MOSQUITO REPELLENTS: CYCLOHEXANEALKANOIC CARBOXAMIDES AS REPELLENTS FOR *AEDES AEGYPTI*, *ANOPHELES QUADRIMACULATUS*, AND *ANOPHELES ALBIMANUS*

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ABSTRACT. Selected carboxamides of cyclohexaneacetic, -propanoic, and -butanoic acids were highly effective repellents for *Aedes aegypti* (L.), *Anopheles quadrimaculatus* Say, or *An. albimanus* (Wiedemann) when tested on cloth. Thirty-seven of 48 amides were Class 5 repellents (effectiveness >21 days) against one or more of the mosquito species and 4 were Class 5 repellents against all 3 species. Twenty-four amides were especially effective against *Ae. aegypti* and *An. quadrimaculatus*, providing >100 days of protection against 1 or both species. 1-(3-Cyclohexyl-1-oxopropyl)- and 1-(4-cyclohexyl-1-oxobutyl)pyrrolidine were the most effective repellents for *Ae. aegypti*, providing 169 and 160 days of protection, respectively. 1-(4-Cyclohexyl-1-oxobutyl)-4-methylpiperazine was the most effective repellent against both anopheline species, providing outstanding protection of 565 days against *An. quadrimaculatus* and 51 days against *An. albimanus*. *An. albimanus* was quite insensitive to most of the test materials; only 4 of the amides were Class 5 repellents against this species of mosquito.

INTRODUCTION

Our recent efforts at synthesis and development of insect repellents have centered primarily around amides of heterocyclic amines (McGovern et al. 1974, 1975, 1978a,b). Certain members of the various amide series were effective repellents and showed exceptional persistence when tested on cloth against mosquitoes. Aliphatic carboxamides with a molecular weight of ca. 225 exhibited the highest levels of repellent activity in 9 of 10 series when tested against *Aedes aegypti* (L.) (McGovern et al. 1975). In addition, certain alicyclic carboxamides of heterocyclic amines, although not possessing exceptional persistence against mosquitoes when tested on clothing, were reported by Schreck et al. (1978, 1979a,b) to be highly effective repellents for the stable fly, *Stomoxys calcitrans* (L.), certain black fly (Simuliidae) species, and 2 species of biting midges of the *Culicoides* genus when tested on skin.

We therefore extended our amide synthesis program to incorporate features of both the aliphatic and alicyclic carboxamides tested previously. We now report repellency data for 48 amides synthesized from 3 cyclohexanealkanoic acids and 4 *N,N*-dialkyl and 12 heterocyclic amines in tests on cloth against *Ae. aegypti*, *Anopheles quadrimaculatus* Say, and *An. albimanus* (Wiedemann).

MATERIALS AND METHODS

CHEMICALS. Amides were synthesized as follows: an anhydrous ether solution of the appropriate acid chloride was slowly added, with stirring, to an anhydrous ether solution of a 2-fold excess of the amine cooled in an ice bath. The reaction mixture was allowed to warm to room temperature and then to stand overnight. The amides were isolated by routine extraction procedures and purified by fractional distillation under high vacuum. Gas chromatographic analysis indicated that the purity of the chemicals was >95%.

MOSQUITO REPELLENCY TESTS. Chemicals were tested as described in McGovern et al. (1975, 1978a). Thus a solution of 1g

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of a test material was applied to a 300 cm^2 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 of ca. 1500 5- to 8-day-old *Ae. aegypti*, *An. quadrimaculatus* or *An. albimanus*. 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 1st bite and to 5 bites in 1 min were recorded. A standard repellent, dimethyl phthalate, was tested concurrently and was effective for 11–21 days (Class 4) against *Ae. aegypti* and *An. quadrimaculatus* but was ineffective against *An. albimanus*; to date there is no suitable standard repellent for use in tests with *An. albimanus*. 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.

RESULTS AND DISCUSSION

Repellency data for 16 carboxamides each of cyclohexaneacetic, cyclohexanepropanoic, and cyclohexanebutanoic acids in tests against 3 species of mosquito are given in Table 1. *An. albimanus* was

Table 1. Repellency of carboxamides to *Aedes aegypti*, *Anopheles quadrimaculatis* and *An. albimanus*.

No.	$-NR_2$	*Ae. aegypti* Class	*Ae. aegypti* Days to 1st bite	*Ae. aegypti* Days to 5 bites	*An. quadrimaculatus* Class	*An. quadrimaculatus* Days to 1st bite	*An. quadrimaculatus* Days to 5 bites	*An. albimanus* Class	*An. albimanus* Days to 1st bite	*An. albimanus* Days to 5 bites
	Group I	C_6H_{11}-C-NR_2 (O)								
1.	1-Pyrrolidinyl	5*	30	38	5*	38	79	1	0	0
2.	1-Piperidinyl	5*	30	38	5*	30	30	1	0	0
3.	Hexahydro-1*H*-1-azepinyl	5*	106	113	5*	22	38	1	0	0
4.	2-Methyl-1-piperidinyl	5*	104	104	5*	111	111	2	1	1
5.	3-Methyl-1-piperidinyl	5*	104	104	5*	22	22	1	0	0
6.	4-Methyl-1-piperidinyl	5*	52	104	5*	0	22	1	0	0
7.	2-Ethyl-1-piperidinyl	5*	104	104	5*	1	22	1	0	0
8.	2,6-Dimethyl-1-piperidinyl	5*	104	104	5*	1	37	1	0	0
9.	1,2,3,6-Tetrahydro-1-pyridinyl	4*	15	15	4*	15	15	1	0	0
10.	4-Methyl-1-piperazinyl	4*	7	15	5*	7	111	1	0	0
11.	4-Morpholinyl	2*	0	1	5*	27	27	1	0	0
12.	2,6-Dimethyl-4-morpholinyl	5*	15	28	5*	35	35	1	0	0
13.	Dimethylamino	2	1	1	2	1	1	2	1	1
14.	Diethylamino	2*	4	4	2*	1	1	1	0	0
15.	Dipropylamino	4*	15	15	3*	8	8	1	0	0
16.	Dibutylamino	3*	0	8	1*	0	0	1	0	0
	Group II	C_6H_{11}-CH_2-C-NR_2 (O)								
17.	1-Pyrrolidinyl	5	108	108	5	21	119	5	21	21
18.	1-Piperidinyl	5	28	108	5	28	28	1	0	0
19.	Hexahydro-1*H*-1-azepinyl	5	21	130	3	1	8	1	0	0
20.	2-Methyl-1-piperidinyl	5	28	102	1	0	0	1	0	0
21.	3-Methyl-1-piperidinyl	5	28	102	1	0	0	1	0	0
22.	4-Methyl-1-piperidinyl	5	28	51	3	8	8	1	0	0
23.	2-Ethyl-1-piperidinyl	5	0	28	3	8	8	1	0	0
24.	2,6-Dimethyl-1-piperidinyl	5	101	108	5	108	108	1	0	0

Table 1. Continued.

No.	-NR_2	*Ae. aegypti* Class	Days to 1st bite	Days to 5 bites	*An. quadrimaculatus* Class	Days to 1st bite	Days to 5 bites	*An. albimanus* Class	Days to 1st bite	Days to 5 bites
25.	1,2,3,6-Tetrahydro-1-pyridinyl	5	8	28	5	28	28	1	0	0
26.	4-Methyl-1-piperazinyl	5	0	105	5	134	175	1	0	0
27.	4-Morpholinyl	2	0	1	4	1	18	1	0	0
28.	2,6-Dimethyl-4-morpholinyl	5	0	105	5	126	126	1	0	0
29.	Dimethylamino	2	1	1	2	1	1	2	1	1
30.	Diethylamino	3	8	8	3	8	8	1	0	0
31.	Dipropylamino	1	0	0	5	28	28	1	0	0
32.	Dibutylamino	1	0	0	1	0	0	1	0	0
	Group III	C_6H_{11}-CH_2CH_2-C-NR_2 (O)								
33.	1-Pyrrolidinyl	5	169	169	5	238	238	4	1	12
34.	1-Piperidinyl	5	29	29	5	7	182	1	0	0
35.	Hexahydro-1*H*-1-azepinyl	5	21	65	5	21	44	1	0	0
36.	2-Methyl-1-piperidinyl	5	7	21	2	1	1	1	0	0
37.	3-Methyl-1-piperidinyl	5	7	21	5	1	126	1	0	0
38.	4-Methyl-1-piperidinyl	3	7	7	1	0	0	1	0	0
39.	2-Ethyl-1-piperidinyl	4	0	12	4	0	12	1	0	0
40.	2,6-Dimethyl-1-piperidinyl	5	0	21	5	126	126	1	0	0
41.	1,2,3,6-Tetrahydro-1-pyridinyl	4	0	12	5	0	93	1	0	0
42.	4-Methyl-1-piperazinyl	3	7	7	5	21	268	4	18	18
43.	4-Morpholinyl	5	61	133	5	238	238	4	12	12
44.	2,6-Dimethyl-4-morpholinyl	5	61	93	5	133	133	1	0	0
45.	Dimethylamino	5	21	21	5	24	24	5	24	24
46.	Diethylamino	5	21	65	5	65	65	1	0	0
47.	Dipropylamino	2	1	1	2	1	1	1	0	0
48.	Dibutylamino	2	1	1	2	1	1	1	0	0
	Group IV	C_6H_{11}-$CH_2CH_2CH_2$-C-NR_2 (O)								
49.	1-Pyrrolidinyl	5	61	160	5	160	160	1	0	0
50.	1-Piperidinyl	5	0	36	5	36	36	1	0	0
51.	Hexahydro-1*H*-1-azepinyl	5	22	22	5	36	134	1	0	0
52.	2-Methyl-1-piperidinyl	1	0	0	1	0	0	1	0	0
53.	3-Methyl-1-piperidinyl	2	0	1	5	36	36	1	0	0
54.	4-Methyl-1-piperidinyl	1	0	0	5	52	94	1	0	0
55.	2-Ethyl-1-piperidinyl	4	13	13	5	36	104	1	0	0
56.	2,6-Dimethyl-1-piperdinyl	2	0	1	5	134	134	1	0	0
57.	1,2,3,6-Tetrahydro-1-pyridinyl	5	0	36	5	134	134	1	0	0
58.	4-Methyl-1-piperazinyl	5	0	51	5	310	565	5	51	51
59.	4-Morpholinyl	5	77	134	5	134	134	1	0	0
60.	2,6-Dimethyl-4-morpholinyl	5	22	22	5	134	134	1	0	0
61.	Dimethylamino	5	42	42	5	24	54	5	24	24
62.	Diethylamino	5	13	113	5	22	22	1	0	0
63.	Dipropylamino	1	0	0	1	0	0	1	0	0
64.	Dibutylamino	1	0	0	1	0	0	1	0	0

* Data from McGovern et al. (1978a, b) are included for comparison.

included in these tests for the 1st time. Published data (McGovern et al. 1978a,b) for 15 cyclohexanecarboxamides (Group I) in tests against *Ae. aegypti* and *An. quadrimaculatus* are included for comparison; data for the *N,N*-dimethyl derivative (no. 13) against *Ae. aegypti* and *An. quadrimaculatus* and all data against *An. albimanus* are new. As can readily be seen, most of the effective repellent activity exhibited by these chemicals was directed against *Ae. aegypti* and *An. quadrimaculatus*. *An albimanus* was impressively insensitive to all but 7 of the repellents.

Chemicals that showed Class 4 or 5 repellency in any of the tests were considered promising repellents. Of the 48 chemicals of Groups II–IV, 39 showed this level of repellency. As we found in our previous studies, the amides derived from the heterocyclic amines were most effective and provided good to outstanding protection against 1 or more mosquito species. Of 36 heterocyclic amine derivatives, 32 were Class 5 repellents against at least 1 species; 20 were Class 5 against 2 species; 2 were Class 5 against all 3 species. The *N,N*-dialkyl amides were somewhat less effective; however 5 of the 12 amides were Class 5 against 1 or more species and 2 of these were Class 5 against all 3 species.

Groups I–IV show 16 homologous series in which the molecular weight was increased by interposing 1, 2, or 3 methylene units between the cyclohexane moiety and the carboxamide function, thus adding more alkane character to the molecule. The correlation between molecular weight and highest repellent activity was less clearly defined in these series than in the alkanoic series, in which amides with a molecular weight of ca. 225, corresponding to 14 carbon atoms or equivalent, provided the longest periods of protection (McGovern et al. 1975). Fourteen of the 16 homologous series had a member whose molecular weight was at or near 225. However, in this study, high levels of repellent activity were often found over a range of molecular weights rather than peaking at or near a particular molecular weight, although this apparently also occurred. Also, because of the limited molecular weight range within an homologous series, the highest level of repellency was sometimes found either with the 1st or last member of that series. This restricted our drawing conclusions about the extent of the "effective" molecular weight range within that particular series. Thus, within the limits of this study, the most effective repellents (>100 days protection) were found in the molecular weight range of ca. 195–237 for *Ae. aegypti* and over the greater range of ca. 195–265 for *An. quadrimaculatus*. Too few promising repellents were found for *An. albimanus* to allow description of a similar "effective" molecular weight range for this species.

For the purpose of discussion, the chemicals in each group have been separated into 3 sections: the first 9 amides of each group can be considered as variations of acylpiperidines; the next 3 have a 2nd heteroatom in the heterocyclic amine moiety; the last 4 are *N,N*-dialkyl amides.

When the effect of increased molecular weight on repellency of the 9 amides in the 1st section of each group was considered, activity generally decreased toward *Ae. aegypti* and increased toward *An. quadrimaculatus*. Against *Ae. aegypti*, the number of repellents providing >100 days protection declined from 6 to 1 (5th bite data) in Groups I to IV; against *An. quadrimaculatus*, the number increased from 1 to 5. The pyrrolidinyl derivatives of cyclohexaneacetic (no. 17), cyclohexanepropanoic (no. 33), and cyclohexanebutanoic (no. 49) acids were exceptions and provided outstanding protection against both *Ae. aegypti* and *An. quadrimaculatus*. No 17 and 33 were also Class 5 and 4 repellents, respectively, against *An. albimanus* and were the only members of these sections to provide more than 1 day's protection against this species. Each was the most effective repellent in its section.

The 2nd section of each group contains derivatives of 4-methylpiperazine, morpholine, and 2,6-dimethylmorpholine

and can be characterized as having a 2nd heteroatom in the ring of the amine moiety. Against *Ae. aegypti*, results with the 4-methylpiperazine derivatives were somewhat variable; no. 26 provided maximum protection of 105 days. The morpholine derivatives were ineffective in Groups I and II but provided 133 and 134 days of protection in Groups III and IV (no. 43 and 59), respectively. Activity of the 2,6-dimethylmorpholine derivatives peaked in Groups II and III; no. 28 provided 105 days protection. Against *An. quadrimaculatus*, all 4-methylpiperazine derivatives provided high levels of protection; no. 10 and 26 provided >100 days, no. 42 >200 days, and no. 58 >500 days of protection. The cyclohexanebutanoic acid derivative (no. 58), which provided 310 days of protection before the 1st bite was recorded and 565 days before 5 bites were recorded, was the most effective repellent we have tested against this species of mosquito. The morpholine derivatives of Groups III and IV (no. 43 and 59) and the 2,6-dimethylmorpholine derivatives of Groups II, III, and IV (no. 28, 44, and 60) also provided noteworthy protection. No. 58 also provided 51 days of protection against *An. albimanus*; this was the highest level of protection against this mosquito found in this study. As was found by McGovern et al. (1978b), amides of this type were more repellent to *An. quadrimaculatus* than to *Ae. aegypti*.

The *N,N*-dialkyl carboxamides (3rd section) generally afforded a much lower level of repellency than did the amides of heterocyclic amines. Only no. 62 provided >100 days protection against 1 mosquito species. Still, the *N,N*-dimethyl carboxamides of Groups III and IV (no. 45 and 61) were Class 5 repellents against all 3 species. Only 2 other Class 5 repellents against *An. albimanus* were found in the entire study. The *N,N*-diethyl derivatives of these same 2 groups (no. 46 and 62) were Class 5 repellents against *Ae. aegypti* and *An. quadrimaculatus*, and the *N,N*-dipropyl derivative of Group II (no. 31) was a Class 5 repellent against *An. quadrimaculatus*. Although the repellency levels of this latter group of chemicals were relatively low, one cannot adequately judge from these data how effective the materials would be if used in applications on human skin. For example, *N,N*-dipropylcyclohexanecarboxamide (no. 15) almost failed to qualify for additional testing under the existing test protocol, yet it has proved to be one of our more promising experimental repellents when subsequently tested on human skin against the stable fly and in field tests against certain biting midges (Schreck et al. 1978, 1979b), the deer fly, *Chrysops atlanticus* Pechuman, and *Ae. taeniorhynchus* (Wiedemann) (Schreck, unpublished data).

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