Studies on the Mating Behavior of the Cockroach, *Nauphoeta cinerea* (OLIVIER) (Dictyoptera: Blaberidae)

IV. Synthesis and Biological Activity of Nauphoetin and Related Compounds

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Nauphoetin, octadecyl (*Z*)-9-tetracosenoate, and related compounds were synthesized and their biological activities were evaluated. Mixing with the wing-raising stimulant of *Nauphoeta cinerea*, nauphoetin and its isomers suppressed wing-raising. The related compounds consisting of saturated esters showed no suppressive activity.

INTRODUCTION

Nauphoetin was isolated and identified as an intermale recognition pheromone from the cuticular wax of male *N. cinerea* (FUKUI and TAKAHASHI, 1983). The pheromone together with the isomers and analogs were synthesized to test the suppressive activity of the wing-raising stimulant produced by male and female conspecifics.

MATERIALS AND METHODS

Synthesis of methyl 9-tetracosenoate. Butyllithium in hexane (15% solution, 4.5 ml) was added into ether solution (50 ml) of pentadecyltriphenylphosphonium bromide (5.26 g, 0.01 mol) under nitrogen with stirring. 8-Carbomethoxyoctanal (1.72 g, 0.01 mol) in ether (20 ml) was added to the above mixture and stirred for 2 hr. After addition of ice water, the mixture was extracted with ether. The product was adsorbed on silicic acid (30 g) and eluted with benzene to give a colorless powder (2.85 g, 75% yield). Two spots of *R_f* 0.3 and 0.45 were identified on a TLC plate impregnated with 30% AgNO₃ developed with 30% benzene in hexane.

Separation of (Z)- and (E)-isomers of methyl 9-tetracosenoate. The above product (1 g) was chromatographed on a 30% AgNO₃ impregnated silicic acid column. Elution with 15% benzene in hexane gave a small amount of (*E*)-isomer (0.2 g, m.p. 31-32°C) and further elution with 30% benzene in hexane gave (*Z*)-isomer (0.7 g, oil).

Methyl (E)-9-tetracosenoate. The reaction product (0.306 g, 0.8 mmol) by WITTIG reaction was heated with thiophenol (0.09 g, 0.8 mmol) and benzene (0.2 mmol) in the presence of azoisobutylnitrile (0.003 g) in a sealed tube at 60°C (SGOUTAS and KUMMEROW, 1969). After cooling, a silver nitrate (0.136 g, 0.8 mmol) solution was

added and extracted with hexane. The product was chromatographed on a 30% AgNO₃ impregnated silicic acid column. Elution with 15% benzene in hexane gave (*E*)-isomer (0.21 g, m.p. 31–32°C) and with an additional 30% benzene in hexane gave a small amount of (*Z*)-isomer (0.04 g, oil).

Synthesis of nauphoetin from (Z)-9-tetracosenoyl chloride and stearyl alcohol. The above methyl (*Z*)-9-tetracosenoate (1.2 g) was hydrolyzed and followed by reaction with thionyl chloride at 70°C. After evaporation of thionyl chloride, the acid chloride was dissolved in benzene. Stearyl alcohol (0.87 g) was added to the benzene solution and boiled for 2 min. The product was collected and crystallized from hexane to give colorless needles (1.5 g, m.p. 34–35°C, 76% yield).

Synthesis of octadecyl (E)-9-tetracosenoate. From methyl (*E*)-9-tetracosenoate (0.3 g) and stearyl alcohol (0.22 g), (*E*)-isomer of nauphoetin (0.4 g, m.p. 45–46°C) was obtained.

Synthesis of saturated and unsaturated esters related to nauphoetin. All esters were prepared by the acid chloride method from the corresponding acids and alcohols. Melting points of the products with the characteristic peaks in mass spectrometry (MS) are shown in Table 1.

Bioassay of the wing-raising stimulants and the depressant. The bioassays were carried by the method previously described (TAKAHASHI and FUKUI, 1980). The wing-raising stimulants (HPLC fr. 2) were dissolved in carbon tetrachloride and prepared 10 mg/ml solution. The number of males raising their wings in the assay was recorded. Nauphoetin, the isomers and the analogs were added to the wing-raising stimulant solution and the wing-raising activity of the mixtures was assayed.

RESULTS AND DISCUSSION

Wing-raising activity of the hydrocarbon mixture (HPLC fr. 2) was found to be 90% (N=50) at the concentration of 10 mg/ml. Addition of different amounts of nauphoetin into the solution decreased the wing-raising activity. Results are shown in Table 2. A solution of the hydrocarbons (10 mg) and nauphoetin (1 mg) in 1 ml of carbon tetrachloride showed no wing-raising activity by bioassay. All synthesized compounds were bioassayed with the same mixing ratio and the results are shown in Table 3. From these results we found that nauphoetin, its (*E*)-isomer and octadecyl (*Z*)-15-tetracosenoate have high depressant activity to the wing-raising stimulants. However, saturated esters corresponding to nauphoetin, octadecyl tetracosanoate and tetracosyl octadecanoate, revealed no depressant activity but, rather, showed 87% and 73% wing-raising activity. On the contrary, a double bond in the alcohol moiety gave intermediate depressant activity. Regardless of the length of the carbon chain in the acid moiety, presence of a double bond is essential for depressant activity. Male-produced mating deterrents have been described in flies, *Glossina morsitans* and *Musca domestica*, and the term "abstinons" has been proposed for these substances (SCHLEIN et al. 1981). The presence of such mating deterrents produced by the male cricket, *Teleogryllus commodus* is critical for the recognition of the sexes (RENCE and LOHER, 1977). Our chemical identification of nauphoetin is the first example of an abstinon or male-produced deterrent in insects.

Table 1. Synthetic compounds tested for depressant activity

Compound	Structure	M.p. (°C)	Characteristic peaks in MS (<i>m/z</i>)
Nauphoetin	$\text{CH}_3-(\text{CH}_2)_{13}-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_{17}-\text{CH}_3$ (<i>Z</i>)	34–35	618(M^+), 348($\text{M}-\text{C}_{18}\text{H}_{37}\text{OH}$) ⁺
(<i>E</i>)-Isomer of nauphoetin	$\text{CH}_3-(\text{CH}_2)_{13}-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_{17}-\text{CH}_3$ (<i>E</i>)	45–47	618(M^+), 348($\text{M}-\text{C}_{18}\text{H}_{37}\text{OH}$) ⁺
Octadecyl	$\text{CH}_3-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_{13}-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_{17}-\text{CH}_3$ (<i>Z</i>)	44–45	618(M^+), 348($\text{M}-\text{C}_{18}\text{H}_{37}\text{OH}$) ⁺
(<i>Z</i>)-15-tetracosenoate	$\text{CH}_3-(\text{CH}_2)_{22}-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_{17}-\text{CH}_3$	66–67	620(M^+), 369($\text{C}_{23}\text{H}_{47}\text{COO}^+\text{H}_2$)
Octadecyl tetracosanoate	$\text{CH}_3-(\text{CH}_2)_{16}-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_{23}-\text{CH}_3$ (<i>Z</i>)	65–67	620(M^+), 285($\text{C}_{17}\text{H}_{35}\text{COO}^+\text{H}_2$)
Tetracosyl octadecanoate	$\text{CH}_3-(\text{CH}_2)_{22}-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_8-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CH}_3$ (<i>Z</i>)	44–46	618(M^+), 250($\text{M}-\text{C}_{23}\text{H}_{47}\text{COOH}$) ⁺
(<i>Z</i>)-9-Octadecenyl tetracosanoate	$\text{CH}_3-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_{23}-\text{CH}_3$ (<i>Z</i>)	41–42	618(M^+), 264($\text{M}-\text{C}_{24}\text{H}_{49}\text{OH}$) ⁺
Tetracosyl	$\text{CH}_3-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CO}\cdot\text{O}\cdot(\text{CH}_2)_{17}-\text{CH}_3$ (<i>Z</i>)	25>	534(M^+), 264($\text{M}-\text{C}_{18}\text{H}_{37}\text{OH}$) ⁺
Octadecyl			
(<i>Z</i>)-9-octadecenoate			

Table 2. Depressant effect of synthetic nauphoetin on the wing-raising stimulant

Test solution (mg/ml CCl ₄)		
Wing-raising stimulant	Nauphoetin	Wing-raising response (%)
10	0	90
10	0.0025	87
10	0.025	33
10	0.5	3
10	1.0	0
10	5.0	0

Substrate for the test: *P. japonica* (♂) antenna.

Fifty *N. cinerea* males were used for the test of each solution.

Table 3. Depressant effect of 1 mg of synthetic compound added to the wing-raising stimulant solution (10 mg/ml CCl₄)

Compound	Wing-raising response (%)
Nauphoetin	0
(<i>E</i>)-Isomer of nauphoetin	0
Octadecyl (<i>Z</i>)-15-tetracosenoate	0
Octadecyl tetracosanoate	87
Tetracosyl octadecanoate	67
(<i>Z</i>)-9-Octadecenyl tetracosanoate	13
Tetracosyl (<i>Z</i>)-9-octadecenoate	0
Octadecyl (<i>Z</i>)-9-octadecenoate	0

Substrate for the test: *P. japonica* (♂) antennae.

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