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Abstract
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Keywords
Sesquiterpene, essential oils, mosquito repellency, quantitative structure–activity

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Quantitative Structure−Activity Relationship of Botanical Sesquiterpenes: Spatial and Contact Repellency to the Yellow Fever Mosquito, *Aedes aegypti*

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The plant terpenoids encompass a diversity of structures and have many functional roles in nature, including protection against pest arthropods. Previous studies in this laboratory have identified naturally occurring sesquiterpenes contained in essential oils from two plants, amyris (*Amyris balsamifera*) and Siam-wood (*Fokienia hodginsii*), that are significantly repellent to a spectrum of arthropod pests. In efforts to further examine the biological activity of this class of compounds 12 of these plant-derived sesquiterpenes have been isolated, purified, and assayed for spatial and contact repellency against the yellow fever mosquito, *Aedes aegypti*. These data were used to develop quantitative structure−activity relationships that identified key properties of the sesquiterpene molecule, including electronic and structural parameters that were used to predict optimal repellent activity. There were notable similarities in the models developed for spatial repellency over five time points and for contact repellency. Vapor pressure was an important component of all repellency models. Initial levels of spatial repellency were also related to polarizability of the molecule and lowest unoccupied molecular orbital (LUMO) energy, whereas the equation for late spatial repellency was dependent on other electronic features, including Mulliken population and electrotopological state descriptors. The model identified for contact repellency was the best fit and most significant model in this analysis and showed a relationship with vapor pressure, Mulliken population, and total energy.

KEYWORDS: Sesquiterpene; essential oils; mosquito repellency; quantitative structure−activity

INTRODUCTION

Since its development in 1953, the synthetic repellent, DEET (*N*,*N*-diethyl-*m*-methylbenzamide), has been a highly effective topical repellent against mosquitoes and other disease-carrying arthropods (*1*); however, complications with toxicity have been reported in some circumstances, especially among children and elderly people (*2*−*4*). Significant amounts of this chemical can be absorbed through the skin (*5*), and there is some evidence of neurotoxicity resulting from high-level exposure to DEET in combination with permethrin and pyridostigmine bromide, all of which were commonly used by soldiers during the Persian Gulf War (*6*). Another limitation is the minimal efficacy against the primary vectors of human malaria parasites, *Anopheles* spp. (*7*). Furthermore, with continued use of only a select number of insecticides and repellents, it is possible that arthropod populations will develop resistance mechanisms. The existence of a DEET-insensitive mutant has been recently documented in *Drosophila melanogaster* (*8*). For all of these reasons, it is advantageous to invest efforts in the development of new technologies for the management of arthropods affecting human and animal health.

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The practice of using plant derivatives, or botanical-based insecticides and repellents, in agriculture dates back two to five millennia in ancient China, Egypt, Greece, and India. Even in Europe and North America, the documented use of botanicals extends back more than 150 years, dramatically predating discoveries of the major classes of synthetic chemical insecticides. Recent studies have further proven the effectiveness of botanicals as alternatives for arthropod repellents (*9*−*12*). Although a limited number of botanical repellents are commercially available, there is intense interest in developing these compounds because of increasing regulation for, and negative public perception of, synthetic chemicals (*13*, *14*). Research and development of alternative repellent compounds for mosquito control would also serve a valuable role in consumer acceptance and the overall movement toward integrated management of arthropods that transmit diseases.

Early studies on the chemical, structural, and physical properties of mosquito repellents showed that measures of volatility (*15*−*18*), such as vapor pressure and boiling point, correlated with repellent responses. Other properties have been examined including partition coefficients, melting points, infrared absorption, viscosity, molecular weights, surface tension, polarizability, and Hammett's substituent constants (*15*). Considerations have been made regarding functional groups as well; Garson and

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Winnike (*17*) noted that compounds containing amides, imides, phenols, alcohols, hydroxy ethers, glycols, and hydroxy esters were active, whereas the parent hydrocarbons were poor repellents (*19*, *20*). Christophers (*16*) reported the repellent activity of alcohol groups, along with aldehydes and phenols and hypothesized that the activity was related to the positioning of the −OH groups. Another study using terpenes found that conversion of the alcohol group to the acetate minimized repellency (*21*), whereas the corresponding ester was superior in activity (*22*).

A selection of plant essential oils that contain significant levels of sesquiterpenes, most notably eremophilane, eudesmane, and germacrane derivatives, have appeared in the literature as effective arthropod repellents (*23*, *24*). Recent studies with a collection of sesquiterpenoids from the heartwood of the Alaska yellow cedar (*Chamaecyparis nootkatensis* D. Don) include testing of nootkatone and valencene-13-ol. Both of these compounds were equally as repellent to *Ixodes scapularis* as DEET (nootkatone, RC_{50} 0.0458 w%/v solution; valencene-13-ol, RC_{50} 0.0712 w%/v solution; DEET, RC_{50} 0.0728 w%/v solution) (*24*). Examination of nootkatone derivatives showed that the ketone group was important for repellent activity to the Formosan subterranean termite (*Coptotermes formosanus* Shiraki). Modification to a 1,10-dihydro- or a tetrahydronootkatone derivative, by reducing the 1,10 double bond, also improved repellency (*25*). Other recently identified sesquiterpenes with repellent activity include callicarpenal and intermedeol, which were isolated from the American beautyberry bush (*Callicarpa americana* L.) and evaluated for activity against mosquitoes and ticks (*26*, *27*). Also, research in our laboratory has reported on the mosquito-repellent properties of elemol, a major component of Osage orange essential oil (*Maclura pomifera* (Raf.) Schneid.) and two sesquiterpene-rich essential oils, amyris (*Amyris balsamifera* L.) and Siam-wood (*Fokienia hodginsii* L.) (*23*, *28*). Further characterization of the bioactivity of these botanical sesquiterpenes by examination of quantitative structure−activity relationships (QSAR) provides insight into the mechanism of action of repellents, as well as a guide to the selection of the most potent compounds.

In this study, we selected 12 sesquiterpenes that share structural similarities and represent a range of mosquito-repellent activities. Individual compounds were tested in a standardized laboratory bioassay, and measures of spatial and contact repellency were observed. These data were analyzed with classic and quantum molecular descriptors, which encompass physical−chemical properties discussed in the literature, as well as structural and electronic features relevant to ligand−receptor interactions.

MATERIALS AND METHODS

Chemicals. Nootkatone **6** (≥99%) (Sigma Aldrich, St. Louis, MO), elemene **4** (≥80%) (Augustus Essential Oils), farnesol **11** (≥95%) (Sigma Aldrich), α-bisabolol **9** (≥95%) (Sigma Aldrich), and *trans*-nerolidol **12** (≥98%) (Fluka Chemie GmbH, Buchs, Switzerland) were purchased from commercial sources. Sufficient quantities of β-eudesmol **2**, elemol **5**, 10-epi-γ-eudesmol **1**, valerianol **3**, α-santalol **13**, turmerone **8**, and fokienol **10** sesquiterpenes were isolated from either technical grade materials or essential oils and then purified in our laboratory. A supply of technical grade, 55% purity elemol (Augustus Essential Oils, Ltd., Hampshire, U.K.) was further purified to ≥95% via column chromatography with silica gel, 40−140 mesh (J. T. Baker, Phillipsburg, NJ), using a hexane/diethyl ether (9:1) mobile phase. A similar approach was used with hexane/acetone/diethyl ether (7:2:1) and hexane/diethyl ether (95:5) solvent systems to isolate and purify fokienol (≥85%) from Siam-wood essential oil, similarly with purified α-santalol (≥85%) from East Indian sandalwood (*Santalum album* L.) essential oil and turmerone (≥70%) from turmeric (*Curcuma longa* Linn.) essential oil. β-Eudesmol, 10-epi-γ-eudesmol, and valerianol were isolated from amyris essential oil (West Indian sandalwood) (Sigma Aldrich) with argentation column chromatography techniques. Multiple columns with 10% silver nitrate impregnated silica gel, +230 mesh (Sigma-Aldrich), were required to attain purity levels of ≥80%. Purity of samples was assessed on a Hewlett-Packard 5890 series II gas chromatograph with a 30 m × 0.25 mm i.d., 0.25 μm, DB-Wax column (Alltech, Deerfield, IL) with flame ionization detection. The injector temperature was 250 °C, and the split valve was opened 1 min after injection. The oven initial temperature was set at 120 °C for 1 min and then increased at 4 °C/min to 236 °C. Confirmation of compound identity was completed on a Hewlett-Packard 5890 series II gas chromatograph interfaced to a Hewlett-Packard 5972 mass selective detector. Mass spectra were recorded from 30 to 550 amu with electron impact ionization at 70 eV. The assignments of chemical identities to the chemical compounds detected were confirmed by comparison of the retention indices with reference spectra in a mass spectral library (Wiley 138K) and comparison to literature sources (*29*, *30*). For select compounds, commercially available analytical standards were used for comparison (β-eudesmol) (Sigma Aldrich).

Hedycaryol **7** was synthesized in the laboratory using elemol as a starting material, in 20% silver nitrate solution and ethyl acetate (*31*). The reaction was run under ice for 48 h, and the hedycaryol product was confirmed by TLC and GC-FID.

Repellency Bioassay. Bioassays were conducted in a static-air chamber (9 × 60 cm section of glass tubing) at a controlled temperature of 26 °C, over a period of 5 days. Yellow fever mosquitoes, *Aedes aegypti* (Liverpool strain), were used from an established laboratory colony. Newly emerged adults were maintained under standard incubator conditions (80% relative humidity and held at 27 °C) and fed a 10% (0.3 M) sucrose solution. Only female mosquitoes were used in testing and were at least 5 days old.

Test solutions were made up in acetone and applied to 9 cm diameter Whatman no. 1 round filter papers (63.6 cm^2). A solvent-only (acetone) control was used for comparison to treatments. The acetone was allowed to evaporate off the filter paper for 5 min prior to testing. A 78.6 $\mu g/cm^2$ rate of exposure was used as it has been used in past studies to effectively measure and compare repellency effects of terpenoids (*23*, *28*). Treated filter papers were placed inside the lids of 9 cm glass Petri dishes, and the dishes were placed over the ends of the glass chamber. A group of 20 female mosquitoes was anesthetized with CO_2 and introduced into the chamber through a 2 cm hole drilled at its midpoint. Mosquito distribution inside the static-air choice-test apparatus was observed at five time points over a total of 180 min. The experimental design was a completely randomized design using five replications of each treatment. Data generated by this study were used to examine two measures of mosquito repellency, percentage (spatial) repellency and contact repellency. Percentage repellency was calculated with the following formula:

$$\text{percentage repellency} = [(\text{no. of individuals in untreated half} - \text{no. of individuals in treated half})/20] \times 100$$

Contact repellency was defined in this assay as avoidance of the treated filter paper (no contact = 100% avoidance) throughout the 180 min observation period and was compared with control treatments, using Fisher's exact test. The reported avoidance frequency is the average of mosquito contact repellency over the total 180 min period (based on six time points).

QSAR Calculations and Model Development. Descriptors were selected to represent molecular properties and features relevant to receptor−ligand interactions and physicochemical properties that could be correlated with repellent activity. Molecular connectivity, total valence connectivity, molar refractivity, molecular topological index, and Wiener index were used to account for the size and shape of the molecule. Both classical and quantum parameters were also examined, including log *P* (octanol−water partition coefficient), Henry's constant, highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), dipole moment, Mulliken population, and polarizability. Descriptors were calculated in GAMESS, through an interface with ChemBio3DUltra 11.0 (CambridgeSoft Corp., Cambridge, MA). Vapor pressures were calculated at 111 °C using ACD/Boiling Point 8.0 (Advanced Chemistry Development, Inc., Ontario, Canada) to distinguish small differences in select compounds. The energy and geometry of each molecule were optimized with a split valence basis set and a polarization function (6-31*d)

calculation. Electrotopological state descriptors (E-state) were calculated in E-Calc (SciVision, Inc., Burlington, MA).

To achieve an accurate model for sesquiterpene repellency, descriptors were analyzed for evidence of intercorrelation, using Pearson and Spearman rank correlation procedures. Parameters that were highly correlated were noted, and only one was used in the final model. A stepwise regression procedure was used to identify key descriptors, prior to final selection of the overall best model selected from a subset regression. Overall fitness of the model was based on AIC values, and potential bias of descriptors was examined by ridge regression. The number of descriptors in the final model was also based on an overall improvement of $\Delta R^2 < 0.02-0.04$ (*32*). Validation of the best-fit models was completed with the leave-one-out method (*33*, *34*):

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

Here

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

All multiple and linear regression procedures were performed on SAS 9.1. Best-fit models were completed for spatial and contact repellency values, independently. Spatial and contact repellency, along with sesquiterpene vapor pressures, were analyzed following log transformation. Predictability of repellency models was also interpreted with an external validation test using a structurally similar sesquiterpene, turmerone.

RESULTS AND DISCUSSION

A selection of 12 sesquiterpenes, including eudesmane, elemane, eremophilane, bisaboene, and germacrane types of compounds, including acyclic and bridged systems (**Figure 1**) were evaluated for repellent activity against *Ae. aegypti*. Overall, hedycaryol and 10-epi-γ-eudesmol showed the highest levels of repellent activity, and elemene and *trans*-nerolidol were the lowest. Repellency values for 10-epi-γ-eudesmol were the most consistent of the compounds tested in this assay. In particular, these compounds show the range observed in spatial and contact activity that was important for the construction of QSAR models for sesquiterpene insect repellency. The molecular descriptors considered in this analysis represent physical−chemical properties, especially those that have been indicated as important for repellent activity, and structural and electronic features relevant to ligand−receptor interactions. Electrotopological descriptors were also considered in model development. Models were developed for each of the spatial repellency time periods.

Both spatial and contact mosquito repellency data (**Table 1**) were used to develop QSAR models capable of predicting the repellent activity of sesquiterpenes. These data show a range of spatial repellency values over multiple time points, as well as contact repellency, and were analyzed separately to select parameters for the best-fit models and validation. The final models for each spatial repellency time point and the contact repellency (shown in **Table 2**) shared several similarities in the final parameters that were selected. Log vapor pressure was identified as an important parameter in all of the models reported, and the Mulliken population of carbon 1 (**Figure 1**) was also important in the majority of the repellency models. Notable differences in final parameters selected for the spatial repellency models are seen in the comparison of the initial spatial repellency time (15 min) against the later time points (60, 90, 120, and 180 min) (**Figure 2**). Models for the early measure of spatial repellency (REP) contained vapor pressure (VP), polarizability (POL), and LUMO parameters (15 min: [log (REP)] = 0.94(±0.09) − 1.01 (±0.16)[log(VP)] + 0.09(±0.03)[LUMO] − 0.64(±0.37) [POL]). Internal and external validation, as well as fitness of the model, provided good evidence of the spatial repellent predictability with the 15 min model ($N = 12$, $F = 16.93$, $R^2 = 0.86$, $Q^2_{\text{LOO}} = 0.61$). Models for the later spatial repellency time points, starting at 60 min, all contained the same selection of parameters including log vapor pressure, Mulliken population of carbon 1, and E-state of carbon 7 (**Figure 1**) (models listed in **Table 2**). Fitness and validation of these models provided good evidence of sesquiterpene spatial repellent predictability (60 min, $N = 12$, $F = 12.20$, $R^2 = 0.82$, $Q^2_{\text{LOO}} = 0.73$; 90 min, $N = 12$, $F = 15.61$, $R^2 = 0.85$, $Q^2_{\text{LOO}} = 0.69$; 120 min, $N = 12$, $F = 13.99$, $R^2 = 0.83$, $Q^2_{\text{LOO}} = 0.72$; 180 min, $N = 12$, $F = 23.88$, $R^2 = 0.89$, $Q^2_{\text{LOO}} = 0.75$). Comparison of the calculated versus observed spatial repellency of turmerone, used for external validation, resulted in residuals ranging from 6.1 to 19.7, with the highest residual resulting from the 90 min model. This was in line with the results from internal validation of the model (i.e., the 90 min model was the weakest late spatial repellency model, $Q^2_{\text{LOO}} = 0.69$).

In addition to the spatial repellency time point models, data collected on mosquito continual avoidance of the sesquiterpene-treated surfaces were also analyzed and used to develop a best-fit model for contact repellency (AVOID). The final model contained vapor pressure, Mulliken population of carbon 1 (MULP-C1), and total energy (TENG) ([log(AVOID)] = −7.42(±0.79) − 0.15(±0.05)[log(VP)] + 0.12(±0.06) [MULP-C1] − 0.01(±0.001) [TENG]). Fitness and cross-validation of this model were the most significant of all the repellency models reported ($N = 12$, $F = 112.89$, $R^2 = 0.97$, $Q^2_{\text{LOO}} = 0.87$), and the residual resulting from the difference in calculated versus observed contact repellency of turmerone was 0.0 (**Table 3**).

The similarities observed in the parameters selected for the best-fit models of sesquiterpene repellency appear to fall into three categories: early spatial repellency, late spatial repellency, and contact repellency. The prediction models in each of these categories differ in terms of either one or two parameters, but all maintain a negative relationship between mosquito repellency and vapor pressure, which is representative of a compounds' volatility. Volatility is widely recognized as an important factor for mosquito repellency as it can affect mosquito responses via chemical contact with the mosquito chemosensory structures. Our results show that repellency increased as the vapor pressure of sesquiterpenes decreased, which suggests that minimal volatility within this class of compounds provided optimal repellency. Experimental data on sesquiterpene gas diffusion coefficients would be useful in future studies to discern the role of volatility in this system. The trend in vapor pressure is maintained even with consideration of any outliers in the ata set, including those compounds with a higher vapor pressure. We believe that this trend is most likely a product of our bioassay system and that interpretation of these results should also include consideration of the repellency assay design (size and static air) used in this study. The size of the bioassay chamber places constraints on the space available for volatilization of candidate repellents. Compounds with lower volatility will be able to maintain a consistently higher concentration on the treated side of the chamber. However, this particular system limits the influence of a number of physical factors on repellency, as well as removes the potential for bias due to other attractants/chemicals involved in mosquito host-seeking. Thus, our bioassay offers more sensitivity to the inherent deterrent activity of sesquiterpenes and allows us to examine electronic and structural properties that are important for receptor−ligand interaction. Other studies that utilize a larger air flow-through system or that incorporate host-generated attractants might show a different relationship, especially with regard to the relationship between spatial repellency and vapor pressure (volatility).

Another interesting outcome of the repellency models is the differentiation in electronic properties between spatial repellency

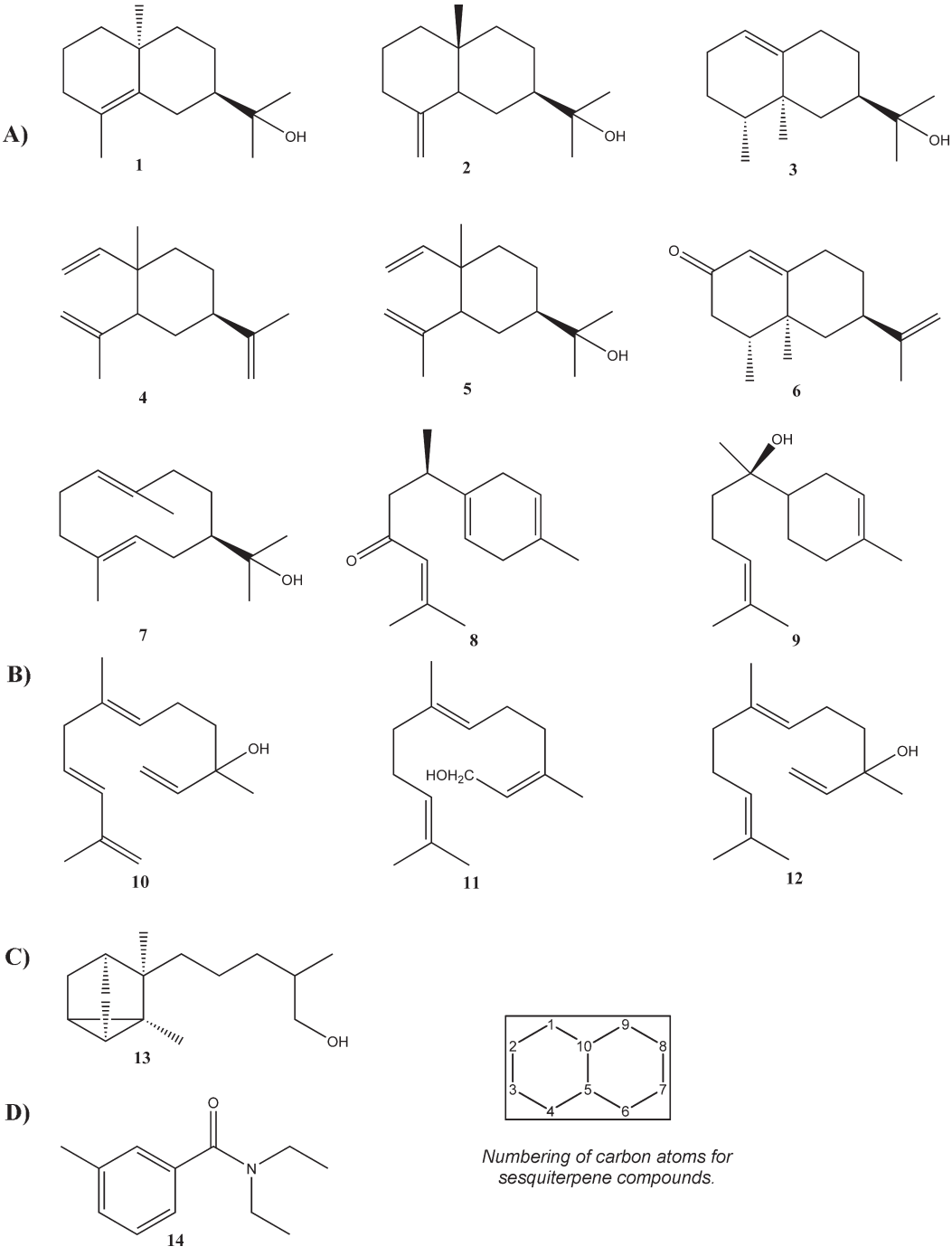

Figure 1. Structures of sesquiterpenes and DEET (*N*,*N*-diethyl-*m*-methylbenzamide): (**A**) eudesmane, elemane, eremophilane, bisaboene, and germacrane compounds; (**B**) acyclic; (**C**) bridged system; (**D**) DEET.

at the early time point, as compared to the late ones. The electronic parameters selected in the early time point model (15 min) included polarizability and LUMO. In these models, the repellent activity increased as polarizability decreased and LUMO increased.

Two specific electronic parameters were identified as important for measures of repellency. One parameter that was present in the late spatial repellency and contact repellency models was Mulliken population at carbon 1. In the 60, 90, 120, and 180 min spatial repellency models and the contact repellency model, repellent activity increased as the Mulliken population (electron density) around carbon 1 (**Figure 1**) increased. The other parameter that appeared in multiple models, primarily the late spatial repellency time point models (60, 90, 120, and 180 min models), was the electrotopological state descriptor at carbon 7 (E-state, carbon 7) (**Figure 1**). These equations show that repellency increases as the electronic accessibility of carbon 7 decreases. In the collection of sesquiterpenes tested, there were different arrangements of the functional/substituent groups at carbon 7, including a hydroxyl group attached to a tertiary carbon that resulted in active repellency. The importance of these two areas on the sesquiterpene molecule shares some similarity to other findings with East Indian sandalwood odor-active groups (*35*), which also contained sesquiterpenes with an electron-donor group (hydroxyl) on the quaternary/tertiary carbon atom. This structural feature was essential for olfactory activity of sandalwood oil, along with the presence of a bulky fragment on the molecule located a distance from the hydroxyl group that serves an electron-acceptor function.

Table 1. Spatial and Contact Repellency of Botanical Sesquiterpenes to the Yellow Fever Mosquito (*Aedes aegypti*)

	av percentage (spatial) repellency[a] at					contact repellency	
treatment[b]	15 min	60 min	90 min	120 min	180 min	avoidance frequency[c]	*P* value[d]
nootkatone	7.7	42.1*	52.3*	51.7*	62.9*	0.6	<0.001
α-santalol	52.3*	65.7*	67.7*	80.5*	87.1*	0.8	<0.001
α-bisabolol	37.4	61.1*	70.5*	83.6*	84.0*	0.8	<0.001
farnesol	20.3	41.9	42.6*	50.7*	68.8*	0.8	<0.001
elemol	39.6	84.2*	86.3*	84.2*	88.9*	0.9	<0.001
β-eudesmol	53.9*	72.3*	88.4*	81.6*	86.1*	0.9	<0.001
hedycaryol	58*	100.0*	92.9*	97.7*	95.3*	0.9	<0.001
valerianol	36.7	72.5*	88.8*	81.9*	84.2*	0.9	<0.001
fokienol	53.9*	81.6*	88.5*	90.8*	93.1*	0.9	<0.001
trans-nerolidol	19.1	62.3*	72.9*	68.7*	66.5*	0.6	<0.001
elemene	−2.9	40.4*	39.2*	43.3*	48.2*	0.1	0.612
10-epi-γ-eudesmol	77.8*	82.2*	88.8*	82.2*	93.3*	0.8	<0.001
DEET	46.7*	71.9*	75.9*	80.2*	82.8*	0.8	<0.001
turmerone[e]	43.4*	62.6*	74.2*	80.4*	88.9*	0.8	<0.001
control	0.7	5.6	−0.1	3.4	14.3	0	

[a] The asterisk indicates significant difference from control (α = 0.05) in Dunnett's test. [b] Treatment applied a concentration of 78.6 μg/cm^2 (from application of 1 mL of a 0.5% solution). [c] Avoidance frequency = average of mosquito contact repellency over 3 h time period (based on six time points). [d] Contact repellency = 100% of the individuals off treated surface. Comparison with control treatments was made using Fisher's exact test. [e] Sesquiterpene used for comparison in model validation.

Table 2. QSAR Models for Spatial and Contact Repellency[a] of Botanical Sesquiterpenes to the Yellow Fever Mosquito (*Aedes aegypti*)

response	best-fit model								
log(spatial repellency)	intercept	log(VP)	Mulliken population-C1	E-state-C7	LUMO	POL	TENG	R^2	Q^2_{LOO}[b]
15 min	0.94 (±0.09)	−1.01 (±0.16)			0.09 (±0.03)	−0.64 (±0.37)		0.86	0.61
60 min	0.22 (±0.44)	−0.20 (±0.04)	0.25	−0.05				0.82	0.73
90 min	0.58 (±0.40)	−0.23 (±0.04)	0.19 (±0.06)	−0.06 (±0.02)				0.85	0.69
120 min	0.84 (±0.36)	−0.22 (±0.04)	0.16 (±0.06)	−0.02 (±0.02)				0.83	0.72
180 min	1.09 (±0.22)	−0.19 (±0.02)	0.12 (±0.04)	−0.003 (±0.01)				0.89	0.75
log(contact repellency) avoidance frequency	−7.42 (±0.79)	−0.15 (±0.05)	0.12 (±0.06)				−0.01 (±0.001)	0.97	0.87

[a] Spatial and contact repellency values reported in **Table 1**. Abbreviations: VP, vapor pressure; LUMO, lowest unoccupied molecular orbital; POL, polarizability; TENG, total energy. [b] Q^2_{LOO}, leave-one-out; 1-PRESS/SSTO.

The contact repellency model (AVOID), which is a measure of continual mosquito avoidance of the treated surfaces over the 180 min observation period, showed the highest level of significance in both internal and external validations (**Tables 2** and **3**). Parameters selected for this model shared similarities with spatial repellency including trends with vapor pressure and Mulliken population of carbon 1. Compounds with lower vapor pressure maintain a higher concentration on the treated filter paper and therefore provide higher contact repellency (avoidance). Increased Mulliken population at carbon 1 again resulted in enhanced repellency. However, this model was unique with respect to one parameter, total energy. Total energy has been interpreted in other QSAR studies to measure stability (*36*), as well as nonspecific interactions (such as solute interactions or membrane flux) (*37*, *38*). The significance of total energy might relate to the difference in stability of the sesquiterpene 10-carbon ring structure. Other studies have noted the germacrene Cope rearrangement to form elemenes (*39*, *40*) and highlighted the importance of steric energies in the transition state (CC). Improved understanding of the contribution of sesquiterpene total energy, as it relates to mosquito repellency, might include further examination of steric energy, rotational energy barriers, and flexibility of active conformers (*41*), as well as other structural and physicochemical parameters, which are relevant to the formation of stable ground states and possible transition state conformations of selected molecules.

Results from these models highlight the importance of electronic properties, especially on two specific areas of the sesquiterpene structure (carbons 1 and 7) that can affect *Ae. aegypti* repellent activity by possibly interacting with an odorant receptor or another component of the olfactory pathway. It also suggests that several different electronic properties of the molecule can help explain the relationship between structure and repellent activity.

The mode of action of mosquito repellency offers numerous challenges, as there are many mechanisms and processes involved in mosquito host-seeking and feeding behaviors. In this study we examined repellency under controlled conditions, specifically a static-air bioassay, to provide information about mosquito avoidance of and movement away from candidate sesquiterpenes. The information generated from these studies was used to develop a QSAR model for repellency. Multiple models of different measures of mosquito repellency (spatial and contact) highlighted the importance of vapor pressure (volatility), as well as electronic and electrotopological descriptors, in predicting repellent activity. Vapor pressure has long been recognized as essential for mosquito repellency, and our study shows that in our static-air chamber and with this class of terpenes, minimal vapor pressure is optimal, because that allows for a sustained gradient in the chamber. Analysis of the electronic and electrotopological properties of the sesquiterpene structure revealed two areas on the molecule that were important to repellent activity and possibly involved in receptor−ligand interactions. The predictive value of

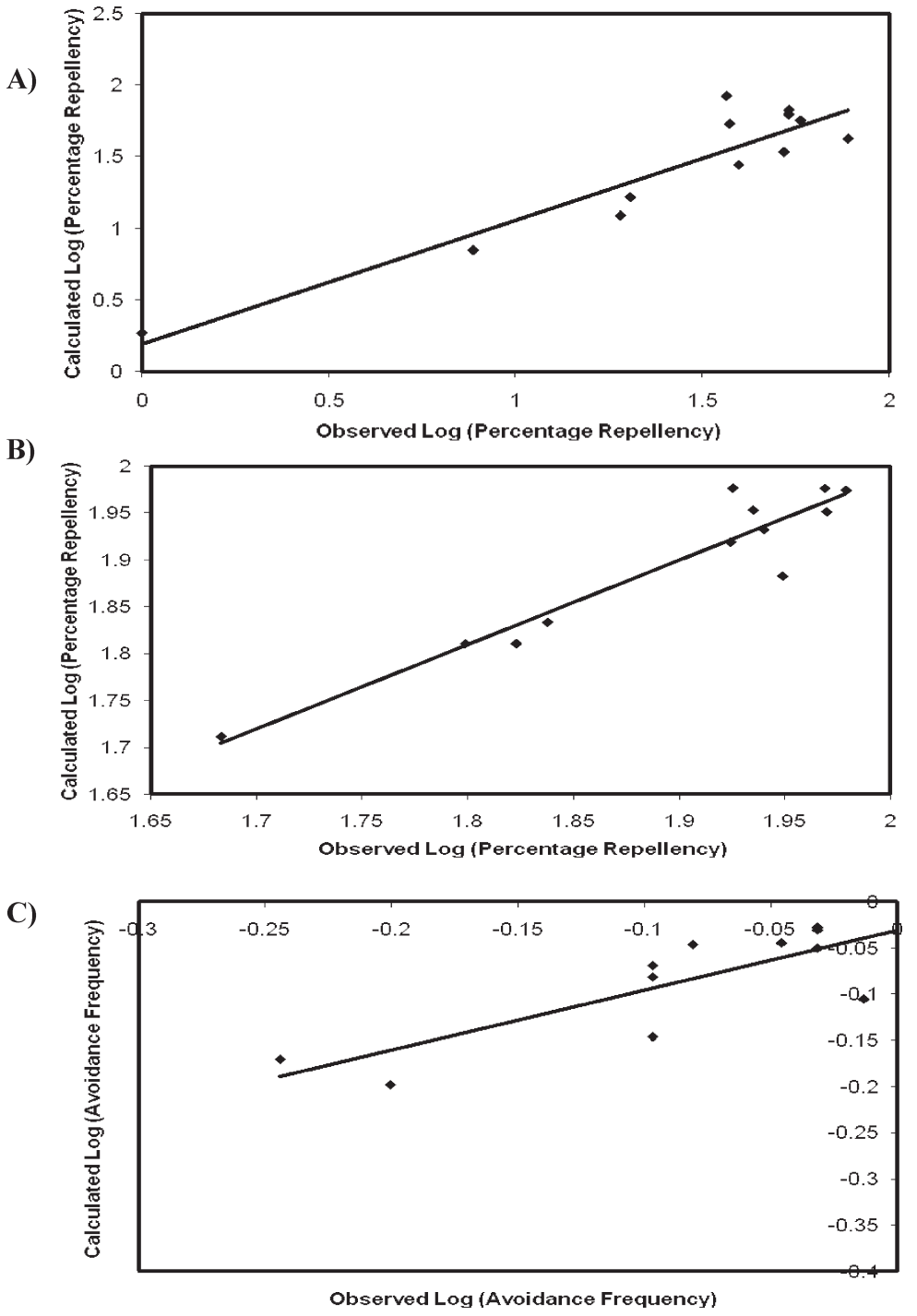

Figure 2. Calculated versus observed spatial and contact repellency values shown for three of the five sesquiterpene repellency models: (**A**) spatial 15 min; (**B**) spatial 180 min; (**C**) contact repellency.

Table 3. Comparison of QSAR Calculated and Observed Values of Turmerone for Spatial and Contact Repellency of Botanical Sesquiterpenes to the Yellow Fever Mosquito (*Aedes aegypti*)

best-fit model	calcd percentage repellency	obsd percentage repellency	residual
spatial repellency			
15 min	34.0	43.4	9.4
60 min	56.5	62.6	6.1
90 min	54.5	74.2	19.7
120 min	73.3	80.4	7.1
180 min	76.5	88.9	12.4
contact repellency			
avoidance frequency	0.8	0.8	0.0

this information can also be utilized to assist in the search for effective natural repellents and the optimization of sesquiterpene structure for insect repellency within a controlled laboratory setting. The main conclusions from this study include (1) in a controlled static-air laboratory bioassay, a minimal sesquiterpene vapor pressure is optimal for *Ae. aegypti* repellency, (2) the electronic and electrotopological properties of carbons 1 and 7 affect activity, and (3) of the 12 plant-derived sesquiterpenes evaluated, hedycaryol and 10-epi-γ-eudesmol resulted in the highest spatial and contact repellency values.

ABBREVIATIONS USED

LUMO, lowest unoccupied molecular orbital; DEET, *N,N*-diethyl-*m*-methylbenzamide; QSAR, quantitative structure−activity relationship; TLC, thin-layer chromatography; GC-FID, gas chromatograph with a flame ionization detector; HOMO, highest occupied molecular orbital; REP, spatial repellency; VP, vapor pressure; POL, polarizability; TENG, total energy; AVOID, contact repellency;

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