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Fatty Acid Esters of Juvenoid Alcohols as Insect Hormonogen Agents (Juvenogens)

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Abstract—A series of 8 new juvenogens (**3**–**10**) was prepared starting from a pair of isomeric insect juvenile hormone bioanalogues (**1** and **2**). The biological activity of the juvenogens **3**–**10** was tested for their effect on reproduction of the blowfly *Neobellieria* (*Sarcophaga*) *bullata* and for the juvenilizing activity on the termite *Prorhinotermes simplex*. Results of biological screening are important in structure–activity studies and promising for potential practical application of some of the juvenogens studied, especially against termites.

Introduction

Fatty acid esters of different alcohols, especially those derived from glycerol, represent a series of compounds occurring in insects.[1] These esters are metabolized by enzymatic system of the insects.[1,2] This mechanism has been taken as a basis for designing and developing a series of hormonogenic fatty acid esters derived from a series of insect juvenile hormone bioanalogues (JHAs; juvenoids).[2] The term insect juvenile hormone bioanalogue describes the organic compound, which mimics the mode of action of the natural insect juvenile hormone, regardless it is structurally different from the natural insect juvenile hormone or structurally similar to it. It has been generally supposed that a biologically active insect juvenile hormone bioanalogue is liberated within the insect body during the introductory step(s) of the metabolic process.

The main advantage of juvenogens consists in their ability to liberate biologically active component (juvenoid) during longer period of time in low concentration, in comparison with an instant application of juvenoid itself. The metabolic degradation of a juvenoid, bearing the carbamate functionality in its molecule [ethyl *N*-{2-{4 - [(2,2 - ethylenedioxycyclohexyl) - methyl]phenoxy}ethyl} carbamate], was already subjected to detailed studies.[3–5] The advantage of juvenogens, compared with that of juvenoids, in the mode of action results in a more effective and more precise treatment of the tested insect species by the biologically active juvenoid. The juvenogen molecule represents a type of practical formulation of the biologically active juvenoid. It is designed and synthesized in a way enabling controlled enzymatic degradation of its molecule in the insect body resulting in slow activation of the biologically active compound during certain time period. In contrary, application of the total quantity of juvenoid topically in one portion results in rapid enzymatic deactivation of juvenoid in insect body. A formation of different types of juvenogens results in a controlled change of physicochemical properties of the compounds. Juvenogens are

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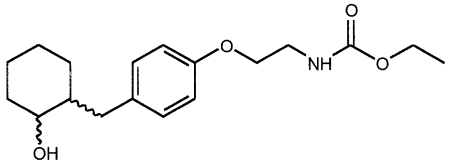
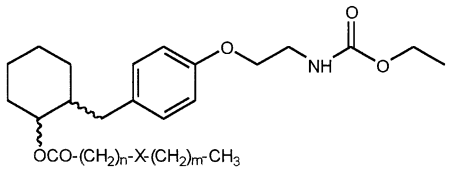


1, *cis*-isomer

2, *trans*-isomer



3, n = 7, X = -CH_2CH_2-, m = 5, *cis*-isomer

4, n = 7, X = -CH_2CH_2-, m = 5, *trans*-isomer

5, n = 7, X = -CH_2CH_2-, m = 7, *cis*-isomer

6, n = 7, X = -CH_2CH_2-, m = 7, *trans*-isomer

7, n = 7, X = -CH=CH-, m = 7, *cis*-isomer

8, n = 7, X = -CH=CH-, m = 7, *trans*-isomer

9, n = 0, X = -CH_2CH_2-, m = 0, *cis*-isomer

10, n = 0, X = -CH_2CH_2-, m = 0, *trans*-isomer

Scheme 1. Transformation of the juvenoids **1** and **2** into the juvenogens **3**–**10**.

designed for oral applications to insects. They are usually bulky molecules, the penetration of which through the insect cuticule may be difficult or even impossible in topical screening tests. Considering all these aspects, juvenogens are more advantageous in oral screening tests than their parent juvenoids. Juvenogens are protected hormonogen forms of juvenoids, adequately stable in the selected environment for the requested period. The structure of juvenogens may positively affect consumption of the treated material by insects, and the transportation of the biologically active component in insect body. Due to the slow liberating of juvenoids by metabolic degradation of the juvenogen molecules, juvenogens are responsible for higher practical effect of the parent juvenoids, which may result even in higher biological activity values displayed by juvenogens in comparison with their parent juvenoids. When termites are considered as target for control, implementation of a bait technology represents a convenient way of environmentally safe termite control. For this purpose, the compounds should be adequately stable, but non-persistent, non-repellent and non-toxic to the target species. The social insects (e.g., termites) then will take the compound (a juvenoid or a juvenogen) up while feeding, and will disseminate the biologically active compound among nestmates.

Previous experience was obtained during investigations of other series of juvenogens, alkyl glycosides[6,7] or glycerides[7,8] derived from juvenoid alcohols. Since the results of introductory studies of juvenogens had been published, a new series of juvenoids has been developed. Some of these new compounds displayed high biological activity towards various insect species.[7] Most satisfactory results were achieved with a series of compounds structurally related to the alcohols **1** and **2** (Scheme 1). This series of juvenoids was subjected to detailed studies, including examples of mammalian toxicity and ecotoxicity.[7,9] Because of the satisfactory results of the screening of these juvenoids, potential juvenogen derivatives of the alcohols **1** and **2** were designed, synthesized and subjected to biological testing. In this study, several targets were achieved and are discussed in this paper: (a) syntheses of fatty acid esters of the juvenoid alcohols **1** and **2** (**3**–**10**); (b) results of biological testing of these juvenogens on the blowfly *Neobellieria (Sarcophaga) bullata* and on the moist-wood subterranean termite *Prorhinotermes simplex*; (c) results of morphological and histological observations performed with the tested blowfly species.

Results and Discussion

A series of 8 new juvenogens was designed, synthesized and bioassayed. Compounds **3**–**10** (Scheme 1) were derived from racemates of the *cis*- and the *trans*-isomers of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}-ethyl} carbamate (**1** and **2**), respectively,[9–11] and from several organic (fatty) acids (Scheme 1). In this investigation, only the racemic compounds **1** and **2** were subjected to the below described synthesis, while the enantiomerically pure juvenogens, corresponding to the racemic structures **3**–**10**, will be subjects of investigation in the near future. The fatty acids used in this investigation were palmitic (hexadecanoic) acid, stearic (octadecanoic) acid, oleic (9*Z*-octadecenoic) acid and butyric (butanoic) acid. The synthesis of the juvenogens **3**–**10** is a simple process, consisting in the reaction of **1** or **2** in an aprotic solution with a fatty acid chloride in the presence of an appropriate base (e.g., pyridine). After stirring of the resulting mixture for 2–6 h, it was worked up by a water treatment, and the products were purified by column chromatography on silica gel, affording the juvenogens **3**–**10** in 85–95% yields.

Biological activity values of the prepared juvenogens **3**–**10** were tested on the blowfly *Neobellieria (Sarcophaga) bullata* (effect on reproduction), and on the moist-wood subterranean termite *Prorhinotermes simplex* (juvenilizing activity). During this study, the following results were obtained.

Neobellieria (Sarcophaga) bullata. In the first step of screening, freshly emerged adult females, not older than 24 h, were topically treated with juvenogens **3**–**10** to study their effect on blowfly reproduction. The tested compounds may act as ovicides reducing egg hatchability due to lethal disorders in embryonic development. Hatchability is defined as a number of larvae

born from eggs deposited in the uterus, and is given as an average value (in percents) calculated on the basis of the studied set of blowfly species. The blowflies are partially viviparous. The ripe eggs from ovaries are transported into uterus, where embryogenesis takes place. Developed larvae are together with empty chorion deposited on the medium (beef liver). The development of the first batch of eggs was not morphologically affected, but the hatchability of eggs in the uterus was lowered. Differences in the activity values of the studied compounds were observed. The lower hatchability is observed the higher is the effect of the tested compounds (**3**–**10**). Therefore, the less satisfactory results were achieved with the juvenogens **3** (80% hatchability) and **6** (90% hatchability), which displayed the weakest effect on hatchability of eggs among the compounds tested (**3**–**10**; Table 1). In contast, treatment of blowfly females with the juvenogens **4** and **5** (i.e., the isomers of **3** or **6**, respectively), resulted in lower hatchability observed (60 and 70% hatchability), which indicated moderate effect. The best ovicidal activity was observed after treatment of blowfly females with the juvenogens **8** (50% hatchability) and **9** (40% hatchability). The activity of their isomers (**7** and **10**) was only average (70% hatchability for either of these compounds). The hatchability effects calculated for the juvenogens **3**–**10** were compared with those calculated for the parent juvenoids **1** and **2** (Table 1). The results clearly show the advantages of the juvenogen molecules **3**–**10** in comparison with their parent juvenoids **1** and **2**. A more rapid metabolic degradation of the juvenoids **1** and **2** in the insect body resulted in low hatchability effect of these compounds in tests with blowflies. A higher hatchability effect of the tested juvenogens was observed during the second oviposition, which indicates that the juvenogens **3**–**10** display no direct effect on DNA and do not act as alkylation or acylation agents. During the second gonotrophic cycle changes caused by the juvenogen treatment were more pronounced. Morphological and histological observations of the ovaries of treated individuals revealed only slight changes in the yolk structure during the first gonotrophic cycle. Some eggs were more round shaped and had no homogenous yolk structure; no visible resorptions were observed. The development of the second egg chambers was markedly affected 10–15 days after juvenogen application. The nuclei of the follicular epithelium, which form monocellular layer under normal conditions, start to divide. Their division is seldom followed by the cell division. The multinuclear layer proliferates towards the inner part of the egg chamber and often fills up the whole egg chamber. Simultaneously, the pycnosis of nuclei and resorption of such a formation follows. Such eggs were never laid. Pictures of histological studies of ovaries, taken by a PC controlled camera after treatment of the blowfly species with juvenogens **3**–**10** are presented in Figures 1–4. Fig. 1 shows normal development of ovaries of *Neobellieria (Sarcophaga) bullata*, when no treatment with juvenogen was applied. All other figures (Figures 2–4) show changes in development of ovaries after application of juvenogens. Figure 2 shows proliferation of follicular epithelium of the second egg chamber, while the first egg chamber located next to it was not damaged at all. Fig. 3 focuses on details of degenerative changes. The content of the egg chamber was seriously disorganized, and remains of trophocytes are recognized in the center of the Figure 3. Figure 4 shows the final state of the resorbed egg chamber. None of the damaged egg chambers shown in Figures 2–4 was able to turn into eggs, which could give rise to a normally developed larva. The number of the normally developed egg chambers was depressed by the effects of the tested

Table 1. The effect of methoprene, the juvenoids **1** and **2** and the juvenogens **3**–**10** on blowfly *Neobellieria* (*Sarcophaga*) *bullata*

Compound	Hatchability (%)
Methoprene	90
1	80
2	90
3	80
4	60
5	70
6	90
7	70
8	50
9	40
10	70

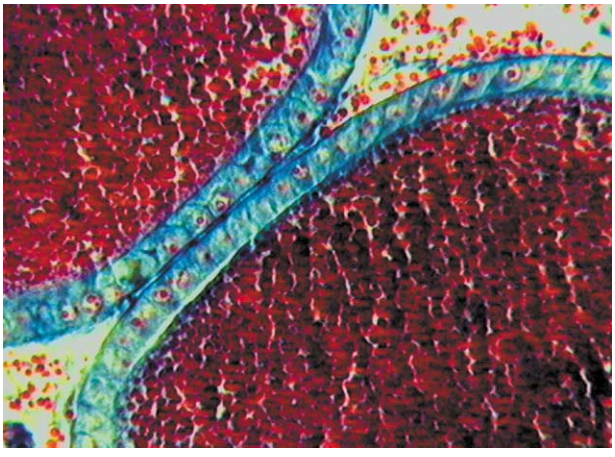


Figure 1. Normal development of ovaries of *Neobellieria* (*Sarcophaga*) *bullata*.

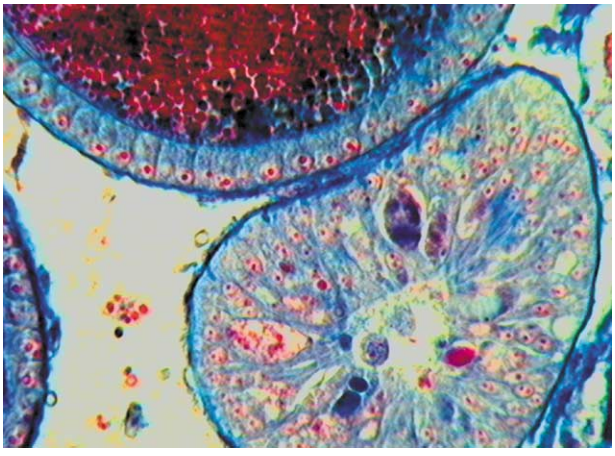


Figure 2. Changes in development of ovaries of *Neobellieria* (*Sarcophaga*) *bullata* after application of juvenogens: Proliferation of follicular epithelium of the second egg chamber.

juvenogens. This result had a practical impact in the depressed number of normally developed larvae achieved by an environmentally safe way. A more detailed study is in progress.

***Prorhinotermes simplex*.** Termites are serious urban, agricultural and forest pests. Effective alternatives to conventional environmentally problematic insecticides for termite control are urgently needed. One of the possibilities is the use of non-toxic insect growth regulators, e.g., juvenoids or juvenogens in baits. One of the juvenoids related to the structures **1** and **2**, ethyl *N*-{2 - {4 - [(2,2 - ethylenedioxycyclohexyl)methyl]phenoxy}ethyl} carbamate, was subjected to regular screening for mammalian toxicity and ecotoxicity with promising results, and a part of the results was published.[7,9] Stable proportion of castes is essential for social homeostasis in termite colonies. A main factor regulating the caste differentiation in termites is juvenile hormone (JH). A treatment with JH mimicking compounds causes a shift in individuals of the worker caste to soldiers.[12–14] These compounds may, therefore, induce differentiation of very high numbers of excessive soldiers which is followed by disruption of the social structure and death of the colony. Regarding this phenomenon, the results achieved by testing the present set of juvenogens as potential termite control agents are generally encouraging. Methoprene and the juvenoids **1** and **2**, employed in testing as a reference compound, used at concentrations of 5 and 500 ppm were almost inactive in both, force-feeding (FF) and choice (C) bioassays (Table 2). Evaluation of the biological activity values of the new compounds showed that treatment at concentrations of 5 ppm was also ineffective in both bioassays. However, slight differences between the compounds were observed in their effects on consumption of treated samples (feeding deterrence or feeding stimulation). In contrast, a treatment with a 500 ppm concentration of the new compounds showed substantial biological activity in the FF bioassay, causing differentiation of a large proportion (39–94%) of pseudergates (worker like individuals) into soldiers (and/or presoldiers and/or pseudergate-soldier intercastes). As these compounds are not toxic, an increased mortality was observed only occasionally and occurred due to disorders during ecdysis. In the C bioassay, juvenogens **3**, **4** and **9** displayed substantial activity values at 500 ppm concentration. In addition, compounds **3** and **9** showed low feeding deterrence or even feeding stimulation (this was found also for compound **7**). In further experiments, these compounds will be candidates for an application in termite control. On the other hand, juvenogens **5**, **6** and **8** seem to act as feeding deterrents and, therefore, will be less suitable as biologically active agents in baits for termite control.

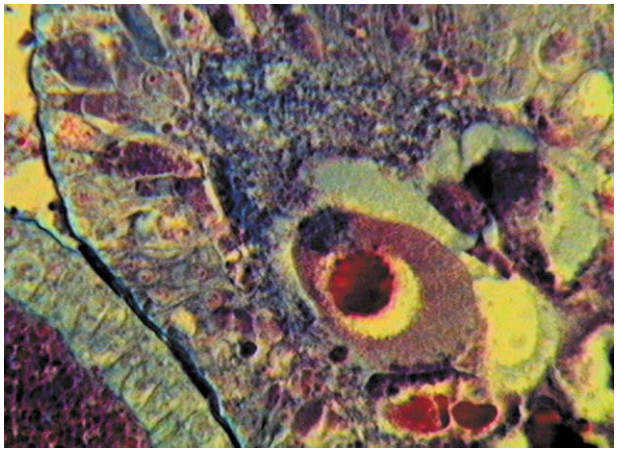


Figure 3. Changes in development of ovaries of *Neobellieria* (*Sarcophaga*) *bullata* after application of juvenogens: Details of degenerative changes (remains of trophocytes in the center).

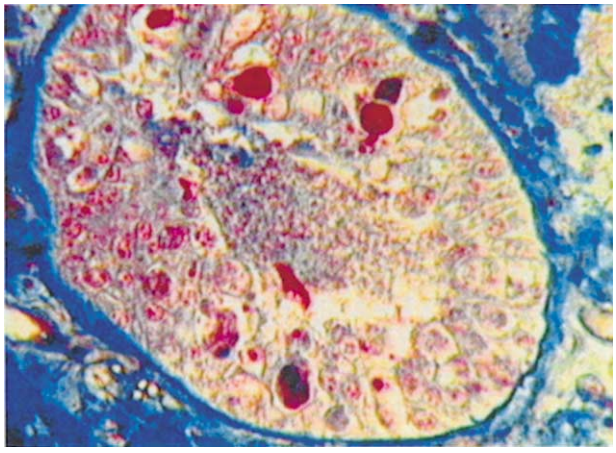


Figure 4. Changes in development of ovaries of *Neobellieria* (*Sarcophaga*) *bullata* after application of juvenogens: Proliferation fills the whole egg chamber.

The results achieved by testing the juvenogens **3**–**10** on blowflies and termites, are generally encouraging. Blowflies are important insect vectors of diseases and cause damages of meat-based food. Design of convenient traps containing food treated with a juvenogen may promote depressing of population densities of this insect pest in the treated area by an environmentally safe way. The results achieved with the termite species clearly demonstrate a convenient possibility for controlling these serious pests on wood and show potential environmentally safe ways of pest management in areas where termites are important pests.

Conclusion

Neobellieria (Sarcophaga) bullata (Table 1)

Since hatchability of eggs of untreated blowfly females ranges between 85 and 95%, the sterilizing activity of compounds **3** and **6** seems to be substantially nil (80 and 90% hatchability). On the contrary, juvenogens **8** and **9** show an effect on egg production and hatching (40 and 50% hatchability). These results encourage further studies directed towards practical application of one of the compounds for blowfly control.

Prorhinotermes simplex (Table 2)

When evaluating the juvenilizing effect of the compounds **3**–**10**, the results achieved in force-feeding (FF) bioassays were highly promising. However, considering

Table 2. The effect of methoprene, the juvenoids **1** and **2** and the juvenogens **3**–**10** on termite *Prorhinotermes simplex*

Compound	Conc (ppm)	Method	Mortality (%)	JH effect (%)	Feeding (%)
Control	0	FF	29.20	0.00	100.00
Methoprene	5	FF	20.80	1.00	68.20
	500	FF	35.00	1.30	48.20
	5	C	34.20	0.00	40.00
	500	C	30.80	0.00	9.30
1	5	FF	20.30	0.00	75.00
	500	FF	15.80	36.50	58.10
	5	C	21.20	0.00	81.20
	500	C	22.10	19.50	60.50
2	5	FF	25.00	0.00	68.60
	500	FF	12.50	39.00	57.40
	5	C	20.50	0.00	76.00
	500	C	26.80	15.70	54.00
3	5	FF	11.70	0.00	74.00
	500	FF	8.30	89.10	39.00
	5	C	16.70	0.00	67.80
	500	C	2.50	80.30	82.30
4	5	FF	9.20	0.00	84.80
	500	FF	54.20	90.10	39.50
	5	C	10.80	0.00	40.40
	500	C	3.30	84.50	22.10
5	5	FF	22.50	0.00	62.70
	500	FF	13.30	39.40	35.40
	5	C	24.20	0.00	20.70
	500	C	30.80	0.00	4.50
6	5	FF	35.80	0.00	56.10
	500	FFF	27.50	70.10	35.20
	5	C	11.70	0.00	39.80
	500	C	25.00	0.00	5.40
7	5	FF	9.20	0.90	81.30
	500	FF	20.80	93.70	38.20
	5	C	10.80	0.00	79.60
	500	C	18.30	18.80	69.60
8	5	FF	10.80	0.00	119.40
	500	FF	0.80	64.70	62.50
	5	C	7.50	0.00	58.83
	500	C	11.70	0.00	1.70
9	5	FF	10.80	0.00	39.00
	500	FF	10.00	86.10	40.30
	5	C	7.50	0.00	58.80
	500	C	0.80	80.70	64.50
10	5	FF	15.00	0.00	86.90
	500	FF	7.50	87.40	36.70
	5	C	15.80	0.00	73.30
	500	C	5.80	10.60	31.40

possible practical application of the studied juvenogens against termites, results achieved in choice (C) tests are more important. Based on the results discussed above, comparisons can be made between the applied methods (FF vs C), focused on compounds displaying highest juvenilizing effects: **3**, **7** and **9** in force-feeding (FF) tests and **3**, **4** and **9** in choice tests. It can be undoubtedly concluded that compounds **3** and **9** should be selected for future studies focussing on practical application of these compounds against termites in bait technology.

If the conclusions based on the results of bioassays performed on blowflies and termites are summarized, the juvenogen **9** seems to meet an optimum combination of physico-chemical properties and high biological activity in tests on the two taxonomically non-related insect pests. Compound **9** is going to become a target of more detailed studies focussed on practical applications. Nevertheless, the juvenogens **3**, **4** and **7** will also be studied in more details in field trials to evaluate their biological activity values with respect to potential practical importance.

Experimental

Analytical methods and instruments

The ^{1}H NMR and the ^{13}C NMR spectra were recorded on a Varian UNITY 500 spectrometer (in an FT mode) at 499.8 and 125.7 MHz in deuteriochloroform using tetramethylsilane ($\delta = 0.0$) as the internal reference. The IR spectra were recorded on a Bruker IFS 88 instrument. The FAB-MS spectra were taken on a VG Analytical ZAB-SEQ instrument. Column chromatography was performed on silica gel (particle size 0.04–0.063 mm; Hermann, Köln-Ehrenfeld, FRG). Thin layer chromatography analyses (TLC) were performed on Silufol (Kavalier, Czech Republic) precoated silica gel TLC sheets using light petroleum ether/diethyl ether

mixture (1:1) as eluent. Analytical high-performance liquid chromatography (HPLC) was performed with a Thermoseparation Products instrument (TSP, USA) equipped with a ConstaMetric 4100 Bio pump (TSP), a SpectroMonitor 5000 UV DAD (TSP), and an evaporative light scattering detector (ELSD; Polymer Laboratories, USA). The system was controlled by a PC 1000 software (TSP) installed to a Pentium PC. A column [250 × 4 (i.d.) mm] filled with Sepharon SGX Si-C18 (particle size 5 μm; Watrex, Czech Republic) was employed for reverse phase HPLC analysis using a methanol/water mixture (4:1) as mobile phase at a flow rate of 0.5 mL min^{-1}. Detection of the compounds during HPLC analysis was achieved using the UV DAD at 220, 254 and 275 nm wavelengths, while UV spectra were taken in the range of 200–300 nm wavelengths. A PC controlled camera, equipped with a LUCIA computer image analysis software (Laboratory Imaging, Czech Republic), was used to perform histological observations on blowflies and to take pictures.

General procedure for the synthesis of fatty acid esters

A solution of the alcohols **1** or **2** (0.884 mmol)[9–11] in benzene (10 mL) and pyridine (0.4 mL) was cooled to 0 °C under vigorous stirring. A fatty acid chloride (1.06 mmol) was added in one portion via a pipette, and the mixture was stirred at 20 °C for 2–6 h. The reaction course was monitored by TLC. The mixture was then poured onto a mixture of ice (20 mL) and hydrochloric acid (1 mL). The organic layer was extracted with ether (3 × 40 mL), and the extract was dried over sodium sulfate. After evaporation of the solvent under reduced pressure, the crude residue was purified by column chromatography on silica gel. The products **3**–**10** were obtained in 85–95% yields. Their analytical data are given below.

3. ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 7.1 Hz, 3H), 1.25 (t, J = 7.1 Hz, 3H), 1.25–1.74 (m, 34H), 1.90 (m, 1H), 2.36 (t, J = 7.7 Hz, 2H), 2.39 (dd, J = 7.9 and 13.6 Hz, 1H), 2.55 (dd, J = 6.8 and 13.6 Hz, 1H), 3.57 (bq, J = 5.3 Hz, 2H), 4.00 (t, J = 5.0 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.90 (dt, J = 2.6, 2.6 and 4.3 Hz, 1H), 5.10 (bt, J = 5.4 Hz, 1H), 6.79 (m, 2H), 7.00 (m, 2H). ^{13}C NMR ($CDCl_3$): 14.10 (q), 14.62 (q), 22.68 (t), 25.29 (t), 27.01 (t), 29.27 (t), 29.33 (t), 29.35 (t), 29.52 (t), 29.62 (t, 2C), 29.65 (t, 2C), 29.68 (t, 2C), 29.69 (t, 2C), 30.00 (t), 31.92 (t), 34.88 (t), 37.76 (t), 40.54 (t), 42.58 (d), 60.93 (t), 67.00 (t), 71.79 (d), 114.32 (d), 129.98 (d), 133.10 (s), 156.76 (s, 2C), 173.33 (s). IR (CCl_4): 3464 (w), 3032 (w), 1729 (s), 1612 (w), 1585 (w), 1510 (s), 1449 (m), 1272 (m) cm^{-1}. FAB-MS (m/z) 560 ($[M+1]^+$, 3), 476 (2), 415 (1), 405 (2), 391 (3), 279 (19), 257 (5), 215 (9), 201 (30), 181 (16), 165 (17), 149 (100), 110 (60). Mp = 78–80 °C. For $C_{34}H_{57}NO_5$ (559.81) calcd 72.94% C, 10.26% H, 2.50% N, found 72.99% C, 10.24% H, 2.48% N.

4. ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 7.1 Hz, 3H), 0.89–1.74 (m, 35H), 1.25 (t, J = 7.1 Hz, 3H), 2.19 (dd, J = 9.3 and 13.6 Hz, 1H), 2.29 (t, J = 7.5 Hz, 2H), 2.84 (dd, J = 3.7 and 13.6 Hz, 1H), 3.57 (bq, J = 5.2 Hz, 2H), 4.00 (t, J = 5.1 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.56 (dt, J = 4.4, 10.1 and 10.1 Hz, 1H), 5.10 (bt, J = 5.3 Hz, 1H), 6.80 (m, 2H), 7.03 (m, 2H). ^{13}C NMR ($CDCl_3$): 14.10 (q), 14.62 (q), 24.53 (t), 25.15 (t), 29.20 (t), 29.28 (t), 29.35 (t, 2C), 29.48 (t, 2C), 29.65 (t, 2C), 29.68 (t, 4C), 29.89 (t), 29.90 (t), 31.93 (t), 34.76 (t), 37.81 (t), 40.54 (t), 43.90 (d), 60.92 (t), 67.03 (t), 76.47 (d), 114.22 (d), 130.18 (d), 132.89 (s), 156.74 (s, 2C), 173.56 (s). IR (CCl_4): 3464 (w), 3033 (w), 1728 (s), 1612 (w), 1585 (w), 1509 (s), 1451 (m), 1243 (s), 1219 (s), 1176 (s) cm^{-1}. FAB-MS (m/z) 560 ($[M+1]^+$, 8), 488 (3), 396 (1), 368 (1), 340 (1), 303 (12), 262 (3), 115 (100), 88 (40). Mp = 67–68 °C. For $C_{34}H_{57}NO_5$ (559.81) calcd 72.94% C, 10.26% H, 2.50% N, found 72.93% C, 10.30% H, 2.51% N.

5. ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 6.8 Hz, 3H), 1.23–1.93 (m, 39H), 1.25 (t, J = 7.2 Hz, 3H), 2.35 (t, J = 7.2 Hz, 2H), 2.39 (dd, J = 8.0 and 13.7 Hz, 1H), 2.55 (dd, J = 6.8 and 13.7 Hz, 1H), 3.57 (bq, J = 5.4 Hz, 2H), 4.00 (t, J = 5.1 Hz, 2H), 4.12 (q, J = 7.2 Hz, 2H), 4.90 (dt, J = 2.6, 2.6 and 4.4 Hz, 1H), 5.11 (bt, J = 5.4 Hz, 1H), 6.79 (m, 2H), 7.00 (m, 2H). ^{13}C NMR ($CDCl_3$): 14.11 (q), 14.60 (q), 20.85 (t), 25.27 (t), 26.97 (t), 29.61 (t, 2C), 29.63 (t, 3C), 29.65 (t, 2C), 29.67 (t, 2C), 29.69 (t, 6C), 31.92 (t), 34.86 (t), 37.76 (t), 40.50 (t), 42.56 (d), 60.92 (t), 66.96 (t), 71.76 (d), 114.26 (d), 129.97 (d), 133.05 (s), 156.66 (s), 156.73 (s), 173.36 (s). IR (CCl_4): 3464 (w), 3032 (w), 1729 (s), 1612 (w), 1585 (w), 1510 (s), 1450 (m), 1243 (s), 1219 (s), 1176 (s) cm^{-1}. FAB-MS (m/z) 588 ($[M+1]^+$, 10), 560 (8), 520 (5), 483 (9), 429 (10), 401 (11), 384 (13), 327 (15), 313 (98), 304 (35), 285 (30), 267 (37), 215 (100), 214 (87), 187 (79). Mp = 78–80 °C. For $C_{36}H_{61}NO_5$ (587.86) calcd 73.55% C, 10.46% H, 2.38% N, found 73.58% C, 10.42% H, 2.40% N.

6. ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 7.6 Hz, 3H), 1.23–2.02 (m, 39H), 1.25 (t, J = 7.1 Hz, 3H), 2.19 (dd, J = 9.3 and 13.6 Hz, 1H), 2.29 (t, J = 7.6 Hz, 2H), 2.84 (dd, J = 3.8 and 13.6 Hz, 1H), 3.57 (bq, J = 5.2 Hz, 3H), 4.00 (t, J = 5.1 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.56 (dt, J = 4.3, 10.1 and 10.1 Hz, 1H), 5.11 (bt, J = 5.2 Hz, 1H), 6.80 (m, 2H), 7.02 (m, 2H). ^{13}C NMR ($CDCl_3$): 14.10 (q), 14.61 (q), 24.52 (t), 25.08 (t), 29.60 (t, 2C), 29.63 (t, 2C), 29.65 (t, 2C), 29.66 (t, 3C), 29.69 (t, 6C), 29.87 (t), 31.86 (t), 34.75 (t), 37.79 (t), 40.53 (t), 43.89 (d), 60.93 (t), 67.00 (t), 76.47 (d), 114. 19 (d), 130.17 (d), 132.86 (s), 156.63 (s), 156.71 (s), 173.59 (s). IR (CCl_4): 3464 (w), 3033 (w), 1729 (s), 1612 (w), 1585 (w), 1509 (s), 1451 (m), 1243 (s), 1219 (s), 1176 (s) cm^{-1}. FAB-MS (m/z) 588 ($[M+1]^+$, 5), 503 (7), 459 (10), 429 (14), 415 (10), 401 (6), 371 (11), 327 (10), 313 (23), 307 (21), 304 (25), 285 (10), 257 (18), 232 (19), 215 (100), 214 (63), 187 (62). Mp = 64–65 °C. For $C_{36}H_{61}NO_5$ (587.86) calcd 73.55% C, 10.46% H, 2.38% N, found 73.53% C, 10.48% H, 2.36% N.

7. ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 7.1 Hz, 3H), 1.25–1.74 (m, 19H), 1.85–1.95 (m, 1H), 1.98–2.05 (m, 2H), 2.36 (t, J = 7.7 Hz, 2H), 2.39 (dd, J = 7.9 and 13.6 Hz, 1H), 2.55 (dd, J = 6.8 and 13.7 Hz, 1H), 3.57 (bq, J = 5.3 Hz, 2H), 4.00 (t, J = 5.1 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.91 (dt, J = 2.7, 2.7 and 4.2 Hz, 1H), 5.12 (bt, J = 5.4 Hz, 1H), 5.31–5.38 (m, 2H), 6.79 (m, 2H), 7.00 (m, 2H). ^{13}C

NMR ($CDCl_3$): 14.08 (q), 14.60 (q), 20.87 (t), 22.66 (t), 25.05 (t), 25.25 (t), 27.00 (t), 27.17 (t), 27.22 (t), 29.14 (t), 29.21 (t), 29.23 (t), 29.31 (t), 29.51 (t), 29.64 (t), 29.70 (t), 29.76 (t), 29.99 (t), 31.89 (t), 34.85 (t), 37.75 (t), 40.53 (t), 42.56 (d), 60.92 (t), 66.99 (t), 71.80 (d), 114.30 (d), 129.72 (d), 129.97 (d), 130.00 (d), 133.07 (s), 156.75 (s, 2C), 173.31 (s). IR (CCl_4): 3464 (w), 3030 (w), 1729 (s), 1653 (w), 1612 (w), 1585 (w), 1510 (s), 1449 (m), 1243 (s), 1220 (s), 1176 (s), 703 (w) cm^{-1}. FAB-MS (*m*/*z*) 586 ($[M+1]^+$, 5), 391 (5), 304 (19), 176 (8), 149 (10), 116 (100), 107 (44), 88 (70). Mp = 39–40 °C. For $C_{36}H_{59}NO_5$ (585.84) calcd 73.80% C, 10.15% H, 2.39% N, found 73.85% C, 10.11% H, 2.42% N.

8. ^1H NMR ($CDCl_3$): 0.90 (t, $J = 7.1$ Hz, 3H), 1.20–1.78 (m, 31H), 1.25 (t, $J = 7.1$ Hz, 3H), 1.91–2.06 (m, 2H), 2.19 (dd, $J = 9.4$ and 13.7 Hz, 1H), 2.35 (t, $J = 7.6$ Hz, 2H), 2.84 (dd, $J = 3.6$ and 13.7 Hz, 1H), 3.57 (bq, $J = 5.4$ Hz, 2H), 4.00 (t, $J = 5.1$ Hz, 2H), 4.12 (q, $J = 7.1$ Hz, 2H), 4.57 (dt, $J = 4.3$, 10.1 and 10.1 Hz, 1H), 5.10 (bt, $J = 5.3$ Hz, 1H), 5.30–5.41 (m, 2H), 6.80 (m, 2H), 7.02 (m, 2H). ^{13}C NMR ($CDCl_3$): 14.09 (q), 14.62 (q), 22.67 (t), 24.53 (t), 25.08 (t), 25.14 (t), 27.17 (t), 27.22 (t), 29.18 (t), 29.31 (t), 29.32 (t), 29.52 (t), 29.60 (t), 29.70 (t), 29.77 (t), 29.89 (t), 31.86 (t), 31.90 (t), 34.75 (t), 37.81 (t), 40.56 (t), 43.90 (d), 60.94 (t), 67.04 (t), 76.48 (d), 114.23 (d), 129.75 (d), 129.99 (d), 130.17 (d), 132.87 (s), 156.50 (s), 156.74 (s), 173.53 (s). IR (CCl_4): 3464 (w), 3032 (w), 1728 (s), 1654 (w), 1612 (w), 1585 (w), 1510 (s), 1451 (m), 1243 (s), 1221 (s), 1176 (s), 705 (w) cm^{-1}. FAB-MS (*m*/*z*) 586 ($[M+1]^+$, 2), 558 (1), 514 (1), 391 (1), 304 (10), 222 (3), 188 (2), 176 (4), 149 (9), 116 (100), 107 (15), 88 (39). Mp = 36–37 °C. For $C_{36}H_{59}NO_5$ (585.84) calcd 73.80% C, 10.15% H, 2.39% N, found 73.84% C, 10.19% H, 2.37% N.

9. ^1H NMR ($CDCl_3$): 1.00 (t, $J = 7.4$ Hz, 3H), 1.20–1.51 (m, 8H), 1.24 (t, $J = 7.2$ Hz, 3H), 1.72 (m, 2H), 1.91 (m, 1H), 2.35 (t, $J = 7.5$ Hz, 2H), 2.39 (dd, $J = 8.2$ and 13.6 Hz, 1H), 2.55 (dd, $J = 6.9$ and 13.6 Hz, 1H), 3.57 (bq, $J = 5.3$ Hz, 2H), 4.00 (t, $J = 5.1$ Hz, 2H), 4.12 (q, $J = 7.2$ Hz, 2H), 4.91 (dt, $J = 2.6$, 2.6 and 4.4 Hz, 1H), 5.11 (bt, $J = 5.3$ Hz, 1H), 6.79 (m, 2H), 7.00 (m, 2H). ^{13}C NMR ($CDCl_3$): 13.78 (q), 14.61 (q), 18.72 (t), 20.86 (t), 25.05 (t), 26.99 (t), 29.99 (t), 36.78 (t), 37.74 (t), 40.52 (t), 42.55 (d), 60.91 (t), 66.99 (t), 71.77 (d), 114.30 (d), 129.97 (d), 133.06 (s), 156.64 (s), 156.74 (s), 173.13 (s). IR (CCl_4): 3464 (w), 3033 (w), 1729 (s), 1612 (w), 1585 (w), 1510 (s), 1243 (s), 1176 (s), 1095 (m) cm^{-1}. FAB-MS (*m*/*z*) 392 ($[M+1]^+$, 35), 304 (21), 231 (8), 154 (9), 137 (9), 116 (100), 107 (21), 88 (42), 71 (13). Mp = 82–84 °C. For $C_{22}H_{33}NO_5$ (391.49) calcd 67.49% C, 8.50% H, 3.58% N, found 67.53% C, 8.47% H, 3.60% N.

10. ^1H NMR ($CDCl_3$): 0.96 (t, $J = 7.4$ Hz, 3H), 1.24 (t, $J = 7.2$ Hz, 3H), 1.26–1.78 (m, 10H), 2.00 (m, 1H), 2.19 (dd, $J = 9.3$ and 13.6 Hz, 1H), 2.27 (t, $J = 7.5$ Hz, 2H), 2.84 (dd, $J = 3.8$ and 13.6 Hz, 1H), 3.57 (bq, $J = 5.3$ Hz, 2H), 4.00 (t, $J = 5.1$ Hz, 2H), 4.12 (q, $J = 7.2$ Hz, 2H), 4.56 (dt, $J = 4.4$, 10.1 and 10.1 Hz, 1H), 5.12 (bt, $J = 5.3$ Hz, 1H), 6.80 (m, 2H), 7.03 (m, 2H). ^{13}C NMR ($CDCl_3$): 13.70 (q), 14.61 (q), 18.60 (t), 24.52 (t), 25.09 (t), 29.91 (t), 31.87 (t), 36.64 (t), 37.84 (t), 40.56 (t), 43.89 (d), 60.92 (t), 67.05 (t), 76.48 (d), 114.25 (d), 130.16 (d), 132.90 (s), 156.66 (s), 156.75 (s), 173.35 (s). IR (CCl_4): 3464 (w), 3034 (w), 1728 (s), 1612 (w), 1585 (w), 1509 (s), 1243 (s), 1177 (s), 1101 (m), 1089 (m) cm^{-1}. FAB-MS (*m*/*z*) 392 ($[M+1]^+$, 17), 320 (6), 304 (11), 222 (6), 133 (6), 116 (100), 107 (37), 88 (45), 71 (13). Mp = 60–62 °C. For $C_{22}H_{33}NO_5$ (391.49) calcd 67.49% C, 8.50% H, 3.58% N, found 67.46% C, 8.46% H, 3.57% N.

Methodology of screening of the juvenogens 3–10 on blowflies

A 0.1% solution of compounds **3**–**10** in acetone was applied on the upper part of the thorax of 40 freshly emerged blowfly females (5 μL per individual). The blowfly females were kept in nylon covered cages together with intact (untreated) males. The insects were fed with water and sugar; after day 4 they were offered beef liver, which later served as an oviposition medium as well. The females were dissected in regular intervals (6, 9, 12, 15 and 21 days after treatment), and the morphology of their ovaries was studied. The shape of eggs, yolk deposition in the first and second egg chamber, as well as signs of resorption were observed. The morphologically changed ovaries were subjected to a histological investigation. Hatchability of eggs was calculated from the number of eggs where a larva was developed and those where development did not take place. The same experimental approach was also used for application of methoprene and the juvenoids **1** and **2** in these tests.

Methodology of screening of the juvenogens 3–10 on termites

The biological activity of two concentrations of methoprene and juvenogens **3**–**10** was tested. Spruce (*Picea abies*) wood samples (30 × 10 × 2 mm) used as feeding substrate were impregnated with 0.4 mL of an acetone solution of the respective compound. Two forms of bioassays were employed: A force-feeding bioassay (FF), where wood samples treated with acetone (control) or with juvenoid/juvenogen were offered to termites and a choice bioassay (C), where the control sample and the juvenoid/juvenogen treated one were offered simultaneously. Groups of 60 pseudergates were exposed to treatments in each test (two replicates, i.e., 120 termites used) in disposable Petri dishes.

The evaluation of results was based on quantification of the juvenilizing effects of the studied compounds, which consisted in the induction of soldier caste differentiation. A percentage of presoldiers, soldiers and/or pseudergate-soldier intercastes was summarized. The mortality rate was calculated from the number of termites at the start and at the end of each experiment. Arithmetical mean of three consecutive weightings of each wood sample (at the beginning and at the end of testing) was used for assessment of feeding (wood consumption) by the insects. The same experimental approach was also used for application of methoprene and the juvenoids **1** and **2** in these tests.

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