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Insect Pest Management Agents: Hormonogen Esters (Juvenogens)

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The chemical part of this investigation focused on designing structures and synthesizing a series of six new esters (juvenogens), derived from biologically active insect juvenile hormone bioanalogues (juvenoids, JHAs) and unsaturated short-chain linear and branched fatty acids for possible application as biochemically targeted insect hormonogen agents. The structures of the new compounds were assigned on the basis of a detailed NMR analysis of their ^{1}H and ^{13}C NMR spectra. The biological part of this investigation focused on introductory biological screening tests with these compounds against the red firebug (*Pyrrhocoris apterus*), termites (*Reticulitermes santonensis* and *Prorhinotermes simplex*), and the blowfly (*Neobellieria bullata*). The biological activity of the juvenogens was studied in relation to the fatty acid functionality in the structures. Notable biological activity in topical tests and medium activity in peroral tests was found for the juvenogens **3** and **7** with *P. apterus*. The compounds **6** and **8** showed the lowest activity in both topical and oral assays with *P. apterus*. Considerable effect of all tested juvenogens was observed in *P. simplex*; however, the juvenogens **5** and **6** (derivatives of the only branched short-chain fatty acid) showed no activity against *R. santonensis*. The effect of the compounds **3**−**8** on larval hatching of *N. bullata* was only moderate (larval hatching 80−90%); however, the proliferation effect caused by **5**, **6**, and **8** is more pronounced than the effect caused by **3**, **4**, and **7**.

KEYWORDS: ***Pyrrhocoris apterus*; *Prorhinotermes simplex*; *Reticulitermes santonensis*; *Neobellieria bullata*; juvenogen; JHA; IGR; IPM**

INTRODUCTION

Recently, we have published results of investigations of a series of eight juvenogens derived from the respective racemic cis and trans isomers of the parent juvenoid (insect juvenile hormone bioanalogue; JHA; IGR; IPM) alcohols (*1*, *2*). Six compounds from this series bore three different saturated fatty acyl functionalities (butanoyl, hexadecanoyl, and octadecanoyl), while the remaining isomeric pair of juvenogens was formed by means of a (9*Z*)-octadec-9-enoyl (oleoyl) functionality. The juvenogen compounds were subjected to a laboratory screening (*1*, *2*) on the termite *Prorhinotermes simplex* and on the blowfly *Neobellieria (Sarcophaga) bullata*. More detailed biological studies on the efficacy of these juvenogens and their parent juvenoids on several termite species have already been reported (*3*, *4*). On the basis of these screening tests, the juvenogens bearing an unsaturated fatty ester functionality were found to display biological activity on all tested termite species and ovicidal activity on blowfly (*1*, *2*, *4*).

The term juvenoid has been used to describe a natural or a synthetic compound which mimics the mode of action of natural insect juvenile hormones (*2*, *5*, *6*). Juvenoids are usually structurally different from juvenile hormones, more convenient for external application on insects, but still relatively easily degradable either by the enzymatic system of insects (biotic factors) or by environmental conditions (abiotic factors) (*7*). Designing and synthesizing juvenogens, biochemically activated hormonogen substances, was the logical next step in developing a convenient tool for suitable and efficient practical application of biologically active juvenoids while respecting the principles of the biology-oriented modeling and the biology-oriented synthesis: i.e., preferably naturally occurring molecules are

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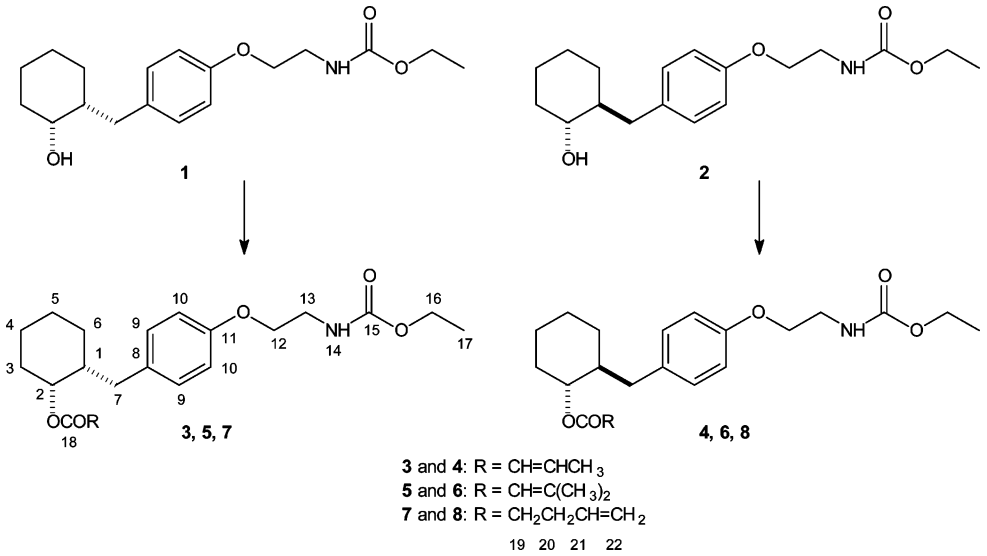
Scheme 1. Biologically Oriented Synthetic Pathway[a]



[a] The numbering of the chain centers is introduced for an easy interpretation of the ^{1}H and ^{13}C NMR spectra.

selected for making juvenogen derivatives of synthetic juvenoids (*8*). Juvenogens act as hormonogen and/or hormonomimetic substances, which are capable of liberating biologically active juvenoids under the effect of biotic or abiotic factors. Juvenogens represent chemical derivatives of juvenoids (e.g., esters of juvenoid alcohols with different naturally occurring or synthetic acids, glycosides of juvenoid alcohols, etc.) (*1*, *2*, *8*, *9*), in which the physicochemical properties of the new substances are modified in comparison with those of the parent juvenoids. This strategy enables targeted design of juvenogens against the selected insect pests and an efficient way for treating them in the laboratory and, if successful, in their natural environment (*2*).

In the former studies (*1*, *2*) of a series of juvenogens, especially butanoate and hexadecanoate esters were found to be biologically active on several termite species. However, the side effect observed with the butanoate esters (the smell of the traces of the butanoic acid), not found during the laboratory tests, was identified during field trials performed in Australia (*10*, *11*). This finding gave increasing importance to the juvenogenic hexadecanoate esters, which displayed no side effects.

Our current project is focused on laboratory investigation of the metabolic pathways of selected juvenogen structures in the termite body by using radiolabeled juvenogen derivatives. One of the suggested ways of investigation also employs compounds bearing radiolabeling in the fatty acyl chain of the juvenogen molecule. Introducing unsaturation to the acyl chain enables easy radiolabeling of the juvenogen molecules with ^{3}H atoms in requested parts of the molecule. For that reason, a new series of six juvenogens (**3**−**8**), which have been synthesized from the parent racemic isomers of juvenoid alcohols (**1** and **2**) and from selected unsaturated short-chain fatty acids, have been subjected to the described study.

The objectives of the present study were: as follows: (a) chemistry, the synthesis of a series of juvenogens **3**−**8** and their structure elucidation; (b) biology, performing laboratory screening tests of the compounds **3**−**8** and their parent juvenoid alcohols **1** and **2**, insect juvenile hormone analogues (JHAs; IGRs), on the red firebug (*Pyrrhocoris apterus*; peroral and topical test), on two termite species (*Reticulitermes santonensis* and *Prorhinotermes simplex*; force-feeding test), and on the blowfly (*Neobellieria bullata*; topical tests); (c) general, evaluation of the introductory biological screening tests.

Evaluation of the ^{1}H and ^{13}C NMR spectra was used in determination of the structure of all new compounds. The unrelated insect species were selected for testing in order to span a wider spectrum of potential targets for possible practical treatment.

MATERIALS AND METHODS

Analytical Methods and Instruments. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE spectrometer (in a FT mode) at 500.13 and 125.8 MHz, respectively, in deuteriochloroform using tetramethylsilane (δ 0.0 for ^{1}H) and the central line of the signal of the solvent (δ 77.0 for ^{13}C) as internal reference. IR spectra were recorded in chloroform on a Bruker IFS 88 instrument. Mass spectra (FAB) were recorded on a VG Analytical 70-250 SE mass spectrometer, ZAB-EQ (BEQQ configuration), at 70 eV. Preparative column chromatography was performed on silica gel type 60 (particle size 0.04−0.063 mm; Fluka, Switzerland). TLC was performed on aluminum sheets precoated with silica gel 60 (Merck, Germany). Analytical HPLC was carried out on a TSP (Thermoseparation Products) instrument equipped with a ConstaMetric 4100 Bio pump and a SpectroMonitor 5000 UV DAD. The analyses of the products were performed on a Sepharon SGX C-18 reverse-phase column (250 × 4 mm, particle size 5 μm; Watrex, Czech Republic) using methanol/water (9/1 v/v) as the mobile phase at 1.0 mL min^{-1}. The eluate was monitored at 220, 254, and 275 nm, and UV spectra were run from 200 to 300 nm.

Synthesis of the Unsaturated Esters 3−8. In a typical experiment (cf. **Scheme 1**), acid chloride (1.0 mmol) was added in one portion, using a pipet, to a cooled and stirred solution (0 °C) of **1** or **2** (0.622 mmol) in toluene (15 mL) and pyridine (0.5 mL). The reaction mixture was stirred at 0 °C for 30 min and then left to stand overnight (12 h) at room temperature. The mixture was poured onto a mixture of ice (20 mL) and hydrochloric acid (1 mL), and the organic compounds were extracted with freshly distilled diethyl ether (3 × 40 mL). The combined extract was dried over sodium sulfate. After the extract was filtered, the solvent was evaporated, and the crude residue was purified by column chromatography on silica gel using a light petroleum/diethyl ether mixture as the mobile phase with a gradient of 2/1 to 1/2. The products **3**−**8** were obtained in 85−93% yields. Their purity was checked by HPLC, and their ^{1}H and ^{13}C NMR data are summarized in **Tables 1** and **2**, while their additional analytical data are presented below.

3: IR (cm^{-1}) 3464 (w), 1722 (s), 1660 (m), 1650 (w), 1510 (s), 1243 (s), 1220 (s), 1188 (s), 1177 (s), 970 (m), 849 (w), 839 (w); FAB-

Table 1. ^{1}H NMR Spectra (Type of Multiplicity of the Signal, Coupling Constant If Applicable) of the Compounds **3**–**8**

atom no.	3	4	5	6	7	8
1	1.68–1.76 (m, 1H)	1.65–1.75 (m, 1H)	1.67–1.74 (m, 1H)	1.64–1.72 (m, 1H)	1.67–1.73 (m, 1H)	1.63–1.72 (m, 1H)
2	4.94 (dt, *J* = 2.5, 2.5, 4.4 Hz, 1H)	4.60 (dt, *J* = 4.6, 10.1, 10.1 Hz, 1H)	4.93 (dt, *J* = 2.5, 2.5, 4.4 Hz, 1H)	4.58 (dt, *J* = 4.3, 10.1, 10.1 Hz, 1H)	4.92 (dt, *J* = 2.6, 2.6, 4.3 Hz, 1H)	4.56 (dt, *J* = 4.4, 10.1, 10.1 Hz, 1H)
3	1.36–1.43 (m, 1H), 1.94 (m, 1H)	1.28–1.36 (m, 1H), 2.03 (m, 1H)	1.35–1.45 (m, 1H), 1.90–1.96 (m, 1H)	1.27–1.35 (m, 1H), 2.03 (m, 1H)	1.35–1.40 (m, 1H), 1.90 (m, 1H)	1.26–1.36 (m, 1H), 1.99 (m, 1H)
4	1.40–1.54 (m, 2H)	1.11 (m, 1H), 1.58–1.63 (m, 1H)	1.43–1.52 (m, 2H)	1.10 (m, 1H), 1.58–1.63 (m, 1H)	1.35–1.51 (m, 2H)	1.10 (m, 1H), 1.57–1.62 (m, 1H)
5	1.23–1.29 (m, 1H), 1.68–1.76 (m, 1H)	1.27–1.36 (m, 1H), 1.65–1.75 (m, 1H)	1.22–1.28 (m, 1H), 1.67–1.74 (m, 1H)	1.27–1.35 (m, 1H), 1.64–1.72 (m, 1H)	1.21–1.26 (m, 1H), 1.67–1.73 (m, 1H)	1.26–1.36 (m, 1H), 1.65–1.74 (m, 1H)
6	1.40–1.54 (m, 2H)	0.98 (m, 1H), 1.65–1.75 (m, 1H)	1.37–1.47 (m, 2H)	0.96 (m, 1H), 1.64–1.72 (m, 1H)	1.35–1.51 (m, 2H)	0.96 (m, 1H), 1.65–1.74 (m, 1H)
7	2.41 (dd, *J* = 7.8, 13.6 Hz, 1H), 2.57 (dd, *J* = 7.1, 13.6 Hz, 1H)	2.22 (dd, *J* = 9.3, 13.6 Hz, 1H), 2.85 (dd, *J* = 3.8, 13.6 Hz, 1H)	2.39 (dd, *J* = 8.1, 13.6 Hz, 1H), 2.57 (dd, *J* = 6.8, 13.6 Hz, 1H)	2.19 (dd, *J* = 9.5, 13.6 Hz, 1H), 2.87 (dd, *J* = 3.5, 13.6 Hz, 1H)	2.39 (dd, *J* = 8.0, 13.7 Hz, 1H), 2.55 (dd, *J* = 6.9, 13.7 Hz, 1H)	2.20 (dd, *J* = 9.2, 13.7 Hz, 1H), 2.84 (dd, *J* = 3.9, 13.7 Hz, 1H)
9	7.00 (m, 2H)	7.03 (m, 2H)	7.00 (m, 2H)	7.03 (m, 2H)	7.00 (m, 2H)	7.03 (m, 2H)
10	6.79 (m, 2H)	6.79 (m, 2H)	6.78 (m, 2H)	6.79 (m, 2H)	6.79 (m, 2H)	6.80 (m, 2H)
12	4.00 (t, *J* = 5.1 Hz, 2H)	4.00 (t, *J* = 5.1 Hz, 2H)	4.00 (t, *J* = 5.1 Hz, 2H)	4.00 (t, *J* = 5.1 Hz, 2H)	4.00 (t, *J* = 5.1 Hz, 2H)	4.00 (t, *J* = 5.1 Hz, 2H)
13	3.57 (bq, *J* = 5.3 Hz, 2H)	3.57 (bq, *J* = 5.5 Hz, 2H)	3.57 (bq, *J* = 5.2 Hz, 2H)	3.57 (bq, *J* = 5.2 Hz, 2H)	3.57 (bq, *J* = 5.5 Hz, 2H)	3.57 (bq, *J* = 5.2 Hz, 2H)
14	5.12 (bt, *J* = 5.5 Hz, 1H)	5.13 (bt, *J* = 5.7 Hz, 1H)	5.12 (bt, *J* = 5.3 Hz, 1H)	5.12 (bt, *J* = 5.3 Hz, 1H)	5.12 (bt, *J* = 5.7 Hz, 1H)	5.12 (bt, *J* = 5.3 Hz, 1H)
16	4.12 (q, *J* = 7.0 Hz, 2H)	4.12 (q, *J* = 7.1 Hz, 2H)	4.12 (q, *J* = 7.0 Hz, 2H)	4.12 (q, *J* = 7.1 Hz, 2H)	4.12 (q, *J* = 7.1 Hz, 2H)	4.12 (q, *J* = 7.1 Hz, 2H)
17	1.25 (t, *J* = 7.0 Hz, 3H)	1.25 (t, *J* = 7.1 Hz, 3H)	1.24 (t, *J* = 7.0 Hz, 3H)	1.25 (t, *J* = 7.1 Hz, 3H)	1.25 (t, *J* = 7.1 Hz, 3H)	1.25 (t, *J* = 7.1 Hz, 3H)
19	5.92 (dq, *J* = 1.7, 1.7, 1.7, 15.5 Hz, 1H)	5.85 (dq, *J* = 1.7, 1.7, 1.7, 15.5 Hz, 1H)	5.77 (m, 1H)	5.69 (m, 1H)	2.41–2.50 (m, 2H)	2.36–2.41 (m, 2H)
20	7.02 (dq, *J* = 6.9, 6.9, 6.9, 15.5 Hz, 1H)	6.96 (dq, *J* = 6.9, 6.9, 6.9, 15.5 Hz, 1H)			2.41–2.50 (m, 2H)	2.36–2.41 (m, 2H)
21	1.92 (dd, *J* = 1.7, 6.9 Hz, 3H)	1.89 (dd, *J* = 1.7, 6.9 Hz, 3H)	1.93 (d, *J* = 1.4 Hz)	1.90 (d, *J* = 1.4 Hz)	5.88 (dd dd, *J* = 5.1, 6.2, 10.3, 17.2 Hz, 1H)	5.84 (dd dd, *J* = 5.1, 6.2, 10.3, 17.2 Hz, 1H)
22			2.21 (d, *J* = 1.3 Hz)	2.17 (d, *J* = 1.3 Hz)	5.04 (ddt, *J* = 1.3, 1.3, 1.7, 10.3 Hz, 1H), 5.11 (dq, *J* = 1.7, 1.7, 1.7, 17.2 Hz, 1H)	5.01 (ddt, *J* = 1.3, 1.3, 1.7, 10.3 Hz, 1H), 5.07 (dq, *J* = 1.7, 1.7, 1.7, 17.2 Hz, 1H)

MS *m*/*z* (%) 412 ([M + Na]$^+$, 9), 390 ([M + H]$^+$, 52), 304 (27), 188 (10), 116 (100), 107 (22), 88 (43), 69 (43). Anal. Calcd for $C_{22}H_{31}NO_5$ (389.48): C, 67.84; H, 8.02; N, 3.60. Found: C, 67.80; H, 8.03; N, 3.58.

4: IR (cm^{-1}) 3463 (m), 1721 (s), 1660 (m), 1650 (w), 1510 (s), 1243 (s), 1220 (s), 1184 (s), 1178 (s), 970 (m), 878 (w), 854 (w); FAB-MS *m*/*z* (%) 412 ([M + Na]$^+$, 1), 390 ([M + H]$^+$, 15), 304 (16), 222 (6), 188 (3), 176 (4), 116 (100), 107 (17), 88 (41), 69 (25). Anal. Calcd for $C_{22}H_{31}NO_5$ (389.48): C, 67.84; H, 8.02; N, 3.60. Found: C, 67.86; H, 8.00; N, 3.57.

5: IR (cm^{-1}) 3464 (w), 3373 (w), 1725 (s), 1720 (s), 1702 (s), 1510 (s), 1244 (s), 1228 (s), 1151 (s), 1143 (s), 989 (m), 853 (m), 842 (w); FAB-MS *m*/*z* (%) 404 ([M + H]$^+$, 5), 304 (12), 188 (2), 116 (100), 107 (25), 88 (53), 83 (54). Anal. Calcd for $C_{23}H_{33}NO_5$ (403.50): C, 68.46; H, 8.24; N, 3.47. Found: C, 68.41; H, 8.25; N, 3.44.

6: IR (cm^{-1}) 3464 (w), 3405 (w), 1726 (s), 1716 (s), 1700 (s), 1509 (s), 1244 (s), 1227 (s), 1149 (s), 879 (w), 854 (w), 833 (w); FAB-MS *m*/*z* (%) 404 ([M + H]$^+$, 4), 304 (7), 188 (4), 116 (100), 107 (22), 88 (58), 83 (64). Anal. Calcd for $C_{23}H_{33}NO_5$ (403.50): C, 68.46; H, 8.24; N, 3.47. Found: C, 68.42; H, 8.26; N, 3.45.

7: IR (cm^{-1}) 3464 (w), 3082 (w), 1729 (s), 1510 (s), 1243 (s), 1220 (s), 1176 (s), 996 (w), 982 (w), 958 (w), 892 (w), 841 (w); FAB-MS *m*/*z* (%) 304 (7), 188 (2), 116 (100), 107 (28), 88 (58). Anal. Calcd for $C_{23}H_{33}NO_5$ (403.50): C, 68.46; H, 8.24; N, 3.47. Found: C, 68.48; H, 8.22; N, 3.46.

8: IR (cm^{-1}) 3464 (w), 3082 (w), 1729 (s), 1509 (s), 1243 (s), 1220 (s), 1176 (s), 991 (m), 878 (w), 854 (w), 834 (w); FAB-MS *m*/*z* (%): 304 (6), 188 (3), 116 (100), 107 (24), 88 (54). Anal. Calcd for $C_{23}H_{33}NO_5$ (403.50): C, 68.46; H, 8.24; N, 3.47. Found: C, 68.48; H, 8.25; N, 3.49.

Experimental Insects. *Pyrrhocoris apterus L.* The juvenile hormone activity was tested on the red firebug (*P. apterus*)—a well-known experimental model (*12*). The cultures of the red firebug were established from insects collected in three locations (Prague, Holoubkov,

Table 2. ^{13}C NMR Spectra (Type of Multiplicity of the Signal) of the Compounds **3**–**8**

atom no.	3	4	5	6	7	8
1	42.67 (d)	43.40 (d)	42.59 (d)	44.08 (d)	42.55 (d)	43.81 (d)
2	71.57 (d)	76.40 (d)	71.04 (d)	75.67 (d)	72.07 (d)	76.75 (d)
3	29.97 (t)	31.85 (t)	30.01 (t)	32.02 (t)	29.98 (t)	31.85 (t)
4	20.83 (t)	24.51 (t)	20.89 (t)	24.57 (t)	20.86 (t)	24.49 (t)
5	25.09 (t)	25.08 (t)	25.08 (t)	25.12 (t)	25.06 (t)	25.04 (t)
6	27.08 (t)	29.87 (t)	27.02 (t)	29.86 (t)	26.99 (t)	29.89 (t)
7	37.76 (t)	37.75 (t)	37.76 (t)	37.74 (t)	37.76 (t)	37.82 (t)
8	133.01 (s)	132.82 (s)	133.16 (s)	132.94 (s)	133.02 (s)	132.78 (s)
9	130.00 (d)	130.19 (d)	130.02 (d)	130.20 (d)	130.02 (d)	130.15 (d)
10	114.19 (d)	114.08 (d)	114.17 (d)	114.06 (d)	114.25 (d)	114.10 (d)
11	156.67 (s)	156.63 (s)	156.93 (s)	156.61 (s)	156.75 (s)	156.66 (s)
12	66.90 (t)	66.91 (t)	66.91 (t)	66.92 (t)	66.96 (t)	66.93 (t)
13	40.46 (t)	40.47 (t)	40.47 (t)	40.47 (t)	40.51 (t)	40.46 (t)
15	156.67 (s)	156.63 (s)	156.93 (s)	156.61 (s)	156.75 (s)	156.66 (s)
16	60.90 (t)	60.91 (t)	60.90 (t)	60.91 (t)	60.96 (t)	60.91 (t)
17	14.60 (q)	14.61 (q)	14.61 (q)	14.61 (q)	14.65 (q)	14.61 (q)
18	166.05 (s)	166.28 (s)	166.32 (s)	166.45 (s)	172.58 (s)	172.75 (s)
19	123.30 (d)	123.08 (d)	116.69 (d)	116.43 (s)	34.01 (t)	33.82 (t)
20	144.15 (d)	144.36 (d)	156.65 (s)	156.41 (s)	29.12 (t)	28.97 (t)
21	17.99 (q)	17.96 (q)	27.45 (q)	27.43 (q)	136.85 (d)	136.73 (d)
22			20.31 (q)	20.20 (q)	115.53 (t)	115.47 (t)

Table 3. Dose (ID 50) of the Compounds **1**–**8** Which Causes 50% Inhibition of Metamorphosis in *Pyrrhocoris apterus*

	ID 50 (μg specimen^{-1})			ID 50 (μg specimen^{-1})	
compd	topical test	peroral test	compd	topical test	peroral test
1	0.005	0.005	**5**	0.029	3.3
2	0.64	0.14	**6**	15	>100
3	0.001	1.0	**7**	0.004	1.1
4	0.26	25.3	**8**	1.6	25

and Hrobce) in the Czech Republic in 2004 and kept on linden seeds (*Tilia cordata* Miller). Tested species were maintained at a constant temperature (27 ± 2 °C) and a constant relative humidity (55 ± 5%) and under a 18:6 light/dark photoperiod.

Termites. All laboratory colonies of termites were reared at a constant temperature (27 ± 1 °C) and in the dark, closed in containers with appropriate humidity. Testing was performed under the same conditions.

(a) Prorhinotermes simplex Hagen. Colonies (D and P/D) of this primitive Rhinotermitid were split off from the original colony collected at Soroa, Pinar del Rio, Cuba, in Dec 1964 and since then maintained in laboratory cultures in Prague.

(b) Reticulitermes santonensis Feytaud. The European subterranean termite is the reference species in European Standards for evaluating wood preservatives (*13*). Colonies (A−C) were collected on the island of Oléron, Department Charente-Maritime, France, in Oct 2005.

Neobellieria bullata Parker. A standard strain of *N. bullata* was obtained from the University of Illinois. Larvae were bred on calf's liver; adults were kept for 4 days after eclosion on sugar and water. Liver was then supplied as a source of proteins and as an oviposition medium as well.

Biological Tests. *Topical test with Pyrrhocoris apterus.* The compounds were diluted in acetone into three concentrations (0.05, 0.5, and 5 mg mL^{-1}), and 1 μL of solution was applied on the freshly molted fifth instar of *P. apterus* (the larvae were not older than 20 h after ecdysis) by Burkhard microapplicator. The control insects were treated with 1 μL of acetone. A total of 5 specimens for each dilution in 3 replicates was used for each treatment: i.e., 60 specimens per each tested compound (*14*). The results are summarized in **Table 3**.

Peroral Test with Pyrrhocoris apterus. For peroral application, a drinking assay (*15*) was used. Each compound was diluted with water into four concentrations (0.025, 0.25, 2.5, and 25 μg in 55 μL; i.e., 0.45, 4.55, 45.45, and 454.55 μg mL^{-1}) and offered to insects during the last larval instar. The dilution rate was based on the earlier observation (*16*) that water consumption by the final nymph stadium of *P. apterus* is approximately 55 μL per individual during the initial 3 days following ecdysis (JH sensitive period). The solutions of the tested compounds, produced by dissolving them in acetone and then mixing with water, and pure water for the controls were offered in glass vials plugged with pieces of cotton wool to 30 individuals placed in Petri dishes in 3 replicates: i.e., 150 specimens (4 concentrations and a control experiment) were used for each tested compound.

The development and mortality of insects were checked daily. The measure of the JHA activity was the degree of inhibition of ecdysis and the morphogenetic disruption following the observed ecdysis. The scoring system indicating formation of perfect adults (0%), intermediates between adult and larval forms (20−80%), and perfect supernumerary larvae (100%) was used. Juvenile hormone activity was expressed in term of ID50, the dose of JHA in micrograms per specimen, which causes 50% inhibition of metamorphosis. The results are summarized in **Table 3**.

Screening Tests with Reticulitermes santonensis and Prorhinotermes simplex. The samples of spruce sapwood (30 × 10 × 2 mm), serving as food source for termites, were treated with 0.4 mL of acetone (control samples) or acetone solutions (0.05−5 mg mL^{-1}) of the test compound. A force-feeding bioassay (*4*) was used, where one wood sample treated with acetone (control) or with juvenoid/juvenogen was offered after conditioning at room temperature for 3 h to termites. Groups of 40 workers (*Reticulitermes*) or 60 pseudergates (*Prorhinotermes*) and/or actively feeding late-instar larvae (ultimate and penultimate larval stages) with visible gut contents were placed in each Petri dish (60 mm diameter) with the bottom covered with 5 mL of moistened fine quartz sand (in 3 replications; i.e., 120−180 termites were used). Samples were weighed three times before impregnation and three times at the end of the experiment after drying for 2 h at temperatures of up to 60 °C. The arithmetical mean of the repeated weightings of each sample was used to assess wood consumption. During the experiments, termite groups were checked regularly for feeding, building activity, and occurrence of presoldiers and/or soldiers. At the final scoring on day 14 (*R. santonensis*) or day 16 (*P. simplex*), the number of soldiers, including presoldiers and/or soldierlike intercastes, both alive or dead, was recorded as soldier induction rate: i.e., juvenilizing effect. The mortality rate was calculated from counts of live termites at the start and the end of the experiment. The results are summarized in **Table 4** (*R. santonensis*) and **Table 5** (*P. simplex*).

Screening Test with Neobellieria bullata. The solutions of all eight compounds in acetone (concentrations 1 and 0.1 mg mL^{-1}) were applied on the upper part of the thorax of 40 freshly emerged blowfly females (5 μL specimen^{-1}). The blowfly females were kept in nylon-covered cages together with intact (untreated) males. Eight females each were dissected at regular intervals (6, 9, 12, 15, and 21 days after treatment), and the morphology of their ovaries was studied. The shapes of the developing eggs and yolk deposition, both in the first and the second egg chambers, as well as signs of resorption were observed. The morphologically changed ovaries were subjected to a histological investigation. Larval hatching of eggs was calculated in dissected uteri from the number of developed larvae and those in which development had not taken place.

RESULTS AND DISCUSSION

The series of six new juvenogens **3**−**8**, derived from the isomeric racemic biologically active juvenoid alcohols **1** and **2**, was prepared (**Scheme 1**), identified, and tested on the red firebug (*P. apterus*; topical and peroral tests; **Table 3**), on two termite species (*R. santonensis* and *P. simplex*; force-feeding test; **Tables 4** and **5**), and on the blowfly (*N. bullata*; morphological and histological study of reproduction; **Figures 1**−**6**). The synthesis of the parent juvenoid alcohols **1** and **2** was already described (*17*−*19*). The esterification reaction is a simple chemical reaction consisting of mixing together a solution of the juvenoid alcohol **1** or **2** in an aromatic solvent (benzene or toluene) with pyridine, used as a base, and the selected acyl chloride, as a reagent, with vigorous stirring and cooling to 0

Table 4. Mortality and Soldier Induction (Juvenilizing Effect) of the Compounds **1**–**8** on *Reticulitermes santonensis*

compd/ treatment[a]	concn (mg mL^{-1})	mortality (%)	juvenilizing effect (%)	wood consumption (%)
control/A	0	7.5	0	100
control/B	0	6.2	0	100
control/C, D	0	8.3	0	100
1/E	0.05	5.0	65.8	not determined
2/F	0.05	0	75.0	50.4
2/G	0.05	12.5	83.5	41.3
3/A	0.05	5.0	22.5	91.6
3/B	0.05	10.0	12.5	80.4
4/A	0.05	11.3	28.8	80.5
4/B	0.05	7.5	77.5	50.9
5/A	0.05	1.3	0	98.1
5/B	0.05	5.0	0	93
5/C	0.05	31	1.2	32.2
5/D	0.5	28.3	35	64.3
5/C	0.5	0	50	26.2
6/A	0.05	3.8	0	90.9
6/B	0.05	2.5	0	100
6/C	0.05	2.5	0	31.3
6/D	0.5	1.7	24	89.1
6/C	0.5	6.2	6.2	31
7/A	0.05	2.5	43.8	65.6
7/B	0.05	15.0	82.5	37.7
8/A	0.05	1.25	51.2	70
8/B	0.05	17.5	75.0	52.4

[a] Legend for treatments: (A) colony A, December 2005; (B) colony B, December 2005; (C) colony C, March 2006; (D) colony C, July 2007; (E) colony A, August 2005; (F) colony C, January 2006; (G) colony B, January 2006.

Table 5. Mortality and Soldier Induction (Juvenilizing Effect) of the Compounds **1**–**8** on *Prorhinotermes simplex*

compd/ treatment[a]	concn (mg mL^{-1})	mortality (%)	juvenilizing effect (%)	wood consumption (%)
control/A	0	20	0	100
control/B	0	20.8	0	100
control/C	0	10.8	0	100
1/C	0.5	7.5	57.5	62.7
1/C	5	21.7	51.7	52.3
2/C	0.5	7.5	49.2	62.1
2/C	5	2.5	71.7	54.8
3/B	0.5	5	74.2	52.1
4/B	0.5	5.8	63.3	47
5/A	0.5	6.7	60	62.1
5/B	0.5	15.8	66.7	45.8
5/A	5	19.2	52.5	48.2
6/A	0.5	8.3	60	65.8
6/B	0.5	6.7	73.7	50.6
6/A	5	16.8	60.8	54.3
7/B	0.5	8.3	59.2	36.6
8/B	0.5	5.8	77.5	39.5

[a] Legend for treatments: (A) colony P/D, January 2006; (B) colony D, February 2006; (C) colony P/D, October 2006.

°C. Subsequent stirring of the reaction mixture at room temperature for an additional time (overnight) and the usual workup of the reaction mixture resulted in a crude residue, which was purified by column chromatography on silica gel, using an ether/light petroleum mixture as a mobile phase, and finally yielded the pure products **3**−**8**. It should be noted that the assignment of the aromatic protons (atoms 9 and 10; **Table 1**) was based on empirical calculations using additive rules (*20*). In addition, differences have been found in the values of the coupling constants for protons of the atoms 12 and 13 (**Table 1**). These observed differences were caused by the fact that the equivalent protons 13 are coupled with the protons 12 and with the proton of the NH group. Due to the natural line width, only average values of coupling constants of protons in these positions can be observed.

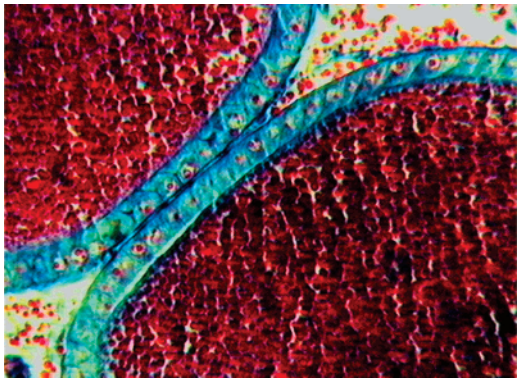


Figure 1. Untreated, normal development of ovaries of *N. bulata* (a reference picture).

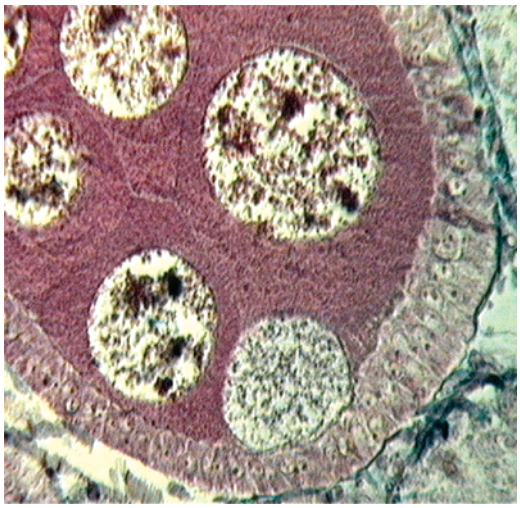


Figure 2. Nucleoli of the follicular epithelium cells divide, and the cell layer adjoining the oocyte is thickened (found with **7**).

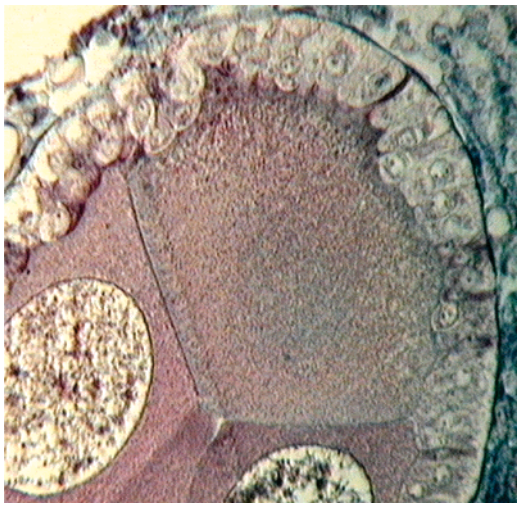


Figure 3. Division of follicular cells in the oocyte region (found with **5**).

Pyrrhocoris apterus. The results of topical and peroral tests are presented, and the target selectivity of this series of compounds was observed (**Table 3**). The juvenogens **3**, **5**, and **7**, derived from **1**, showed biological activity at least 2 orders of magnitude higher than that of the corresponding derivatives **4**, **6**, and **8**, synthesized from **2**. In both tests, compounds **3** and **7** were the most effective compounds. In turn, the juvenogens

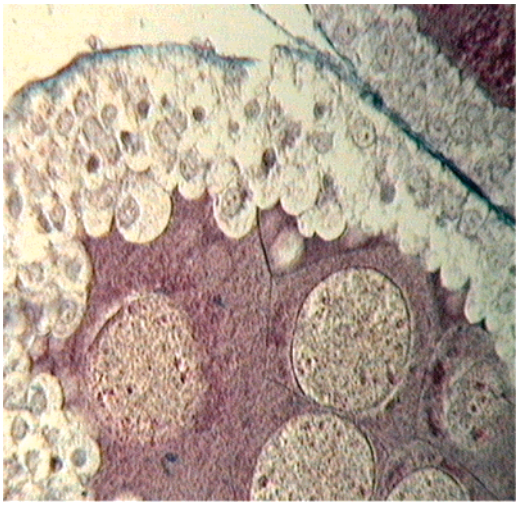


Figure 4. Newly formed follicular cells migrate into the region of nutritive cells and oocyte (found with **5**).

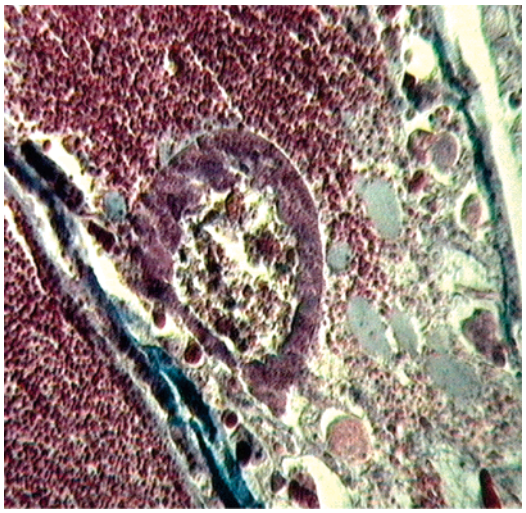


Figure 5. Resorption of the content of egg chamber starts (found with **8**).

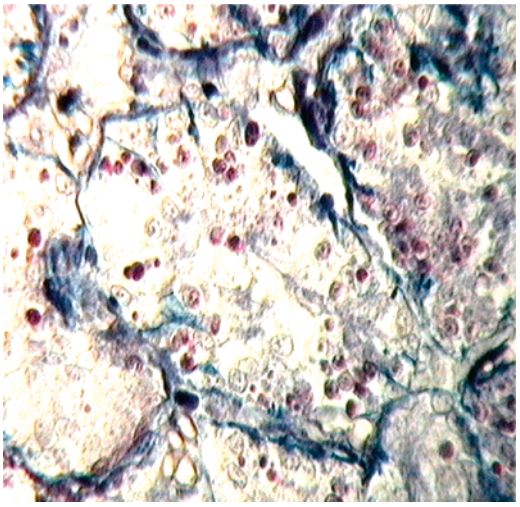


Figure 6. Complete resorption of the egg chamber content; vitelline membrane envelops the remains of the follicular cell nuclei (found with **6**).

6 and **8** were found to be the least effective. The main effect of the given series of juvenogen esters on *P. apterus* was found by topical application (*5*, *21*). However, the juvenogen esters have never been intended for use against phytophagous insect species such as *P. apterus* due to the physicochemical properties of these compounds. The very low polarity of the juvenogen esters does not allow efficient application of these compounds as systemic insect pest management agents, but they can be more advantageously used in termite control (cf. below).

Reticulitermes santonensis. The mortality in treatments, generally ranging from 0 to 17.5%, exceeded 25% in two cases only (cf. **Table 4**). In the control experiments, the mortality was kept below 10%. The experimental compounds, except for compounds **5** and **6**, can be classified as highly effective against *R. santonensis* with a soldier induction rate comparable to that of other hormone mimics studied so far (*3*, *4*). In several cases, as many as 75% of individuals molted into presoldiers within 14 days. Only the compound **3** seems to have lower juvenilizing activity (below 25%). Target specificity of this series of compounds was also observed (**Table 4**). Compounds **5** and **6** proved repeatedly to be inactive at the lower dose (0.05 mg mL^{-1}), without displaying any significant impact on termite mortality. However, at higher concentration (0.5 mg mL^{-1}), the compounds became active, even though their juvenilizing effect did not exceed 50%. In light of these results, screening of the compounds **1**−**8** was also performed with lower termite species (*P. simplex*).

Prorhinotermes simplex. The data presented (**Table 5**) are from three subsequent treatments (A−C) of groups of termites taken from two different colonies (D and P/D). All tested compounds showed notable juvenilizing effects ranging from 49% (juvenoid **2**) to 77.5% (juvenogen **8**). The parental juvenoid alcohols **1** and **2** and other types of their derivatives had been already tested on *P. simplex* from different colonies in the previous studies, when their soldier initiation was found to be slightly lower at the same concentration of 0.5 mg mL^{-1} (*1*) or slightly lower but still sufficient at a concentration of 0.05 mg mL^{-1} (*4*).

The juvenilizing effect of the tested compounds **1**−**8** on *P. simplex*, within the range of 50−78% at the concentration 0.5 mg mL^{-1}, is convincing enough and may be explained by the nature of the species used. Despite using termites from different colonies and different times of treatments, the results are very uniform, in contrast to the case of the genus *Reticulitermes*, where the results of testing with termites of the same species but from different colonies may diverge (*22*). The mortality in present tests with *P. simplex* was relatively low. The mortality around 20% in any of the experimental groups can be well explained by early formation of supplementary reproductives killed in mutual conflicts or by the nestmates and is not due to the effects of juvenogens, at least not within the test period of 16 days. The juvenogens **8**, **3**, and **6** displayed the highest observed juvenilizing effect on the colony D (**8**, 77.5%; **3**, 74.2%; **6**, 73.7%; **Table 5**).

Neobellieria bullata. During the first gonotrophic cycle, no changes were observed in the structure of the egg chamber components; the larval development is close to the normal one (80−90%). Histological observation of dissected ovaries 12−13 days after application of tested compounds revealed multiplication of nucleoli in follicular cells followed by a nucleus division, and often a division of a follicular cell itself. This process appeared mainly in the region of the oocyte (follicular cells migrated into the ooplasma; however, no aberrant nuclei or number of multinuclear cells were observed). No pathological changes were observed in the nuclei of the cytoplasma of nutritive cells. Rapid resorption of the whole egg chamber followed.

Described changes in the ovarian development were observed in all studied compounds, with exception of the juvenogen **8**, where the process of nuclei division allowed multiple, seldom later pycnotic nuclei formation. The proliferation never proceeded further into the inner part of the egg chamber, as was observed with the compounds studied earlier (*1*). In the present tests, some oocytes contained the yolk spheres; however, the yolk deposition was never normal. Germarium was not affected. The results following from a subsequent histological study are presented in **Figures 1**−**6**. The proliferation effect caused by the juvenogens **5**, **6**, and **8** is more pronounced than the effect caused by **3**, **4**, and **7**.

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