demonstrate that the electronic effects of thymol, carveol, carvacryl compounds are important for explaining toxicity.

![Figure 4. Numbering of the atoms for thymol, carvacryl, and carveol compounds. These numbers correspond to the order they were placed into the Z-matrix.](image-url)
Thymol Compounds

$y = 15.32x - 85.51$

$R^2 = 0.97$

Figure 5. Linear correlation between thymol compounds’ house fly toxicity and Mulliken population around atom 13.

Thymol Compounds

$y = -18.37x + 110.64$

$R^2 = 0.83$

Figure 6. Linear correlation between thymol compounds’ house fly toxicity and Mulliken population around atom 11.
Thymol Compounds

\[ y = 38.15x - 235.01 \]
\[ R^2 = 0.92 \]

Mulliken Population at Atom #12

Figure 7. Linear correlation between thymol compounds' house fly toxicity and Mulliken population around atom 12.

Carvacrol Compounds

\[ y = -9.96x + 56.90 \]
\[ R^2 = 0.78 \]

Mulliken Population at Atom #6

Figure 8. Linear correlation between carvacrol compounds' house fly toxicity and Mulliken population around atom 6.
Carvacrol Compounds

\[ y = -148.64x + 925.01 \]

\[ R^2 = 0.86 \]

Figure 9. Linear correlation between carvacrol compounds' house fly toxicity and Mulliken population around atom 12.

Carveol Compounds

\[ y = -312.63x + 1948.25 \]

\[ R^2 = 0.86 \]

Figure 10. Linear correlation between carveol compounds' house fly toxicity and Mulliken population around atom 6.
Conclusion

No relationship was found between parameters for all the monoterpenoids (and their derivatives) and their toxicity; however we did find relationships for the structure characteristics of sub-groups and their toxicity. Since the sub-groups are not as large or diverse as the whole group the monoterpenoids, further compounds are needed to truly test the validity of these relationships. These smaller sets of relationships give us a good starting point to develop more robust QSARs and also can be used to increase the insecticidal effectiveness of compounds with in the sub-groups.

Geraniol compounds were the only set of monoterpenoids to show a relationship between toxicity and the classical parameters studied. Those classic parameters all encoded information on size and shape of the ester functional group. If these correlations hold true when more compounds are added, we will know that there is an optimal size and shape requirement for that part of the molecule that must be met for the compound to exert its toxic effect on house flies. Since there is a parabolic relationship, we can already predict the optimum toxicity for these compounds. To increase geraniol compounds’ toxicity, other regions of the molecules need to be modified.

For thymol, carvone, and carvacrol compounds, Mulliken population around certain atoms in the molecules showed a strong correlation with their toxicity. Mulliken population, which represents the probability of electron population around the atoms in the molecule, may explain electrostatic interactions of the monoterpenoids to a receptor. Regardless of the actual mechanism, the electronic effects of the molecule are important for their toxicity. The classical parameters revealed no correlation with these compounds’ toxicity nor any structural parameter examined. This indicates we can modify the –OH region of the molecule. Because size and shape of that part of the molecule does not seem to be important for toxicity, we can add a functional group at that part of the molecule to change the Mulliken population around certain atoms to increase toxicity. In the future, more compounds with different functional groups need to be examined in order to truly validate these QSARs.

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Reference