Overcrowding Factors of Mosquito Larvae. VIII. Structure–Activity Relationship of Methyl 2-Alkylalkanoates against Mosquito Larvae

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Twenty methyl 2-ethylalkanoates, methyl 2-butylalkanoates, and methyl 2-hexylalkanoates, with even numbers of carbon atoms and a total number of carbon atoms from 12 to 24 in their acid moieties, were prepared and bioassayed against first instar *Culex pipiens quinquefasciatus* Say. The methyl 2-alkylalkanoates with a total number of carbon atoms from 14 to 22, in general, showed a high level of biological activity in inhibiting adult emergence. With a few exceptions, the biological activity was dependent upon the total number of carbon atoms in the acid moiety of the esters. The activity of the methyl 2-alkylalkanoates was invariably higher than that of the corresponding 2-alkylalkanoic acids. The increase in activity by esterification was more distinct for the less active acids than for the more active acids. These compounds were much more effective than petroleum oils routinely used in mosquito control programs.

The overcrowding factors of mosquito larvae, consisting of branched-chain carboxylic acids and branched-chain hydrocarbons, are produced by older larvae of the southern house mosquito *Culex pipiens quinquefasciatus* Say in overcrowded cultures. These factors play a role in regulating populations of younger larvae by either killing them or suppressing their development (Ikeshoji and Mulla, 1970a,b, 1974a; Hwang et al., 1976).

Investigations on the biological activity of various branched-chain carboxylic acids revealed that 2- and 3-alkylalkanoic acids manifested a high level of activity (Ikeshoji and Mulla, 1974b; Hwang et al., 1974a). Systematic studies on the structure–activity relationship of 2-alkylalkanoic acids showed that 2-ethyl-, 2-butyl-, and 2-hexylalkanoic acids with a total number of carbon atoms from 14 to 18 generally exhibited good activity (Hwang et al., 1974b).

Some of the analogues of the overcrowding factors show good biological activity in the range of 0.5–5 parts per million (ppm) (Hwang et al., 1974a,b, 1976; Ikeshoji and Mulla, 1974b), whereas the effective rates of petroleum oils routinely used for mosquito control are in the order of 20–50 ppm (Hagstrum and Mulla, 1968). The overcrowding factors are much more active than petroleum oils and thus offer an additional tool for possible control of pest and vector mosquitoes.

To find more active compounds related to the overcrowding factors, we embarked upon the synthesis of various derivatives of the alkylalkanoic acids for biological studies. Here we report the biological activity and structure–activity relationship of methyl 2-alkylalkanoates against first instar larvae of *C. p. quinquefasciatus*.

EXPERIMENTAL SECTION

2-Ethyl-, 2-butyl-, and 2-hexylalkanoic acids having even numbers of carbon atoms and a total number of carbon atoms from 12 to 24 were synthesized according to the procedure of Hwang et al. (1974a,b). These 2-alkylalkanoic acids were esterified with diazomethane in ether. After removal of ether and excess diazomethane, the crude products were distilled in vacuo to yield pure methyl 2-alkylalkanoates.

First-instar larvae of *C. p. quinquefasciatus* were used in assessing the biological activity of the methyl 2-alkylalkanoates. The bioassay procedure was reported by Hwang et al. (1974a,b). Briefly, 20 larvae were placed in 200 ml of water in 11-cm diameter glass custard dishes and fed with a mixture of ground rabbit pellets and yeast. Serially diluted acetone solutions of the methyl 2-alkylalkanoates were added to the dishes which were then kept at 27 ± 1 °C under a photoperiod of 14 h. The bioassays were continued until adult emergence. The tests were run in duplicate and repeated at least twice. Checks were treated with an equal volume of acetone and were run in duplicate in each experiment. Mean percent emergence was plotted against concentration in parts per million on log-probit paper, and the points (3–4 points) were fitted with a straight line from which lethal concentrations in part per million inhibiting the emergence of 50% of the population (LC_{50}) were determined. Slopes of probit regression lines were also calculated (Finney, 1952).

RESULTS AND DISCUSSION

Table I shows uncorrected boiling points of the methyl 2-alkylalkanoates. The ir spectra of these esters generally showed maximum absorption at 1740 and 1175 cm^{-1} (film). Elemental analysis data were within ±0.4% of the theoretical values. GLC analysis of the esters showed at least 95% purity. All methyl 2-alkylalkanoates, except methyl 2-hexyloctanoate, are racemic compounds; the prefix *dl* is omitted.

Twenty methyl 2-alkylalkanoates synthesized were studied for biological activity against first instar larvae, and the developmental events were followed to the time of emergence (Table I). Methyl 2-ethyldecanoate (**1**), methyl 2-ethyldodecanoate (**2**), methyl 2-ethyltetradecanoate (**3**), and methyl 2-ethylhexadecanoate (**4**) showed moderate activity. Methyl 2-ethyloctadecanoate (**5**) exhibited the greatest activity among the methyl 2-ethylalkanoates. As the molecular weight of the esters increased, the activity drastically decreased in methyl 2-ethylicosanoate (**6**) and methyl 2-ethyldocosanoate (**7**).

Methyl 2-butyloctanoate (**8**) did not show good activity; however, the activity increased as the molecular weight increased. Thus, methyl 2-butyldecanoate (**9**) and methyl 2-butyldodecanoate (**10**) were more active than their lower homologue. The activity increased further in methyl 2-butyltetradecanoate (**11**), methyl 2-butylhexadecanoate (**12**), and methyl 2-butyloctadecanoate (**13**). The latter three esters were the most active among all compounds tested. Thereafter, the activity suddenly diminished when the molecular weight increased to methyl 2-butylicosanoate (**14**).

The lowest homologue of the methyl 2-hexylalkanoates was methyl 2-hexylhexanoate (**8**, methyl 2-butyloctanoate)

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Table I. Boiling Points and Biological Activity of Methyl 2-Alkylalkanoates

Compds	R	$\begin{matrix} R \\ \diagdown \\ \diagup \\ R' \end{matrix}$CHCO$_2CH_3$ R′	Bp, °C (mm)	LC_{50}, ppm	Slope of probit regression lines[a]
1	C_2H_5	n-C_8H_{17}	81-82 (0.6)	5.5	3.2
2	C_2H_5	n-$C_{10}H_{21}$	99-101 (0.43)	2.5	1.7
3	C_2H_5	n-$C_{12}H_{25}$	126-128 (0.5)	3.0	2.3
4	C_2H_5	n-$C_{14}H_{29}$	143-144 (0.5)	5.0	2.9
5	C_2H_5	n-$C_{16}H_{33}$	160 (0.4)	1.5	1.2
6	C_2H_5	n-$C_{18}H_{37}$	173-175 (0.45)	10.0	
7	C_2H_5	n-$C_{20}H_{41}$	188-191 (0.4)	>20.0	
8	n-C_4H_9	n-C_6H_{13}	67-70 (0.35)	10.0	5.0
9	n-C_4H_9	n-C_8H_{17}	87-91 (0.4)	2.5	3.4
10	n-C_4H_9	n-$C_{10}H_{21}$	114-116 (0.55)	3.5	2.8
11	n-C_4H_9	n-$C_{12}H_{25}$	152-154 (0.6)	0.6	1.1
12	n-C_4H_9	n-$C_{14}H_{29}$	153-154 (0.4)	0.9	1.2
13	n-C_4H_9	n-$C_{16}H_{33}$	167-170 (0.4)	0.5	1.0
14	n-C_4H_9	n-$C_{18}H_{37}$	186-189 (0.4)	>20.0	
15	n-C_6H_{13}	n-C_6H_{13}	84-86 (0.3)	2.6	1.7
16	n-C_6H_{13}	n-C_8H_{17}	109-112 (0.3)	1.0	1.2
17	n-C_6H_{13}	n-$C_{10}H_{21}$	128-130 (0.35)	1.0	1.8
18	n-C_6H_{13}	n-$C_{12}H_{25}$	146-148 (0.3)	2.5	1.4
19	n-C_6H_{13}	n-$C_{14}H_{29}$	165-166 (0.3)	1.5	1.5
20	n-C_6H_{13}	n-$C_{16}H_{33}$	170-174 (0.4)	1.0	1.0

[a] Finney (1952).

which did not show good activity as described previously. However, as the molecular weight increased, the activity increased in methyl 2-hexyloctanoate (15), methyl 2-hexyldecanoate (16), and methyl 2-hexyldodecanoate (17). The activity decreased somewhat in methyl 2-hexyltetradecanoate (18) but increased again in methyl 2-hexylhexadecanoate (19) and methyl 2-hexyloctadecanoate (20).

As in the case of 2-alkylalkanoic acids (Hwang et al., 1974b), the biological activity of the methyl 2-alkylalkanoates was significantly influenced by the molecular weight of the compounds.

Figure 1 shows the structure–activity relationship of the three series of methyl 2-alkylalkanoates. In general, those methyl 2-alkylalkanoates with a total number of carbon atoms from 14 to 22 in their acid moieties showed better activity than other esters. However, methyl 2-ethylicosanoate (6) had 22 carbon atoms in its acid moiety but did not show a high level of activity. On the other hand, methyl 2-hexyloctadecanoate (20) possessed 24 carbon atoms but exhibited good activity. Excluding these exceptions, the biological activity appeared to be dependent upon the total number of carbon atoms in the acid moiety of the methyl 2-alkylalkanoates.

The size of substituents on the acid moiety of the esters in some cases affected the activity significantly. In comparing the activity of the esters having the same number of carbon atoms in their acid moieties, the methyl 2-alkylalkanoates with 14, 16, and 20 carbon atoms in their acid moieties showed almost the same level of activity among their structural isomers. The activity of the esters with 18 carbon atoms varied somewhat, 2-butyl (11) and 2-hexyl isomers (17) being more active than the 2-ethyl isomer (4). Similarly, among C-22 esters, methyl 2-butyloctadecanoate (13) and methyl 2-hexylhexadecanoate (19) were more active than their structural isomer, methyl 2-ethylicosanoate (6). Among C-24 esters, methyl 2-hexyloctadecanoate (20) was more active than its isomers, methyl 2-ethyldocosanoate (7) and methyl 2-butylicosanoate (14). In considering these esters as derivatives of straight-chain alkanes with a methoxycarbonyl group attached to the C-3, C-5, and C-7 positions of the alkane chain, the position of methoxycarbonyl substitution influenced the activity markedly in methoxycarbonylheptadecanes (4, 11, and 17), methoxycarbonylhenicosanes (6, 13, and 19), and methoxycarbonyltricosanes (7, 14, and 20). In these cases, the C-5 and C-7 substitutions were more effective in contributing to a high level of activity than the C-3 substitution.

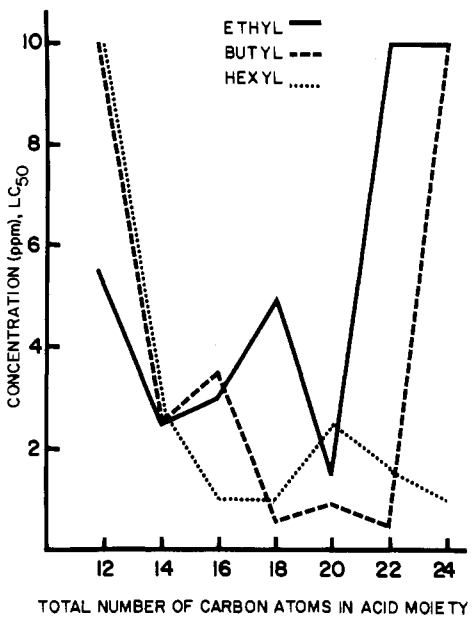

Figure 1. Structure-activity relationship of methyl 2-alkylalkanoates.

Figures 2–4 show comparative biological activity of methyl 2-ethylalkanoates, methyl 2-butylalkanoates, and methyl 2-hexylalkanoates with their corresponding 2-alkylalkanoic acids (Hwang et al., 1974b). In every case, the esters were more active than the corresponding acids. The increase in biological activity on esterification of 2-alkylalkanoic acids to methyl alkylalkanoates was more apparent in the less active acids than in the more active acids. For example, when active 2-ethyloctadecanoic acid was converted to its methyl ester, the LC_{50} value decreased from 2.2 to 1.5 ppm (see Figure 2). In contrast, the activity of 2-butyloctadecanoic acid (LC_{50} >50 ppm) was increased

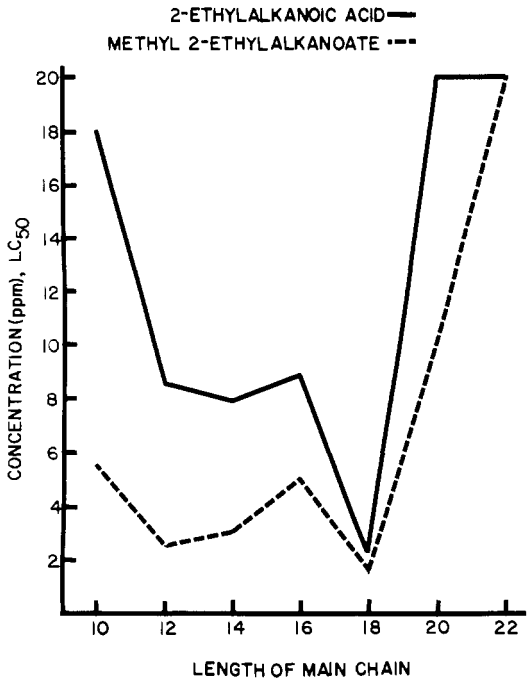

Figure 2. Comparison of the activity of 2-ethylalkanoic acids and their methyl esters.

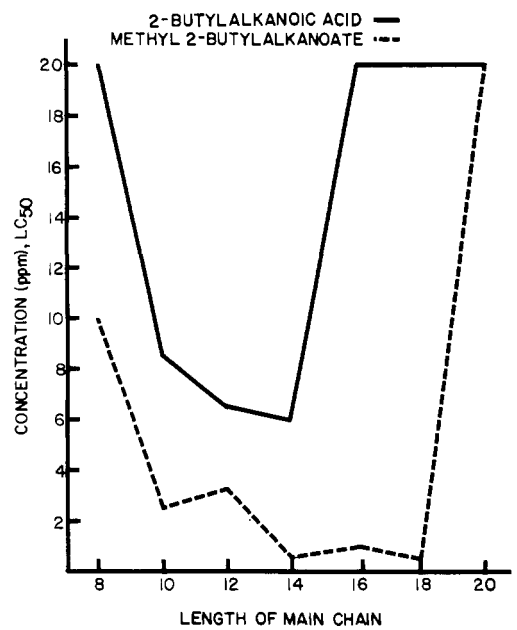

Figure 3. Comparison of the activity of 2-butylalkanoic acids and their methyl esters.

markedly by esterification, resulting in obtaining active methyl 2-butyloctadecanoate (LC_{50} 0.5 ppm) (see Figure 3).

When comparing methyl 2-ethylalkanoates with their corresponding 2-ethylalkanoic acids (Figure 2), the pattern of the activity of the esters generally corresponded to those of the acids. The activity of the methyl 2-butylalkanoates and the methyl 2-hexylalkanoates, however, deviated from that of their parent acids after the length of the main chain reached 14 and 12, respectively (Figures 3 and 4).

Understandably, a compound with a smaller LC_{50} value and a larger value in the slope of probit regression line is

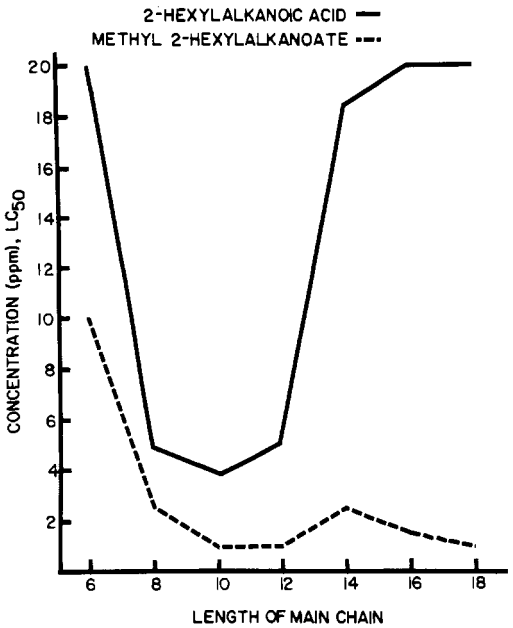

Figure 4. Comparison of the activity of 2-hexylalkanoic acids and their methyl esters.

a better larvicide. This is because a compound with a larger value in the slope of probit regression line shows higher mortality at a lower concentration than a compound with a smaller value. For example, compound **17** (LC_{50} 1 ppm) was more active than compound **20** (LC_{50} 1 ppm) because the former shows a larger value in the slope than the latter. Both the LC_{50} and the slope of probit regression line, therefore, serve as useful criteria in determining the activity of these compounds.

The esterification of 2-alkylalkanoic acids produced more active methyl 2-alkylalkanoates. The process also transformed inactive acids into active esters. Because these substances are considerably stable in experimental media and show much higher activity than most of the petroleum hydrocarbon mosquito larvicides currently in use (Hagstrum and Mulla, 1968), it is possible to utilize them for the control of mosquito larvae in the future.

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LITERATURE CITED

Finney, D. J., "Probit Analysis", Cambridge University Press, London, 1952, pp 20–27.
Hagstrum, D. W., Mulla, M. S., *J. Econ. Entomol.* **61**, 220 (1968).
Hwang, Y.-S., Mulla, M. S., Arias, J. R., *J. Agric. Food Chem.* **22**, 400 (1974a).
Hwang, Y.-S., Mulla, M. S., Arias, J. R., *J. Agric. Food Chem.* **22**, 1004 (1974b).
Hwang, Y.-S., Mulla, M. S., Arias, J. R., Majori, G., *J. Agric. Food Chem.* **24**, 160 (1976).
Ikeshoji, T., Mulla, M. S., *J. Econ. Entomol.* **63**, 90 (1970a).
Ikeshoji, T., Mulla, M. S., *J. Econ. Entomol.* **63**, 1737 (1970b).
Ikeshoji, T., Mulla, M. S., *Environ. Entomol.* **3**, 482 (1974a).
Ikeshoji, T., Mulla, M. S., *Environ. Entomol.* **3**, 487 (1974b).

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