COMPOUNDS AFFECTING MOSQUITO OVIPOSITION: STRUCTURE-ACTIVITY RELATIONSHIPS AND CONCENTRATION EFFECTS

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ABSTRACT. Evidence is reviewed for a dose-dependent reversal of response in mosquito oviposition, in which a compound that attracts or stimulates oviposition may repel or deter oviposition at a higher concentration. On the basis of a review of structure-activity relationships in compounds affecting mosquito oviposition, 5 hexanoic acid derivatives were selected for field tests with *Aedes aegypti* ssp. *formosus* in Kenya. Egg counts were increased most by methyl hexanoic acid and 5-methyl-2-hexanone, and the presence or absence of a methyl branch affected egg numbers more than the difference between a carbonyl and a carboxylic acid functional group. Hexanoic acid increased egg counts at low release rates, but decreased them at higher release rates.

INTRODUCTION

A review of compounds that modify mosquito oviposition behavior (Bentley and Day 1989) reveals certain molecular features commonly found among active compounds. On this basis, we have selected compounds likely to affect oviposition and therefore to be of potential value in ovitraps, and we have investigated their performance in the field. Bentley and Day (1989) deal with "attractants" and "repellents" separately. In selecting candidate compounds, we have considered these 2 categories together, to allow for possible dose-dependent reversal of effect. As well as depending on the mosquito's physiological state (Klowden and Blackmer 1987) and input from other sensory modalities (Kennedy 1978, O'Gower 1963, Bentley and Day 1989), the olfactory response may also show a dose-dependent reversal of effect (Dethier 1947). This reversal is recognized by those working with insect pheromones (e.g., Kaae et al. 1973), but has sometimes escaped the attention of others working on insect semiochemicals. It is demonstrated in dose-response curves such as those of Rodrigues (1980) for the olfactory response of *Drosophila melanogaster* Meigen to various volatile chemicals. As the dose or concentration of the semiochemical increases, the insect's response initially becomes positive, but then decreases as the dose rises still further, eventually becoming negative. With increasing dose, the activity of the compound changes from attraction to repellency, or from stimulation to deterrency. Recognition of any dose-dependent reversal of effect is important for both screening and formulation of behaviorally active compounds.

Do mosquito chemosensory responses show dose-dependent reversal of this kind? Müller's (1968) dose-response curves for *Aedes aegypti* (Linn.) show that they can, although they may not do so in every case (Laurence and Pickett 1985). Some studies designed to identify mosquito oviposition "attractants" or "repellents," even if they have not considered concentration effects explicitly, provide indirect evidence for dose-dependent reversal of effect. For example, Perry and Fay (1967) showed that ethyl acetate, methyl propionate and methyl butyrate enhanced oviposition rates at low concentrations. Increasing the concentration increased enhancement up to a critical value, above which higher concentrations led to a reversal in the response. In a field study Maw (1970) reported that decanoic acid reduced oviposition by *Culex restuans* Theobald at 150 ppm. This effect was lost over time, and treated pools showed enhanced oviposition 10–14 days after addition of 150 ppm. This may be due to decreasing concentration or, alternatively, to the action of bacteria producing a metabolite of decanoic acid. Workers studying oviposition repellents sometimes give a threshold for effect and occasionally they record a positive oviposition response at concentrations below the threshold for repellency, but as they are seeking repellents, attractancy at low doses may go unnoticed. For example, Hwang et al. (1980) and Kramer et al. (1980), in experiments on ovipositional repellency of carboxylic acids, reported that butanoic acid at concentrations less than $6 \times 10^{-3}\%$ caused a positive oviposition response in *Culex pipiens fatigans* Wied. (= *Cx. quinquefasciatus* Say), and *Culiseta incidens* (Thomson) showed a highly significant positive oviposition response at $6 \times 10^{-3}\%$ ($P < 0.01$); these compounds were repellents at higher concentrations. Schultz et al. (1982) found octanoic acid to have a negative effect on oviposition by *Culex tarsalis* Coq. and *Culex peus* Speiser (= *Cx. stigmatosoma* Dyar) at concentrations above 15 ppm (semi-field conditions) and 100 ppm (field conditions) but at lower concentrations oviposition was enhanced. Similar results were obtained by Schultz et al. (1982) with nonanoic acid, which was a repellent or deter-

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rent above 25 ppm (field conditions). Hwang et al. (1982) working on C5-C13 acids found significant repellency only above a critical concentration, below which enhanced values were often recorded. For example, the critical concentration was $<1 \times 10^{-2}$ M for *Cx. quinquefasciatus* with pentanoic acid or heptanoic acid, and $<1 \times 10^{-3}$M for *Ae. aegypti* with hexanoic acid, or decanoic acid, or C11 and C13 acids. Hwang et al. (1984) record the oviposition repellent effect of *trans*-octadec-9-enoic acid and *trans*-octadec-11-enoic acid with *Cx. quinquefasciatus* as being absent at concentrations of 1×10^{-5} M and below, although positive values for the ratio of eggs laid relative to the control indicate that these compounds may actually enhance oviposition.

STRUCTURE-ACTIVITY RELATIONSHIPS: SELECTION OF COMPOUNDS FOR TESTING

One of the few structure-activity studies is that of Ikeshoji and Mulla (1974) in which only repellent effects were sought and concentration effects were not considered. To allow for possible dose-dependent reversal of effect, we consider attractants and repellents together (c.f. Bentley and Day 1989). When this is done, many compounds affecting mosquito oviposition are seen to show the following features in common:

1) The molecules have polar and nonpolar regions and are often straight chain hydrocarbons. Such surface active agents would move to lipid-water interfaces. Molecules that elicit olfactory responses in animals are commonly of this type (Beets 1970).

2) Many active compounds possess a carboxylic acid or carbonyl functional group. The evidence suggests that either may confer activity, the level of which is then determined by other factors such as chain length, molecular weight, methyl branching, concentration and the species involved.

3) A chain length of 6–10 carbon atoms seems to be optimal. Ikeshoji and Mulla (1974) surveyed 124 alkyl carbonyl compounds and found 9 carbon length compounds such as 8-methyl nonanone most active. Hwang et al. (1982) and Schultz (1982) found 8–10 carbon compounds, such as nonanoic acid of the carboxylic acids, and ethyl nonanoate of the fatty acid esters, most active.

4) Branching has been reported to increase activity. Possession of a methyl branch was shown by Ikeshoji and Mulla (1974) and Bentley et al. (1981) to increase the activity of the compound relative to its unbranched parent. Workers reporting branched compounds as active include Gjullin and Johnson (1965), Perry and Fay (1967), Ikeshoji et al. (1979), Hwang et al. (1980, 1982) and Kramer et al. (1980).

MATERIALS AND METHODS

Compounds chosen for our field work had the same parent compound, hexane, but differed in their functional group or in possession of a methyl branch. This allowed some comparison of the possible effects of these groups without the added complication of increased chain length. Hexanoic acid has been reported by Hwang et al. (1982) as a repellent for *Ae. aegypti* above 1×10^{-3} M, as well as for *Cx. quinquefasciatus* above 1×10^{-2} M and for *Cx. tarsalis* above 1×10^{-2} M. It was therefore selected as the compound on which to test the dose-response relationship.

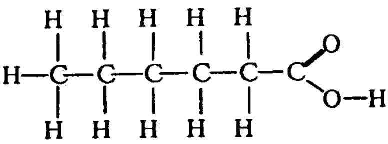
The following chemicals were used (Fig. 1): hexanoic acid and methyl hexanoic acid (Sigma Chemicals); hexanoic acid, ethyl ester (Aldrich Chemicals); 2-hexanone and 5-methyl-2-hexanone (BDH).

The study was carried out between December 26, 1988 and January 2, 1989 on the Kenya coast in Kwale District near Ukunda township (4° 10′ S, 39° 20′ E.). To explore effects of the chemical in the vapor phase rather than through contact mechanisms, test chemicals (a volume of the pure chemical equivalent to 1×10^{-3} moles) were placed in a gauze-covered glass tube, 5 cm high × 24 mm diam, in the center of the oviposition dish so that mosquitoes could not contact them. Control dishes had no chemical in the glass tube. The dishes, purchased locally, were of green plastic, 20 cm diam × 5 cm deep, and each contained 100 ml tap water. To explore the dose-response effect, we varied the effective rate of release of the test vapor by using a variety of exit tube diameters; the tubes had corks with different diameters of glass tubing through them.

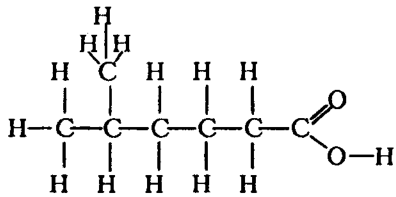
The dishes were set out, 2 m apart in a completely randomized design, between 1700 and 1800 h each evening around habitations and in nearby bush where rubbish was thrown. Early next morning, the number of eggs laid in each dish was counted. *Aedes* eggs were laid almost exclusively in refuse areas, and natural accumulations of water in discarded containers had large numbers of culicine larvae. To identify the ovipositing species, a proportion of eggs found in the dishes were allowed to develop to the adult. All that emerged proved to be *Ae. aegypti* ssp. *formosus* Walker.

RESULTS AND DISCUSSION

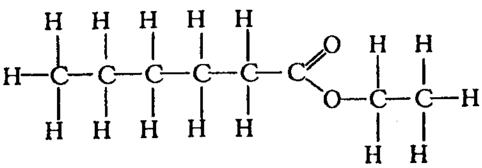
Effects of different chemicals on oviposition: The results of an experiment with 10 replicates



Hexanoic acid



5-methyl-hexanoic acid



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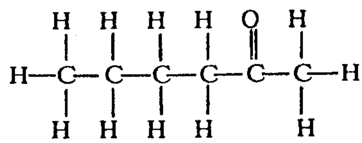
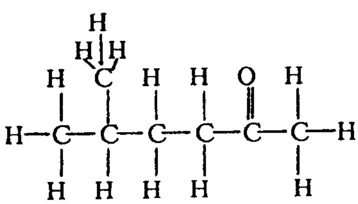


2-hexanone



5-methyl-2-hexanone

Fig. 1. Structure of chemicals used.

Table 1. Egg counts in treated (containing 1×10^{-3} moles of a test compound) and control dishes, for an experiment performed on December 31, 1988.

Replicate no.	Treatment					Control	
	A	B	C	D	E		
1	13	36	200	110	98	25	44
2	30	22	21	36	120	9	26
3	60	48	77	20	27	56	13
4	42	76	16	34	76	7	6
5	31	65	10	11	11	16	12
6	22	25	31	28	37	9	5
7	22	29	20	34	18	31	23
8	11	28	16	8	129	3	13
9	58	40	28	9	68	31	—
10	15	37	10	74	59	—	—
Total	304	406	429	364	643	329	
Mean	30.4	40.6	42.9	36.4	64.3	19.3	
SE	5.6	5.6	18.5	10.2	13.2	3.6	

A = hexanoic acid; B = methyl hexanoic acid; C = hexanoic acid, ethyl ester; D = 2-hexanone; E = 5-methyl-2-hexanone. — indicates missing values; these dishes were overturned by baboons.

Table 2. Two way analysis of variance comparing the effects of possessing a methyl branch with the effects of the type of functional group.

Methyl branch	Functional group COOH	Functional group C=O	Totals
Yes	15.750	16.961	32.711
No	14.147	14.174	28.321
Totals	29.897	31.135	61.032

Sources of variation	DF	SS	MS	F
Between rows (effect of methyl group)	1	0.488	0.488	5.304*
Between columns (effect of functional group)	1	0.045	0.045	NS
Interaction (methyl × functional group)	1	0.029	0.029	NS
Error	36	3.321	0.092	
Total	39	3.883		

* $P < 0.05$.

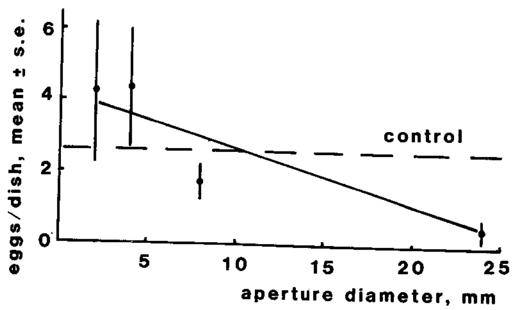


Fig. 2. Variation of mean egg count ±SE with effective rate of release: hexanoic acid, 19×10^{-3} moles per tube. The horizontal dashed line represents the mean egg count for the control dishes (SE = 1.24).

for each chemical and 20 for the control are shown in Table 1. The data did not conform to a normal distribution. Nonparametric analysis using the Mann-Whitney U test revealed that counts in dishes with treatments B (methyl hexanoic acid) and E (5-methyl-2-hexanone) differed significantly from the control at the 5% level ($P = 0.004$ and 0.002 respectively), whereas treatments A (hexanoic acid), C (hexanoic acid, ethyl ester) and D (2-hexanone) did not ($P = 0.186$, $P = 0.183$ and $P = 0.114$ respectively).

By comparing chemicals A, B, D and E, it was possible to separate the effects of a methyl branch and of different functional groups. Table 2 shows the two-way analysis of variance that was performed after log transformation of the data; the figures in the 2 × 2 contingency table were the sum of the logged values from Table 1. Possession of a methyl group is thus seen to affect oviposition more strongly than the 2 different functional groups.

Dose-response effect: This relationship was explored on January 2, 1989, using oviposition dishes set out 2 m apart with tubes containing 1×10^{-3} moles of hexanoic acid. The vapor release rate was varied by using corks with 2, 4 or 8 mm diam holes or a 24 mm diam opening (no cork). There were 8 replicates for each dose and 16 for the controls, which had a tube but no chemical present.

Egg numbers decreased with increasing release rate (Fig. 2). The slope of the linear regression of egg counts on hole diameter was −0.361, indicating a reversal of effect from enhancing to reducing oviposition rates with increase in the effective concentration of hexanoic acid. The significance of this cannot be tested using conventional parametric tests because the data are not seen to be normally distributed. A randomization test (Walters 1981) of the null hypothesis of zero slope of egg count on hole diameter gave a one-sided significance level of 3%.

Overall egg counts were considerably lower than those of preceding days, and the results for the 24-mm treatment might seem to contradict those for hexanoic acid in Table 2. Such day-to-day variation in dose-response thresholds is not unexpected in the field, where weather can influence the effective concentration (Elkinton and Cardé 1984).

CONCLUSIONS

Earlier studies have shown that compounds affecting mosquito oviposition are often straight-chain hydrocarbons 6–10 carbons long. In many cases they have polar and nonpolar regions on the molecule, and activity is enhanced by carboxylic acid or carbonyl functional groups, and by the presence of a methyl branch. Various compounds based on hexane, selected on this basis, are here shown to be behaviorally active for the oviposition of *Ae. aegypti* ssp.

formosus. Experiments using hexanoic acid at various release rates showed dose-dependent reversal of effect, with enhancement at low doses and inhibition at higher doses. At the release rate used here, 5-methyl hexanoic acid and 5-methyl-2-hexanone were most active in enhancing oviposition. A methyl branch is seen to affect the response more than the nature of the functional group, indicating that C=O and COOH are equally active functional groups in these behaviorally active compounds, and that addition of a methyl branch may significantly increase activity.

The dose-dependent reversal of response, demonstrated here for hexanoic acid, has implications for the interpretation of field and laboratory tests. When tested at a standard dose, it is expected that the more effective compounds, that is those with lower response thresholds, may repel or deter, whereas the less effective compounds, those with higher response thresholds, may attract or stimulate oviposition. The most effective compounds may therefore elicit egg counts that are lower than those elicited by less effective test compounds, and perhaps lower than those of the controls. Any comparison between compounds at the same dose, including this one, would be of greater value if accompanied by a set of dose-response curves, made under comparable conditions, for all the compounds. Without this family of dose-response curves, it is not safe to assume that the compound with the highest oviposition score is the compound with the lowest threshold, or the highest potential as an attractant or repellent.

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