similar to that of 4,6-octadien-3-one (Buttery, 1978), but sufficient differences exist to make the assignment doubtful. The other, at 71.06 min, appears to be a monounsaturated compound, possibly 2-octen-4-one.

Pyrrole is the only nitrogen-containing constituent fully identified. A peak at 120.85 min contains two compounds, one of which is definitely biphenyl. When the biphenyl MS is subtracted from the combined MS, a MS identical with that of indole remains. However, indole's retention time is approximately 3 min longer. The m/e intensities for 116, 117, and 118 are 11, 100, and 11%, respectively, ruling out the related benzyl cyanide and isocyanide, as well as the methyl cyano- and isocyanobenzenes, which typically have 116 intensities equal to 40–80% of the 117 intensity. The preparation of isoindole has recently been reported (Bonnett et al., 1973), but besides showing considerably lower stability than indole, it reportedly has an m/e 118 intensity that is 26% of the 117 intensity.

Preliminary bioassay results were largely inconclusive. Some indication of female moth preference for corn silk concentrate during oviposition was noted on occasion, but responses were inconsistent. Future testing design will include correction of several problem areas. The number of samples, including blanks, will be reduced to two, or at most three. The closed chamber, which rapidly became permeated with the silk volatiles, will be replaced with a larger laminar flow chamber, and the question of insect food preference "imprinting" during larval growth stages will be considered.

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Overcrowding Factors of Mosquito Larvae. 11. Biological Activity of 2-Halooctadecanoic Acids and Alkyl 2-Halooctadecanoates against Mosquito Larvae

Yih-Shen Hwang,* H. A. Navvab-Gojrati,[1] and Mir S. Mulla

As part of our studies on the synthesis of active analogues of the overcrowding factors of mosquito larvae, 2-chloro-, 2-bromo-, and 2-iodooctadecanoic acids and their methyl, ethyl, and isopropyl esters were synthesized and evaluated for their larvicidal activity against first instars of the southern house mosquito, *Culex pipiens quinquefasciatus* Say. Based on the evaluation, the structure–activity relationship of the compounds was investigated. 2-Bromooctadecanoic acid and its methyl, ethyl, and isopropyl esters were the most active larvicides over 2-chloro- and 2-iodooctadecanoic acids and their esters. The activity of the 2-halogenated carboxylic acids and esters, except for the 2-bromo analogues, generally declined in the order of acids, methyl, ethyl, and isopropyl esters. The activity did not have clear-cut relationships with the van der Waals radii and electronegativities of the halogen atoms attached to the acids and esters.

In our search for finding active analogues of the overcrowding factors of mosquito larvae, we previously reported the synthesis and structure–activity relationship of 2-alkylalkanoic acids and 3-methylalkanoic acids and their esters against first-instar larvae of the southern house mosquito *Culex pipiens quinquefasciatus* Say (Hwang, 1976; Hwang et al., 1974a,b, 1976, 1978). These studies revealed that alkanoic acids and esters with certain main-chain lengths and with an ethyl, butyl, or hexyl group at the C-2 position or a methyl group at the C-3 position in the carbon chain manifested a high level of larvicidal activity (Hwang and Mulla, 1976a).

In investigating bromine analogues of the overcrowding factors, we found that 2-bromoalkanoic acids from C-14

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to C-18 and methyl 2-bromoalkanoates from C-10 to C-18 showed a high level of activity, thus demonstrating once again the relationship between the biological activity and the lengths of the carbon chain in carboxylic acids (Hwang and Mulla, 1976b). Among the acids and their methyl esters, 2-bromooctadecanoic acid and methyl 2-bromooctadecanoate possessed the highest activity against mosquito larvae. In connection with this work, we synthesized other halogen analogues of 2-bromooctadecanoic acid and their methyl, ethyl, and isopropyl esters and investigated the larvicidal activity of these compounds. Here we report the biological activity and structure–activity relationship of 2-chlorooctadecanoic acid, 2-bromooctadecanoic acid, and 2-iodooctadecanoic acid and their methyl, ethyl, and isopropyl esters against first-instar larvae of *C. pipiens quinquefasciatus*.

EXPERIMENTAL SECTION

Synthesis. Previously, 2-bromoalkanoic acids and their esters were prepared by the modified Hell–Volhard–Zelinsky reaction from their corresponding alkanoic acids (Cason et al., 1953; Hwang and Mulla, 1976b). However, this procedure not only suffered from relatively low yield but gave exclusively 2-halogenated products only in the case of bromination, variable selectivity in chlorination, and no reaction at all in iodination (Fieser and Fieser, 1961; March, 1968). In the present studies, a more efficient procedure for the α-halogenation of acyl halides was adopted using *N*-bromosuccinimide (NBS), *N*-chlorosuccinimide (NCS), and molecular iodine as halogenating agents and thionyl chloride as solvent. Although NBS and NCS are known as efficient halogenating agents, their usage in direct halogenation of acyl chlorides had not been reported until Harpp et al. (1975) disclosed that α-halogenation of hexanoyl chloride and other low-molecular-weight acyl chlorides could be facilitated by these agents. In the present work, this procedure was used in α-halogenation of octadecanoyl chloride.

All melting points and boiling points were uncorrected. All 2-halooctadecanoic acids and their methyl, ethyl, and isopropyl esters are racemates; for convenience, the prefix *dl* is omitted. These compounds were more than 95% pure in GLC analysis on a silicon gum rubber UCC-W 982 column. Elementary microanalyses were conducted by Chemalytics, Inc., Tempe, Ariz.

2-Chlorooctadecanoic Acid. Octadecanoic acid (28.4 g, 0.1 mol) and thionyl chloride (28.8 mL, 0.4 mol) were stirred and heated under reflux for 30 min. To the cooled reaction mixture containing octadecanoyl chloride, NCS (26.7 g, 0.2 mol), thionyl chloride (20 mL), and concentrated hydrochloric acid (7 drops) were added successively. The mixture was then heated under reflux for 2 h. The solvent was removed, and the solid (byproduct succinimide) was filtered and washed with carbon tetrachloride. The filtrate was concentrated and distilled in vacuo to give 2-chlorooctadecanoyl chloride (29.9 g, 89% yield): bp 178–179 °C (0.5 mm); IR (neat) 1790 cm^{-1}; NMR (neat) δ 4.49 (t, 1, J = 6 Hz, CHClCOCl).

2-Chlorooctadecanoyl chloride (17.9 g) was stirred overnight with water (100 mL) at room temperature. The separated solid was collected and recrystallized from ethanol or petroleum ether to give pure 2-chlorooctadecanoic acid (12.2 g, 81% yield): mp 64.5–65.5 °C [lit. mp 66 °C (Guest, 1947)]; IR (CCl_4) 3300–2500 and 1735 cm^{-1}.

Anal. Calcd for $C_{18}H_{35}O_2Cl$: C, 67.79; H, 11.06; Cl, 11.12. Found: C, 67.58; H, 10.91; Cl, 11.05.

Methyl, Ethyl, and Isopropyl 2-Chlorooctadecanoates. 2-Chlorooctadecanoyl chloride (4 g each) was separately poured into methanol, ethanol, and isopropyl alcohol (50 mL each). The mixtures were stirred overnight at room temperature. The alcohols were removed. The residues were dissolved in ether (50 mL each) and washed successively with an aqueous sodium bicarbonate solution and water three times each. After drying over sodium sulfate, the ether solutions were evaporated to yield almost quantitatively methyl 2-chlorooctadecanoate: bp 140–143 °C (20 μm) [lit. bp 190–200 °C (6 mm) (Guest and Goddard, 1944)]; IR (neat) 1755 cm^{-1}; ethyl 2-chlorooctadecanoate: bp 153–155 °C (60 μm); IR (neat) 1760 cm^{-1}; and isopropyl 2-chlorooctadecanoate: bp 160–162 °C (60 μm); IR (neat) 1750 cm^{-1}.

2-Bromooctadecanoic Acid. Octadecanoyl chloride was prepared as described above from octadecanoic acid (28.4 g, 0.1 mol), carbon tetrachloride (10 mL), and thionyl chloride (28.8 mL, 0.4 mol). To the cooled reaction mixture, NBS (21.4 g, 0.12 mol), carbon tetrachloride (50 mL), and 48% hydrobromic acid (7 drops) were added successively. The mixture was refluxed and worked up as previously described to give 2-bromooctadecanoyl chloride [NMR (neat) δ 4.50 (t, 1, J = 6 Hz, CHBrCOCl)], which, without purification, was treated with water to give 2-bromooctadecanoic acid (30.8 g, 85% yield): mp 55–57 °C [from C_2H_5OH, lit. mp 60 °C (Ucciani et al., 1967)]; IR (Nujol) 3300–2500 and 1710 cm^{-1}.

Anal. Calcd for $C_{18}H_{35}O_2Br$: C, 59.49; H, 9.71; Br, 21.99. Found: C, 59.28; H, 9.90; Br, 21.75.

Methyl, Ethyl, and Isopropyl 2-Bromooctadecanoates. 2-Bromooctadecanoyl chloride (4 g each) was separately treated with methanol, ethanol, and isopropyl alcohol (50 mL each) to yield almost quantitatively methyl 2-bromooctadecanoate [bp 155–157 °C (0.2 mm); lit. bp 160–162 °C (0.2 mm) (Ucciani et al., 1967)]; IR (neat) 1750 cm^{-1}], ethyl 2-bromooctadecanoate [bp 173–177 °C (1.6 mm); IR (neat) 1745 cm^{-1}], and isopropyl 2-bromooctadecanoate [bp 177–178 °C (1.5 mm); IR (neat) 1750 cm^{-1}].

2-Iodooctadecanoic Acid. A mixture of octadecanoic acid (28.4 g, 0.1 mol) and thionyl chloride (60 mL, 0.83 mol) was stirred and heated under reflux for 2 h. To the cooled mixture, iodine (15.23 g, 0.12 g-atom) was added. The reaction mixture was again stirred and refluxed for 4 h. Thionyl chloride was removed by evaporation, and the excess iodine was filtered and washed with carbon tetrachloride. The filtrate was washed five times with an aqueous sodium thiosulfate solution and dried over sodium sulfate. Removal of the solvent gave 2-iodooctadecanoyl chloride [NMR (neat) δ 4.63 (t, 1, J = 7 Hz, CHICOCl)], which, without purification, was treated with water to give 2-iodooctadecanoic acid (33.2 g, 81% yield): mp 64–66 °C [from petroleum ether, lit. mp 67 °C (Frewing, 1944)]; IR (Nujol) 3300–2500 and 1700 cm^{-1}.

Anal. Calcd for $C_{18}H_{35}O_2I$: C, 52.68; H, 8.60; I, 30.93. Found: C, 52.75; H, 8.44; I, 30.65.

Methyl, Ethyl, and Isopropyl 2-Iodooctadecanoates. 2-Iodooctadecanoyl chloride (4 g each) was separately treated with methanol, ethanol, and isopropyl alcohol (50 mL each) and worked up as described above to give almost quantitatively methyl 2-iodooctadecanoate [bp 163–164 °C (50 μm); IR (neat) 1750 cm^{-1}], ethyl 2-iodooctadecanoate [bp 166–170 °C (30 μm); IR (neat) 1745 cm^{-1}], and isopropyl 2-iodooctadecanoate [bp 180–182 °C (0.3 mm); IR (neat) 1740 cm^{-1}].

Bioassay. The 2-halooctadecanoic acids and their esters were bioassayed against first-instar larvae of *C. pipiens quinquefasciatus* as reported elsewhere (Hwang et al., 1974a). The bioassay data using percent mortalities at various concentrations were analyzed for the log-probit

Table I. Biological Activity of 2-Halooctadecanoic Acids and Methyl, Ethyl, and Isopropyl 2-Halooctadencanoates against First-Instars of *C. pipiens quinquefasciatus*

$$n\text{-}C_{16}H_{33}\overset{X}{\underset{|}{C}}HCO_2R$$

	R = H				R = CH_3				R = C_2H_5				R = $CH(CH_3)_2$			
X	no.	LC_{50}	LC_{90}	slope[a]	no.	LC_{50}	LC_{90}	slope	no.	LC_{50}	LC_{90}	slope	no.	LC_{50}	LC_{90}	slope
Cl	1	0.7	2.5	2.4	4	0.6	1.7	2.7	7	1.9	9.5	1.8	10	2.1	>10.0	1.7
Br	2[b]	0.6	2.7	1.9	5[b]	0.9	1.5	6.0	8	0.6	1.0	5.1	11	0.6	0.9	7.2
I	3	1.5	2.8	4.8	6	4.8	>10.0	0.5	9	5.8	>10.0	0.6	12	>10.0	>10.0	1.8

[a] Slope of probit regression line. [b] Hwang and Mulla, 1976b.

regression analysis with a Compucorp Model 145E computor. The biological activity of the test compounds was expressed in terms of lethal concentrations in part per million inhibiting the emergence of 50 and 90% of the population (LC_{50} and LC_{90}).

RESULTS AND DISCUSSION

Table I shows the larvicidal activity of the 2-halooctadecanoic acids and their methyl, ethyl, and isopropyl esters against first-instars of *C. pipiens quinquefasciatus*.

In the series of chlorinated carboxylic acid and its esters, 2-chlorooctadecanoic acid (**1**) and methyl 2-chlorooctadecanoate (**4**) showed considerable activity, the ester being slightly more active than the acid. When the size of the alcohol moieties of the alkyl 2-chlorooctadecanoates increased from ethyl to isopropyl groups, the activity decreased. Thus, ethyl 2-chlorooctadecanoate (**7**) and isopropyl 2-chlorooctadecanoate (**10**) showed diminished activity.

2-Bromooctadecanoic acid (**2**) was as active as its chloro analogue. The activity of this acid was previously reported (Hwang and Mulla, 1976b). Esterification of the acid resulted in slightly increasing the activity of the resultant esters; therefore, methyl 2-bromooctadecanoate (**5**), ethyl 2-bromooctadecanoate (**8**), and isopropyl 2-bromooctadecanoate (**11**) were considerably active with LC_{50} and LC_{90} at 0.6–0.9 and 0.9–1.4 ppm, respectively. The activities of these esters were at the same level and were not influenced by different sizes of alcohol moieties in the esters.

2-Iodooctadecanoic acid (**3**) was considerably active. The activity decreased considerably by esterification of the acid to its esters. Thus, methyl 2-iodooctadecanoate (**6**) and ethyl 2-iodooctadecanoate (**9**) were only moderately active, and isopropyl 2-iodooctadecanoate (**12**) was almost inactive.

The more active halogen-substituted carboxylic acids and esters, such as compounds **1**, **2**, **3**, **4**, **5**, **8**, and **11**, generally showed larger slopes of probit regression lines, while the less active esters, **6**, **7**, **9**, **10**, and **12**, exhibited smaller slopes.

Figure 1 shows the structure–activity relationship of 2-halooctadecanoic acids and their esters, in which the biological activity in terms of LC_{90} and LC_{50} was plotted against the size of the R group [H, CH_3, C_2H_5, or $CH(CH_3)_2$] in these compounds. In comparing the three series of halogenated carboxylic acids and esters, the series of brominated carboxylic acid and esters was the most active over the other two series of compounds. The activity of the brominated compounds was not affected too much by the R group. The second active series of compounds was the chlorinated acid and esters, the activity of which generally decreased considerably as the R group increased. The decrease of activity was more pronounced in the LC_{90} than in the LC_{50}. The iodinated compounds were the least active, and their activity diminished drastically as the R

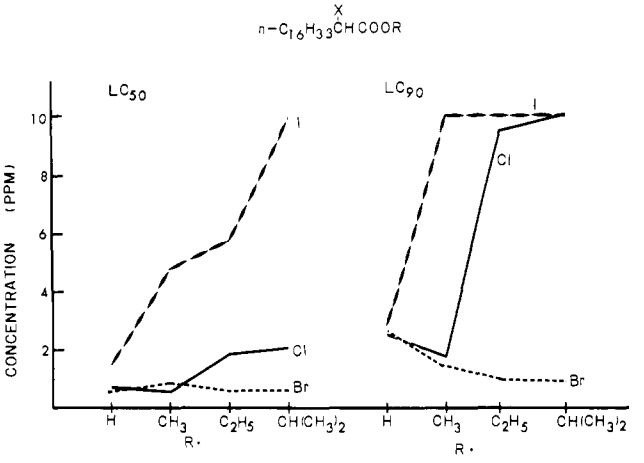

Figure 1. Structure–activity relationship of 2-halooctadecanoic acids and their esters in terms of LC_{50} and LC_{90}.

group increased. Except for the bromo analogues, the general overall trend was that the activity of the halogenated carboxylic acids and esters decreased while the size of R group increased from hydrogen to methyl, ethyl, and isopropyl groups.

The activity of the 2-halogenated carboxylic acids and esters increased in the following order: 2-iodooctadecanoic acid and esters < 2-chlorooctadecanoic acid and esters < 2-bromooctadecanoic acid and esters. It was suspected that this order might have some relationship with the size of the halogen atoms attached to the C-2 position of the acids and esters. According to Pauling (1960), the van der Waals radii of halogens attached to a carbon atom are, however, in the order of iodine (2.2 Å), bromine (2.0 Å), and chlorine (1.8 Å). Therefore, no close relationship could be found between the van der Waals radii of halogen atoms and the biological activity of the 2-halogenated carboxylic acids and esters. Furthermore, the electronegativities of halogen atoms increase in the order of iodine (2.5), bromine (2.8), and chlorine (3.0) (Gould, 1959). The biological activity of the 2-halogenated carboxylic acids and esters, again, did not seem to be directly related to the electronegativities. The high level of activity of the bromo-substituted compounds might be due to unknown factors unrelated to the physical parameters described above.

In studying the biological activity of the 2-halooctadecanoic acids and their methyl, ethyl, and isopropyl esters, we found that, among the compounds studied, 2-bromooctadecanoic acid and its methyl, ethyl, and isopropyl esters were the most active larvicides against *C. pipiens quinquefasciatus*. The biological activity of the 2-halogenated acids and esters, except the bromo analogues, generally declined in the order of acids, methyl, ethyl, and isopropyl esters. The biological activity did not have clear-cut relationships with the van der Waals radii and the electronegativities of the halogen atoms attached to the acids and esters.

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Multiple Forms of Rat Liver Glutathione *S*-Transferases: Specificity for Conjugation of *O*-Alkyl and *O*-Aryl Groups of Organophosphorus Insecticides

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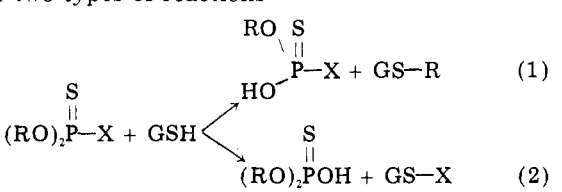
Multiple forms of glutathione *S*-transferases were partially purified from rat liver, using 1-chloro-2,4-dinitrobenzene as the substrate to monitor activity. Their properties were studied, especially with regard to the specificity for *O*-alkyl and *O*-aryl conjugations of organophosphorus insecticides. Substrates studied were diazinon and methyl parathion as well as 3,4-dichloronitrobenzene and methyl iodide. Based upon the nature of the binding to a DEAE–cellulose column, pH–activity relationships, and preference for alkyl and aryl conjugations, the rat liver enzymes were classified into two major groups, each of which was further separated on CM–cellulose and hydroxylapatite columns. The multiple forms showed distinctive or overlapping properties. Their identity with those reported previously is discussed.

Glutathione *S*-transferases (EC 2.5.1.18) are involved in the metabolism of xenobiotics as well as in the binding of diversified groups of chemicals (see reviews by Arias et al., 1976; Jakoby et al., 1976a,b; Jakoby and Keen, 1977). These enzymes are also responsible for the detoxification of certain pesticides (Yang, 1976). With organophosphorus (OP) insecticides as substrates, glutathione *S*-transferases catalyze two types of reactions



where R = alkyl, X = "leaving group" and aryl group.

Whether the alkyl conjugation (eq 1) and the "leaving group" conjugation (eq 2) are catalyzed by the same enzyme has been a question for many years (Yang, 1976). In the case of rat liver supernatant, Hollingworth et al. (1973) reported that two distinct enzymes were involved in the two reactions. In contrast, Motoyama and Dauterman (1977) reported that both types of reactions were catalyzed by the same housefly glutathione *S*-transferase and they found that the reaction ratio varied markedly depending upon the alkyl and the "leaving group" structures of the OP substrates. However, Usui et al. (1977a) purified several glutathione *S*-transferases from the fat body of the the American cockroach and demonstrated that these enzymes had overlapping specificities. The same workers (Usui et al., 1977b) also separated several forms of glutathione *S*-transferases from rat liver, which showed overlapping specificities for the OP insecticides. Although not yet established, it appears that the mode of interaction between glutathione *S*-transferases and OP insecticides varies according to the structure of the substrates as well as the source and form of the enzymes.

The present study was undertaken in an attempt to clarify the apparent contradictions. Multiple forms of rat liver glutathione *S*-transferases were separated and their properties were studied especially with emphasis on alkyl and aryl group conjugations.

METHODS AND MATERIALS

Chemicals. DEAE–cellulose (DE52) and CM–cellulose were obtained from Pharmacia Fine Chemicals. Hydroxylapatite (Bio-Gel HTP) was obtained from Bio-Rad Laboratories. Precoated silica gel plates (Polygram Sil N-HR) (0.25 mm) for the thin-layer chromatography were obtained from Brinkmann Instruments. Sources of the substrates used were as follows: 1-chloro-2,4-dinitrobenzene (DNCB) from Aldrich Chemical Co., 1,2-dichloro-4-nitrobenzene (DCNB) from Eastman-Kodak,

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