

## 7,11-Dimethyloctadecane: An Ovipositional Attractant for *Aedes aegypti* Produced by *Pseudomonas aeruginosa* on Capric Acid Substrate

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Previous works have shown that *Pseudomonas aeruginosa* produces ovipositional attractants for several species of mosquitos on capric acid substrate in laboratory and field. In the present work, we isolated an attractant for *Aedes aegypti* from the bacterial culture medium by thin layer and gas chromatography, and identified it by mass spectroscopy as 7,11-dimethyloctadecane. This identification was confirmed with synthesis of this hydrocarbon by two methods, Grignard coupling reaction and a combination of acetylation and Wittig reaction. The synthetic one showed the identical mass-fragmentation pattern and the GC-retention time with those of the isolated one. However, there was some discrepancy of attractancy between the isolated and synthetic ones; 6.9 times at a most attractive dosage vs. 2.9 times at 0.4 mg/slide respectively. A minute attractive component included in the isolated fraction or the differential attractancy of the diastereomers of the synthetic hydrocarbon is suggested for the discrepancy.

### INTRODUCTION

Maw<sup>1)</sup> found *n*-capric acid to make artificial field water attractive for oviposition of *Culex restuans* THEOBALD within several days after the treatment. He also proved in the laboratory that the treated water became attractive to other mosquitos such as *Aedes aegypti* (L.), *Culex pipiens* L. and *Culex tarsalis* Coq. and incriminated *Pseudomonads* for production of the ovipositional attractants. Maw and Bracken<sup>2)</sup> used this property in developing effective artificial oviposition sites for *A. restuans*. Ikeshoji, Saito and Yano<sup>3)</sup> developed the Maw's finding further by isolating the responsible bacterium, *Pseudomonas aeruginosa*, and producing the attractants for *A. aegypti* and

*Culex pipiens molestus* FORSKAL *in vitro* on capric acid substrate.

In the present work, we have isolated the attractant for *A. aegypti* by thin layer and gas chromatography, identified the chemical structure by mass spectroscopy, and confirmed the identification by synthesis.

### MATERIALS AND METHODS

#### 1. Production, Fractionation and Bioassay of the Attractant

One liter medium of 1% capric acid and 0.8% Difco nutrient broth in 10 vials was inoculated with *Pseudomonas aeruginosa* (isolated from the capric acid-treated field water as previously reported by Ikeshoji *et al.*<sup>3)</sup>) and incubated at 37°C for 4 days. The bacterial cultures were pooled and extracted with 2 liters of ether. The ether extract was washed with a half volume of 10 N NaOH solution saturated with

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NaCl three times to eliminate the remaining capric acid and other fatty acids. The washed extract, after condensation *in vacuo*, was chromatographed on four plates of silicagel with the developing solvent of hexane and ether 9 : 1. The plates were scraped into four zones, the *R<sub>f</sub>* values of 1–0.9, 0.9–0.7, 0.7–0.1 and 0.1–0, which were eluted with ether for bioassay and further chemical analysis.

For bioassay, a portion of the eluate was dropped onto a slide glass, which covered treated-face-down a 5-cm-diam petridish lined inside with a strip of filter paper and containing 6 ml of tap water. The dishes covered with the treated and untreated slides were set in a 30-cm cubic cage accommodating about 100 of *A. aegypti* in the dark overnight. The attractancy was expressed by the average ratios of the numbers of eggs laid between the treated and untreated dishes. Because the analytical fractions could not be defined quantitatively, they were bioassayed at decimally diluted three or four concentrations, which usually covered a repelling high and undetectably low concentrations to mosquitos. Each test was replicated several times.

## 2. Gas-chromatographic Isolation and Mass-spectroscopic Identification

The eluate of the front zone was further chromatographed with a Shimadzu GC-4B gas chromatography equipped with a FID and a 3-mm wide and 3-m long stainless column packed with 5% OV 101 on 60–80 mesh Chromosorb W. By programming the column temperature from 100 to 230°C at a rate of 2°C/min, some 10 peaks were resolved. By attaching a splitter on the column (1 : 9 ratio) four zones were preparatively collected by several injections.

For analytical purpose, on the other hand, a 20-m glass capillary column coated with OV 101 was used operated at the oven temperature of 180–200°C at 1°C/min. For mass-spectroscopic identification of each resolved compound, a Shimadzu LKB GC-MS 9000 mounted with the same stainless column was used at 180°C of the column temperature, 290°C of the separator temperature and 70 eV of the electron energy.

## 3. Synthesis of 7,11-Dimethyloctadecane

This was synthesized by the following two methods.

### 4. Method I. Grignard Coupling Reaction (Fig. 1)

#### 4.1 Carboethoxymethylenetriphenylphosphorane (1)

This was prepared from triphenylphosphine and ethyl bromoacetate according to the procedure previously described.<sup>4)</sup> Mp 120–122°C (lit. mp 116–117°C).

#### 4.2 Ethyl 3-methyl-2-nonenolate (2)

A solution of 74 g (0.21 mol) of the compound (1), 21 g (0.16 mol) of 2-octanone and 10 g (0.08 mol) of benzoic acid in 500 ml of toluene was refluxed with stirring for 20 hr. The mixture was then washed with 10% NaHCO<sub>3</sub>, followed by 5% HCl and water. After removal of the solvent, dry petroleum ether was added to the residue until precipitation ceased. The solid material, triphenyl phosphine oxide, was filtered off. The filtrate was dried, evaporated and the residue was distilled *in vacuo* to afford (2) as a colorless, oily mixture of E and Z isomers (E/Z=6/4), bp 85–90°C (2.5 mm), 19.5 g (60.5 %). IR  $\nu_{\max}$ : 1720 (C=O), 1642 (C=C), 1210 (C–O–C ester), 860 cm<sup>-1</sup>. MS: *m/e* 198 (M<sup>+</sup>), 169, 157. <sup>1</sup>HNMR  $\delta_{\text{TMS}}^{\text{CDCl}_3}$ : Z, 1.87 (3H, d (*J*=1.5 Hz)), 5.65 (olefinic proton); E, 2.15 (3H, d (*J*=1.5 Hz)), 5.66 (olefinic proton).

#### 4.3 3-Methyl-2-nonen-1-ol (3)

A solution of 2.85 g (0.075 mol) of LiAlH<sub>4</sub> in 70-ml dry ether was refluxed for 1 hr with stirring. To this was added slowly 17 g (0.086 mol) of the compound (2) in 100 ml of dry ether under gentle refluxing, and the mixture was stirred for 1 hr at room temperature and refluxed for additional 1 hr. After cooling, the reaction mixture was diluted with 300 ml of ether, and then treated with 15–20 ml of water. The resulted mixture was filtered with Celite to remove the precipitation (Al(OH)<sub>3</sub>), and the filtrate was dried, evaporated and the residue was distilled *in vacuo* to afford the compound (3) as a colorless, oily mixture of E and Z isomers, bp 80–85°C (2.5 mm), 12.5 g (92%). IR  $\nu_{\max}$ : 3300 (OH), 1660 (C=C), 1,000 (alk. C–O) cm<sup>-1</sup>. <sup>1</sup>HNMR  $\delta_{\text{TMS}}^{\text{CDCl}_3}$ : E, 1.69 (3H, d (*J*=1.5 Hz)), 4.11 (2H, d (*J*=7 Hz)); Z, 1.77 (3H, d (*J*=1.5 Hz)), 4.28 (2H, d (*J*=7 Hz)).

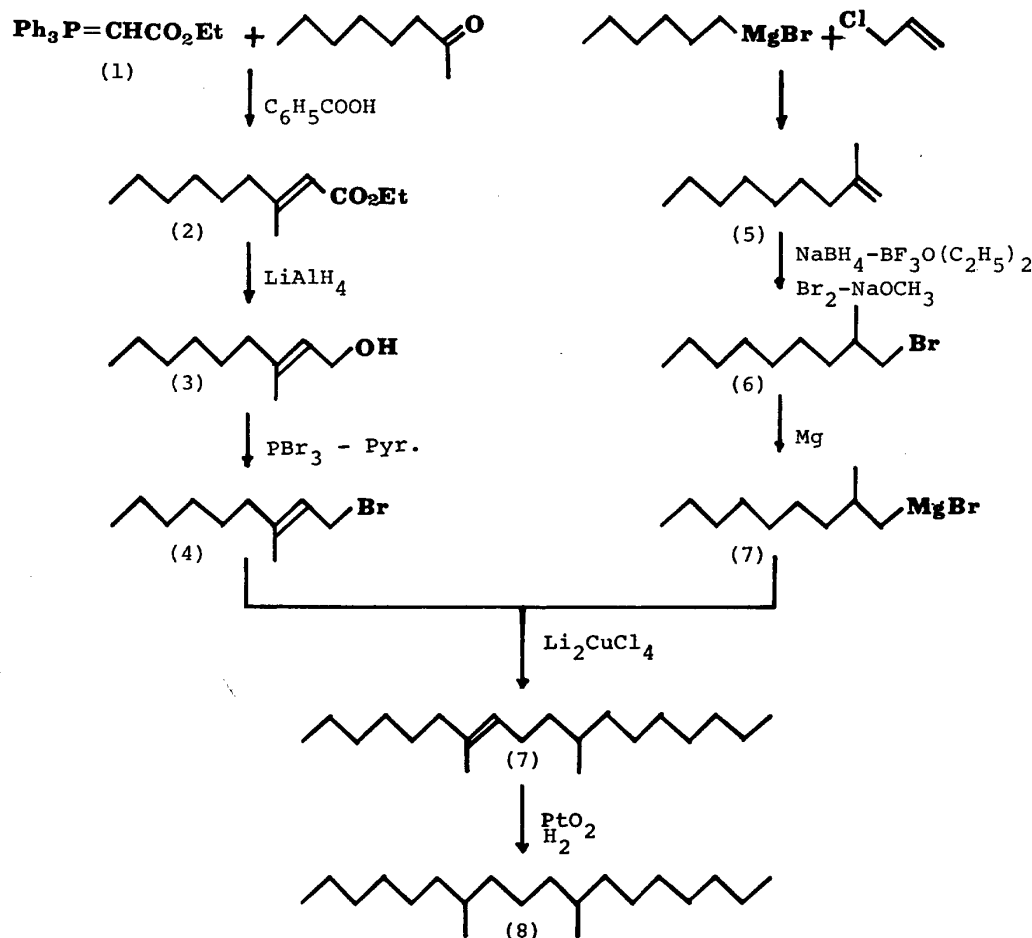


Fig. 1 Synthesis of 7,11-dimethyloctadecane (8) by Grignard coupling reaction (Method I).

#### 4.4 1-Bromo-3-methyl-2-nonene (4)

To a chilled solution of 11 g (0.07 mol) of the compound (3) and 2.5 g of pyridine in 80 ml of dry ether was added dropwise 99.5 g of phosphorous tribromide (0.035 mol) over a period of 30 min at 5–10°C, and stirred for 1 hr. The mixture was poured into ice water and extracted with ether. The ether solution was washed with water,  $\text{NaHCO}_3$  and saturated salt solution, then dried. After removal of the solvent, the residue was distilled *in vacuo* to afford the compound (4) as a colorless oily mixture of E and Z isomers, bp 73–76°C (2.5 mm), 10.5 g (68%). IR  $\nu_{\text{max}}$ : 1660, 1202, 842, 595  $\text{cm}^{-1}$ . MS:  $m/e$  218, 220 ( $\text{M}^+$ ).  $^1\text{H}$ NMR  $\delta_{\text{TMS}}^{\text{CDCl}_3}$ : E, 1.73 (3H, d ( $J=1.5$  Hz)), Z, 1.79 (3H, d ( $J=1.5$  Hz)), 3.98 (2H, d ( $J=7$  Hz)), 5.57 (1H,  $J=7$  Hz).

#### 4.5 2-Methyl-1-nonene (5)

*n*-Hexyl magnesium bromide was prepared in the usual way from 7.44 g (0.31 mol) of Mg,

50 g (0.304 mol) of *n*-hexyl bromide and 120 ml of dry ether. To the Grignard reagent was added dropwise 28 g (0.31 mol) of methallyl chloride, and the mixture was refluxed for 1 hr with stirring. After standing for 20 hr at room temperature, the mixture was poured into a mixture of  $\text{NH}_4\text{Cl}$  and ice water. The organic layer was separated and the aqueous layer was extracted with 50 ml of ether. The combined ether solution was washed with 100 ml of saturated salt solution, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and the ether was evaporated. The residue was distilled *in vacuo* to afford a colorless oil (5), bp 160–166°C, 20 g (46.2%). The infrared spectrum of this compound was identical with that of authentic sample.<sup>5)</sup>

#### 4.6 1-Bromo-2-methylnonane (6)

To a stirred mixture of 1.14 g (0.03 mol) of  $\text{NaBH}_4$  and 14 g (0.1 mol) of the compound (5) in 60 ml of dry THF was added dropwise 6 ml of  $\text{BF}_3\text{O}(\text{C}_2\text{H}_5)_2$  over 30 min and stirred for

1.5 hr at room temperature. After addition of 5 ml of methanol, this was chilled to 0°C and 9 ml of bromine was gently added. To this was added dropwise over a period of 1.5 hr a sodium methoxide solution prepared from 5 g of sodium and 60 ml of methanol. The solution was diluted with 50 ml of *n*-hexane, 20 ml of water and saturated  $K_2CO_3$  solution and extracted with ether. The ether solution was washed with water, saturated salt solution and saturated  $NaHCO_3$ , dried over  $Na_2SO_4$  and the ether was evaporated. The residue was distilled *in vacuo* to afford (6), bp 75–80°C (2.5 mm), 15.7 g (71%). IR  $\nu_{max}$ : 1460, 1380, 1230, 722, 655, 620  $cm^{-1}$ . MS:  $m/e$  222, 220 ( $M^+$ ). NMR  $\delta_{TMS}^{CDCl_3}$ : 0.94 (3H, d ( $J=7$  Hz)), 3.33 (2H, d ( $J=7$  Hz)).

#### 4.7 7,11-Dimethyl-7-octadecene (7) and 7,11-dimethyloctadecane (8)

To 40-ml dry THF solution of 8 g (0.0365 mol) of the compound (4) were added Grignard reagent, which was prepared from 9 g (0.0407 mol) of the compound (6), 1 g (0.0417 mol) of Mg and 40 ml of dry THF, and 2 ml of 0.1 M  $Li_2CuCl_4$  solution. After stirring for 15 hr at room temperature, the reaction mixture was slowly poured into iced dilute sulfuric acid and extracted with ether. The ether extract was washed with water and saturated salt solution, dried over  $Na_2SO_4$  and ether was evaporated.

The residue was distilled *in vacuo* to afford a pale yellow viscous oil (7), bp 145–152°C (2.5 mm), 120–123°C (0.25 mm), 6.3 g (61.1%). MS:  $m/e$  280 ( $M^+$ ). NMR  $\delta_{TMS}^{CDCl_3}$ : 4.95 (olefinic proton).

Fifty mg of platonic oxide in 70 ml of ethanol containing catalytic amounts of acetic acid was shaken in presence of hydrogen and added with 1.2 g of the compound (7). The resulted mixture was stoichiometrically hydrogenated in 3 hr under atmospheric pressure and room temperature. After removal of the solvent and the catalyst, the residue was dissolved in 30 ml of ether, washed with saturated salt solution, dried over  $Na_2SO_4$  and the ether was evaporated. The residue was distilled *in vacuo* to afford the compound (8) in pure state, bp 143°C (3.0 mm), 1.0 g (84%). IR  $\nu_{max}$ : 2950, 2880, 1465, 1382, 727  $cm^{-1}$ . MS:  $m/e$  282 ( $M^+$ ).

#### 5. Method II. Combination of Acetonylation and Wittig Reaction (Fig. 2)

##### 5.1 3-Methylnonan-1-ol (9)

Two and half g of palladium carbon (5%) was shaken in 85 ml of ethanol in presence of hydrogen, and added with 8.4 g of 3-methylnonen-1-ol (3). The resulted mixture was hydrogenated under same condition as described above. Within 5 hr, the theoretical amounts of hydrogen was absorbed. After

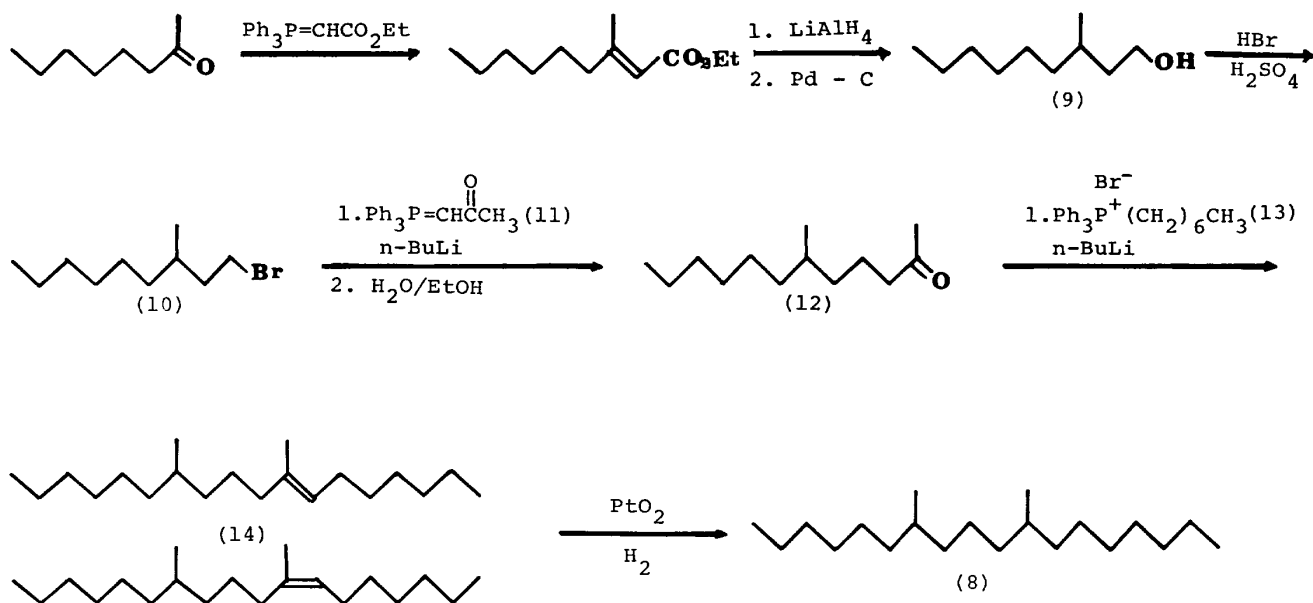


Fig. 2 Synthesis of 7,11-dimethyloctadecane by a combination of acetonylation and Wittig reaction (Method II).

removal of the solvent and the catalyst, the residue was distilled *in vacuo* to give the colorless oil (9) in almost quantitative yield (8.3 g), bp 82–83°C. IR  $\nu_{\max}$ : 3310 (OH), 1460, 1387, 1058 (alk. C–O)  $\text{cm}^{-1}$ . NMR  $\delta_{\text{TMS}}^{\text{CDCl}_3}$ : 0.84 (3H, d ( $J=7$  Hz)), 3.46 (2H, t ( $J=7$  Hz)), 3.74 (1H, b.s.,  $-\text{CH}_2\text{OH}$ ).

#### 5.2 1-Bromo-3-methylnonane (10)

A mixture of 3.5 g (0.022 mol) of the compound (9), 8.6 g (0.05 mol) 48% hydrobromic acid and 1.4 ml of conc. sulfuric acid was refluxed for 6 hr. The resulted mixture was diluted with 30 ml of water, and extracted with 50 ml of ether. The ether extract was washed with 10%  $\text{Na}_2\text{CO}_3$  and saturated salt solution and dried. After removal of the solvent, the residue was distilled *in vacuo* to give (10), bp 66–69°C (3 mm), 2.8 g (56%). IR  $\nu_{\max}$ : 2950, 2890, 1380, 655, 572  $\text{cm}^{-1}$ . MS:  $m/e$  222, 220 ( $\text{M}^+$ ). NMR  $\delta_{\text{TMS}}^{\text{CDCl}_3}$ : 0.94 (3H, d ( $J=7$  Hz)), 3.33 (2H, d ( $J=7$  Hz)).

#### 5.3 Acetylmethylenetriphenylphosphorane (11)<sup>6)</sup>

Acetonyltriphenylphosphonium chloride (11a) was prepared from the reaction of triphenyl phosphine with chloroacetone in chloroform, mp 237–238°C (lit. mp 234–237°C). A mixture of (11a) 18 g (0.05 mol) and 19% aqueous  $\text{Na}_2\text{CO}_3$  was shaken for 8 hr. The crystal was collected and recrystallized from methanol–water to afford (11), 13 g (81.3%), mp 207°C (lit. 205–216°C).

#### 5.4 *n*-Heptyltriphenylphosphonium bromide (13)<sup>7)</sup>

A mixture of 27 g (0.151 mol) of *n*-heptyl bromide and 39.6 g (0.151 mol) of triphenyl phosphine was refluxed in 150 ml of xylene with stirring for 27 hr. After removal of the solvent, the viscous residue was washed with ether and stood in a refrigerator to give the crystalline product, yields 57.6 g (86.8%), mp 171–174°C (lit. mp 171–174°C).

#### 5.5 6-Methyldodecan-2-one (12)

A stirred suspension of 4.3 g (0.014 mol) of the powdered compound (11) in 50 ml of dry THF was cooled in a dry ice–acetone bath at  $-85^\circ\text{C}$  under nitrogen atmosphere, and dropwise added with 7.6 ml (0.014 mol) of 15% *n*-BuLi in hexane. After stirring for 15 min, 2.5 g (0.011 mol) of the compound (10) in 15 ml of dry THF was added to the above ylide solution. The mixture was stirred at  $0^\circ\text{C}$  for

4 hr, whereupon the color of the ylide anion was nearly discharged. The solvent was removed under reduced pressure and the residue was dissolved in 30 ml of ethanol, followed by the addition of 20 ml of water till approaching the cloud point. The resulted solution was heated for 22 hr at  $70\text{--}80^\circ\text{C}$  with stirring, then poured into a saturated salt solution and extracted twice with 60 ml of *n*-hexane. The extracts were dried, the solvent was removed *in vacuo* and the residue was treated with petroleum ether to remove  $\text{Ph}_3\text{PO}$ . After cooling, the precipitated  $\text{Ph}_3\text{PO}$  was filtered off and the filtrate was evaporated *in vacuo* to give an oily residue. Distillation of the residue afforded 2-g (92%) of colorless oil (12), bp  $88^\circ\text{C}$  (2.5 mm). IR  $\nu_{\max}$ : 1720 (C=O), 1170  $\text{cm}^{-1}$ . MS:  $m/e$  198 ( $\text{M}^+$ ), 183, 180, 85, 58, 43. NMR  $\delta_{\text{TMS}}^{\text{CDCl}_3}$ : 0.89 (6H, 2 $\text{CH}_3$ –), 2.12 (3H, s), 2.40 (2H, t ( $J=7$  Hz)).

#### 5.6 8,12-Dimethyl-7-octadecane (14)

To a stirred suspension of 6.4 g (0.0145 mol) of the compounds (13), in 30 ml of dry ether, was added dropwise 8.9-ml (0.0145 mol) hexane solution of 15% *n*-BuLi at  $0^\circ\text{C}$ . After 1-hr stirring, 2.4 g (0.0121 mol) of the compound (12) in 10 ml of dry ether was slowly added over a period of 20 min. The reaction mixture was again stirred for 3 hr at room temperature under nitrogen atmosphere. This was then poured into 30 ml of water and neutralized with a dilute HCl. The aqueous layer was extracted with 20 ml of ether. The ether solution was pooled, washed with water, dried and evaporated. The residue was distilled *in vacuo* to afford 3.1 g (92%) of the compound (14), bp  $108\text{--}123^\circ\text{C}$ , as an approximately equimolecular mixture of E and Z isomers. IR  $\nu_{\max}$ : 2953, 2900, 1463, 1380  $\text{cm}^{-1}$ . MS:  $m/e$  280 ( $\text{M}^+$ ), 182, 126, 98, 84. NMR  $\delta_{\text{TMS}}^{\text{CDCl}_3}$ : 4.93 (olefinic proton).

#### 5.7 7,11-Dimethyloctadecane (8)

The compound (14) was hydrogenated with platinum oxide as in Method I to afford the compound (8), bp  $143^\circ\text{C}$  (3 mm). The mass spectrum of the compound (8) was almost identical with that of natural product.

## RESULTS

### 1. Chromatographic Isolation and Mass-spectroscopic Identification

When the ether eluates of four *tlc* zones were bioassayed against four species of mosquitos,

the front zone eluate was 4.9 times as attractive as the untreated water against *A. aegypti*, and 2 to 7 times attractive against *Culex pipiens pallens* Coq., *C. p. molestus* and *Aedes albopictus* SKUSE. Nevertheless, the last three species of mosquitos were not studied further in the present work. The eluates of the other three *tlc* zones were only 2.5, 1.8 and 0.6 times attractive against *A. aegypti* respectively. In fact, the last eluate was quite repellent because of

slight inclusion of capric acid inspite of the alkaline wash of the ether extract.

The analytical gas chromatogram of the front zone eluate is shown in Fig. 3, and the identified chemical names are given to the corresponding peaks. The 1st GC-zone shown in Fig. 3 including a large quantity of *n*-heptadecane, *n*-heptadecene and 7-methyloctadecane was not attractive at any concentrations tested, but rather repellent showing 0.4-time attrac-

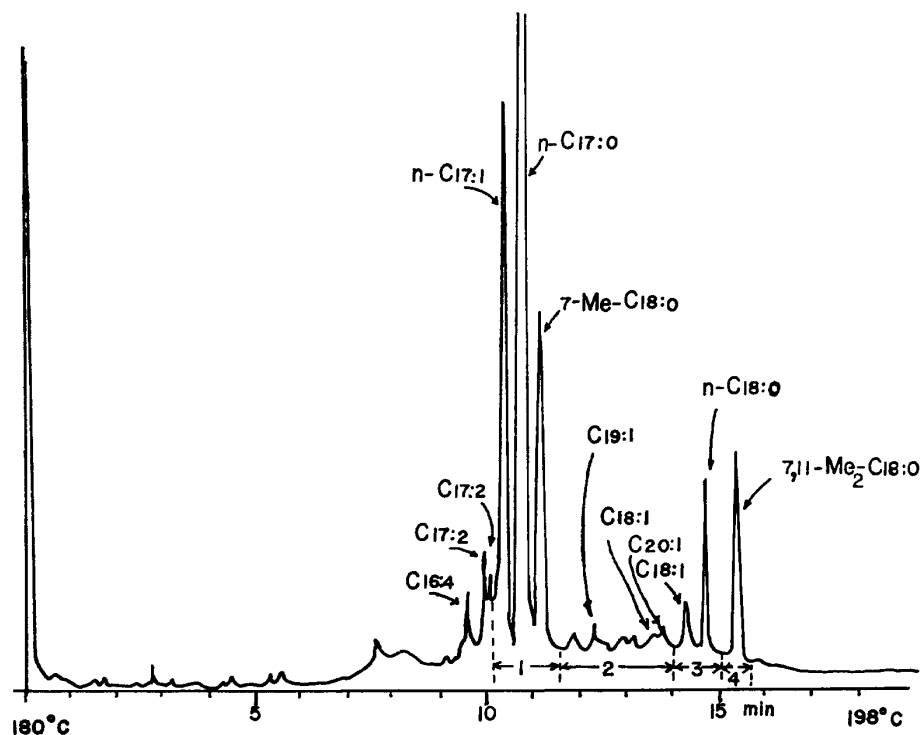


Fig. 3 Gas chromatogram of the hydrocarbon eluate of the frontal *tlc*-zone which was extracted from the bacterial culture medium.

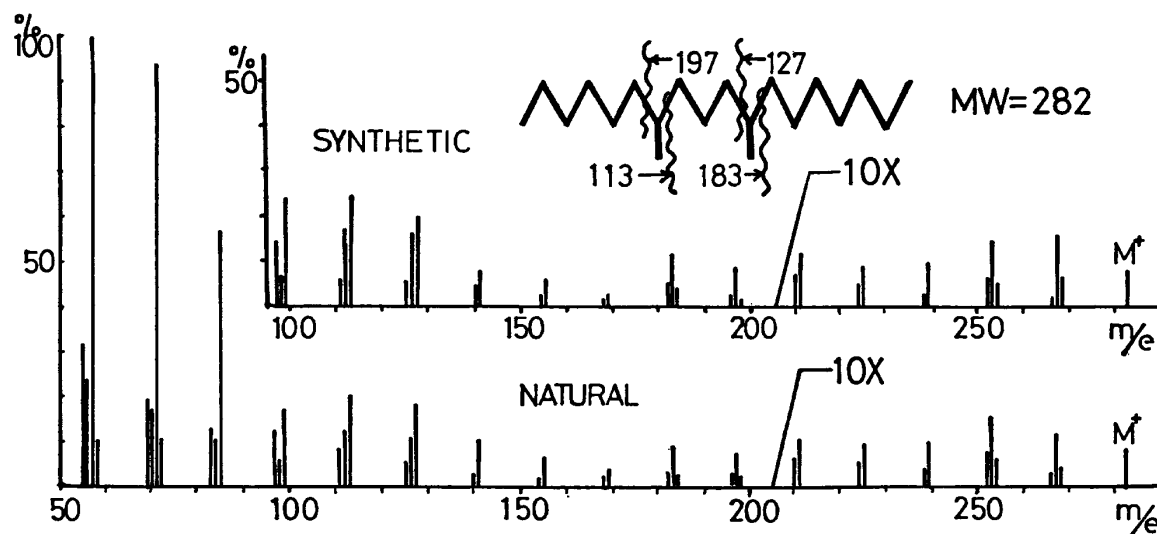


Fig. 4 Mass spectra of the isolated and synthetic 7,11-dimethyloctadecanes.

tancy index. The 2nd GC-zone including minor quantity of octadecane, nondecane and eicocene was 2.2 times attractive at most. The 3rd GC-zone including *n*-octadecane and some *n*-octadecene was 3.8 times attractive. Whereas, the final GC-zone including 7,11-dimethyloctadecane was 6.7 times attractive. The mass spectrum of the isolated 7,11-dimethyloctadecane is shown in Fig. 4 with that of synthetic one for comparison. The molecular ions  $m/e=282$  in both spectra are conspicuous and the fragmentation patterns are similar with the relatively intense fragments of  $m/e=113, 137, 183$  and  $197$ , all of which represent cleavage on either side of the two methyl branches.  $[M-CH_3]^+$  and  $[M-2CH_3]^+$  are also strong. These fragments do not occur with the corresponding straight-chain hydrocarbons.

## 2. Ovipositional Attractancy of the Synthetic Hydrocarbons

To confirm the attractancy of 7,11-dimethyloctadecane and to explain some attractancy of the preceding poorly resolved 2nd and 3rd GC-zones, we synthesized and obtained from other sources some of the major saturated hydrocarbons identified and all geometrical isomers of *cis*- and *trans*-octadecenes. The synthetic 7,11-dimethyloctadecane, which had been purified by 30%  $AgNO_3$ -silicagel *tlc* to eliminate the unsaturated hydrocarbons and further fractionated by GC under the same preparative condition was 2.9 times attractive at the approximate dosage of 0.4 mg/slide when tested at several concentrations.

*n*-Heptadecane and *n*-octadecane at 0.05 mg, 0.2 mg and 0.5 mg/slide were quite repellent showing 0.3 to 1-time attractancy indexes. None of the octadecenes and *cis*-8-hexadecene showed attractancy at the above concentrations. Therefore, saturated and unsaturated straight-chain hydrocarbons were unlikely responsible for the attractancy of the 2nd and 3rd GC-zones. The synthetic 7-methyloctadecane was attractive showing 1.9 times at 0.05 mg/slide. The attractancy of this hydrocarbon included in the 1st GC-zone was apparently masked by the massive quantity of the repellent *n*-heptadecane.

## DISCUSSION

### 1. Natural Occurrence and Possible Biosynthetic Process of the Monomethyl Hydrocarbons

Methyl-branched hydrocarbons occur extensively in natural lipids.<sup>8)</sup> However, the abundant occurrence of C17-C20 methyl-branched and straight-chain hydrocarbons as shown in the present analysis are restricted only to the aquatic protists such as bacteria and blue green algae. Particularly, such Cyanophycophyta as *Anacystis cyanea*, *Lyngbya aestuarii*, *Nostoc* spp., *Chroococcus turgidus*, *Phormidium luridum* and *Anabaena variabilis* produce 7-methyl and 8-methylheptadecane in large quantity.<sup>9-12)</sup> The bacterium, *Vibrio marinus* also produces *n*-heptadecane. Together with *n*-octadecane and *n*-heptadecane, earth worm contains a series of isomeric methylheptadecanes, which presumably originates from the bacteria acting on humus in soil.<sup>14)</sup> Therefore, the production of these so-called aquatic hydrocarbons by *Pseudomonas aeruginosa* should not be strange.

Concerning the biosynthesis of these monomethyl hydrocarbons from capric acid substrate in the bacterial medium would probably take the following process. First, the capric acid is degraded to caprylic acid, which then forms *n*-heptadecene and *n*-heptadecane with capric acid in head-in-head condensation followed by decarboxylation. Meanwhile, *n*-octadecane is synthesized similarly but without decarboxylation. According to Albro and Dittmer<sup>13)</sup> the proportion of decarboxylation following the head-to-head condensation is dependent on the concentration of acetic acid involved in the bacterial medium. In this experiment, acetic acid should be abundant on capric acid degradation in the medium. One portion of 7-octadecene then would be saturated to form *n*-octadecane, and the other portion methylated at carbon 7 to form 7-methyloctadecane with methionine. However, a plausible explanation for biosynthetic process of 7,11-dimethyloctadecane is not possible at present.

### 2. Comparison of the Isolated and Synthetic 7,11-dimethyloctadecanes

No plausible explanation of the biosynthetic

process and no occurrence of 7,11-dimethyloctadecane hitherto might suggest our misinterpretation of the unresolved peak of the GC-zone 4. In fact, Han *et al.*<sup>11)</sup> could recognize the presence of an equimolecular mixture of 7-methylheptadecane and 8-methylheptadecane in *Nostoc* hydrocarbon only by comparing their retention times with the synthetic 7,9-dimethylhexadecane, since the mixture gave the identical mass-fragmentation pattern with that of the dimethylhexadecane.

However, this was not the case in our analysis. The synthetic 7,11-dimethyloctadecane gave the same retention time with the corresponding isolated peak, but the synthetic 7-methyl and 8-methylnonadecanes were retained a little longer than the dimethyloctadecane was. Besides, these methylnonadecanes were not attractive individually and in mixture at any concentration tested.

The best attractancy of the synthetic dimethyloctadecane was 2.9 times at the approximate dosage of 0.4 mg/slide, in contrast to 6.9 times of the isolated one. This discrepancy could be explained with undetected minor inclusion of an attractant in the GC-zone 4, and/or the differential attractancy of the optical isomerism of the isolated and synthetic dimethyloctadecanes. This study is in progress.

#### ACKNOWLEDGEMENT

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#### 要 約

#### Capric acid を基質として *Pseudomonas aeruginosa* が生産するネッタイシマカの産卵誘引物質: 7, 11-Dimethyloctadecane

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直島好伸, 上田博夫

Maw (1970) はカプリン酸を処理した野外水が多種類の蚊を誘引し産卵させることを発見した。池庄司ら (1975) はカプリン酸処理水から *Pseudomonas aeruginosa* を分離し, *in vitro* で *A. aegypti* (ネッタイシマカ) に対する産卵誘引物質を産生させた。

本報は培地から, その誘引物質をエーテル抽出し, 脂肪酸を鹼化, 塩析により除去後, 薄層およびガスクロマトグラフィーで分離し, 質量スペクトルで, 7, 11-dimethyloctadecane と同定した。さらに, 二つの方法でこれを合成し, 確認した。

しかし, 生物検定の結果, 7, 11-dimethyloctadecane のみを含むと思われる分画は水道水と比較して, 6.7 倍の誘引性を示したが, 合成物質は 2.9 倍の誘引性しか示さなかった。この誘引性の差についてはさらに検討中である。

細菌, 藍藻などの水生生物による monomethyl hydrocarbons の生産は一般的であり, 本実験でも, 多量の *n*-heptadecane, *n*-octadecane の他 7-methyloctadecane が分離され, とりわけ合成した 7-methyloctadecane は, 1.9 倍の産卵誘引性を示した。