

## Overcrowding Factors of Mosquito Larvae. VI. Structure-Activity Relationships of 2-Substituted Aliphatic Carboxylic Acids against Mosquito Larvae

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To study the structure-activity relationships of 2-substituted aliphatic carboxylic acids structurally related to the overcrowding factors of mosquito larvae, 20 2-ethyl-, 2-butyl-, and 2-hexyl-substituted carboxylic acids having even numbers of carbon atoms and a total number of carbon atoms from 12 to 14 were synthesized and evaluated for their biological activity against young larvae of *Culex pipiens quinquefasciatus* Say. 2-Ethyltadecanoic acid, 2-butyltadecanoic and 2-butyltetradecanoic acids, and 2-hexyldecanoic

acid showed the greatest activity in the 2-ethyl-, 2-butyl-, and 2-hexyl series, respectively. In all three series, the 2-substituted carboxylic acids having a total number of carbon atoms from 14 to 18 generally exhibited good activity; however, 2-ethyltadecanoic acid was an exception. The level of biological activity seemed to be dependent upon the total number of carbon atoms in the carboxylic acids regardless of the size of the substituents.

Mosquito larvae overcrowded in laboratory cultures produce overcrowding factors which are responsible for regulating young larval populations. These factors possess toxicity and growth-retarding activity against younger larvae of several species of mosquitoes (Ikeshoji and Mulla, 1970a,b). Chemical studies revealed that the major constituents of the overcrowding factors were two straight-chain and two branched-chain hydrocarbons (Ikeshoji and Mulla, 1974a). The branched-chain hydrocarbons, 8-methylnonadecane and 7-methyloctadecane, were synthesized and bioassayed. They manifested considerable activity against the southern house mosquito *Culex pipiens quinquefasciatus* Say (Y.-S. Hwang, unpublished data). Some other constituents, mainly 2-methyl- and 2-ethyl-substituted aliphatic carboxylic acids, were also found in the natural extract of overcrowded larval cultures (Ikeshoji and Mulla, 1974a). To ascertain the activity of substituted carboxylic acids, various carboxylic acids were obtained or prepared and bioassayed for their biological activity against mosquito larvae (Ikeshoji and Mulla, 1974b; Hwang *et al.*, 1974).

Biological activity of straight-chain aliphatic carboxylic acids (both saturated and unsaturated) against mosquito larvae has been studied. The queen substance, 9-oxodecatrienoic acid, and related carboxylic acids were tested against immature stages of *Aedes aegypti* (L.) (Quraishi, 1972; Quraishi and Thorsteinson, 1965). Linoleic and linolenic acids were found to show some activity at very high and impractical dosages against *A. aegypti* (Saxena and Thorsteinson, 1971). Caproic acid was found to be a good larvicide at 150 and 300 ppm (Maw, 1970). The activity levels of these straight-chain carboxylic acids are much lower than those of the branched-chain carboxylic acids studied in this laboratory.

For systematic studies on the structure-activity relationships of substituted aliphatic carboxylic acids against mosquito larvae, 20 2-ethyl-, 2-butyl-, and 2-hexyl-substituted long-chain carboxylic acids having even numbers of carbon atoms and a total number of carbon atoms from 12 to 24 were synthesized, and their activity against mosquito larvae was investigated.

### EXPERIMENTAL SECTION

**General Procedures for Preparing 2-Substituted Carboxylic Acids.** The procedures described by Hwang *et al.* (1974) were followed. In brief, sodium ethoxide solution was prepared by dissolving sodium (0.1 g-atom) in abso-

lute ethanol (100 ml). Into this solution, diethyl ethyl-, butyl-, or hexylmalonate (0.1 mol) was added. An alkyl bromide (0.1 mol) was then added into the resulting solution. The reaction mixture was heated under reflux for 5 hr and worked up as previously described to yield a disubstituted malonic ester (50-70% yield).

The diethyl dialkylmalonate (50 mmol) thus obtained was refluxed with 50% aqueous potassium hydroxide solution (200 ml) for 8-12 hr. The reaction mixture was worked up to give a dialkylmalonic acid which was decarboxylated at 180°. Distillation or recrystallization from acetone of the crude product yielded a pure 2-substituted carboxylic acid (80-90% yield). The ir spectra (Nujol or film) of these acids generally showed maximum absorptions at about 3400-2100, 1700, 1420, 1290, 1240, and 950  $\text{cm}^{-1}$ . The nmr spectra ( $\text{CCl}_4$ ) generally showed signals at about  $\delta$  0.91 (t,  $\text{CH}_3$ ), 1.29-1.89 (m,  $\text{CH}_2$ ), 2.30 (m, CH), and 12.18 ppm (s,  $\text{CO}_2\text{H}$ ). Elemental analysis data were within  $\pm 0.4\%$  of the theoretical values. All carboxylic acids described herein are racemic compounds; the prefix *dl* is omitted. Table I shows the physical properties of the 2-substituted carboxylic acids.

**Bioassay Methods.** First-instar larvae of *C. p. quinquefasciatus* were used in assessing the biological activity of 2-substituted carboxylic acids. Twenty larvae, less than 24-hr old, were placed in Pyrex custard dishes containing 200 ml of tap water. The larvae were fed with a mixture of ground rabbit pellets and yeast (3:1). The larval dishes were placed in a room at a temperature of  $27 \pm 1^\circ$  and a photoperiod of 14 hr. The loss of water was replenished every other day. The testing compounds were dissolved in acetone and serially diluted. No more than 1 ml of these solutions was added to the test containers. Checks were treated with equal volumes of acetone only. The bioassays were continued until adult emergence and repeated at least twice with three replicates in each test. The level of activity was determined by the magnitude of emergence. Mean per cent emergence was plotted against concentration (parts per million) on log-probit paper, and the points were fitted with a straight line from which lethal concentrations (parts per million) for 50 and 90% inhibition of emergence ( $\text{LC}_{50}$  and  $\text{LC}_{90}$ ) were determined.

### RESULTS AND DISCUSSION

The biological activity of the synthesized 2-ethyl-, 2-butyl-, and 2-hexyl-substituted aliphatic carboxylic acids against larvae of *C. p. quinquefasciatus* is listed in Table I. The biological activity is expressed as  $\text{LC}_{50}$  and  $\text{LC}_{90}$  in parts per million.

Seven 2-ethyl-substituted carboxylic acids were synthesized, ranging from 2-ethyldecanoic acid (1) to 2-ethyldo-

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Table I. Physical Properties and Biological Activity of 2-Substituted Aliphatic Carboxylic Acids

Compd	R	R'	$\begin{array}{c} \text{R} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{R}' \end{array} \text{CHCO}_2\text{H}$		Mp, °C		Concn, ppm	
			Bp, °C (mm)	Lit.	Obsd	Lit.	LC <sub>50</sub>	LC <sub>90</sub>
1	C <sub>2</sub> H <sub>5</sub>	<i>n</i> -C <sub>8</sub> H <sub>17</sub>	119–121 (0.2)	105–108 (0.5) <sup>a</sup>			18.0	44.0
2	C <sub>2</sub> H <sub>5</sub>	<i>n</i> -C <sub>10</sub> H <sub>21</sub>	125–128 (0.2)	130 (0.15) <sup>b</sup>			8.5	23.0
3	C <sub>2</sub> H <sub>5</sub>	<i>n</i> -C <sub>12</sub> H <sub>25</sub>	154–160 (0.3)	178–179 (3) <sup>c</sup>			7.8	17.0
4	C <sub>2</sub> H <sub>5</sub>	<i>n</i> -C <sub>14</sub> H <sub>29</sub>	165–170 (0.4)	167–170 (2.5) <sup>c</sup>	36–36.5	23–24 <sup>c</sup>	8.4	17.0
5	C <sub>2</sub> H <sub>5</sub>	<i>n</i> -C <sub>16</sub> H <sub>33</sub>	172–174 (0.25)	182 (0.5) <sup>d</sup>	45–46	49–49.5 <sup>d</sup>	2.2	6.2
6	C <sub>2</sub> H <sub>5</sub>	<i>n</i> -C <sub>18</sub> H <sub>37</sub>	180–182 (0.06)		53–54		>25.0	>25.0
7	C <sub>2</sub> H <sub>5</sub>	<i>n</i> -C <sub>20</sub> H <sub>41</sub>	208–212 (0.12)		56–57		>25.0	>25.0
8	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	103–106 (0.15)				25.0	>25.0
9	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	<i>n</i> -C <sub>8</sub> H <sub>17</sub>	139–140 (0.4) <sup>e</sup>	132 (0.2) <sup>b</sup>			8.6	38.0
10	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	<i>n</i> -C <sub>10</sub> H <sub>21</sub>	160–161 (0.4) <sup>e</sup>	175–176 (3) <sup>c</sup>			6.4	13.0
11	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	<i>n</i> -C <sub>12</sub> H <sub>25</sub>	170–172 (0.1)	180–184 (4) <sup>c</sup>	27–28	23–24 <sup>c</sup>	6.0	13.0
12	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	<i>n</i> -C <sub>14</sub> H <sub>29</sub>	185–186 (0.15)		39–40	39.1–39.6 <sup>f</sup>	34.0	>25.0
13	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	<i>n</i> -C <sub>16</sub> H <sub>33</sub>	189–192 (0.07)	197 (0.5) <sup>d</sup>	48–48.5	49.5–50 <sup>d</sup>	>50.0	>50.0
14	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	<i>n</i> -C <sub>18</sub> H <sub>37</sub>	203–204 (0.07)		54–55		32.0	>50.0
15	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	132–135 (0.35)	120 (0.1) <sup>b</sup>			4.8	18.0
16	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	<i>n</i> -C <sub>8</sub> H <sub>17</sub>	130 (0.05)	165–168 (2) <sup>c</sup>			3.8	10.0
17	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	<i>n</i> -C <sub>10</sub> H <sub>21</sub>	178–181 (0.6)	182–184 (0.5) <sup>c</sup>			5.0	22.0
18	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	<i>n</i> -C <sub>12</sub> H <sub>25</sub>	173–175 (0.12)		36–36.5	36–36.1 <sup>f</sup>	18.5	>50
19	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	<i>n</i> -C <sub>14</sub> H <sub>29</sub>	180–181 (0.05)		45–46	44.6–45.5 <sup>f</sup>	40.0	>25
20	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	<i>n</i> -C <sub>16</sub> H <sub>33</sub>	195–196 (0.05)	210 (0.5) <sup>d</sup>	52–52.5	52.8–53.5 <sup>d</sup>	7.4	25

<sup>a</sup> Nikishin *et al.*, 1961. <sup>b</sup> Asinger *et al.*, 1963. <sup>c</sup> Stanley *et al.*, 1929. <sup>d</sup> Weitzel and Wojahn, 1950. <sup>e</sup> Hwang *et al.*, 1974. <sup>f</sup> Breusch and Ulusoy, 1953.

cosanoic acid (7). The activity of this series of carboxylic acids increased as the length of the main chain increased. The lowest homolog, 2-ethyldecanoic acid (1), exhibited some activity; however, the LC<sub>50</sub> was greater than 10 ppm. 2-Ethyltridecanoic (2), 2-ethyltetradecanoic (3), and 2-ethylhexadecanoic (4) acids showed increased activity with LC<sub>50</sub> below 10 ppm and LC<sub>90</sub> below 25 ppm. The greatest activity of this series of compounds was shown by 2-ethyltetradeconoic acid (5) being the most effective of all acids synthesized and evaluated. Biological activity of this acid (5) against first-instar larvae of *C. p. quinquefasciatus* was also reported elsewhere (Ikeshoji and Mulla, 1974b). The results of these two tests agree with each other. The activity of 2-ethyl-substituted carboxylic acids then suddenly decreased as the length of the main chain increased further. 2-Ethyleicosanoic (6) and 2-ethyltetracosanoic (7) acids both showed LC<sub>50</sub> greater than 25 ppm.

The 2-butyl-substituted carboxylic acids synthesized ranged from 2-butyldecanoic acid (8) to 2-butyleicosanoic acid (14). 2-Butyldecanoic acid (8) did not show good activity as was the case with 2-ethyldecanoic acid (1). Similarly, the activity of this series of acids increased sharply as the length of the main chain increased. 2-Butyltridecanoic acid (9) showed somewhat greater biological activity than the acid 8, but 2-butyltridecanoic (10) and 2-butyltetradecanoic (11) acids exhibited the greatest activity among the 2-butyl-substituted acids. Biological activity of the acids 9 and 10 against larvae of *C. p. quinquefasciatus*, *C. tarsalis* Coquillett, *Anopheles albimanus* Wiedemann, and *A. aegypti* was also reported elsewhere (Ikeshoji and Mulla, 1974b; Hwang *et al.*, 1974), which agreed with the present studies. The activity of this series of acids then diminished drastically as the main chain length increased further; 2-butylhexadecanoic (12) and 2-butyltetradeconoic (13) acids showed the least activity in this series. The highest homolog, 2-butyleicosanoic acid (14), however, showed greater activity than the acid 13.

2-Hexyl-substituted carboxylic acids showed the same relationship between activity and main chain length. This series of acids ranged from 2-hexyldecanoic acid (15) to 2-hexyltetradeconoic acid (20). Although 2-butyldecanoic acid (2-hexylhexanoic acid (8)), which could also be classified as a 2-hexyl-substituted carboxylic acid, did not possess good activity, its three higher homologs, 2-hexyltridecanoic (15), 2-hexyltridecanoic (16), and 2-hexyltridecanoic (17) acids, showed good activity. Of these three acids, the acid 16 exhibited the greatest activity, LC<sub>50</sub> and LC<sub>90</sub> being about 4 and 10 ppm, respectively. As the main chain length increased, the activity started to decline in 2-hexyltridecanoic acid (18) and reached the lowest level in 2-hexylhexadecanoic acid (19). It was interesting to note that the activity increased again as the main chain length increased to 2-hexyltetradeconoic acid (20).

Figure 1 shows the structure-activity relationship of the three series of 2-substituted carboxylic acids. All three series of carboxylic acids showed the same trend. These carboxylic acids having a total number of carbon atoms from 14 to 18, in general, exhibited good biological activity. The only exception was 2-ethyltetradeconoic acid (5) which had 20 carbon atoms but showed high activity. Excluding this acid, the activity appeared to be dependent upon the total number of carbon atoms in the carboxylic acids.

The size of substituents on the carboxylic acids did not affect the activity significantly. As far as these three series of acids were concerned, there was no indication that any of the series of acids studied possessed better activity than the others. However, 2-methyl-substituted aliphatic carboxylic acids were reported to have very weak activity (Ikeshoji and Mulla, 1974b; Hwang *et al.*, 1974).

In comparing the activity of the 2-substituted carboxylic acids having the same number of carbon atoms and in considering these carboxylic acids as derivatives of straight chain hydrocarbons with carboxyl group attached

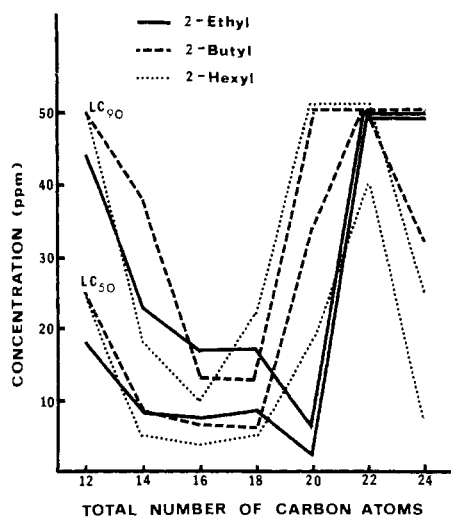


Figure 1. Structure-activity relationship of 2-substituted aliphatic carboxylic acids.

to the 3, 5, or 7 position in the hydrocarbon chain, the position of carboxyl substitution did not influence the activity markedly. For instance, 2-ethyltetradecanoic (3), 2-butyl dodecanoic (10), and 2-hexyldecanoic (16) acids exhibited almost the same level of activity regardless of the position of the carboxyl group. However, being more active than its structural isomers, 2-butylhexadecanoic (12) and 2-hexyltetradecanoic (18) acids, 2-ethyloctadecanoic acid (5) was again an exception to this regularity.

Since the 2-substituted carboxylic acids are insoluble in water, they form a thin-layer film on the top of water in the custard dishes during the bioassay tests. It was reported that the activity of 3-methyloctadecanoic and 2,3-dimethyloctadecanoic acids could be enhanced manifold by using emulsifiable preparations (Ikeshoji and Mulla, 1974b). It is, therefore, likely that the activity of the most active carboxylic acids studied here may be further enhanced by formulating them in suitable solvents and surface active agents.

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## Implication of Mycotoxins for Human Health

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The veterinary problem, Turkey X disease, led to the discovery of aflatoxins and to studies which demonstrated that toxins in the absence of visible molds could produce effects (carcinogenesis and organ damage) far removed in time from the cause. Only a few cases of acute mycotoxicoses in humans have been recorded. To shed some light on the risk to man from ingestion of aflatoxins in his food supply, published data on comparative metabolism, primate studies, inadvertant human

incidents, and epidemiology are analyzed and presented in common terms to facilitate inter-comparison. Although the data raise some questions, they presently provide the best available basis for estimating risk. The greatest attention has been given to aflatoxins, but other mycotoxins potentially capable of causing damage on chronic ingestion have also been considered. Among them are patulin, penicillic acid, trichothecenes, luteoskyrin, and citrinin.

The association of microbial toxins in foods or feed with human and animal disease has generally been based on symptoms occurring shortly after ingestion, providing a readily ascertainable cause-and-effect relationship. There are not many such situations involving molds. The first followed fast on the early developments in the field of microbiology. Ergot alkaloids and ergot poisoning, the first mold toxin and mycotoxicosis so recorded, were associated with growth of the mold *Claviceps purpurea* as early as

1711 (Barger, 1931). More than 200 years later two epidemic incidents occurred within a few years of each other. In Japan the "yellow rice disease," which caused many deaths, was associated with invasion of the rice by a number of molds including *Penicillium islandicum*, *P. citrinum*, and *P. citreo-viride* (Saito *et al.*, 1971; Uruguchi, 1971); in Russia, an even more severe loss of life was caused by ingestion of grain which had been invaded by *Fusarium tricinctum* (= *F. sporotrichioides*) (Joffe, 1971).

Both incidents were caused by dislocation of normal food supplies due to wartime situations: rice from South-east Asia stored under poor conditions was imported into Japan in unusually large quantities to make up for the Japanese production deficit; grain, which could not be harvested the previous fall because all available Russian manpower was at the fighting front, was salvaged from

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