

MOSQUITO REPELLENTS: MONOCARBOXYLIC ESTERS OF ALIPHATIC DIOLS

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ABSTRACT. Selected monocarboxylic esters of aliphatic diols that contained an alicyclic group in the acyl portion of the molecule were effective repellents for *Aedes aegypti*, *Anopheles quadrimaculatus* and *An. albimanus* when tested on cloth. Although 25 of the esters provided >21 days of protection against all 3 species, the repellent potency of the 143 hydroxyesters that were tested was found to vary with mosquito species. *Anopheles quadrimaculatus* was especially susceptible to this type of structure; 112 of the esters provided >21 days of protection and 56 provided >100 days of protection. *Aedes aegypti* was moderately susceptible to these repellents; 72 esters provided >21 days of protection. *Anopheles albimanus* was the most difficult mosquito to repel. Only 29 of the esters provided >21 days of protection.

INTRODUCTION

Chemicals that contain an alicyclic group have been shown to be effective repellents for a variety of biting insects.

Certain alicyclic carboxamides were effective mosquito repellents when applied to cloth (McGovern et al. 1978a, 1978b, 1980) or when applied topically to human skin (Smith et al. 1976, Schreck and McGovern 1985). In addition, they were effective topical repellents for black-flies (Schreck et al. 1979b, Lacey et al. 1982), biting midges (Schreck et al. 1979a), sand flies (Schreck et al. 1982), and the stable fly, *Stomoxys calcitrans* (Linn.) (Schreck et al. 1978).

The hydroxyester, particularly those synthesized from alicyclic carboxylic acids and aliphatic diols, was another type of compound that showed promise as an insect repellent. Schreck et al. (1978) found selected hydroxyesters to be effective stable fly repellents and Schreck et al. (1986) found 2-hydroxyethyl cyclohexanecarboxylate was much more effective than *N,N*-diethyl-*m*-toluamide, deet, as a repellent for the deer fly, *Chrysops atlanticus* Pechuman. Little else has been done to examine the efficacy of that type of hydroxyester as repellents for other species. In order to explore their potential as mosquito repellents, we synthesized 13 series of monohydroxyesters of alicyclic carboxylic acids and tested them on cloth against *Aedes aegypti* (Linn.), *Anopheles quadrimaculatus* (Say), and *An. albimanus* (Wiedemann). Data from these tests are reported here.

MATERIALS AND METHODS

Chemicals. The hydroxyesters were synthesized in the laboratory by one of two methods.

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In the first method, a carboxylic acid was allowed to react in refluxing benzene with a diol that was present in excess (1:6). The reaction was acid catalyzed (para-toluenesulfonic acid) and the water by-product was removed as formed. The reaction was terminated when the theoretical amount of water was collected in a water separator. The products were isolated by pouring the cooled reaction mixture into water and washing the separated organic layer sequentially with a 5% sodium hydroxide solution and a saturated salt solution. After drying, the solvent was filtered and removed under reduced vacuum. The resultant crude product was purified by fractional distillation under high vacuum. In the second method, a carboxylic acid chloride was added dropwise into a cold, well-stirred solution of excess diol and pyridine. Suitable solvents included benzene, ether or acetone. After the addition was completed, the reaction mixture was allowed to warm to room temperature and then was refluxed for 3 hr. Isolation and purification of the esters was carried out as described in Method 1 with the following modifications. In the reactions carried out in acetone, the solvent was removed under reduced pressure prior to the water wash. After pouring the crude reaction mixture into water, the organic phase was taken up in ether. A washing with 5% hydrochloric acid was added to the washing sequence in all of the syntheses.

The purity of the test materials was determined to be >95% by gas chromatographic analysis.

Each series in the study was made up of 11 esters of 13 different alicyclic carboxylic acids. The number of carbon atoms in the acid moiety varied from 6 to 10 and, in the diol moiety, they varied from 2 to 6.

Mosquito repellency tests. Chemicals were tested as described in McGovern et al. (1978a). Thus, a solution of 1 g of a test material was applied to a 300 cm² area of a cotton stocking. After 2 hr, the treated stocking was placed over


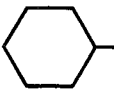
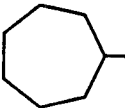
an untreated nylon stocking on the arm of a human subject and exposed for 1 min in a cage containing *Ae. aegypti*, *An. quadrimaculatus* or *An. albimanus* of mixed sexes of which ca. 1500 were 5- to 8-day-old blood-hungry females. Test exposures were repeated at 24 hr and then at weekly intervals until 5 bites were received in the 1-min test period. Numbers of days to the first bite and to 5 bites in 1 min were recorded. A standard repellent, dimethyl phthalate, was tested concurrently. Dimethyl phthalate is effective against *Ae. aegypti* and *An. quadrimaculatus* and its performance against those species is used as an indication of their biting pressure at that time, however, it is ineffective against *An. albimanus*. Although the procedure is not consistently part of the bioassay, when laboratory-reared mosquitoes are challenged with an arm in an untreated stocking, biting rates are usually >60 bites per min. A number of test materials were evaluated during each test period and at no time was a lack of biting pressure observed. Effectiveness of the candidate repellents was rated (based on the time until 5 bites were received) as follows: Class 1, 0 day protection; Class 2, 1-5 days; Class 3, 6-10 days; Class 4, 11-21 days; Class 5, >21 days. Repellents that provide 11 days or more of protection (Class 4) are considered promising.

RESULTS AND DISCUSSION

Each of the tables is divided into 3 sections where the diol moieties (R') are grouped according to structural similarities. The effect of chain length on repellency can be observed in the primary hydroxyl section where a homologous series of alcohol groups are listed. The effect of hydroxyl placement on repellency can be observed in the secondary hydroxyl section as well as by comparing that data with data from esters containing the diol moieties No. 2 or No. 3. The effect on repellency of adding unsaturation to the diol moiety can be observed by comparing the data in the third section with that from the esters of diol No. 3. The alicyclic acid moieties (R) are similarly grouped on the tables to allow comparisons of the effect of structural differences on repellency.

Duration of repellency data for 3 series (A, B, and C) of hydroxyesters in which the size of the alicyclic ring structure was varied is presented in Table 1. The isomeric esters, A4, B3, and C2, with a molecular formula of C₁₁H₂₀O₃, were the only such grouping to show Class 4 or greater repellency for all of the 3 mosquito species. In Series A and B, repellency began with the esters A2 and B1, which have 9 carbon atoms, and ended with the 12-carbon atom esters (A5 and

Table 1. Repellency of selected alicyclic monoesters of aliphatic diols toward *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Anopheles albimanus*.

No.	R'	RCOOR'								
		Series A			Series B			Series C		
		No. of days to the fifth bite								
		R								
										
		Ae.a.	An.q.	An.a.	Ae.a.	An.q.	An.a.	Ae.a.	An.q.	An.a.
Primary hydroxyl										
1.	—CCOH	1	1	0	36	35	39	39	39	0
2.	—CCCOH	93	93	27	33	47	0	43	152	43
3.	—CCCCOH	16	58	16	13	27	24	43	72	0
4.	—CCCCCOH	16	249	16	1	0	1	147	187	0
5.	—CCCCCCOH	0	0	0	0	0	0	39	101	0
Secondary hydroxyl										
6.	—C—COH—C	15	124	8	27	27	1	62	117	8
7.	—CC—COH—C	16	16	16	47	47	24	78	187	0
8.	—C—COH—CC C	0	1	0	20	20	0	124	187	0
9.	—C—COH—C C	0	1	1	22	22	22	77	107	0
Primary hydroxyl plus unsaturation										
10.	—CC=CCOH	16	23	16	53	152	62	0	402	0
11.	—CC≡CCOH	16	37	9	8	514	8	29	875	0

B4). Thus, the repellency observed in those series appears to follow the volatility of the compounds, and it can be assumed that the cyclopentyl and cyclohexyl structures did not contribute in a unique way to the observed repellency, even though the non-planar rings exist in different spatial configurations. However, in Series C, Class 5 repellency for *Ae. aegypti* and *An. quadrimaculatus* continued through compound C5. The next homolog in the series was tested and repellency fell to zero days for all 3 mosquito species. If repellency in the cycloheptyl series followed only the volatility of the compounds, as appears to have occurred in Series A and B, one would expect activity to end at approximately the molecular weight of compound C3. Apparently, the 7-membered ring itself is a contributing factor in the extended repellency that was observed.

In comparing the effect of hydroxyl placement on the repellency of the 3-carbon-atom diol derivatives, the esters derived from 1,3-propanediol (A2, B2, and C2) were generally superior repellents to those that were derived from 1,2-propanediol (A6, B6, and C6). Similar comparisons with the esters of the 4-carbon atom diols showed a change in effectiveness as one proceeds from Series A to Series C. In Series A, ester A3, which has a primary hydroxyl group, was usually

the superior repellent. In Series B, esters containing a secondary hydroxyl group (B7, B8, and B9) were slightly superior or about equal in repellency to that shown by compound B3. In Series C, the esters with the secondary hydroxyl were decidedly superior repellents for *Ae. aegypti* and *An. quadrimaculatus*, the only species affected.

The third section of Table 1 contains esters of 4-carbon-atom diols that contain olefinic and acetylenic unsaturation. Comparisons can be made with the data from esters of the alcohol No. 3. Mixed results were observed depending on the type of unsaturation and which alicyclic group was involved. The major effect of adding unsaturation was the increased persistence against *An. quadrimaculatus* found in Series B and C. Ester C11 was still effective after 2 years and esters C10 and B11 were effective for over 1 year.

Repellency data for 3 series of esters that contain a cyclopentyl group are given in Table 2. The only difference between the series is the addition of 1 and 2 methylene groups between the cyclopentyl ring and the carboxyl group (Series D and E).

Esters A3, D2, and E1 were the only group of isomeric compounds ($C_{10}H_{18}O_3$) in the first section of the table that showed Class 4 or greater

Table 2. Repellency of selected alicyclic monoesters of aliphatic diols toward *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Anopheles albimanus*, cyclopentyl homologs.

		RCOOR'								
		No. of days to the fifth bite								
		R								
		Series A ^a			Series D			Series E		
No.	R'	Ae.a.	An.q.	An.a.	Ae.a.	An.q.	An.a.	Ae.a.	An.q.	An.a.
<i>Primary hydroxyl</i>										
1.	—CCOH	1	1	0	15	7	0	28	35	28
2.	—CCCOH	93	93	27	33	33	20	35	79	79
3.	—CCCCOH	16	58	16	0	150	0	14	100	7
4.	—CCCCCOH	16	249	16	0	33	0	7	79	7
5.	—CCCCCOH	0	0	0	0	22	0	0	8	0
<i>Secondary hydroxyl</i>										
6.	—C—COH—C	15	124	8	13	13	17	28	35	17
7.	—CC—COH—C	16	16	16	15	21	0	21	79	7
8.	—C—COH—CC	0	1	0	15	15	0	21	76	7
9.	$\begin{array}{c} \text{C} \\ \\ \text{—C—COH—C} \end{array}$	0	1	1	8	8	0	21	79	7
<i>Primary hydroxyl plus unsaturation</i>										
10.	—CC=CCOH	16	23	16	22	114	0	28	284	7
11.	—CC≡CCOH	16	37	9	8	144	0	35	240	24

^a Series repeated from Table 1 for convenience of comparison.

repellency against all of the 3 species. Other than that observation, there is little indication of a consistent effect on repellency resulting from changes in the alicyclic moiety. However, within a series, particularly in Series D and E against *An. quadrimaculatus*, an increase, a peaking, and a subsequent decline in repellency occurred as the length of the diol moiety was increased.

The effect of hydroxyl placement differed from the results presented in Table 1 only in the results observed with the 4-carbon-atom diol derivatives. Derivatives with a secondary hydroxyl group in Series D and E showed enhanced repellency toward *Ae. aegypti* and reduced repellency toward *An. quadrimaculatus* when compared with the results from esters D3 and E3.

The addition of unsaturation in Series D did not have much effect on repellency, in contrast to what was generally observed with the isomeric compounds of Series B in Table 1. However, the addition of unsaturation in Series E increased repellency against all of the 3 species; this is also in contrast to the observations with the isomeric compounds of Series C in Table 1.

Repellency data for 4 series of esters contain-

ing a cyclohexane ring, which differ among themselves only in the number of methylene groups attached to the ring, are presented in Table 3.

Repellent activity in section 1 (primary hydroxyl) did not go beyond that observed with the esters of the 4-carbon-atom diol. Esters derived from ethylene glycol (No. 1) were especially effective repellents for all of the species. There seems to be a volatility effect in the *Ae. aegypti* repellency data. Numbers of compounds showing Class 4 or greater repellency systematically declined beginning with compound B3. Its molecular formula of C₁₁H₂₀O₃ was the upper limit for effective repellency as one proceeds from Series B through Series H. Data from the tests with *An. quadrimaculatus* suggest somewhat different parameters for receptor response to repellents in that species. Although the length of the acyl moiety continually increased by a methylene group going from Series B to Series H, repellent activity against *An. quadrimaculatus* abruptly ended in each series when the length of the diol moiety exceeded 4 carbon atoms. These results suggest that, in this instance, it is the length of a portion of the mole-

Table 3. Repellency of selected alicyclic monoesters of aliphatic diols toward *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Anopheles albimanus*, cyclohexyl homologs.

		RCOOR'											
		No. of days to the fifth bite											
		R											
		Series B ^a			Series F			Series G			Series H		
No.	R'	Ae.a.	An.q.	An.a.	Ae.a.	An.q.	An.a.	Ae.a.	An.q.	An.a.	Ae.a.	An.q.	An.a.
<i>Primary hydroxyl</i>													
1.	—CCOH	36	35	39	55	55	33	27	125	33	8	69	40
2.	—CCCOH	33	47	0	50	78	1	1	255 ^c	1	1	119	0
3.	—CCCCOH	13	27	24	1	240 ^b	0	1	269 ^c	1	1	240	0
4.	—CCCCCOH	1	0	1	0	0	0	0	0	0	0	0	0
5.	—CCCCCOH	0	0	0	0	0	0	0	0	0	0	0	0
<i>Secondary hydroxyl</i>													
6.	—C—COH—C	27	27	1	39	39	0	0	102	0	0	39	0
7.	—CC—COH—C	47	47	24	29	46	0	8	106	0	29	39	0
8.	—C—COH—CC	20	20	0	64	64	0	0	0	0	0	0	0
9.	—C—COH—C	22	22	22	76	178	35	59	69	35	1	310 ^c	0
<i>Primary hydroxyl plus unsaturation</i>													
10.	—CC=CCOH	53	152	62	8	234 ^b	0	8	508 ^b	8	8	999 ^c	0
11.	—CC≡CCOH	8	514 ^b	8	0	522 ^c	0	0	1438 ^d	0	0	508 ^b	0

^a Series repeated from Table 1 for convenience of comparison.

^b Same number of days until the first bite was recorded.

^c Days until the first bite was recorded: G2, 240; G3, 240; H9, 257; H10, 771; F11, 508.

^d 60 days until the first bite; >1000 days until the second bite.

Table 4. Repellency of selected alicyclic monoesters of aliphatic diols toward *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Anopheles albimanus*, substituted cyclohexyl and bicyclo compounds.

No.	R'	RCOOR'																	
		No. of days to the fifth bite																	
		Series B ^a		Series I		Series J		Series K		Series L		Series M							
Ae.a.	An.q.	Ae.a.	An.q.	Ae.a.	An.q.	Ae.a.	An.q.	Ae.a.	An.q.	Ae.a.	An.q.	Ae.a.	An.q.	An.a.					
1.	-CCOH	36	35	39	33	47	45	33	33	0	33	40	9	30	16	0	16	30	0
2.	-CCCOH	33	47	0	33	61	24	20	55	33	14	97	24	76	187	0	59	101	59
3.	-CCCCOH	13	27	24	22	210 ^b	15	22	210 ^b	0	50	240 ^b	0	23	287	0	41	101	31
4.	-CCCCCOH	1	0	1	0	0	1	0	0	0	0	0	0	23	287 ^b	0	0	108	0
5.	-CCCCCOH	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	101	0
6.	-C-COH-C	27	27	1	22	32	22	15	22	1	22	1	1	39	187	0	41	87	0
7.	-CC-COH-C	47	47	24	29	39	0	39	39	0	29	53	0	76	187	14	59	73	59
8.	-C-COH-CC C	20	20	0	42	41	0	74	74	0	42	41	0	64	64	0	42	41	0
9.	-C-COH-C 	22	22	22	22	22	22	36	41	0	35	35	29	46	61	0	59	66	59
10.	-CC=CCOH	53	152	62	97	514 ^b	80	101	135	0	53	270 ^b	62	46	187	0	66	66	59
11.	-CC≡CCOH	8	514 ^b	8	8	270 ^b	8	101	402 ^c	0	8	514 ^b	0	0	111	0	41	101	17

* Series repeated from Table 1 for convenience of comparisons.

^b Same number of days until the first bite was recorded.^c 395 days until first bite was recorded.

cule and the functionalities found therein that are critical for effective receptor interaction and that the length of the acyl portion of the molecule (R) does not seem to be critical. Curiously, similar results were not observed with the cyclopentyl series in Table 2.

It was again observed, where comparisons could be made, that esters of the 3-carbon-atom diol with a primary hydroxyl group were superior repellents to those with a secondary hydroxyl group. In general, results with the esters of the 4-carbon-atom diols paralleled results found with the analogous compounds in Table 2. Esters F9 and G9 were particularly noteworthy in providing persistent levels of repellency against all of the species.

Anopheles quadrimaculatus was greatly affected by the addition of unsaturation to the diol in these series. Compounds H10 and G11 provided outstanding persistence, lasting 2.7 and 3.9 years, respectively, until the fifth bite was received. In addition, several of the esters in the table showed exceptional persistence against *An. quadrimaculatus* before the first bite was received. For many, it took as long to receive the first bite as it took to receive 5 bites. Compound G11 allowed a first bite at 60 days but then went >1000 days before a second bite was recorded. Compound B10 was the only ester in this section that showed significant repellency for the other 2 species.

Data presented in Table 4 permits observations on the repellency effect of adding unsaturation and a methyl substituent to the alicyclic group. Esters in the first section that contain a cyclohexane ring provided Class 4 or greater repellency against *Ae. aegypti* and *An. quadrimaculatus* through the 4-carbon-atom diol homolog. An abrupt loss of repellency was observed with the next higher homolog, as was seen in Table 3. The structural changes on the cyclohexane ring, Series I-K, affected only *An. quadrimaculatus* in a consistent manner. In 2 instances there was little noticeable effect; in all other instances there was an increase in repellency. In Series L and M, the acid moiety included a bicyclo structure and, as was observed in the series that contained a cyclopentyl or cycloheptyl group, effective repellency (Class 4 or greater) was observed with the higher homologs.

The hydroxyl placement in these series generally affected *Ae. aegypti* and *An. quadrimaculatus* in a manner similar to that observed in Tables 2 and 3. Results with *An. albimanus* were inconsistent, although esters I9, K9 and M9 were Class 5 repellents and each had an unsaturated alicyclic moiety.

Adding unsaturation into the diol moiety frequently caused an increase in the duration of

repellency, especially against *An. quadrimaculatus*; however, in the bicyclo series, the large increase in persistence observed in other series does not occur and repellency usually declined. Nevertheless, L10, L11, M10 and M11 are still very good repellents for *An. quadrimaculatus*. However, when the unsaturation was added to the bicyclo group, a significant increase in the number of effective repellents for *An. albimanus* was observed. Series L has one Class 4 repellent, while Series M has one Class 4 and five Class 5 repellents for that species. As was seen with the compounds in Table 3, many of the esters showed prolonged persistence before the first bite from *An. quadrimaculatus* was recorded.

The acid and diol moieties that were most effective against each species are shown in Fig. 1. The number of esters that contained that moiety and were Class 5 repellents are listed beside each structure. The maximum number of possibilities is 11 for the acid moiety and 13 for the diol moiety. The preponderance of 7-carbon-atom R groups in the acid moieties and the diversity of their structures is noteworthy. Another noteworthy feature is that esters derived from 1,3-propanediol head the list of effective structures for all 3 mosquito species. Three other diol moieties are among the most effective for 2 of the species.




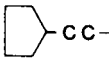

In considering the broad spectrum nature of the compounds, 25 of the esters (17.5%) were Class 5 repellents for all 3 species. Twenty of those repellents were derived from 4 of the 11 diols listed in the tables. They included six 2-hydroxy-1-methylpropyl (No. 9), five 2-hydroxyethyl (No. 1), five 3-hydroxypropyl (No. 2), and four 4-hydroxy-2-butenyl (No. 10) esters. When viewed from the contribution of the acid moiety, 5 each were found in Series I and M and 4 were found in Series B. In considering the effect of the compounds on the individual species, *An. quadrimaculatus* was found to be most susceptible to this type of repellent and *An. albimanus* was found to be least susceptible. Seventy-two (50.3%) were Class 5 repellents for *Ae. aegypti*, 112 (78.3%) for *An. quadrimaculatus*, and 29 (20.3%) for *An. albimanus*. The repellents were exceptionally persistent against *An. quadrimaculatus*; 56 lasted >100 days and 9 of those lasted >500 days. The 2 most effective repellents for each species from this study and the number of days until the first and fifth bites were recorded are: *Ae. aegypti*, 5-hydroxypentyl cycloheptanecarboxylate, C4 (0 and 147 days) and 2-hydroxybutyl cycloheptanecarboxylate, C8 (103 and 124 days); *An. quadrimaculatus*, 4-hydroxy-2-butenyl 3-cyclohexylpropionate, G11 (60 and 1,438 days) and 4-hydroxy-2-butenyl 4-cyclohexylbutyrate, H10 (771 and 999 days); *An. albimanus*, 4-hydroxy-2-butenyl 3-cyclohexene-1-

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



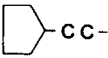

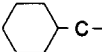
Alicyclic Groups (R)

Diol Moieties (R')

Aedes aegypti

	10		8	-CCCOH	10
	9		8	-CC-COH-C	10
	8			$\overset{\text{C}}{\text{C}}\text{-COH-C}$	10
				-CCOH	9

Anopheles quadrimaculatus

	11		9	-CCCOH	14
	11		9	-CCCCOH	14
	10		9	-CC=CCOH	14
	9			-CC≡CCOH	13
				-CC-COH-C	12

Anopheles albimanus


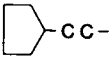



	5		3	-CCCOH	8
	5		3	-CCOH	6
	5			$\overset{\text{C}}{\text{C}}\text{-COH-C}$	6

Fig. 1. Most effective alicyclic groups and diol moieties of selected hydroxyester repellents and the number of Class 5 repellents (>21 days persistence) having that structural feature as part of the molecule.

carboxylate, I10 (8 and 80 days) and 3-hydroxypropyl 3-cyclopentylpropionate, E2 (14 and 79 days).

The chemicals reported here are experimental and are not recommended for general use.

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