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Overcrowding Factors of Mosquito Larvae. V. Synthesis and Evaluation of Some Branched-Chain Fatty Acids against Mosquito Larvae

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Overcrowding factors of mosquito larvae contained minute quantities of branched-chain fatty acids. Seven 2- and 3-substituted fatty acids were synthesized and evaluated for their biological activity against larvae of *Culex pipiens quinquefasciatus* Say, *C. tarsalis* Coquillett, *Anopheles albimanus* Wiedemann, and *Aedes aegypti* (L.). 2-Methylnonanoic acid (**1b**) and 2-methyloctadecanoic acid (**2b**) showed weak activity. 3-Methyloctadecanoic acid (**3b**) and 2,3-dimethyloctadecanoic acid (**4b**) possessed potent activity. 2-Butyldecanoic acid (**5b**), 2-butyldodecanoic acid (**6b**), and 2-butyl-4-methylundecanoic acid (**7b**) showed considerable activity. A methyl group at the 3 position or an *n*-butyl group at the 2 position of long-chain carboxylic acids seemed essential in obtaining good activity.

Mosquito larvae overcrowded in laboratory cultures showed increased mortality and slow development. Emergence of smaller adults from overcrowded larvae was also observed (Ikeshoji, 1965). The autoregulating properties of chemical factors in the culture water of overcrowded larvae of *Culex pipiens quinquefasciatus* Say were demonstrated, and these chemical factors were designated as *overcrowding factors of mosquito larvae* (Ikeshoji and Mulla, 1970).

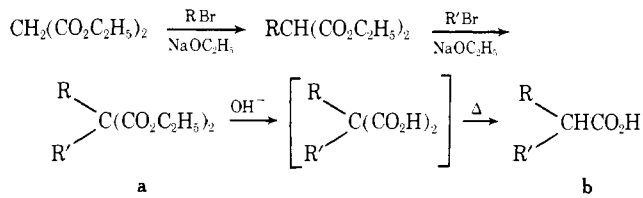
During the isolation and identification of these overcrowding factors, the mass spectra of the crude natural products indicated the presence of branched-chain fatty acids in minute amounts (Ikeshoji and Mulla, 1974). Along with the studies on the isolation and identification of overcrowding factors, attempts were made to synthesize a series of substituted long-chain fatty acids. The biological activity of these acids as related to their structures was investigated. This report presents the synthesis of these branched-chain fatty acids and their biological activity against mosquito larvae.

EXPERIMENTAL SECTION

Synthesis. The compounds synthesized and evaluated for activity are shown in Scheme I. The malonic ester condensation was used to prepare these fatty acids. Alkyl bromides were prepared and treated with diethyl malonate in absolute ethanol in the presence of sodium ethoxide to yield monosubstituted malonic esters which, upon further alkylation with alkyl bromides under the same conditions, afforded disubstituted malonic esters. Saponification and subsequent thermal decarboxylation of these mono- and disubstituted malonic esters yielded the desired branched-chain fatty acids.

Scheme II shows the method for preparing 2-bromoheptadecane which is not readily available. 2-Heptadecanone (**9**) was previously prepared by Cason *et al.* (1949) through the condensation of dimethylcadmium and palmitoyl chloride in dry benzene; however, the yield of the pure ketone was only 55%. In the present report, a method of forming methyl ketone *via* β-keto sulfoxide was adopted (Corey and Chaykovsky, 1964). A nearly quantitative yield was obtained in following this method. Thus, methyl palmitate was treated with methylsulfinyl carbanion in dimethyl sulfoxide to give methylsulfinylmethyl *n*-pentadecyl ketone (**8**) which upon hydrogenolysis with aluminum amalgam in aqueous tetrahydrofuran yielded the ketone **9**. Reduction of **9** with lithium aluminum hydride afforded 2-heptadecanol (**10**). In order to avoid the formation of isomeric secondary bromides during the conversion of the secondary alcohol **10** into the corresponding bromide **12**, **10** was first treated with *p*-toluenesulfonyl chloride to form 2-heptadecyl tosylate (**11**) which was then allowed to react with sodium bromide in dimethylformamide to give 2-bromoheptadecane (**12**).

Scheme I. Synthesis of Various Branched-Chain Fatty Acids



1. R = CH_3; R′ = *n*-C_7H_{15}
2. R = CH_3; R′ = *n*-$C_{16}H_{33}$
3. R = H; R′ = *n*-$C_{15}H_{31}C(CH_3)H$
4. R = CH_3; R′ = *n*-$C_{15}H_{31}C(CH_3)H$
5. R = *n*-C_4H_9; R′ = *n*-C_8H_{17}
6. R = *n*-C_4H_9; R′ = *n*-$C_{10}H_{21}$
7. R = *n*-C_4H_9; R′ = *n*-$C_7H_{15}C(CH_3)HCH_2$

Methylsulfinylmethyl n-Pentadecyl Ketone (8). Methylsulfinyl carbanion solution was prepared from sodium hydride (9.6 g, 0.4 mol) and anhydrous dimethyl sulfoxide (200 ml) under dry nitrogen. Into this solution, anhydrous tetrahydrofuran (200 ml) was added. The resulting solution was cooled in an ice bath and kept stirring during the addition of methyl palmitate (54.1 g, 0.2 mol). The ice

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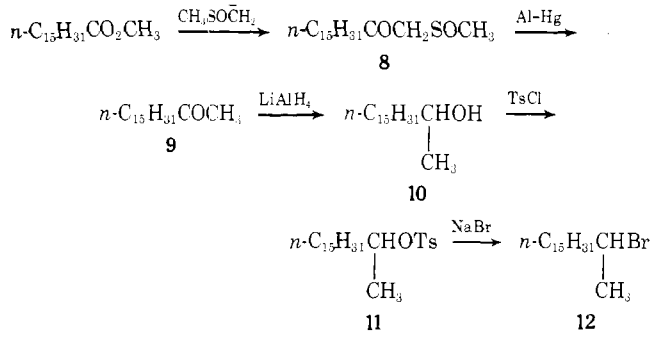
Scheme II. Synthesis of 2-Bromoheptadecane



bath was removed, and stirring was continued for 30 min. The reaction mixture was then poured into water (1.2 l.), acidified with hydrochloric acid to pH 3, and extracted three times with chloroform. The combined extracts were washed with water and dried ($MgSO_4$). Evaporation of the chloroform solution gave crude methylsulfinyl *n*-pentadecyl ketone (63.0 g, 99.5% yield) as a white crystalline solid, mp 92°. Two recrystallizations from ethyl acetate gave the pure ketone: mp 93°; ir ($CHCl_3$) 2950, 1720, 1480, 1380, 1310, and 1050 cm^{-1}; nmr ($CDCl_3$) δ 0.87 (t, 3), 1.26 (large peak, 26), 2.62 (t, 2), 2.68 (s, 3), and 3.75 (s, 2) ppm.

Anal. Calcd for $C_{18}H_{36}O_2S$: C, 68.30; H, 11.46; S, 10.13. Found: C, 68.21; H, 11.28; S, 10.11.

2-Heptadecanone (9). Into a solution of methylsulfinylmethyl *n*-pentadecyl ketone (31.7 g, 0.1 mol) in tetrahydrofuran (1.62 l.) and water (0.18 l.), strips of aluminum amalgam, prepared by immersing aluminum foil (27 g, 1 g-atom, *ca.* 10 × 1 cm) in a 2% aqueous solution of mercuric chloride, were added. The mixture was kept stirring while heat was evolving. After evolution of heat subsided, the reaction mixture was heated under reflux for 2 hr. The mixture was then filtered, and the filtered solids were washed with warm tetrahydrofuran. The filtrate was concentrated to remove most of the tetrahydrofuran. The residue was extracted three times with ether, and the combined ether extracts were washed with water and dried (Na_2SO_4). Evaporation of ether gave 2-heptadecanone (25.0 g, 98% yield) as a white crystalline solid, mp 47°, which was distilled *in vacuo* giving the pure ketone: bp 149–150° (0.4 mm); mp 48–49° [lit. bp 156–158° (10.5 mm) (Weitzel and Wojahn, 1951), mp 45.0–46.5° (Cason *et al.*, 1949), or 48–48.5° (Weitzel and Wojahn, 1951)].

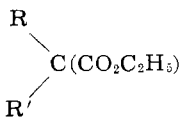
2-Heptadecanol (10). Into a stirred slurry of lithium aluminum hydride (1.05 g, 28 mmol) in ether (100 ml), a solution of 2-heptadecanone (25.4 g, 0.1 mol) in ether (200 ml) was added. The reaction mixture was stirred and heated under reflux for 1 hr. Dilute sulfuric acid (10%, 300 ml) was added into the mixture to dissolve the solids. The ether layer was separated, and the aqueous layer was extracted twice with ether. The combined ether solutions were washed successively with water, saturated aqueous sodium bicarbonate solution, and water. After drying (Na_2SO_4), the ether solution was evaporated to give crude 2-heptadecanol (25.3 g, 98.6% yield), mp 40°, which was distilled *in vacuo* to give the pure alcohol: bp 134–135° (0.12 mm); mp 42° [lit. bp 158° (0.5 mm), mp 35–36° (Weitzel and Wojahn, 1951)].

2-Bromoheptadecane (12). 2-Heptadecanol (106.5 g, 0.42 mol) was dissolved in dry pyridine (700 ml), and the solution was cooled to 0°. Into this solution, *p*-toluenesulfonyl chloride (160.2 g, 0.84 mol) was added all at once. After solution was complete, the mixture was placed in a refrigerator (0–5°) for 65 hr. The reaction mixture was then poured with stirring into ice water (4.2 l.), and the resultant suspension was extracted three times with ether. The combined ether extracts were washed three times each with cold diluted hydrochloric acid (1:1) and cold water. After drying (Na_2SO_4), the ether solution was evaporated under reduced pressure at room temperature to yield crude 2-heptadecyl tosylate (169.5 g, 98% yield): mp 48–50°; ir (nujol) 1600, 1310, 1300, 1200, 1180, 920, 820, and 675 cm^{-1}. The tosylate was used in the following reaction without purification.

2-Heptadecyl tosylate (169.5 g, 0.41 mol) was added with stirring into a solution of sodium bromide (42.2 g, 0.41 mol) in *N,N*-dimethylformamide (1.9 l.). After the tosylate completely dissolved, the reaction mixture was allowed to stand at room temperature for 90 hr. The mixture was then poured into ice water (7 l.) and extracted three times with ether. The combined ether extracts were washed with water and dried (K_2SO_4). Evaporation of ether gave crude 2-bromoheptadecane (122 g) which was distilled *in vacuo* yielding the pure product (116 g, 89% yield): bp 139–142° (0.18 mm) [lit. bp 117–122° (0.056 mm) (Kuhn *et al.*, 1936)].

General Method of Preparing Substituted Malonic Esters. Sodium (0.1 g-atom) was added into absolute ethanol (100 ml), and the solution was cooled to 40–50° after solution was complete. Into this sodium ethoxide solution, diethyl malonate or a diethyl alkylmalonate (0.1 mol) was added with stirring. An alkyl bromide (0.1 mol) was then added dropwise into the resulting clear solution. The reaction mixture was refluxed for 5 hr and distilled to remove ethanol. Water was added to the residue, and the resulting mixture was extracted three times with ether. The combined ether extracts were washed once with dilute hydrochloric acid and three times with water. After drying (Na_2SO_4), the ether solution was evaporated. The residue was fractionally distilled *in vacuo* to give a pure mono- or disubstituted malonic ester (48–91% yield). The ir spectra

Table I. Physical Properties of Substituted Malonic Esters



Compd no.	R	R′	Bp, °C (mm) Obsd	Bp, °C (mm) Lit.	Elemental comp.	*Anal.* Calcd C	Calcd H	Found C	Found H
1a	CH_3	n-C_7H_{15}	168–171 (23)		$C_{15}H_{28}O_4$	66.14	10.36	66.40	10.41
2a	CH_3	n-$C_{16}H_{33}$	190–193 (0.3)	202 (0.5)[a]	$C_{24}H_{46}O_4$	72.31	11.63	72.39	11.66
3a	H	n-$C_{15}H_{31}C(CH_3)H$	169–170 (0.1)	180 (0.3)[b]	$C_{24}H_{46}O_4$	72.31	11.63	72.59	11.47
4a	CH_3	n-$C_{15}H_{31}C(CH_3)H$	173 (0.1)		$C_{25}H_{48}O_4$	72.76	11.72	73.06	11.60
5a	n-C_4H_9	n-C_8H_{17}	171–175 (3.7)	181 (10)[c]	$C_{19}H_{36}O_4$	69.47	11.05	69.48	11.06
6a	n-C_4H_9	n-$C_{10}H_{21}$	170–176 (1.3)	181–183 (4)[d]	$C_{21}H_{40}O_4$	70.74	11.31	70.82	10.92
7a	n-C_4H_9	n-$C_7H_{13}C(CH_3)HCH_2$	135–136 (0.2)		$C_{21}H_{40}O_4$	70.74	11.31	70.81	11.08

[a] Weitzel and Wojahn, 1950. [b] Kuhn *et al.*, 1936. [c] Asinger *et al.*, 1963. [d] Stanley *et al.*, 1929.

Table II. Physical Properties of Substituted Fatty Acids

Compd no.	R	R'	Bp, °C (mm) Obsd	Bp, °C (mm) Lit.	Mp, °C Obsd	Mp, °C Lit.	Elemental comp.	Anal. Calcd C	Anal. Calcd H	Anal. Found C	Anal. Found H
1b	CH_3	*n*-C_7H_{15}	160 (25)	149 (14)[a]			$C_{10}H_{20}O_2$	69.72	11.70	69.98	11.41
2b	CH_3	*n*-$C_{16}H_{33}$	180–185 (0.7)	176 (0.5)[b]	55	55[b]	$C_{19}H_{38}O_2$	76.45	12.83	76.71	12.98
3b	H	*n*-$C_{15}H_{31}C(CH_3)H$			52–53	50.8–51.3[c]	$C_{19}H_{38}O_2$	76.45	12.83	76.51	12.76
4b[d]	CH_3	*n*-$C_{15}H_{31}C(CH_3)H$			48–54	63–64[e]	$C_{20}H_{40}O_2$	76.86	12.90	77.20	12.91
5b	*n*-C_4H_9	*n*-$C_{18}H_{17}$	139–144 (0.4)	132 (0.2)[e]			$C_{13}H_{28}O_2$	73.63	12.36	73.92	12.10
6b	*n*-C_4H_9	*n*-$C_{10}H_{21}$	148–154 (0.23)	175–176 (3)[f]			$C_{16}H_{32}O_2$	74.94	12.58	75.05	12.65
7b[g]	*n*-C_4H_9	*n*-$C_7H_{15}C(CH_3)HCH_2$	150–151 (0.3)				$C_{16}H_{32}O_2$	74.94	12.58	75.42	12.71

[a] Conia, 1954. [b] Weitzel and Wojahn, 1950. [c] Cason *et al.*, 1949. [d] Mixture of threo and erythro forms. [e] Asinger *et al.*, 1963. [f] Stanley *et al.*, 1929. [g] Mixture of four isomers.

of these malonic esters generally showed maximum absorptions at 1720 and 1240 cm^{-1}. All nmr spectra conformed to the structures. Table I shows the physical properties of the substituted malonic esters synthesized.

General Methods of Preparing Substituted Fatty Acids. A mono- or a disubstituted malonic ester (50 mmol) was heated under reflux with 50% (w/w) aqueous potassium hydroxide solution (200 ml) for 8–12 hr. The mixture was stirred during the saponification. Enough water was added into the mixture to dissolve the acid salt. The resulting solution was washed once with ether and acidified with hydrochloric acid. The separated substituted malonic acid was extracted three times with ether. The ether extracts were combined and dried (Na_2SO_4). Evaporation of the ether solution gave a crude substituted malonic acid which, without purification, was heated to *ca.* 180° until evolution of carbon dioxide ceased to yield a substituted fatty acid (78–99% yield). The crude acid thus obtained was purified by vacuum distillation or recrystallization from acetone. All acids were in the *dl* form. The ir spectra of these acids generally showed maximum absorption at 3400–2100, 1700, 1420, 1290, 1230, and 930 cm^{-1}. All nmr and mass spectra conformed to the structures. Table II shows the physical properties of the branched-chain fatty acids synthesized.

Bioassay Procedures. First- and fourth-instar larvae of *Culex pipiens quinquefasciatus* Say and *Aedes aegypti* (L.) and fourth-instar larvae of *C. tarsalis* Coquillett and *Anopheles albimanus* Wiedemann were used to evaluate the biological activity of the branched-chain fatty acids. In all cases, bioassays were continued until adult emergence.

Twenty first-instar larvae of *C. p. quinquefasciatus* and *A. aegypti*, 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 kept at a constant temperature of 27 ± 1° and under a photoperiod of 14 hr. The loss of water was replenished at 2-day intervals. The late fourth-instar larvae of all four species of mosquitoes were also handled in the same manner as described for the first-instar larvae.

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. Mean per cent emergence was plotted on Ld-p paper, and from the resulting lines, lethal concentrations (LC) were determined. The biological activity was thus measured in terms of inhibition of emergence of adults resulting from treated larvae.

RESULTS AND DISCUSSION

Table III shows the biological activity of the synthesized branched-chain fatty acids against four species of mosquitoes. The biological activity is expressed as LC_{50} and LC_{90}. Two 2-methyl-substituted carboxylic acids (**1b** and **2b**), regardless of their chain lengths, showed a low level of activity against three species of mosquitoes. Although these two acids showed some activity against *C. tarsalis*, their LC_{50} values were greater than 10 ppm, evidencing that methyl substitution at the 2 position did not invest significant larvicidal activity.

3-Methyloctadecanoic acid (**3b**) exhibited a higher level of activity. The first-instar larvae of *C. p. quinquefasciatus* were quite susceptible to this acid whereas the fourth-instar larvae of the species tested were susceptible to a lesser extent. Introduction of another methyl group at the 2 position (compound **4b**) decreased its activity somewhat. Since octadecanoic acid had very weak larvicidal activity against *C. p. quinquefasciatus* (Ikeshoji and Mulla, 1974), the presence of a methyl group at the 3 position of octadecanoic acid contributed to the high activity.

Table III. Biological Activity of Synthesized Branched-Chain Fatty Acids against Mosquito Larvae

Compd no.	Activity, ppm, against larvae of: *C. p. quinquefasciatus* 1st LC_{50}	*C. p. quinquefasciatus* 1st LC_{90}	*C. p. quinquefasciatus* 4th LC_{50}	*C. p. quinquefasciatus* 4th LC_{90}	*C. tarsalis* 4th LC_{50}	*C. tarsalis* 4th LC_{90}	*A. albimanus* 4th LC_{50}	*A. albimanus* 4th LC_{90}	*A. aegypti* 1st LC_{50}	*A. aegypti* 4th LC_{50}
1b	>25.0		>50.0		11.0	>50.0	>25.0			
2b	>25.0		>50.0		11.0	>50.0	>10.0			
3b	0.2	0.7	3.0	25.0	1.7	10.0	1.1	15.0	2.0	
4b	0.5	2.5	5.0	40.0	1.1	15.0	6.0	65.0	1.0	
5b	4.5	13.0	9.0	18.0	2.8	18.0	6.0	19.0	>10.0	>10.0
6b	9.2	16.0	10.0	33.0	2.8	16.0	2.4	10.0	>10.0	>10.0
7b	3.0	12.0	9.2	23.0	3.2	30.0	2.7	12.0	>10.0	>10.0

Substitution of an *n*-butyl group at the 2 position also invested good activity in the carboxylic acids **5b, 6b,** and **7b.** The LC_{50} values of these three acids were about the same; therefore, the 4-methyl group in compound **7b** did not contribute to the activity. All branched-chain fatty acids showed almost the same level of activity against all four species of mosquitoes tested.

It can be concluded that a methyl group at the 3 position or an *n*-butyl group at the 2 position in long-chain carboxylic acids is essential in obtaining good biological activity against mosquito larvae.

These branched-chain fatty acids manifest only toxic effects on the mosquitoes bioassayed. Growth-retarding activity and emergence of smaller adults from larvae were not observed in these tests. Ikeshoji and Mulla (1974) ascribed the growth-retarding features of the overcrowding factors of mosquito larvae to the presence of branched-chain hydrocarbons. They also proposed that the branched-chain fatty acids interfered with biosynthesis of lipids from straight-chain fatty acids in the larval cuticles and therefore acted as antimetabolites against straight-chain fatty acids. As a result of this antimetabolite action, mosquito larvae formed water-permeable cuticles which might cause their sudden death immediately after ecdysis.

Some of the branched-chain fatty acids tested here show good biological activity against mosquitoes at low concentrations; therefore, they offer good potential for the control of mosquitoes.

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