

# Structure-Activity Correlations for Derivatives of Siglure: Attractants for *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae)<sup>1</sup>

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**ABSTRACT** A previously described olfactometer for *Oryctes rhinoceros* (L.) was used to show that *cis*-siglure (1-methylpropyl *cis*-6-methyl-3-cyclohexene-1-carboxylate) inhibits the effects of moderately attractive *trans*-siglure. In the course of structure-activity investigations, several new *O. rhinoceros* attractants were discovered. Two compounds, sigluric acid (*trans*-6-methyl-3-cyclohexene-1-carboxylic acid) and its methyl ester equaled or exceeded the activity of the currently used attractant, ethyl chrysanthemumate, in olfactometer tests. Modifications to the ring structure, the *trans*-6-methyl group, or the 3-double bond caused either greatly diminished or complete loss of activity. Similarly, *cis*-isomers were generally less attractive than the corresponding *trans*-isomers. The shape and size of the ester group had a dramatic effect on the activity of the compound. Straight-chain lengths of more than three carbon atoms or a three-carbon chain with a methyl group in other than the 1 position were inactive.

*Oryctes rhinoceros* L. has been a pest of the coconut palm in the South Pacific for over half a century (Catley 1969). A joint United Nations-South Pacific Commission project was initiated in 1966 to develop effective control methods. As part of their concentrated effort, this group developed methodology to screen chemicals in the field for potential use as attractants. The discovery of an effective chemical attractant was considered useful in population monitoring, in studying the effects of other control methods, and in certain situations as a direct control measure. During these tests, two effective attractants, ethyl dihydrochrysanthemumate (Barber et al. 1971) and the currently used ethyl chrysanthemumate (Maddison et al. 1973), were discovered, as were several compounds of weak to moderate activity. One of these, 1-methylpropyl *trans*-6-methyl-3-cyclohexene-1-carboxylate (siglure, Fig. 1A) was moderately attractive in both field (Maddison 1973) and laboratory tests (Vander Meer et al. 1979); however, field tests of a 1:1 mixture of the *cis*- and *trans*- isomers gave no activity (Maddison 1973). These data led to a laboratory investigation of the effects of *cis*-, *trans*- isomerism, and to structure-activity studies regarding derivatives of siglure. We report here the results of this study.

## Materials and Methods

Adult *O. rhinoceros* used in bioassays were obtained from the Fiji Department of Agriculture, Koronivia, Fiji Islands, or from the UNDP/FAO project for research on the Control of the Coconut Palm Rhinoceros Beetle, Apia, Western Samoa, or by rearing field-collected larvae and pupae in the laboratory. The bioassays were done by using the Y-olfactometer and procedure described by Vander Meer et al. (1979).

Each group of 25 to 35 beetles, with approximately equal numbers of males and females, was assigned an

olfactometer. A complete trial consisted of two groups of beetles being put through their assigned olfactometers with the sample on one side, followed by a second testing period with the sample on the opposite side. The results for the two groups were added (to eliminate a bias inherent in the olfactometers and in the groups of beetles) and analyzed statistically by  $\chi^2$  tests, assuming a null hypothesis of equal distribution and one degree of freedom (Vander Meer et al. 1979). Testing was carried out twice a day, between 9:00 and 11:00 a.m., and 3:00 and 5:00 p.m. If the  $\chi^2$  value was  $>3$  or if the sample was part of a homologous series, the trial was repeated. Compounds that were attractive (Tables 1-5) based on the  $\chi^2$  test ( $P \geq 0.05$ ) were ranked by Duncan's multiple range test (Statistical Analysis System at Northeast Regional Data Center, Cary, N.C.).

*cis*-Siglure (Fig. 1B) was prepared in a modification of the synthesis employed by Green and Beroza (1959). *trans*-Siglure was heated in a pressure bottle for 4 h at 150°C with 25% methanolic potassium hydroxide. Excess alcohol was removed from the cooled reaction mixture under reduced pressure by using a rotary evaporator. The residue was taken up in water and extracted with ether. The water layer was acidified with dilute sulfuric acid and extracted with ether. *cis*-Enriched 6-methyl-3-cyclohexene-1-carboxylic acid (ca. 60 to 65% *cis*-) was recovered from the ether layer after drying and solvent removal. Additional enrichment was obtained by cooling the liquid acid in a freezer and seeding with a crystal of pure *trans*- acid. Filtration of the precipitated *trans*- acid left a filtrate containing ca. 75 to 80% *cis*- acid. *cis*-Enriched siglure was prepared via the acid chloride using standard procedures. Final purification was accomplished by distillation utilizing a spinning band column (Nester-Faust NFA-200 Annular Still). Gas-chromatographic analysis was performed with a Varian Aerograph model 2740 instrument equipped with a flame ionization detector and fitted with a stainless-steel column (1.52 m by 2 mm ID) packed with 3% G.C. SE 30 on 100/120-mesh Varaport 30. Temperature of the injection port was 230°C, and that of the detector was 270°C; nitrogen carrier gas flow rate was 30 ml/min.

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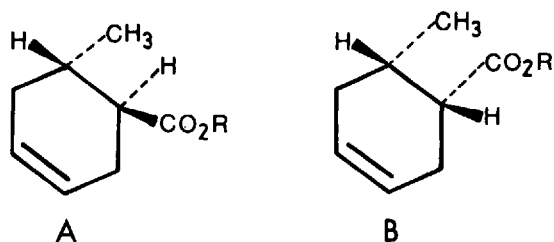


FIG. 1. General structures for *trans*- (A) and *cis*- (B) esters of sigluric acid. Structures for *trans*- and *cis*-siglure equal A and B when R = 1-methylpropyl.

Methyl *cis*-6-methyl-3-cyclohexene-1-carboxylate was prepared in the same manner as *cis*-siglure, and in addition, was used as the source of *cis*-sigluric acid used in the synthesis (via the acid chloride) of other *cis*-homologs. *cis*-Sigluric acid was obtained by saponification of the *cis*-methyl ester with 10% methanolic potassium hydroxide at room temperature. Re-esterification of a small portion of the recovered acid showed that no epimerization had occurred during saponification. The *cis*-acid chloride was prepared via the standard thionyl chloride reaction at room temperature and was used immediately in the ester synthesis by slow addition to a cooled solution of the appropriate alcohol and pyridine in anhydrous ether. The crude esters were isolated by standard extraction techniques, and final purification was accomplished by column chromatography with Florisil. Hexane and 1 and 3% ether-hexane were used sequentially as eluants.

*Trans*- homologs that were not available from other studies were prepared from the *trans*- acid by standard methods. Esters of other cyclohexanecarboxylic or cyclohexenecarboxylic acids were synthesized by standard esterification procedures, using acids either synthesized for another study (Valega and Beroza 1967) or obtained commercially and then purified by column chromatography as described above. The epoxy compounds were synthesized by the standard reaction with *meta*-chloroperbenzoic acid in methylene chloride at 0 to 5°C. Purification was accomplished by column chromatography as described above. Isomeric purity of all *cis*- and *trans*-compounds was determined by gas-chromatographic analysis. This analysis showed all of the chemicals tested were >95% pure.

In all cases, 0.01 ml of a 10% ether solution was applied to a 2.0-cm filter paper disc. The disc was placed in an Erlenmeyer flask topped with a drechsel bottle head and connected to the appropriate side of the olfactometer air flow system (Vander Meer et al. 1979). In measuring the effects of mixtures of *cis*- *trans*- isomers, the total amount of material applied to the filter paper disc was kept constant.

### Results and Discussion

Our initial tests with *trans*-siglure (Fig. 1A) indicated that it was moderately attractive to *O. rhinoceros* (Vander Meer et al. 1979). Field tests of pure *trans*-siglure supported our laboratory data (Maddison 1973); however, a 1:1 *cis*-, *trans*- mixture was not found to be

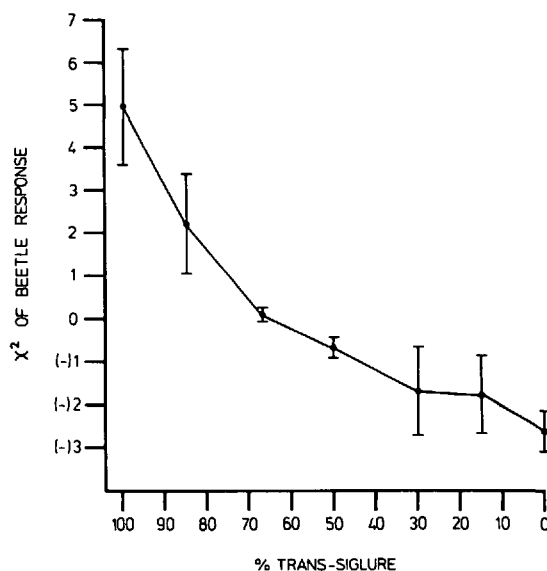


FIG. 2. Attraction of *O. rhinoceros* to mixtures of *trans*- and *cis*-siglure (mean  $\chi^2 \pm$  SD for two trials; mean sample size  $\pm$  SD for each trial was  $114 \pm 7$ ). When  $\chi^2 = 3.84$ ,  $P = 0.05$ . Percent beetles attracted: 100% *trans*- =  $60.1 \pm 2.3$ ; 85% =  $56.6 \pm 2.8$ ; 70% =  $50.7 \pm 2.2$ ; 50% =  $46.2 \pm 1.0$ ; 30% =  $43.9 \pm 2.8$ ; 15% =  $44.0 \pm 0.0$ ; 100% *cis*- =  $42.4 \pm 0.9$ .

attractive to beetles in field tests. Laboratory tests were conducted to determine the attractant qualities of *cis*-siglure (Fig. 1B) and mixtures of *cis*- and *trans*-siglure. The results (Fig. 2) showed that pure *cis*-siglure was a weak repellent and exerted a depressant effect on the attractive qualities of the *trans*- isomer greater than expected for simple dilution effects (Vander Meer et al. 1979). These data explain the field results obtained by Maddison (1973) for the 1:1 *cis*-, *trans*-siglure mixture. *O. rhinoceros* has already been shown to distinguish between two diastereomers of ( $\pm$ )-des-N-morphinan (Vander Meer et al. 1979), and it has been well documented that insects are capable of differentiating geometrical isomers (Roelofs and Comeau 1971). In addition, geometrical isomers of sex pheromones in several Lepidoptera act as potent inhibitors of pheromone activity (Bierl et al. 1974, Tumlinson et al. 1974).

Table 1 shows data for straight and branched chain alkyl esters of *trans*-sigluric acid. We discovered that the attraction of *O. rhinoceros* to *trans*-sigluric acid esters increased relative to the control with diminishing chain length and steric bulk, starting with *trans*-siglure itself. Although the trend was toward increased attraction, only the *trans*-methyl ester was statistically more attractive than the other esters (Duncan's multiple range test). *Trans*-siglure apparently contains the structural limits to beetle attraction for the various alcohol moieties used in this study. Esters having alcohol moieties with straight-chain lengths of more than three carbons were inactive, as were esters that had a methyl group in other than the 1-position, or that contained a group larger than a single methyl in the 1-position of the alcohol moiety. *trans*-

**Table 1.** Olfactory response of *O. rhinoceros* to straight- and branched-chain alkyl esters of *trans*-sigluric acid

R (Fig. 1A)	% <sup>a</sup>	$\chi^2$ (n) <sup>b</sup>
-H	67.6 $\pm$ 0.2 (A)	16.1 $\pm$ 0.2 (131)
-CH <sub>3</sub>	69.5 $\pm$ 1.2 (A)	21.0 $\pm$ 2.4 (139)
-CH <sub>2</sub> CH <sub>3</sub>	63.6 $\pm$ 0.2 (B)	9.9 $\pm$ 0.3 (125)
-CH(CH <sub>3</sub> ) <sub>2</sub>	61.6 $\pm$ 1.8 (B,C)	7.1 $\pm$ 1.9 (133)
-(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	60.5 $\pm$ 0.3 (B,C)	6.0 $\pm$ 0.6 (136)
-CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	60.1 $\pm$ 2.3 (B,C)	4.9 $\pm$ 2 (119)
-C(CH <sub>3</sub> ) <sub>3</sub>	55.6	1.7 (135)
-(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	46.7	(-) 0.6 (135) <sup>c</sup>
-CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	47.0	(-) 0.5 (132) <sup>c</sup>
-(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	49.3	0.0 (140)
-(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	50.0	0.0 (140)
-CH <sub>2</sub> CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	53.1	0.5 (128)
-CH(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	50.0	0.0 (140)
-C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	43.5	(-) 2.4 (138) <sup>c</sup>
-CH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	56.7	2.0 (120)

<sup>a</sup>Mean percent response and SD for two bioassay trials, or the percent for a single trial when  $\chi^2 < 3.0$ . Compounds followed by a common letter are not significantly different at the  $P = 0.05$  level (Duncan's multiple range test).

<sup>b</sup>Mean  $\chi^2$  and SD for two bioassay trials or the  $\chi^2$  of a single trial when  $\chi^2 < 3.0$ ; n indicates sample size for each trial. When  $\chi^2 = 3.84$ ,  $P = 0.05$ .

<sup>c</sup>Negative sign indicates a negative response.  $\chi^2$  is always positive.

**Table 2.** Olfactory response of *O. rhinoceros* to alkyl esters of *cis*-sigluric acid

R (Fig. 1B)	% <sup>a</sup>	$\chi^2$ (n) <sup>b</sup>
-H	52.2	0.2 (115)
-CH <sub>3</sub>	59.1 $\pm$ 0.7 (C)	4.5 $\pm$ 0.6 (138)
-CH <sub>2</sub> CH <sub>3</sub>	60.2 $\pm$ 1.3 (B,C)	4.7 $\pm$ 1.2 (113)
-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	47.1	(-) 0.5 (136) <sup>c</sup>
-(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	44.0	(-) 1.9 (132) <sup>c</sup>
-CH(CH <sub>3</sub> ) <sub>2</sub>	50.0	0.0 (136)
-CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	42.4 $\pm$ 1.0	(-) 2.7 $\pm$ 0.7 (116) <sup>c</sup>

<sup>a</sup>Mean percent response and SD for two bioassay trials or the percent for a single trial when  $\chi^2 < 3.0$ . Compounds with a common letter are not significantly different at the  $P = 0.05$  level (Duncan's multiple range test).

<sup>b</sup>Mean  $\chi^2$  and SD for two bioassay trials or the  $\chi^2$  of a single trial when  $\chi^2 < 3.0$ ; n indicates sample size for each trial. When  $\chi^2 = 3.84$ ,  $P = 0.05$ .

<sup>c</sup>Negative sign indicates a negative response.  $\chi^2$  is always positive.

Sigluric acid itself was statistically as attractive as the methyl ester (Duncan's multiple range test), showing that an ester group was not necessary for attraction.

Because some of the compounds listed in Table 1 are superior attractants to *trans*-sigluric acid, we also evaluated the activity of the corresponding *cis*- isomers (Table 2). The *cis*- methyl and ethyl esters, analogues of the two *trans*- esters with the highest activity (Table 1), were still significantly attractive; however, the other *cis*- analogues showed no attractant activity. Activity for sigluric acid changed dramatically from highly significant attraction for the *trans*- isomer ( $P < 0.001$ ) to no attraction for the *cis*- isomer. We also tested a 1:1 mixture of the *cis*- and *trans*- methyl esters ( $\chi^2 = 3.5 \pm 3.0$ ,  $n$

**Table 3.** Olfactory response of *O. rhinoceros* to selected esters of *trans*-sigluric acid

R (Fig. 1A)	% <sup>a</sup>	$\chi^2$ (n) <sup>b</sup>
Allyl	59.7	1.3 (67)
2-Chloroethyl	51.5	0.1 (66)
2-Methoxyethyl	54.4	0.5 (68)
1-Cyclopropylethyl	50.0	0.0 (58)
Cyclohexyl	48.4	(-) 0.1 (64) <sup>c</sup>
Cyclopentyl	46.3	(-) 0.4 (67) <sup>c</sup>
Benzyl	51.9	0.1 (54)

<sup>a</sup>Percent response for a single bioassay trial.

<sup>b</sup> $\chi^2$  for a single bioassay trial; n = sample size. When  $\chi^2 = 3.84$ ;  $P = 0.05$ .

<sup>c</sup>Negative sign indicates a negative response.

= 139;  $57.6 \pm 3.5\%$ ) of sigluric acid and found significant reduction of activity from that of the pure *trans*- isomer (Duncan's multiple range test).

Several miscellaneous esters of *trans*-sigluric acid were bioassayed. Data for these are shown in Table 3. None had significant attraction for the *O. rhinoceros*. Even those esters that met the chainlength requirement, e.g., allyl, were not attractive.

To investigate the structural effects of ring substituents, we concentrated on the *trans*- methyl ester since changes in attraction would be most readily observed. Table 4 shows comparative results obtained after modification of the ring and its substituents while the methyl ester moiety was held constant. None of the modifications provided a superior attractant. If the 6-methyl group or double bond were removed (i.e., methyl 3-cyclohexene-1-carboxylate and methyl *trans*-2-methylcyclohexanecarboxylate), activity was completely lost; however, if both were removed (methyl cyclohexanecarboxylate) the compound still maintained some activity ( $P \sim 0.05$ ). Substitution of an additional methyl group (methyl 4,6-dimethyl-3-cyclohexene-1-carboxylate) or replacing the methyl with an ethyl group (methyl *trans*-6-ethyl-3-cyclohexene-1-carboxylate) resulted in loss of activity. Similarly, if the methyl group was shifted to another ring carbon (methyl 1-methyl-3-cyclohexene-1-carboxylate) activity was lost. Epoxidation of the double bond (methyl 3,4-epoxy-*trans*-6-methyl cyclohexanecarboxylate) also resulted in loss of activity, but when a chlorine was attached to one of the double-bond carbons [methyl (3 or 4)-chloro-6-methyl-3-cyclohexene-1-carboxylate], some activity ( $P = 0.05$ ) was retained. These data indicated that the 6-methyl group and the 3-double bond were both essential for maximum activity. Methyl cyclohexanecarboxylate lacked both the double bond and methyl group and did not have any *cis*,- *trans*- isomerism, yet it did maintain some activity ( $P \sim 0.05$ ).

Three similar structural modifications were available for the *trans*- ethyl ester and *trans*-sigluric acid. Results with the ethyl ester (Table 5) were consistent with those found for the methyl esters. Removal of the 6-methyl group (ethyl 3-cyclohexene-1-carboxylate) or the 3-double bond (ethyl *trans*-2-methylcyclohexanecarboxylate) eliminated attraction. However, when both methyl group

Table 4. Olfactory response of *O. rhinoceros* to the modifications of the methyl ester of *trans*-sigluric acid

Compound	% <sup>a</sup>	$\chi^2(n)^b$
R = CH <sub>3</sub> (Fig. 1A)	69.5 ± 1.2 (A)	21.0 ± 2.4 (139)
Methyl cyclohexane = carboxylate	57.8 ± 0.4 (C)	3.3 ± 0.4 (136)
Methyl 3-cyclohexene-1-carboxylate	51.6	0.1 (124)
Methyl <i>trans</i> -2-methyl = cyclohexanecarboxylate	48.3	-0.1 (120) <sup>c</sup>
Methyl <i>trans</i> -6-ethyl-3-cyclohexene-1-carboxylate	54.2	0.9 (118)
Methyl 4,6-dimethyl-3-cyclohexene-1-carboxylate	52.5	0.3 (122)
Methyl 3-(or 4) chloro-6-methyl-3-cyclohexene-1-carboxylate	59.4 (C)	3.8 (106)
Methyl 1-methyl-3-cyclo = hexene-1-carboxylate	46.0	(-)0.7 (138) <sup>c</sup>
Methyl 3,4-epoxy- <i>trans</i> -6-methylcyclohexane carboxylate	53.5	1.1 (128)

<sup>a</sup>Mean percent response and SD for two bioassay trials or the percent for a single trial when  $\chi^2 < 3.0$ . Compounds followed by a common letter are not significantly different at the  $P = 0.05$  level (Duncan's multiple range test).

<sup>b</sup>Mean  $\chi^2$  and SD for two bioassay trials or the  $\chi^2$  of a single trial when  $\chi^2 < 3.0$ ;  $n$  indicates sample size for each trial. When  $\chi^2 = 3.84$ ,  $P = 0.05$ .

<sup>c</sup>Negative sign indicates a negative response.  $\chi^2$  is always positive.

Table 5. Olfactory response of *O. rhinoceros* to the structural modifications of *trans*-sigluric acid and its ethyl ester

Compound	% <sup>a</sup>	$\chi^2(n)^b$
R = CH <sub>3</sub> CH <sub>2</sub> (Fig. 1A)	63.6 ± 0.2 (B)	9.9 ± 0.3 (125)
Ethyl 3-cyclohexene-1-carboxylate	50.8	0. (122)
Ethyl cyclohexane carboxylate	59.2 ± 4.2 (C)	3.7 ± 2.2 (132)
Ethyl <i>trans</i> -2-methylcyclohexanecarboxylate	52.4	0.3 (124)
R = H (Fig. 1A)	67.6 ± 0.2 (A)	16.1 ± 0.2 (131)
3-Cyclohexene-1-carboxylic acid	51.6	0.1 (128)
Cyclohexanecarboxylic acid	51.5	0.1 (130)
2-Methylcyclohexane carboxylate acid	60.5 ± 0.7 (B,C)	5.4 ± 0.4 (131)

<sup>a</sup>Mean percent response and SD for two bioassay trials or the percent of a single trial when  $\chi^2 < 3.0$ . Compounds followed by a common letter are not significantly different at the  $P = 0.05$  level (Duncan's multiple range test).

<sup>b</sup>Mean  $\chi^2$  and SD for two bioassay trials or the  $\chi^2$  of a single trial when  $\chi^2 < 3.0$ ;  $n$  indicates sample size for each trial. When  $\chi^2 = 3.84$ ,  $P = 0.05$ .

<sup>c</sup>Negative sign indicates a negative response.  $\chi^2$  is always positive.

and double bond were removed (ethyl cyclohexanecarboxylate), significant activity ( $P = 0.05$ ) was still observed. The situation for *trans*-sigluric acid was different. Removal of the 6-methyl group (3-cyclohexene-1-carboxylic acid) eliminated attraction. However, in contrast to the ester situation, when only the 3-double bond was removed (2-methylcyclohexanecarboxylic acid) significant attraction ( $P > 0.05$ ) remained, and when both the 6-methyl and double bond were removed (cyclohexanecarboxylic acid) the compound showed no attraction.

The results obtained for the *trans*-esters in Table 1 might simply reflect their increasing vapor pressure as the ester group becomes smaller. However, we included four butyl esters in this study which differed only in the arrangement of the carbon atoms (Table 1). These isomers would not be expected to have significantly different vapor pressures, yet only one of them was attractive to *O. rhinoceros*. Further, *trans*-sigluric acid, a solid

with a much lower vapor pressure than its esters, was highly attractive ( $P > 0.001$ ). Similarly, the largely neutral or minor effects of the *cis*-isomers (Table 2) and the effects of removing the 6-methyl group and 3-double bond (Tables 4 and 5) lead us to conclude that the observed behavioral effects are not predicated on the physical movement of the chemicals to the beetles' antennal sensilla.

We have discovered a number of *O. rhinoceros* attractants, two of which equaled the activity of the currently used trap attractant, ethyl chrysanthemumate (percent positive response = 70.2,  $n = 94$ ,  $\chi^2 = 15.4$  [Vander Meer et al. 1979, and Maddison 1973]). It is tempting to postulate that the results were due to the degree of fit into specific sensilla-active sites. Those compounds that were not attractive might not fit adequately and were not sensed by the beetles. However, without the appropriate electroantennogram work, we

can only present these structure-behavior relationships without a mechanistic explanation of the results.

### Acknowledgment

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