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# Insect Juvenile Hormone Analogues and Their Biological Activity on Sea Lice (*Lepeophtheirus salmonis*)

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A number of synthetic compounds that exhibit juvenile hormone activity when tested on the insect species *Tenebrio molitor* have been shown to inhibit the development of the sea lice species *Lepeophtheirus salmonis*. The testing was carried out on both isolated nauplius larvae and live Atlantic salmon (*Salmo salar*).

**KEYWORDS: Fish farming; Atlantic salmon; *Salmo salar*; sea lice; *Lepeophtheirus salmonis*; juvenile hormone analogues**

## INTRODUCTION

Fish farming has become a very important source of fish for human consumption. Because of the high population density, farmed fish are particularly susceptible to infections, and disease control is an important part of this activity. In this respect the frequent occurrence of infestations by ectoparasites constitutes a serious problem, giving rise to considerable economic loss to the fish farmers. The ectoparasites affect the health of farmed fish in both freshwater and seawater environments. The crustacean species called sea lice are among the most harmful of the ectoparasites. Thus, infestations by the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus* are among the most important disease problems in farming of salmonids, particularly for the Atlantic salmon (*Salmo salar*) and the rainbow trout (*Oncorhynchus mykiss*) (*1*). The costs associated with treatment and lower classification ratings of slaughtered fish as well as reduced feed intake contribute to the economic loss caused by sea lice infestation. Furthermore, current research has shown that sea lice infestations could be the main reason for the decline in wild salmonid stock that has been observed in Ireland and Norway in recent years (*2*, *3*).

The life cycle of the sea lice does not involve an intermediate host in the metamorphosis to adult lice. It commences with the mature egg hatching to the nauplius I stage larvae that molts further through the nauplius II into the copepodid, the infestive stage. The copepodid settles normally to the ventral surface and on the fins of the fish, where molting continues successively through four chalimus stages into the pre-adult stage, in which the parasite moves freely on the skin of the host. Adult males may mate with pre-adult females, which therefore may already be pregnant when finally molting to adult. Completion of the life cycle normally takes ~6 weeks at a water temperature between 9 and 12 °C.

At present the most common treatment of sea lice infestastions involves bathing or immersing the fish in solutions of insecticides such as organophosphates or pyrethroids, which are toxic to the ectoparasites. There are several disadvantages with this method. Organophosphates are toxic to humans as well and must therefore be treated with caution; moreover, overdosing has resulted in fish mortality on several occasions. On the other hand, pyrethroids are not acutely toxic to humans, but they are toxic to fish. Another approach involves the use of the chitin synthesis inhibitor teflubenzuron, which by oral administration has been found to be effective against sea lice (*4*). However, the inhibitors have no effect against adult stages of the sea lice, and rapid metabolism in the fish requires repeated treatments. Longer protection against infestation is obtained with in-feed use of emamectin benzoate (Slice), a second-generation avermectin pesticide (*5*, *6*).

There is clearly a need for improved means of controlling sea lice infestations on fish. One possible solution would be compounds that exert antiparasitic activity by interrupting the metamorphosis of the sea lice. Juvenile hormones (JH) are among the hormones that control the metamorphosis of arthropods. The effect of JH on the metamorphosis of insects has been thoroughly studied (*8*), but less so in crustacean species; however, methyl farnesate has been identified in several crustacean species as a compound affecting the metamorphosis (*9*−*12*). Insects treated with an excess of JH at an early stage of development remain juvenile or develop into sterile adult insects. Several structurally different compounds have been shown to exert such an effect, and some of these so-called juvenoids are used commercially for insect control. Examples are methoprene (**1**), hydroprene (**2**), fenoxycarb (**3**), and pyriproxyfen (**4**) (**Figure 1**).

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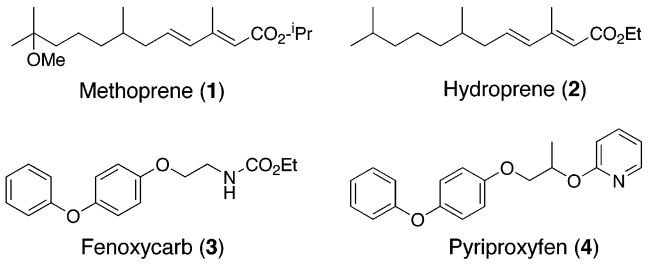



**Figure 1.** Examples of commercially available juvenoids.

In the present work we report on several compounds with juvenile hormone properties in insects that are biologically highly active against sea lice by interrupting the metamorphosis.

## MATERIALS AND METHODS

**Preparation of Compounds.** The compounds tested in the present work are compiled in **Figure 2**. In addition, we tested the commercially available juvenoids **1** and **3**.

The ethers **6**, **11**, **12**, and **14** are known compounds that were prepared according to the literature (*13*). The other aromatic ethers recorded in **Figure 2** were prepared in the same way by reaction of the appropriate alkyl bromide with a DMF solution of the sodium salt of either a hydroxypyridine or a phenol derivative at room temperature. The thioethers were prepared according to a similar procedure; in separate experiments 3-pyridinethiyllithium and 2-thiophenethiyllithium reacted with 1-bromo-3,7-dimethyloctane to yield the thioethers **7** and **15**, respectively. The phenoxy ethers **19** and **20** were prepared from 2-bromo-1-(4-phenoxyphenoxy)ethane (*14*) by reaction with 2- and 3-hydroxypyridine, respectively. Similarly, 4-phenoxybenzyl bromide (*15*) was transformed to the ether **21** with 3-hydroxypyridine. The products were purified by column chromatography, followed by short path distillation or recrystallization, and characterized spectroscopically. The yields of purified ethers ranged from 50 to 90%. The alkylpyridine derivative **8** was prepared from 3-cyanopyridine in 30% overall yield as shown in **Scheme 1**.

The cyclopropane derivatives **13** and **18** were prepared as outlined in **Scheme 2**.

Linalool was converted in excellent yield to the dichlorocyclopropane derivative **22**, as reported in the literature (*16*). Reduction with sodium in liquid ammonia furnished the cyclopropyl alcohol **23**, which was transformed to the bromide **24** in 94% overall yield. Only one isomer is formed, and by comparison of the NMR spectra with those of geranyl bromide and similar compounds, the *E*-configuration has been assigned (*17*). Reaction of the bromide with 3-hydroxy-6-methylpyridine in the usual way afforded the ether **13**, as the *E*-isomer. The hydroprene analogue **18** was prepared by first transforming the diethylacetal of citronellal to the dichlorocyclopropane derivative **25**, which was subsequently reduced and hydrolyzed to the aldehyde **26** in 71% overall yield. The aldehyde underwent a Wittig reaction with the ylid **27**, generated from (*E*)-4-bromo-3-methyl-2-butenoic acid (*18*) and triphenylphosphine. The acid was initially obtained as a mixture of stereoisomers, which was isomerized by benzenthiol to the 2*E*,4*E*-isomer **28** (*19*). Finally, esterification with diazomethane afforded the methyl ester **18**.

**Experimental Procedures.** *General.* The NMR spectra were recorded on Varian Gemini 200 or Varian Mercury-300 instruments using $CDCl_3$ as solvent and TMS as an internal standard. IR spectra were recorded on a Perkin-Elmer Paragon 500 FT spectrometer. MS spectra were recorded on a GC-MS JEOL DX-303. For analytical GLC a 25 m SP 2100 capillary column was used.

*2-Methyl-5-(3,7-dimethyloctyloxy)pyridine (**10**). General Ether and Thioether Synthesis.* To a stirred MeOH solution of NaOMe (2 M, 10 mL, 20 mmol) was added 2.18 g (20 mmol) of 3-hydroxy-6-methylpyridine. After 15 min of stirring at room temperature, the solvent was evaporated under reduced pressure. The sodium salt was dissolved in dry DMF (20 mL), and 4.42 g (20 mmol) of 1-bromo-3,7-dimethyloctane was added. The mixture was stirred at room temperature for 16 h and then diluted with diethyl ether to precipitate NaBr. The organic layer was separated and washed successively with 10% aqueous NaOH and water and dried ($MgSO_4$). Evaporation and short path distillation at 131 °C/0.02 mmHg gave 4.39 g (88%) of the ether **10** as a colorless oil: $^1$H NMR $\delta$ 0.87 (d, $J$ = 6.6 Hz, 6 H), 0.94 (d, $J$ = 6.6 Hz, 3 H), 1.10−1.23 (m, 3H), 1.23−1.38 (m, 3 H), 1.46−1.72 (m, 3 H), 1.77−1.88 (m, 1 H), 2.48 (s, 3H), 3.96−4.04 (m, 2 H), 7.04 (d, $J$

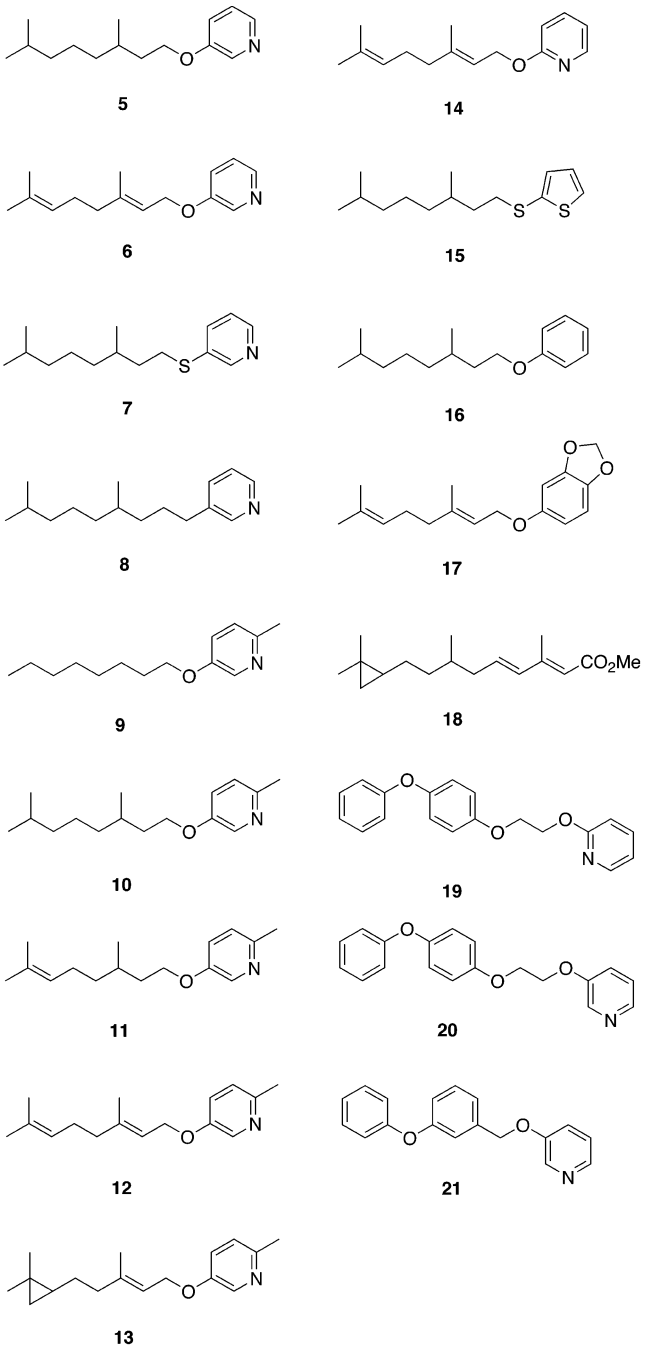
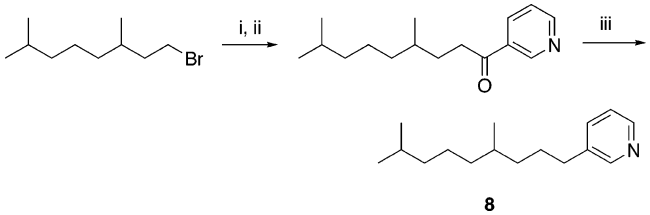


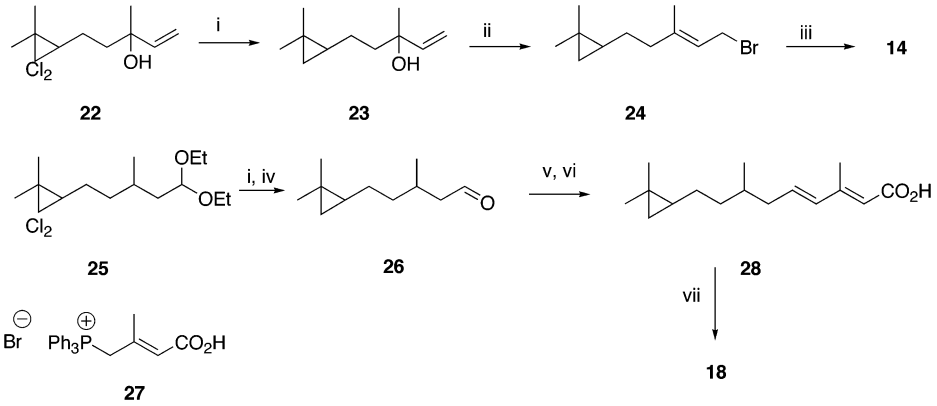

**Figure 2.** Compounds tested in this study.

**Scheme 1.** Synthesis of Compound **8**[a]




[a] Reagents and conditions: i, Mg, ether; ii, HBr; iii, (a) 3-cyanopyridine, (b) 2 N HCl, $H_2O$, D; $N_2H_2$, KOH D.

**Scheme 2.** Synthesis of the Cyclopropane Derivatives **14** and **18**[a]




[a] Reagents and conditions: i, Na, $NH_3$(l), $NH_4Cl$; ii, HBr; iii, NaH, DMF, 3-hydroxy-6-methylpyridine; iv, 2 N HCl, 2-propanol; v, **27**, NaOMe, MeOH; vi, PhSH; vii, $CH_2N_2$, $Et_2O$.

= 8.4 Hz, 1H), 7.10 (dd, *J* = 2.6, 8.4 Hz, 6H), 8.18 (d, *J* = 2.6 Hz, 1 H); $^{13}$C NMR $\delta$ 19.72, 22.70, 22.81, and 23.40 ($CH_3$ × 4), 24.76 ($CH_2$) and 28.06, 29.85 (CH × 2), 36.24, 37.35, 39.32, and 66.86 ($CH_2$ × 4), 122.15, 123.38, and 136.79 (CH × 3), 150.16 and 153.33 (C × 2); IR 1605 (m), 1580 (s), 1505 (s) cm$^{-1}$; HRMS (ESI) found ($M^+$) 249.2088, $C_{16}H_{27}NO$ requires 249.2092.

*3-(3,7-Dimethyloctyloxy)pyridine (**5**).* Reaction of 3-hydroxypyridine and 1-bromo-3,7-dimethyloctane according to the general procedure gave the ether **5** as an oil in 88% yield: $^1$H NMR $\delta$ 0.87 (d, *J* = 6.6 Hz, 6H), 0.95 (d, *J* = 6.2 Hz, 3H), 1.12−1.38 (m, 6H), 1.46−1.74 (m, 3H), 1.78−1.90 (m, 1H), 4.00−4.07 (m, 2H), 7.20 (m, 2H), 8.20 (dd, *J* = 2.1, 4.0 Hz, 1H), 8.31 (bdd, *J* = 1.1, 2.1 Hz, 1H); $^{13}$C NMR $\delta$ 19.78, 22.75, and 22.85 ($CH_3$ × 3), 24.80 ($CH_2$), 28.13 and 29.95 (CH × 2), 36.25, 37.41, 39.38, and 66.88 ($CH_2$ × 4), 121.30, 123.96, 138.16, and 141.97 (CH × 3), 155.45 (C); IR 3047 (m), 1584 (m), 1570 (m), 1467 (s) cm$^{-1}$; HRMS found ($M^+$) 235.1940, $C_{15}H_{25}NO$ requires 235.1936.

*3-(3,7-Dimethyloctylthio)pyridine (**7**).* Reaction of 3-mercaptopyridine (*20*) and 1-bromo-3,7-dimethyloctane according to the general procedure gave the thioether **7**, as an oil in 80% yield: $^1$H NMR $\delta$ 0.86 (d, *J* = 6.6 Hz, 6H), 0.89 (d, *J* = 6.6 Hz, 3H), 1.11−1.32 (m, 6H), 1.42−1.72 (m, 4H), 2.84−3.02 (m, 2H), 7.21 (dd, *J* = 4.8, 8.0 Hz, 1H), 7.63 (ddd, *J* = 1.4, 2.2, 8.0 Hz, 1H), 8.41 (bd, *J* = 4.0 Hz, 1H), 8.56 (bd, *J* = 1.4 Hz, 1H); $^{13}$C NMR $\delta$ 19.46, 22.75, and 22.85 ($CH_3$ × 3), 24.78 ($CH_2$), 28.11 (CH), 31.71 ($CH_2$), 32.32 (CH), 36.34, 37.01, and 39.33 ($CH_2$ × 3), 123.72 (CH), 134.39 (C), 136.79, 146.89, and 149.95 (CH × 3); IR 3020 (m), 1570 (m), 1560 (m), 1475 (s) cm$^{-1}$; HRMS found ($M^+$) 251.1703, $C_{15}H_{25}NS$ requires 251.1707.

*3-(4,8-Dimethyl-1-nonyl)pyridine (**8**).* 3,7-Dimethyl-1-octylmagnesium bromide was prepared from Mg (2.7 g) and 1-bromo-3,7-dimethyloctane (22.10 g, 0.10 mol) in dry ether (50 mL). The Grignard reagent was cooled in an ice bath, and 3-cyanopyridine (9.37 g, 0.09 mol) in dry ether (100 mL) was added dropwise with stirring. The slurry was stirred at room temperature for an additional 17 h and cooled in ice. A saturated aqueous solution of $NH_4Cl$ (100 mL) and 12 M HCl (25 mL) were added successively, and the mixture was stirred at room temperature for 7 h. The ether phase was separated and discarded, and the aqueous solution was heated under reflux for 14 h. The reaction mixture was made alkaline to litmus with aqueous NaOH, and the product was isolated with ether in the usual way. Evaporation of ether and short path distillation, 98 °C (bath)/0.01 mmHg, furnished *4,8-dimethyl-1-(3-pyridinyl)-1-nonanone* (9.10 g, 41%). The solution of the ketone (1.98 g, 8 mmol), $N_2H_2$ (99%, 1.20 g, 24 mmol) and KOH (85%, 1.00 g, 16 mmol) in triethylene glycol (15 mL) was heated to 180−190 °C for 5 h and left at room temperature overnight. Brine (125 mL) was added and the product extracted with $CH_2Cl_2$. The dried ($K_2CO_3$) extract was evaporated and the residue distilled to give 1.40 g (74%) of compound **8**: bp 70−72 °C/0.002 mmHg; $^1$H NMR $\delta$ 0.85 (d, *J* = 1.8 Hz, 3H), 0.86 (d, *J* = 1.8 Hz, 6H), 1.0−1.7 (m, 12H), 2.59 (t, *J* = 8.0 Hz, 2H), 7.22 (dd, *J* = 4.7, 7.7 Hz, 1H), 7.51 (d, *J* = 7.7 Hz, 1H), 8.44 (d, *J* = 4.7 Hz, 2H); $^{13}$C NMR $\delta$ 19.77, 22.77, and 22.87 ($CH_3$ × 3), 24.91, 28.13, and 28.81 ($CH_2$ × 3), 32.78 (CH), 33.52, 36.72, 37.34, and 39.47 ($CH_2$ × 4), 123.50 and 136.25 (CH × 2), 138.35 (C), 147.02 and 149.80 (CH × 2); IR (film) 3082, 3025, 1574, 1559, 1463 cm$^{-1}$; HRMS found ($M^+$) 233.2144, $C_{16}H_{27}N$ requires 233.2143.

*2-Methyl-5-octyloxypyridine (**9**).* Reaction of 3-hydroxy-6-methylpyridine and 1-bromooctane according to the general procedure gave the ether **9**, as a liquid in 68% yield: $^1$H NMR $\delta$ 0.88 (t, *J* = 6.6 Hz, 3H), 1.24−1.39 (m, 8H), 1.40−1.50 (m, 2H), 1.46−1.72 (m, 3H), 1.77 (b quintet, *J* = 6.6 Hz, 2H), 2.47 (s, 3H), 3.96 (t, *J* 6.6 = Hz, 2H), 7.04 (d, *J* = 8.4 Hz, 1H), 7.10 (dd, *J* = 2.9, 8.4 Hz, 1H), 8.17 (d, *J* = 2.9 Hz, 1H); $^{13}$C NMR $\delta$ 14.20 ($CH_3$), 22.76 ($CH_2$), 23.40 ($CH_3$), 26.07 ($CH_2$), 29.33 ($CH_2$ × 2), 29.44 ($CH_2$), 31.91 and 68.59 ($CH_2$ × 2), 122.16, 123.41, and 136.76 (CH × 3), 150.16 and 153.38 (C × 2); IR 1605 (m), 1580 (s), 1505 (s) cm$^{-1}$; HRMS found ($M^+$) 221.1777, $C_{14}H_{23}NO$ requires 221.1779.

*2-(3,7-Dimethyloctylthiol)thiophene (**15**).* Reaction of 2-thiophenethiyllithium, from 1.93 g (23 mmol) of thiophene (*21*), with 5.00 g (23 mmol) of 1-bromo-3,7-dimethyloctane in ether and dry DMF (10 mL, 1:1) gave, after flash chromatography ($SiO_2$) and short path distillation at 98 °C (bath)/0.02 mmHg, 3.1 g (53%) of the sulfide **15**: $^1$H NMR $\delta$ 0.86 (d, *J* = 6.6 Hz, 9H), 1.06−1.32 (m, 6H), 1.38−1.70 (m, 4H), 2.70−2.88 (m, 2H), 6.95 (dd, *J* = 3.7, 5.5 Hz, 1H), 7.09 (dd, *J* = 1.1, 3.7 Hz, 1H), 7.31 (dd, *J* = 1.1, 5.5 Hz, 1H); $^{13}$C NMR $\delta$ 19.51, 22.77, and 22.87 ($CH_3$ × 3), 24.78 ($CH_2$), 28.12 and 32.00 (CH × 2), 36.69 ($CH_2$), 37.04 ($CH_2$ × 2), 39.37 ($CH_2$), 127.56, 128.99, and 133.30 (CH × 3), 135.20 (C); IR 3070 (w), 1464 (m), 1215 (m), 697 (m) cm$^{-1}$; HRMS (EMI), found ($M^+$) 256.1319, $C_{14}H_{24}S_2$ requires 256.1319.

*(3,7-Dimethyl-1-octyloxy)benzene (**16**).* Reaction of phenol and 1-bromo-3,7-dimethyloctane according to the general procedure gave the ether **16** as an oil in 86% yield: $^1$H NMR $\delta$ 0.92 (d, *J* = 6.6 Hz, 6H), 0.99 (d, *J* = 6.2 Hz, 3H), 1.12−1.44 (m, 6H), 1.52−1.78 (m, 3H), 1.80−1.94 (m, 1H), 3.97−4.07 (m, 2H), 6.90−7.00 (m, 3H), 7.26−7.35 (m, 2H); $^{13}$C NMR $\delta$ 19.86, 22.79, and 22.89 ($CH_3$ × 3), 24.86 ($CH_2$), 28.17 and 30.08 (CH × 2), 36.45, 37.50, 39.45, and 66.38 ($CH_2$ × 4), 114.71, 120.63, and 129.56 (CH × 3), 159.35 (C); IR (film) 3061, 3040, 1600, 1586, 1497, 1473, 1244, 752, 690 cm$^{-1}$. Anal. Calcd for $C_{16}H_{26}O$: C, 81.99; H, 11.18. Found: C, 81.69; H, 11.12.

*4-(3,7-Dimethyl-1-octa-2,6-dienyloxy)-1,2-(methylenedioxy)benzene (**17**).* Reaction of 3,4-methylenedioxyphenol and 1-bromo-3,7-dimethylocta-2,6-diene according to the general procedure gave the ether **17** as a liquid in 50% yield: $^1$H NMR $\delta$ 1.60 (s, 3H), 1.68 (s 3H), 1.71 (s, 3H), 2.05−2.15 (m, 4H), 4.46 (d, *J* = 6.6 Hz, 2H), 5.09 (t, *J* = 6.9 Hz, 1H), 5.46 (t, *J* = 6.6 Hz, 1H), 5.90 (s, 2H), 6.34 (dd, *J* = 2.4, 8.4 Hz, 1H), 6.51, (d, *J* = 2.4 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 1H); $^{13}$C NMR $\delta$ 16.63, 17.88, 25.85, 26.51, 39.74, 66.04, 98.53, 101.25, 106.18, 108.09, 119.80, 124.02, 131.99, 141.28, 141.75, 148.38, 154.64; IR 3066 (w), 1665 (m), 1632 (m), 1608 (m), 1500 (s), 1485 (s), 1377 (m), 1179 (s) cm$^{-1}$; HRMS (EMI), found ($M^+$) 274.1565, $C_{17}H_{22}O_3$ requires 274.1568.

*1-(4-Phenoxyphenoxy)-2-(2-pyridyloxy)ethane (**19**).* Reaction of 2-hydroxypyridine and 2-bromo-1-(4-phenoxyphenoxy)ethane (*14*) ac-

cording to the general procedure gave the ether **19** in 80% yield: mp 85−87 °C; IR (KBr) 3032 (w), 3006 (w), 1615 (m), 1583 (s), 1578 (s), 1490 (s) $cm^{-1}$; $^1H$ NMR (200 MHz) $\delta$ 4.22 (t, $J$ = 4.9 Hz, 2H), 4.58 (t, $J$ = 4.9 Hz, 2H), 6.69−7.21 (m, 11H), 7.48 (m, 1H), 8.06 (m, 1H); $^{13}C$ NMR (50 MHz) $\delta$ 62.42 and 65.33 ($CH_2$ × 2), 109.13, 113.53, 114.67, 115.36, 118.40, 120.09, 127.15, 136.09, and 144.09 (CH × 9), 147.50 (C × 2), 152.50 and 160.50 (C × 2); HRMS (EMI), found ($M^+$) 307.1215, $C_{17}H_{22}O_3$ requires 307.1208.

*1-(4-Phenoxyphenoxy)-2-(3-pyridyloxy)ethane (**20**).* Reaction of 3-hydroxypyridine and 2-bromo-1-(4-phenoxyphenoxy)ethane (*14*) according to the general procedure gave the ether **20** in 80% yield: mp 56−57 °C; IR (KBr) 3030 (w), 3004 (w), 1616 (m), 1583 (s), 1579 (s), 1490 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 4.27−4.31 (m, 2H), 4.32−4.36 (m, 2H), 6.88−7.16 (m, 7H), 7.18−7.32 (m, 4H), 8.24 (dd, $J$ = 1.5, 4.5 Hz, 1H), 8.37 (dd, $J$ = 0.6, 2.7 Hz, 1H); $^{13}C$ NMR (75 MHz) $\delta$ 67.06 ($CH_2$), 115.91, 117.84, 120.84, 121.56, 122.68, 123.94, 129.73, 138.16, and 132.61 (CH × 9), 150.83, 154.81, 154.98, and 158.39 (C × 4); HRMS (EMI), found ($M^+$) 307.1203, $C_{17}H_{22}O_3$ requires 307.1208.

*3-(3-Phenoxybenzyloxy)pyridine (**21**).* Reaction of 3-hydroxypyridine and 3-phenoxybenzyl bromide (*15*) according to the general procedure gave the ether **21** in 80% yield: mp 44−46 °C; IR (KBr) 3029 (w), 3004 (w), 1617 (m), 1581 (s), 1577 (s), 1490 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 5.09 (s, 2H), 6.86−6.98 (m, 3H), 6.98−7.10 (m, 3H), 7.12−7.20 (m, 2H), 7.22−7.32 (m, 3H), 8.16 (bs, 1H), 8.29 (bs, 1H); $^{13}C$ NMR (75 MHz) $\delta$ 70.00 ($CH_2$), 117.67, 118.49, 119.17, 121.80, 122.05, 123.60, 123.96, 129.87, and 130.13 (CH × 9), 138.16 (C), 138.19 and 142.36 (CH × 2), 154.67, 156.83, and 157.73 (C × 3); HRMS (EMI), found ($M^+$) 277.1104, $C_{18}H_{15}NO_2$ requires 277.1103.

*5-(2,2-Dimethylcyclopropyl)-3-methyl-1-penten-3-ol (**23**).* A solution of 11.9 g (50 mmol) of the alcohol **22** (*16*) in ether (35 mL) was added to a stirred solution of 5.0 g (0.22 mol) of Na in liquid ammonia (125 mL) kept at −78 °C. Stirring was continued at this temperature for an additional 2h followed by addition of solid $NH_4Cl$. Ammonia was evaporated and the residue extracted with ether and dried ($MgSO_4$). Evaporation of the solvent gave 8.3 g (98%) of the alcohol **23**: bp 82−84 °C/10 mmHg; $^1H$ NMR $\delta$ −0.15 (bs, 1 H), 0.37 (bs, 2 H), 1.04 (s, 6 H), 1.23 (s, 3 H), 1.3−1.6 (m, 4 H), 1.67 (bs, 1 H), 4.9−6.2 (m, 3 H); $^{13}C$ NMR $\delta$ 15.65, 20.10, 22.96, 24.46, 25.01, 27.76, 27.98, 42.93, 73.38, 111.71, 145.43; IR 3400 (s), 1650 (m) $cm^{-1}$. Anal. Calcd for $C_{11}H_{20}O$: C, 78.51; H, 11.98. Found: C, 78.42; H, 12.02.

*1-Bromo-5-(2,2-dimethylcyclopropyl)-3-methyl-2-pentene (**24**).* To 8.4 g (50 mmol) of the alcohol **23**, cooled in ice, was added 50 mL of 48% aqueous HBr. The mixture was stirred at this temperature for 30 min and then diluted with water. The product was extracted with $CH_2Cl_2$, and the extract was washed with saturated $NaHCO_3$ (aqueous) and dried ($MgSO_4$). Evaporation and short path distillation of the residue gave 11.1 g (96%) of the bromide **24** as a liquid, which was used without further purification: $^1H$ NMR $\delta$ −0.12 (bs, 1 H), 0.40 (bs, 2 H), 1.03 (s, 6 H), 1.73 (bs, 3 H), 1.2−2.3 (m, 4 H), 3.95 (d, $J$ = 8.5 Hz, 2 H), 5.53 (bt, $J$ = 8.5 Hz, 1 H); IR 1655 (m) $cm^{-1}$.

*5-[5-(2,2-Dimethylcyclopropyl)-3-methylpent-2-enyloxy]-2-methylpyridine (**13**).* Reaction of 3-hydroxy-6-methylpyridine and the bromide **24** according to the general procedure gave the ether **13** as a liquid in 80% yield: $^1H$ NMR $\delta$ − 0.10 (dd, $J$ = 4.4, 9.1 Hz, 1 H), 0.34−0.52 (m, 2 H), 1.01 (s, 3 H), 1.04 (s, 3 H), 1.36−1.52 (m, 2 H), 1.74 (s, 3 H), 2.13 (t, $J$ = 7.6 Hz, 1 H), 2.19 (t, $J$ = 7.6 Hz, 1 H), 2.49 (s, 3 H), 4.55 (d, $J$ = 6.6 Hz, 2 H), 5.48 (t, $J$ = 6.6 Hz, 1 H), 7.05 (d, $J$ = 8.4 Hz, 1 H), 7.12 (dd, $J$ = 2.9, 8.4 Hz, 1 H), 8.18 (d, $J$ = 2.9 Hz, 1 H); $^{13}C$ NMR $\delta$ 15.69 (C), 17.00 ($CH_3$), 19.95 ($CH_2$), 20.18 and 23.48 ($CH_3$ × 2), 24.57 ($CH_2$), 27.82 ($CH_3$), 28.38 and 40.27 ($CH_2$ × 2), 65.54 (C−O), 118.90, 122.68, 123.42, and 136.73 (CH × 4), 142.31, 150.16, and 153.04 (C × 3); IR 3043 (w), 1670 (w), 1600 (w), 1575 (m), 1456 (m), 1375 (m), 1127 (s), 1065 (s) $cm^{-1}$; HRMS found ($M^+$) 259.1937, $C_{17}H_{25}NO$ requires 259.1936.

*5-(2,2-Dichloro-3,3-dimethylcyclopropyl)-1,1-diethoxy-3-methylpentane (**25**).* To a mixture of 45.6 g (0.20 mol) of racemic citronellal diethyl acetal, 95.6 g (0.80 mol) of $CHCl_3$, and 1.0 g of triethylbenzylammonium chloride in $CH_2Cl_2$ (25 mL), cooled in ice, was added with stirring 50% aqueous NaOH (100 mL). The mixture was stirred vigorously for 16 h at room temperature and then diluted with water. The product was extracted with $CH_2Cl_2$, and the extract was washed with saturated aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). Evaporation of solvents and distillation gave 52.3 g (84%) of the acetal **25** as a 6:4 mixture of diastereoisomers: bp 104−106 °C/0.01 mmHg; $^1H$ NMR $\delta$ 0.91 (d, $J$ = 6.2 Hz, 3H), 1.08 (t, ($J$ = 6.6 Hz, 1H), 1.13 (s, 3H), 1.18 (t, $J$ = 6.9 Hz, 6H), 1.31 (s, 3H), 1.35−1.48 (m, 5H), 1.60−1.68 (m, 2H), 3.46−3.72 (m, 4H), 4.57 (bs, 1H); $^{13}C$ NMR $\delta$ 15.52, 17.26, and 19.94 ($CH_3$ × 3), 23.13 ($CH_2$), 25.10 ($CH_3$), 28.47 (C), 29.15 (CH), 36.11 ($CH_2$), 38.96 (CH), 40.64 ($CH_2$), 60.81 and 61.12 (diasteroisomeric $CH_2$−O), 72.23 ($CCl_2$), 101.64 (CH); IR 1456 (m), 1375 (m), 1129 (s), 1065 (s), 999 (m), 835 (m) $cm^{-1}$. Anal. Calcd for $C_{15}H_{28}Cl_2O_2$: C, 57.87; H, 9.06. Found: C, 57.81; H, 8.80.

*5-(2,2-Dimethylcyclopropyl)-3-methylpentanal (**26**).* Reduction of the acetal **25** as described for compound **23** gave *5-(2,2-dimethylcyclopropyl)-1,1-diethoxy-3-methylpentane* in 94% yield as a 6:4 mixture of diastereoisomers: bp 85−87 °C/9 mmHg; $^1H$ NMR $\delta$ −0.13 (dd, $J$ = 4.0, 4.4 Hz, 1H), 0.32−0.48 (m, 2H), 0.91 (d, $J$ = 6.6 Hz, 3H), 1.01 (s, 3H), 1.02 (s, 3H), 1.20 (t, $J$ = 6.9 Hz, 6H), 1.24−1.46 (m, 5H), 1.57−1.72 (m, 2H), 3.44−3.76 (m, 4H), 4.59 (t, $J$ = 6.2 Hz, 1H); $^{13}C$ NMR $\delta$ 15.52 (C), 15.66 ($CH_3$), 20.04 ($CH_2$), 20.15 and 20.21 ($CH_3$ × 2), 25.21 (CH), 27.25 ($CH_2$), 27.93 ($CH_3$), 29.29 (CH), 37.99 and 40.83 ($CH_2$ × 2), 60.71 and 61.08 (diasteroisomeric $CH_2$−O), 101.77 (CH); IR 3052 (w), 1456 (m), 1375 (m), 1126 (s), 1065 (s), 1000 (m) $cm^{-1}$. Hydrolysis of the acetal with 2 N HCl in 2-propanol gave the aldehyde **26** in 90% yield: bp 85−87 °C/9 mmHg; $^1H$ NMR $\delta$ −0.13 (dd, $J$ = 3.6, 8.4 Hz, 1H), 0.32−0.48 (m, 2H), 0.97 (d, $J$ = 6.6 Hz, 3H), 1.01 (s, 3H), 1.02 (s, 3H), 1.20−1.48 (m, 4H), 2.02−2.15 (m, 1H), 2.16−2.30 (m, 1H), 2.35−2.45 (m, 1H), 9.76 (t, $J$ = 2.3 Hz, 1H); $^{13}C$ NMR $\delta$ 15.52 (C), 19.79 ($CH_2$), 20.09 and 24.68 ($CH_3$ × 2), 24.73 (CH), 27.31 ($CH_2$), 27.74 ($CH_3$), 28.23 (CH), 37.58 and 51.33 ($CH_2$ × 2), 203.27 (CH═O); IR 3052 (w), 2712 (m), 1727 (s), 1456 (m), 1376 (m) $cm^{-1}$; HRMS found ($M^+$) 166.1356, $C_{11}H_{18}O$ requires 166.1357.

*Methyl 9-(2,2-Dimethylcyclopropyl)-3,7-dimethyl-2E,4E-nonadienoate (**18**).* Reaction of the aldehyde **26** with the ylid **27** (*19*) gave the acid **28** as a mixture of stereoisomers. The mixture was isomerized with thiophenol according to the literature (*19*), furnishing the 2*E*,4*E*-isomer in 33% overall yield: mp 37−40 °C; IR 3500 (s), 1690 (s), 1635 (m), 1610 (m) $cm^{-1}$; $^1H$ NMR $\delta$ −0.15 (bs, 1H), 0.38 (bs, 2H), 0.92 (d, $J$ = 5.5 Hz, 3H), 1.03 (s, 6H), 1.35 (s, 5H), 1.9−2.2 (m, 2H), 2.28 (s, 3H), 5.69 (bs, 1H), 6.12 (bs, 2H), 12.23 (s, 1H). Treatment of the acid with diazomethane in the usual way gave a quantitative yield of the methyl ester **18** as a liquid: $^1H$ NMR $\delta$ −0.14 (dd, $J$ = 3.6, 8.4 Hz, 1H), 0.32−0.48 (m, 2H), 0.89 (d, $J$ = 6.6 Hz, 3H), 1.02 (s, 3H), 1.03 (s, 3H), 1.18−1.46 (m, 5H), 1.52−1.66 (m, 1H), 1.95−2.06 (m, 1H), 2.12−2.25 (m, 1H), 2.28 (s, 3H), 3.70 (s, 3H), 5.70 (bs, 1H), 6.11 (bs, 2H); $^{13}C$ NMR $\delta$ 14.08, 15.53, 19.80, 19.88, 19.95, 20.06, 24.97, 27.82, 33.24, 37.34, 40.57, 51.08, 117.30, 134.88, 136.56, 153.08, 167.89 (C═O); IR 3042 (w), 1716 (s), 1636 (w), 1612 (m), 1456 (m), 1376 (m), 1356 (m), 1239 (m), 1156 (s) $cm^{-1}$; HRMS found ($M^+$) 264.2103, $C_{17}H_{28}O_2$ requires 264.2089.

**Biological screening.** *Screening for Juvenile Hormone Activity. The Tenebrio Test.* This test was carried out in the usual way (*8*). Newly molted (4−8 h) *Tenebrio molitor* pupae were held until the following molt, and juvenile hormone activity was recorded by observing the immature characters, using methyl farnesate as reference compound (*8*).

*Screening against Sea Lice. A. Testing on Isolated Nauplius Larvae.* Adult females of *L. salmonis* with mature egg strings were collected from Atlantic salmon reared in seawater. They were kept in 0.5−2 L tanks with aerated seawater for hatching of the eggs, and 20−119 newly hatched larvae of the nauplius I stage were collected and distributed into containers (50−400 mL Petri dishes or beakers). The compounds to be tested (**Figure 2**) were applied as acetone solutions at concentrations ranging from 0.14 to 1.0 ppm, and the vitality and mortality of the nauplii were monitored for up to 7 days. After specific exposure times, the nauplii were filtered and transferred to containers with clean seawater. Acetone was added to the control groups at the same concentration as that used for the group exposed to the highest compound concentration. After 6−7 days, the nauplii of the control groups had developed into live and active copepodids. For compounds with low or no effect the presence of large numbers of empty cuticles and vital nauplii with upward swimming movement was typically

**Table 1.** Effect of Different Juvenoids on the Development from Nauplius I to Copepodid[a]

| test compd | exposure time (h) | total no. of nauplii at start | % of nauplii developed to copepodid (mean ± SEM) | % reduction in development realtive to control |
|---|---|---|---|---|
| **5** | 24 | 92 | 9 ± 4 | 91 |
| **6** | 24 | 71 | 18 ± 6 | 81 |
| **7** | 24 | 91 | 0 | 100 |
| **7** | 1 | 66 | 5 ± 3 | 95 |
| **9** | 24 | 119 | 6 ± 2 | 94 |
| **10** | 24 | 102 | 4 ± 5 | 95 |
| **12** | 24 | 89 | 6 ± 2 | 94 |
| **16** | 24 | 91 | 16 ± 9 | 83 |
| **17** | 24 | 68 | 10 ± 6 | 90 |
| **19** | 24 | 88 | 7 ± 6 | 68 |
| **20** | 24 | 88 | 0 | 100 |
| **21** | 24 | 82 | 0 | 100 |
| fenoxycarb | 24 | 93 | 12 ± 5 | 88 |
| deltamethrin | 24 | 90 | 0 | 100 |
| dichlorvos | 1 | 91 | 0 | 100 |
| control | | 90 | 96 ± 1 | |

[a] Compound concentration, 1.0 ppm. Exposure time, 1 and 24 h. Each value is the average of three parallels. Seawater salinity, 2.3–3.4%. Temperature, 10–16 °C.

observed. The endpoint in the initial screening was based on reduced mobility, reduced molting as observed by the amount of empty cuticles, or mortality of nauplii. In other experiments the fraction of nauplius I larvae that developed to copepodid was used as endpoint. The development took 3−7 days depending on the seawater temperature and salinity.

In the first initial experiment juvenoid compound **8** was tested at a concentration of 0.7 ppm. After 14 days of exposure, the fraction of nauplii developed to copepodid was evaluated. Most of the nauplius larvae in the control group had developed into copepodid, and lots of empty cuticles from molting were observed. Larvae exposed to compound **8** were similar to control.

The activity of compounds **5**, **6**, and **12** was further tested at concentrations of 0.01, 0.05, and 0.3 ppm and exposure times of 1,5, and 24 h. Significant reduction of copepodids compared to controls was observed for all compounds at 1.0 ppm and 1 h of exposure and even at 50 ppb and 24 h of exposure time for compounds **5** and **12**.

*B. Testing on Fish.* Atlantic salmon, artificially infested with sea lice (*L. salmonis*), was distributed to 7 tanks with 17 fish in each. The seawater temperature was 8−9 °C and salinity, 3.0−3.4%. The day before treatment the total number of sea lice was counted, with differentiation between chalimus and pre-adult stages. The sea lice were present as 79% chalimus I and II stages and 21% pre-adults originating from an earlier infestation. The mean was 15.0 sea lice per fish [standard error mean (SEM) = 0.7], based on counts on 12 fish per group. The compounds to be tested were dissolved in an emulsifying mixture of *N*-methyl-2-pyrrolidinone, *N*-octyl-2-pyrrolidinone, and polyoxyl 35 castor oil (approximately 1:1:2) and added to the tanks until a concentration of 2.0 ppm was reached. During treatment the water flow to the tank was stopped. Exposure time was 3 h except for compound **20**, which caused loss of equilibrium in the fish and was stopped after 1 h. The sea lice were counted on days 7 and 14 after treatment. The mean number of sea lice on days 7 and 14 was based on counts from five fish per group per time point. The rise in number of sea lice in the control is probably due to some young chalimus being overlooked initially because of the small size. At day 7 chalimus III and IV stages were the most abundant in all groups, whereas at day 14 pre-adults dominated. The results are recorded in **Table 2**.

The effect of methoprene (**1**) on sea lice infested Atlantic salmon was examined in a different way. The salmon was infested with copepodids and distributed with 45 fish (60−70 g) in each of two tanks of 500 L with running seawater (10 °C). The flow of water was regulated to give a minimum 70% saturation of oxygen at the outlet. A 1.7 L acetone solution containing **1** at a concentration of 5000 ppm

**Table 2.** Bath Treatment of Sea Lice Infested Salmon with Juvenoids[a]

| test compd | mean no. of sea lice | | | % reduction of sea lice relative to control, day 14 |
|---|---|---|---|---|
| | day 0 | day 7 | day 14 | |
| control | 15.0 | 21.4 | 30.8 | |
| **5** | 15.0 | 17.0 | 15.8 | 49 |
| **11** | 15.0 | 14.8 | 12.4 | 60 |
| **12** | 15.0 | 14.8 | 18.4 | 40 |
| **13** | 15.0 | 8.4 | 11.6 | 62 |
| **18** | 15.0 | 14.8 | 23.6 | 23 |
| **20** | 15.0 | 11.8 | 21.8 | 29 |

[a] Compound concentration, 2.0 ppm. Exposure time, 3 h (compound **20**, 1 h). Reduction of sea lice after 14 days.

**Table 3.** Bath Treatment of Sea Lice Infested Salmon with Methoprene (**1**)[a]

| sea lice stage | mean ± SEM | | | |
|---|---|---|---|---|
| | control | | methoprene (**1**) | |
| | day 0 | day 14 | day 0 | day 14 |
| chalimus | 27 ± 2.5 | 0 | 41 ± 7.3 | 0 |
| pre-adult | 0 | 19 ± 2 | 0 | 7.1 ± 1.8 |
| adult | 0 | 2.6 ± 0.7 | 0 | 2.0 ± 0.4 |

[a] Mean number of sea lice per fish before treatment and after 14 days.

was added continuously by a pump to one of the tanks during all 14 days. The other tank was for control. All stages of sea lice on the salmon were counted on 5 fish per group before treatment and on 10 fish on day 14 after treatment. The results are recorded in **Table 3**.

*Statistics.* Statistical analysis was performed using NCSS 2000 (Number Cruncher Statistical Systems, Kaysville, UT). The level of significance was set at $\alpha = 0.05$.

## RESULTS AND DISCUSSION

The use of free-living nauplii going through two moltings to copepodid is convenient as a screening system for compounds with a potential for interrupting the metamorphosis. The experiments are influenced by the quality of eggs and the seawater, especially temperature and salinity. A prerequisite for success is the presence of a high number of live vital copepodids in the control group at the conclusion of the test.

The compounds screened for inhibitory effect on the metamorphosis of the parasite, *L. salmonis*, all exhibited juvenile hormone activity in insects as observed from the Tenebrio test (see above). In addition, the organophosphate dichlorvos, for many years used in salmon farming for removing sea lice, was included for comparison, and likewise the pyrethrin analogue deltamethrine, which presently is the drug of choice used by most fish farmers in Norway for combating sea lice.

Results of the initial testing are recorded in **Table 1**. The compounds inhibited the process of molting and phase transformation from nauplius I to copepodid, resulting in larvae that were either dead or had reduced mobility. Compounds **7**, **20**, and **21**, deltamethrin, and dichlorvos inhibited completely the development to copepodids, but compounds **5**, **8**, **9**, **10**, and **12** also exhibited significant activity.

The results from **Table 1** do not allow a serious structure−activity discussion, but they give some indications. It seems that a pyridine ring alkyl-substituted at the 3-position is a preferred structural feature. On the other hand, whether the alkyl group is attached to the pyridine ring directly or through an oxygen or sulfur atom seems to be less significant. Concerning the alkyl group we assumed from tests on insects that it should preferably have a length of 8−10 carbon atoms; otherwise, the

results of **Table 1** indicate that the number of double bonds and branched methyl groups is less important for activity.

However, for a compound to be of use for combating sea lice it must be efficacious and safe when applied to live salmon. Testing on live fish infested with sea lice required multigram quantities of the compounds to be tested. Because the project had a practical goal, the ease of preparation of the compound and economical aspects were considered when the candidates were chosen. Consequently, the thioether **7** was excluded, whereas compounds **5**, **12**, and **20** were chosen on the basis of the initial test results (**Table 1**). Furthermore, promising results from testing on two other crustacean species, namely, the barnacles *Ballanus ballanoides* and *Ballanus improvisus* (*22*), and the availability of sufficient quantities led us to include compounds **11**, **13**, and **18** as well (**Figure 2**). Note that compound **11** is structurally very similar to **10**, an obvious candidate for this testing. Furthermore, the commercially available juvenoid methoprene was included in the testing on fish.

From the testing on live fish (**Table 2**) the best results were recorded for compounds **11** and **13** with 60 and 62% reductions of sea lice, respectively, compared with the control. ANOVA analysis of sea lice numbers on day 14 revealed significant differences ($p < 0.05$). Using Duncan's multiple-comparison test, compounds **5**, **11**, and **13** were significantly different from the control; by comparison, the reductions in number of adult females were 82% for compound **13**, 73% for compound **5**, and 64% for compound **11**. As shown in **Table 3**, continuously treating sea lice infested salmon with water containing methoprene at low concentrations resulted in a significant reduction of sea lice numbers ($p = 0.0001$) after 14 days, whereas no significant change was observed in the control group ($p = 0.07$). A number of factors may induce differences in results from the two testing systems. All stages of the parasite will be present when sea lice infested fish in an open water system are tested, and consequently migration and attachment of drifting copepodids throughout the experiment may take place. Furthermore, the compounds may also be absorbed by the fish to a certain degree and thereby exert a systemic effect on attached sea lice.

Our results show that many of the compounds tested, including the juvenoid methoprene, interrupt the metamorphosis of sea lice. This supports the notion that the parasite is sensitive to the external application of compounds that are known to affect the metamorphosis of insects. Moreover, the activities of the juvenoids compare favorably with those of the organophosphate dichlorvos and the pyrethroid deltamethrin, which are used commercially for treatment of sea lice infestations. Hence, it seems that juvenoids have a potential for being developed into a practical method for control of parasitic marine crustacean species. Furthermore, the compounds reported in the present paper are easily prepared on a large scale for practical use.

## LITERATURE CITED


(1) Pike, A. W.; Wadsworth, S. L. Sealice on salmonids: their biology and control. *Adv. Parasitol.* **1999**, 233−337.

(2) Grimnes, A.; Jakobsen, P. J. The physiological effects of salmon lice (*Lepeophtheirus salmonis*) infection on post smolt of atlantic salmon (*Salmo salar*). *J. Fish Biol.* **1996**, *48*, 1179−1194.

(3) Tully, O.; Poole, W. R.; Wheland, K. R. Infestation parameters for *Lepeophtheirus salmonis* parasite on sea trout, *Salmo trutta* L., off the west coast of Ireland during 1990 and 1991. *Aquacult. Fish. Manage.* **1993**, *24*, 545−555.

(4) Ritchie, G.; Ronsberg, S. S.; Hoff, K. A.; Branson, E. J. Clinical efficacy of teflubenzuron (Calicide) for the treatment of *Lepeoptheirus salmonis* infestations of farmed Atlantic salmon *Salmo salar* at low water temperatures. *Dis. Aquat. Organ.* **2002**, *51*, 101−6.

(5) Ramstad, A.; Colquhoun, D. J.; Nordmo, R.; Sutherland, I. H.; Simmons, R. Field trials in Norway with Slice (0.2% emamectin benzoate) for the oral treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*. *Dis. Aquat. Organ*. **2002**, *50*, 29−33.

(6) Stone, J.; Sutherland, I. H.; Sommerville, C.; Richards, R. H.; Varma, K. J. Field trials to evaluate the efficacy of emamectin benzoate in the control of sea lice, *Lepeoptheirus salmonis* (Krøyer) and *Caligus elongates* (Nordmann), infestations in Atlantic salmon *Salmo salar* L. *Aquaculture* **2000**, *186*, 205−19.

(7) Fingerman, M. The endocrine mechanisms of crustaceans. *J. Crust. Biol*. **1987**, *7*, 1−24.

(8) Slama, K.; Romanuk, M.; Sorm, F. *Insect Hormones and Bioanalogues*; Springer-Verlag: New York, 1974.

(9) Laufer, H.; Sagi, A.; Ahl, J. S. B.; Homola, E. Methyl farnesoate appears to be a crustacean reproductive hormone. *Invertebr. Reprod. Dev.* **1992**, *22*, 17−20.

(10) Laufer, H.; Ahl, J.; Rotllant, G.; Baclaski, B. Evidence that ecdysteroids and methyl farnesoate control allometric growth and differentiation in a crustacean. *Insect Biochem. Mol. Biol*. **2002**, *32*, 205−210.

(11) Homola, E.; Chang, E. S. Methyl farnesoate: Crustacean juvenile hormone in search of functions. *Comp. Biochem. Physiol.* **1997**, *117B*, 347−356.

(12) Charmantier, G.; Charmantier-Daures, M.; Aiken, D. E. Larval development and metamorphosis of the American lobster *Homarus americanus* (crustacean, decapoda): effect of eystalk ablation and juvenile hormone injection. *Gen. Comp. Endocrinol.* **1988**, *70*, 319−333.

(13) Solli, H.; Madsen, H. B.; Holst, P. L.; Klemmensen, P. D. Pyridyl terpenoid ethers with high juvenile hormone activity. *Pestic. Sci.* **1976**, *7*, 503−511.

(14) O'Brien, E. M.; Tipton, K. F.; Meroni, M.; Dostert, P. Inhibition of monoamine oxidase by clorgyline analogs. *J. Neural Transm., Suppl.* **1994**, *41*, 295−305.

(15) Surman, M. D.; Mulvihill, M. J.; Miller, M. J. Regio- and stereoselective ring openings of 3-aza-2-oxabicyclo[2.2.1]hept-5-ene systems with copper catalyst-modified Grignard reagents: Application to the synthesis of an inhibitor of 5-lipoxygenase. *J. Org. Chem.* **2002**, *67*, 4115−4121.

(16) Kleveland, K.; Skattebøl, L.; Sydnes, L. K. Reactions of dihalocarbenes with allylic alcohols. *Acta Chem. Scand*. **1977**, *B31*, 463−468.

(17) Gosselin, P.; Maignan, C.; Rouessac, F. Stereospecific synthesis of homogeranic and homoneric acids. *Synthesis* **1984**, 876−881.

(18) Habbachi, M.; Gaudemar, M. Silicon in organic synthesis: An improved 4- of 2-alkenoic acids. *Synthesis* **1983**, 745−746.

(19) Henrick, C. A.; Willy, E. W.; Baum, J. W.; Baer, T. A.; Garcia, B. A.; Mastre, T. A.; Chang, S. M. Stereoselective synthesis of alkyl (2*E*,4*E*)- and (2*Z*,4*E*)-3,7,11-trimethyl-2,4-dodecadienoates. Insect growth regulators with juvenile hormone activity. *J. Org. Chem*. **1975**, *40*, 1−7.

(20) Fuerst, H.; Heltzig, M.; Goebel, W. Preparation of pyridine-3-sulfonyl chloride and pyridine-3-thiol. *J. Prakt. Chem. (Leipzig)* **1967**, *36*, 160−164.

(21) Jones, E.; Moodie, I. M. 2-Thiophenethiol. *Org. Synth.* **1970**, *50*, 104−106.

(22) Kleveland, K.; Nilsen, N. O.; Stenstrøm, Y.; Skattebøl, L. Unpublished results.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