conditioning solution produced the first two, very large peaks.) The reproducibilities of the absorbances of the two standards are shown in Table II. The 2σ values for the X_1 and X_2 standards were essentially the same, ±0.008 and ±0.010 absorbance unit, respectively. Table III lists the final results for a series of commercial, spray-coated samples, replicated on three separate days with fresh standards. The 95% confidence limits are ±0.4%, the same as yielded by the phosphorus method and significantly better than the earlier limits of ±0.6% shown in Table I.

To evaluate the applicability of the method to the new Dasanit + Di-Syston 7.5–7.5% granular formulation, three samples were prepared in the laboratory by the previously reported solution coating technique (Talbott et al., 1972). Di-Syston was applied at concentrations which were below, equal to, and above levels which might be expected from commercial production, and matching amounts of Dasanit were added. These samples were analyzed by four different analysts, using separate weighings of samples and standards.

The sample extracts were run against both Di-Syston and mixed Dasanit–Di-Syston reference standards. The data shown in Table IV indicate essentially no differences in accuracy and precision using either standard. Hence, for all practical purposes the Dasanit does not interfere with the Di-Syston. (The fact that the precision standard deviation is better than that shown in Table III is attributed to a higher degree of homogeneity in solution-coated material.)

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Overcrowding Factors of Mosquito Larvae. VII. Preparation and Biological Activity of Methyloctadecanes and Methylnonadecanes against Mosquito Larvae

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All structural isomers of 7-methyloctadecane and 8-methylnonadecane, two components of the overcrowding factors of mosquito larvae, were bioassayed for their biological activity against first- and fourth-instar larvae of *Culex pipiens quinquefasciatus* Say. Straight-chain alkanes from C_{11} to C_{20} were also evaluated for comparison. The branched-chain alkanes in general were more active than the straight-chain alkanes. Among the alkanes studied, 3-methyloctadecane and 3-, 4-, 7-, and 9-methylnonadecanes showed the greatest activity with LC_{50} 1–2 ppm and LC_{90} about or less than 10 ppm. These branched-chain alkanes showed greater activity than most petroleum hydrocarbon larvicides utilized in mosquito control today. At sublethal concentrations, the branched-chain alkanes showed good growth retarding activity against the immature stages of the mosquito.

Older larvae of the southern house mosquito, *Culex pipiens quinquefasciatus* Say, under overcrowded conditions elaborate a number of chemicals known as overcrowding factors which play a role in regulating mosquito larval populations. These factors manifest toxic and growth-retarding effects in younger larvae (Ikeshoji and Mulla, 1970a,b). In fractionating the overcrowded cultures of mosquito larvae, Ikeshoji and Mulla (1974) obtained a mixture of active components consisting of carboxylic acids and hydrocarbons which could be separated by gas chromatography. Mass spectrometric studies showed that the hydrocarbon fraction contained heptadecane, octadecane, 7-methyloctadecane, and 8-methylnonadecane.

Petroleum hydrocarbons have been known as mosquito-control agents for more than a half-century (Balfour, 1913; Takatsuki, 1917; Hagstrum and Mulla, 1968). These control agents, however, have been used as mixtures, and no efforts have been made to isolate and identify the active compounds. Moreover, the rates of application for satisfactory control of mosquitoes are quite high. Quraishi and Thorsteinson (1967) investigated toxicity of several pure alkanes and alkenes (both ranging from C_7 to C_{16}) against *Aedes aegypti* (L.) larvae and found that nonene was the only one which gave high mortality of larvae and pupae at the very high dosage of 500 ppm.

In studies on the isolation and identification of the overcrowding factors of mosquito larvae, Ikeshoji and Mulla (1974) reported the biological activity of pure hydrocarbons such as octadecane, 3-methyloctadecane, 3-methylnonadecane, and 9-methylnonadecane against young larvae of *C. p. quinquefasciatus*. These three branched-chain alkanes were the structural isomers of the naturally occurring 7-methyloctadecane and 8-methylnonadecane which were not available for bioassay at that time.

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As a continuance of the studies described above, the present investigations were initiated to ascertain the biological activity of these naturally occurring branched-chain alkanes and their isomers and to study their structure–activity relationship. In this paper, we report the preparation and biological activity of 7-methyloctadecane and 8-methylnonadecane, two major components of the overcrowding factors of mosquito larvae, and their structural isomers. We also report the biological activity of straight-chain alkanes ranging from C_{11} to C_{20}, including naturally occurring heptadecane and octadecane, the other two components of the overcrowding factors.

EXPERIMENTAL SECTION

Synthesis. All possible structural isomers of methyloctadecanes and methylnonadecanes were either purchased or synthesized. Namely, 2-, 3-, and 4-methyloctadecanes, 2-, 3-, 4-, and 9-methylnonadecanes, and all straight-chain alkanes were obtained from ICN K&K Laboratories and purified by fractional distillation in vacuo. The others were synthesized according to the following procedure. The Grignard reaction of alkylmagnesium bromides with methyl ketones yielded tertiary alcohols which upon dehydration and subsequent hydrogenation gave various methyl-substituted saturated hydrocarbons. Compounds synthesized and their starting materials were as follows: 5-methyloctadecane from 1-bromotridecane and 2-hexanone, 6-methyloctadecane from 1-bromododecane and 2-heptanone, 7-methyloctadecane from 1-bromohexane and 2-tridecanone, 8-methyloctadecane from 1-bromodecane and 2-nonanone, 9-methyloctadecane from 1-bromooctane and 2-undecanone, 5-methylnonadecane from 1-bromotetradecane and 2-hexanone, 6-methylnonadecane from 1-bromotridecane and 2-heptanone, 7-methylnonadecane from 1-bromododecane and 2-octanone, 8-methylnonadecane from 1-bromoheptane and 2-tridecanone, and 10-methylnonadecane from 1-bromononane and 2-undecanone. Except 10-methylnonadecane, all compounds were racemates. The prefix *dl* is omitted.

General Procedure for Preparing Methyloctadecanes and Methylnonadecanes. Into a Grignard reagent prepared from a 1-bromoalkane (0.25 mol) and magnesium turnings (0.25 g-atom) in anhydrous ether (60 ml), a solution of a 2-alkanone (0.25 mol) in anhydrous ether (60 ml) was added at 0°C. The reaction mixture was stirred at room temperature for 2 hr and poured on cracked ice (100 g). Enough dilute sulfuric acid was added to dissolve the separate solids. The ether layer was separated, and the aqueous layer was extracted twice with ether. The combined ether solutions were washed once with water, twice with saturated aqueous sodium bicarbonate solution, and again once with water. The ether solution was dried (Na_2SO_4). Evaporation of the solvent left a crude product which on fractional distillation in vacuo gave a pure methyldialkylcarbinol (52–69% yield).

A solution of the methyldialkylcarbinol (0.13 mol) and *p*-toluenesulfonic acid monohydrate (0.02 mol) in benzene (150 ml) was refluxed for 1 hr during which water was azeotropically distilled. The solution was washed successively once with water, twice with 10% aqueous sodium carbonate solution, and again once with water. After it was dried (K_2CO_3), the benzene solution was evaporated. The residue was distilled in vacuo to give a mixture of methylalkenes (75–91% yield).

The mixture of methylalkenes (0.05 mol) was hydrogenated in isopropyl alcohol (50 ml) in the presence of palladium on carbon (5%, 2 g). Removal of the catalyst and the solvent gave a methylalkane which was purified by fractional distillation in vacuo (81–99% yield). Table I shows the boiling points and mass spectrometric data of the synthesized methyloctadecanes and methylnonadecanes. Elemental analysis data were within ±0.5% of the theoretical values.

Table I. Boiling Point and Mass Spectrometric Data of Synthesized Methyloctadecanes and Methylnonadecanes

Compound	Bp, °C (mm), obsd	*m/e*
Methyloctadecane[a]		
5-	139–141 (0.85)[b]	268, 253, 210, 84
6-	144–148 (1.5)	268, 253, 196, 98
7-	134–136 (0.6)	268, 253, 182, 112
8-	142–145 (1.1)	268, 253, 168, 126
9-	140–145 (1.0)	268, 253, 154, 140
Methylnonadecane		
5-	150–153 (1)[c]	282, 267, 224, 84
6-	139–140 (0.5)	282, 267, 210, 98
7-	153–155 (1.1)	282, 267, 196, 112
8-	145–147 (0.6)	282, 267, 182, 126
10-	147–150 (1)	282, 267, 154

[a] Synthesis and physical properties of the methyloctadecanes were reported by Sörensen and Sörensen (1948), but the boiling points were not given. [b] Lit. bp 322.5°C (760 mm) (Terres et al., 1959). [c] Lit. bp 336°C (760 mm) (Terres et al., 1959).

Analysis. Gas–liquid chromatographic analyses of straight- and branched-chain alkanes were performed on a Hewlett-Packard Model 5750B gas chromatograph with a flame ionization detector and a Model 7123A recorder. A 6 ft × 0.125 in. o.d. (wall thickness 0.016 in.) stainless steel column packed with 10% silicon gum rubber UCC-W982 on 80–100 mesh, acid-washed, DMCS-treated Chromosorb W was used in the chromatograph. Operating conditions were: injection port temperature, 310°C; detector temperature, 350°C; column temperature, starting at 100, 120, 150, or 200°C depending on the molecular weights of the compounds at 2°C/min programmed rate; carrier gas (N_2) flow rate, 28 ml/min at 60 psi; hydrogen flow rate, 39 ml/min; air flow rate, 400 ml/min; electrometer range, 10^4; recorder attenuation, ×8; sample size, 0.2–0.4 μl; and recorder speed, 0.25 in./min. Integration was carried out by triangulation. The purity of all but one of the alkanes was over 95%.

Bioassay Procedures. The biological activity of the alkanes was measured in terms of inhibition of emergence of adult mosquitoes resulting from treated larvae and expressed as LC_{50} and LC_{90} in parts per million. The bioassay procedures were essentially similar to those reported previously (Hwang et al., 1974a,b). Briefly, 20 first- or fourth-instar larvae of *C. p. quinquefasciatus* were placed in Pyrex custard dishes containing 200 ml of tap water. The larvae were fed a mixture of ground rabbit pellets and yeast (3:1). The larval dishes were placed in a room at a constant temperature of 27 ± 1°C and under a photoperiod of 14 hr. Lost water was replenished at 2-day intervals.

The 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. Tests were in duplicate and repeated two or three times. The lethal concentrations (LC) of the compounds were obtained by plotting the mean

Table II. Biological Activity (ppm) of Branched- and Straight-Chain Alkanes against Larvae of *C. p. quinquefasciatus*

Compound	Larval instar			
	1st		4th	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Methyloctadecane				
2-	6.5	27.0		
3-	1.0	5.0	>40.0	>40.0
4-	35.0	>25.0		
5-	>10.0	20.0	20.0	>20.0
6-	10.0	<20.0	>20.0	>20.0
7-	11.0	44.0		
8-	8.0	15.0	40.0	>40.0
9-	10.0	20.0	>20.0	>20.0
Methylnonadecane				
2-	7.0	15.0	>40.0	>40.0
3-	1.0	5.0		
4-	2.1	11.0	>40.0	>40.0
5-	5.0	10.0	10.0	>20.0
6-	20.0	>20.0	>20.0	>20.0
7-	1.5	10.0	>40.0	>40.0
8-	12.0	34.0		
9-	1.6	12.0	20.0	>25.0
10-	10.0	<20.0	>20.0	>20.0
Straight-chain alkanes				
n-$C_{11}H_{24}$	10.0	>20.0	>20.0	>20.0
n-$C_{12}H_{26}$	20.0	>20.0	>20.0	>20.0
n-$C_{13}H_{28}$	>20.0	>20.0	>40.0	>40.0
n-$C_{14}H_{30}$	40.0	>40.0	>40.0	>40.0
n-$C_{15}H_{32}$	10.0	>20.0	40.0	>40.0
n-$C_{16}H_{34}$	20.0	>20.0	40.0	>40.0
n-$C_{17}H_{36}$	10.0	20.0	20.0	>40.0
n-$C_{18}H_{38}$	20.0	>40.0		
n-$C_{19}H_{40}$	>25.0	>25.0		
n-$C_{20}H_{42}$	>25.0	>25.0		

percent inhibition of emergence on a log dosage–response paper.

The growth-retarding activity of some of these compounds at sublethal concentrations was assessed by recording the number of larvae in each stadium every 2 or 3 days. Compositions by stage in untreated and treated cultures are presented against time. Three compounds were studied for their growth-retarding effects. Since the growth-retarding effects of all three showed similar trends, results for only one of these are presented.

RESULTS AND DISCUSSION

The biological activity of the branched- and straight-chain hydrocarbons is shown in Table II. In general, the branched-chain alkanes were more active than the straight-chain alkanes, and the first-instar larvae were more susceptible than the fourth-instar larvae. This was also true with the branched-chain carboxylic acids which were more toxic than the straight-chain acids and also more active against the first-instar than the fourth-instar larvae (Hwang et al., 1974a).

Among the eight methyloctadecanes tested, all except 4-methyloctadecane showed considerable activity against young larvae. The position of methyl substituent on the carbon chain had some influence on their activity. 3-Methyloctadecane was the most active among these isomers whereas 4-methyloctadecane showed a low level of activity. 7-Methyloctadecane which showed a good level of activity is one of the components of the overcrowding factors of mosquito larvae isolated from overcrowded cultures (Ikeshoji and Mulla, 1974).

Some methylnonadecanes exhibited higher activity than the alkanes described above. 3-, 4-, 7-, and 9-methylnonadecanes showed good activity against first-instar larvae with LC_{50} of 1–2 ppm. 2-, 5-, 8-, and 10-methylnonadecanes possessed some activity whereas 6-methylnonadecane showed a low level of activity. Similar to methyloctadecanes, the position of the methyl group in methylnonadecanes also influenced the activity. 8-Methylnonadecane which was found to have some activity in the present study is one of the components of the overcrowding factors (Ikeshoji and Mulla, 1974).

The straight-chain alkanes, from undecane to eicosane, did not show a high level of activity (see Table II). Undecane, pentadecane, and heptadecane exhibited some degree of biological activity. Heptadecane and octadecane were found in the overcrowded cultures of mosquito larvae (Ikeshoji and Mulla, 1974). By topical and surface applications, Quraishi and Thorsteinson (1967) evaluated straight-chain alkanes and alkenes having 7–16 carbon atoms, but the dosages employed were extremely high (5 μl/10 ml for surface application), much higher than the rates used in our studies on branched- and straight-chain alkanes.

In the evaluation of petroleum hydrocarbon mosquito larvicides, several authors reported a low level of susceptibility of younger instars (Hagstrum and Mulla, 1968; Micks et al., 1968). Only older instars were quickly killed by these oils. To increase the spectrum of activity of petroleum hydrocarbon larvicides, it will be desirable to increase the content of branched-chain alkanes such as those showing much greater levels of activity than the larvicidal oils.

Ikeshoji and Mulla (1970b) reported that, in addition to toxic effects, the overcrowding factors also manifest growth-retarding activity against mosquito larvae. One unit of the overcrowding factors (the amount of crude ether extract isolated from 300 ml of culture containing 1500 to 2000 larvae) permitted only 0.6% of the first-instar larvae to pupate on the 16th day after treatment, whereas 68% of the untreated larvae pupated with the median pupation time being 10 days.

In the present studies, 4-, 7-, and 9-methylnonadecanes were bioassayed for growth-retarding activity. Among these alkanes, 7-methylnonadecane at 0.5- and 1.0-ppm concentrations was chosen to demonstrate this kind of activity (Figure 1). In the histogram, percent composition of immature stages of mosquitoes following treatment of first-instar larvae is compared with those from the untreated. The resulting adult emergence is shown cumulatively during the study period.

One day after hatching, 12.5% of the untreated first instars became second instars. After 5 days, most of the surviving untreated larvae were in the third stage. In 7 days, 42.5% of these larvae pupated, and the same percentage of the insects emerged into adults after 9 days. All surviving insects became adults 14 days after hatching.

The first-instar larvae which had been treated with 0.5 ppm of 7-methylnonadecane developed slower than the untreated. As the untreated, almost all of the treated larvae became third instars after 5 days. However, after 7 days, 2.5% still remained as second instars, 42.5% remained as third instars, 17.5% were in the fourth stage, and 27.5% pupated in the treated units, showing delay in development as compared to the untreated. It took 21 days for all surviving insects treated with 0.5 ppm of 7-methylnonadecane to emerge into adults as compared to 14 days for the untreated.

The treatment with 1.0 ppm of 7-methylnonadecane drastically delayed development. Five days after hatching, most of the treated larvae were still in the second stage. Even after 7 days, a small proportion (12.5%) of the larvae were fourth instars, 7.5% pupated, and the rest were still in the second and third stages. Only 5% of the insects

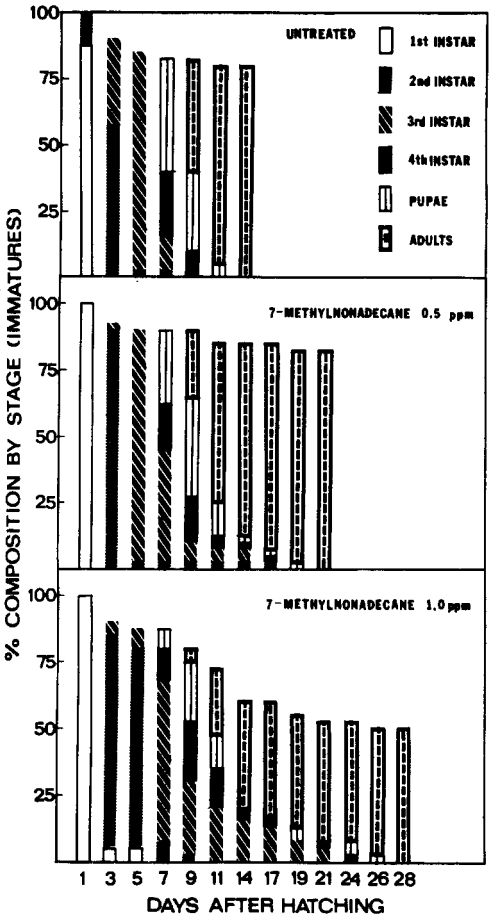

Figure 1. Growth-retarding effects of 7-methylnonadecane when first-instar larvae of *C. p. quinquefasciatus* were treated.

emerged into adults 9 days after hatching. Thereafter the percentage of adults increased while those of immatures decreased steadily. Twenty-eight days were needed for complete adult emergence as compared with 14 and 21 days for the untreated and 0.5-ppm treatment, respectively. This compound at 1.0 ppm produced 50% mortality until adult emergence. Mortality occurred mostly in the larval stages although some pupal mortality was also observed.

The other active branched-chain alkanes, such as 4- and 9-methylnonadecanes, also showed similar growth-retarding effects against mosquitoes. The results described above are presented only as an example of the growth-retarding effects of these branched-chain alkanes on immature mosquitoes.

These overcrowding factors are considered to be highly specific against mosquito larvae. From all indications, they possess little or no toxicity to game and beneficiary life, thus showing no adverse effects on nontarget organisms in aquatic habitats. These compounds, therefore, offer a good potential for specific and safe control of mosquito populations.

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