

Novel Juvenogens (Insect Hormonogenic Agents): Preparation and Biological Tests on *Neobellieria bullata*

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The development of new types of environmentally safe insecticides has been advantageous to replace toxic and persistent insecticides, which have adverse effects on animals and humans. Our effort in this field was aimed at the synthesis of insect juvenile hormone analogues, juvenoids, nontoxic for warm-blooded animals and fish, which can become advanced compounds in regulating many aspects in insect physiology. Structure modification of juvenoids via juvenogen derivatives (hormonogenic substances) plays an important role in controlling the juvenoid liberation rate in the insect digestive system and can also play an important role in the mode of action toward different arthropod groups, with focus on insect pest species. For that reason, juvenoids were esterified with hexadecanoic, butanoic, and 3-methylbut-2-enoic acids. A total of 12 new compounds with potential insecticidal activity were synthesized and characterized, and the results of biological screening tests on blowflies *Neobellieria (Sarcophaga) bullata* were presented. Results of the inhibitory activity of the tested compounds on the reproduction of the blowflies were also presented.

KEYWORDS: *Neobellieria bullata*; insecticide; juvenoid; juvenogen; hormonogen

INTRODUCTION

A considerable number of insect species have been competing with man for food sources (e.g., stored-product pests, aphids, locusts, orchard pests, etc.) or have been vectors of serious diseases (e.g., flies, mosquitoes, etc.). The application of insecticides is believed to be one of the major factors behind the increase of agricultural productivity in the 20th century and up to now. More food is needed because of the increasing human population (1). Unfortunately, nearly all insecticides have the potential to significantly alter ecosystems; many of them are toxic to humans, and others concentrate in the food chain. Therefore, it is necessary to obtain a balance between of the agricultural, environmental, and health issues when using insecticides in practice. To replace toxic and persistent insecticides, which have adverse effects on animals and/or humans, designing and developing new types of environmentally safe insecticides is highly recommended (2).

Our effort in this field has been aimed at the synthesis of insect juvenile hormone analogues, juvenoids, which display no or very low toxicity toward warm-blooded animals and fish, and, therefore, are considered to be environmentally safe insecticides. Juvenile hormones are the key compounds in the regulation of many aspects of insect physiology (3, 4). Juvenoids 1–4 bear a

free hydroxyl functionality, which may be further subjected to chemical (5, 6) or biochemical modifications (6–9), resulting in the design of novel application forms of juvenoids, juvenogens. The approach to the synthesis of juvenogen esters has been based on the knowledge of biodegradation of esters in the insect digestive system, where they are cleaved into the biologically active alcohols and organic (natural) acids (5, 6, 10). Retrosynthetically, the corresponding alcohols were highly biologically active juvenoids (1–4) (11), while the acids were selected from the group of natural fatty acids, already successfully tested as juvenogen esters derived from another series of promising juvenile hormone analogues (10, 12). The choice of organic acids used for esterification of juvenoids may play an important role in the rate of liberation of the biologically active component from the juvenogen molecules and may also play a key role in the mode of action of the compounds toward the selected insects (6, 10, 12; cf. also our results presented below). Three new series of potential insecticidal agents 5–16 were synthesized and characterized. The results of biological screening tests on blowflies *Neobellieria (Sarcophaga) bullata* are also presented in this paper. The juvenogens are considered to become potential biochemical pesticides, because rather than controlling target pests through direct toxicity, juvenogens control the insect development and reproduction. Prepared compounds display ovicidal activity against *N. bullata*, similarly as it had already been studied with earlier juvenogens (10, 12). Generally, the mode of action of these juvenogens leads to physiological and morphological changes of

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Table 1. ^1H and ^{13}C NMR Spectra of the Compounds 1–4

carbon atom number (number of hydrogen atoms)	compound			
	1	2	3	4
1 (1H)	1.62 (m) ^a /43.64 (d) ^b	1.46 (m) ^a /47.05 (d) ^b	1.65 (m) ^a /43.66 (d) ^b	1.48 (m) ^a /47.09 (d) ^b
2 (1H)	3.78 (dt, $J_{2,3} = 2.1$ Hz, $J_{1,2} = 4.5$ Hz) ^a /68.48 (d) ^b	3.28 (dt, $J_{2,3} = 9.8$ Hz, $J_{1,2} = 4.4$ Hz) ^a /74.47 (d) ^b	3.78 (dt, $J_{2,3} = 2.6$ Hz, $J_{1,2} = 4.5$ Hz) ^a /68.50 (d) ^b	3.28 (dt, $J_{2,3} = 9.5$ Hz, $J_{1,2} = 4.2$ Hz) ^a /74.51 (d) ^b
3 (2H)	1.45 (m) and 1.77 (m) ^a /33.28 (t) ^b	1.26 (m) and 1.98 (m) ^a /35.81 (t) ^b	1.47 (m) and 1.76 (m) ^a /33.27 (t) ^b	1.26 (m) and 1.97 (m) ^a /35.81 (t) ^b
4 (2H)	1.45 (m) and 1.57 (m) ^a /20.33 (t) ^b	1.09 (m) and 1.58 (m) ^a /25.42 (t) ^b	1.48 (m) and 1.59 (m) ^a /20.35 (t) ^b	1.08 (m) and 1.58 (m) ^a /25.45 (t) ^b
5 (2H)	1.23 (m) and 1.68 (m) ^a /25.29 (t) ^b	1.24 (m) and 1.71 (m) ^a /24.88 (t) ^b	1.24 (m) and 1.70 (m) ^a /25.31 (t) ^b	1.24 (m) and 1.70 (m) ^a /24.90 (t) ^b
6 (2H)	1.43 (m) ^a /26.37 (t) ^b	1.63 (m) and 0.89 (m) ^a /29.96 (t) ^b	1.42 (m) ^a /26.37 (t) ^b	1.62 (m) and 0.89 (m) ^a /30.01 (t) ^b
7a,b (2H)	2.48 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7a} = 7.6$ Hz) and 2.65 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7b} = 7.6$ Hz) ^a /37.76 (t) ^b	2.33 (dd, $J_{7a,7b} = 13.5$ Hz, $J_{1,7a} = 9.0$ Hz) and 3.08 (dd, $J_{7a,7b} = 13.5$ Hz, $J_{1,7b} = 4.0$ Hz) ^a /38.08 (t) ^b	2.48 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7a} = 7.6$ Hz) and 2.65 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7b} = 7.6$ Hz) ^a /37.76 (t) ^b	2.32 (dd, $J_{7a,7b} = 13.5$ Hz, $J_{1,7a} = 8.9$ Hz) and 3.06 (dd, $J_{7a,7b} = 13.5$ Hz, $J_{1,7b} = 4.2$ Hz) ^a /38.08 (t) ^b
8	a/133.77 (s) ^b	a/133.40 (s) ^b	a/133.29 (s) ^b	a/132.96 (s) ^b
9 (2H)	7.08 (m) ^a /130.07 (d) ^b	7.09 (m) ^a /130.35 (d) ^b	7.08 (m) ^a /130.02 (d) ^b	7.08 (m) ^a /130.32 (d) ^b
10 (2H)	6.84 (m) ^a /114.58 (d) ^b	6.82 (m) ^a /114.48 (d) ^b	6.84 (m) ^a /114.35 (d) ^b	6.85 (m) ^a /117.40 (d) ^b
11	a/156.43 (s) ^b	a/156.42 (s) ^b	a/156.35 (s) ^b	a/156.35 (s) ^b
12 (2H)	4.47 (dq, $J_{12,18} = 0.6$ Hz, $J_{12,18} = 0.6$ Hz, $J_{12,14} = 1.7$ Hz) ^a /71.80 (t) ^b	4.47 (dq, $J_{12,18} = 0.6$ Hz, $J_{12,18} = 0.6$ Hz, $J_{12,14} = 1.4$ Hz) ^a /71.73 (t) ^b	5.17 (dq, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,14} = 1.8$ Hz) ^a /67.24 (t) ^b	5.17 (dq, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,14} = 1.8$ Hz) ^a /67.24 (t) ^b
13	a/152.98 (s) ^b	a/153.01 (s) ^b	a/156.73 (s) ^b	a/156.77 (s) ^b
14 (1H)	6.06 (m) ^a /115.70 (d) ^b	6.06 (m) ^a /115.63 (d) ^b	5.82 (m) ^a /117.40 (d) ^b	5.82 (m) ^a /117.40 (d) ^b
15	a/166.54 (s) ^b	a/166.56 (s) ^b	a/165.93 (s) ^b	a/165.94 (s) ^b
16 (2H)	4.18 (q, $J_{6,17} = 7.1$ Hz) ^a /59.81 (t) ^b	4.18 (q, $J_{6,17} = 7.1$ Hz) ^a /59.82 (t) ^b	4.17 (q, $J_{6,17} = 7.1$ Hz) ^a /59.97 (t) ^b	4.17 (q, $J_{6,17} = 7.1$ Hz) ^a /59.97 (t) ^b
17 (3H)	1.29 (t, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz) ^a /15.67 (q) ^b	1.29 (t, $J_{16,17} = 7.1$ Hz) ^a /14.27 (q) ^b	1.29 (t, $J_{16,17} = 7.1$ Hz) ^a /14.28 (q) ^b	1.29 (t, $J_{16,17} = 7.1$ Hz) ^a /14.28 (q) ^b
18 (3H)	2.19 (dt, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz) ^a /15.67 (q) ^b	2.20 (bd, $J_{12,18} = 1.4$ Hz) ^a /15.68 (q) ^b	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz) ^a /21.32 (q) ^b	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz) ^a /21.33 (q) ^b

^a ^1H NMR signal (type of multiplicity; coupling constants, if applicable). ^b ^{13}C NMR signal (type of multiplicity).

Table 2. ^1H NMR Spectra of the Compounds 5–8

carbon atom number (number of hydrogen atoms)	compound			
	5 ^a	6 ^a	7 ^a	8 ^a
1 (1H)	1.65–1.73 (m)	1.61–1.66 (m)	1.64–1.71 (m)	1.58–1.64 (m)
2 (1H)	4.92 (dt, $J_{2,3} = 2.6$ Hz, $J_{2,3} = 2.6$ Hz, $J_{1,2} = 4.4$ Hz)	4.56 (dt, $J_{2,3} = 10.0$ Hz, $J_{2,3} = 10.0$ Hz, $J_{1,2} = 4.3$ Hz)	4.92 (dt, $J_{2,3} = 2.6$ Hz, $J_{2,3} = 2.6$ Hz, $J_{1,2} = 4.4$ Hz)	4.56 (dt, $J_{2,3} = 10.1$ Hz, $J_{2,3} = 10.1$ Hz, $J_{1,2} = 4.3$ Hz)
3 (2H)	1.20–1.39 (m) 1.86–1.94 (m)	1.25–1.34 (m) 1.97–2.03 (m)	1.32–1.43 (m) 1.88–1.95 (m)	1.24–1.35 (m) 1.95–2.00 (m)
4 (2H)	1.40–1.53 (m)	1.07–1.12 (m), 1.55–1.61 (m)	1.42–1.57 (m)	1.04–1.13 (m), 1.55–1.63 (m)
5 (2H)	1.20–1.39 (m) 1.65–1.73 (m)	1.25–1.34 (m) 1.66–1.74 (m)	1.19–1.28 (m) 1.64–1.73 (m)	1.24–1.35 (m) 1.64–1.72 (m)
6 (2H)	1.40–1.53 (m)	0.94–0.97 (m), 1.63–1.74 (m)	1.40–1.52 (m)	0.90–0.98 (m), 1.63–1.70 (m)
7a,b (2H)	2.39 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 7.9$ Hz) 2.55 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 6.8$ Hz)	2.20 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 9.2$ Hz) 2.84 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 3.8$ Hz)	2.37 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 8.1$ Hz) 2.55 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 6.7$ Hz)	2.18 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 9.3$ Hz) 2.84 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 3.7$ Hz)
9 (2H)	7.00 (m)	7.03 (m)	6.99 (m)	7.01 (m)
10 (2H)	6.80 (m)	6.81 (m)	6.83 (m)	6.84 (m)
12 (2H)	4.46 (dq, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,14} = 1.4$ Hz)	4.47 (dq, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,14} = 1.4$ Hz)	5.16 (dq, $J_{12,18} = 0.9$ Hz, $J_{12,18} = 0.9$ Hz, $J_{12,18} = 0.9$ Hz, $J_{12,14} = 1.8$ Hz)	5.17 (dq, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,14} = 1.8$ Hz)
14 (1H)	6.06 (m, $5 \times J = 1.5$ Hz)	6.06 (m, $5 \times J = 1.5$ Hz)	5.81 (m, $5 \times J = 1.6$ Hz)	5.81 (m, $5 \times J = 1.6$ Hz)
16 (2H)	4.17 (q, $J_{16,17} = 7.1$ Hz)	4.18 (q, $J_{16,17} = 7.1$ Hz)	4.17 (q, $J_{16,17} = 7.1$ Hz)	4.18 (q, $J_{16,17} = 7.1$ Hz)
17 (3H)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)
18 (3H)	2.19 (dt, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{12,18} = 1.4$ Hz)	2.20 (dt, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{12,18} = 1.4$ Hz)	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{12,18} = 1.5$ Hz)	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{12,18} = 1.5$ Hz)
2' (2H)	2.35 (t, $J_{2',3'} = 7.5$ Hz)	2.27 (t, $J_{2',3'} = 7.5$ Hz)	2.36 (t, $J_{2',3'} = 7.5$ Hz)	2.28 (t, $J_{2',3'} = 7.5$ Hz)
3'–15' (26H)	1.2–1.39 (m)	1.17–1.35 (m)	1.19–1.41 (m)	1.16–1.33 (m)
16' (3H)	0.88 (t, $J_{15',16'} = 7.1$ Hz)	0.88 (t, $J_{15',16'} = 7.1$ Hz)	0.88 (t, $J_{15',16'} = 7.1$ Hz)	0.88 (t, $J_{15',16'} = 7.1$ Hz)

^a ^1H NMR signal (type of multiplicity; coupling constants, if applicable).**Table 3.** ^1H NMR Spectra of the Compounds 9–12

carbon atom number (number of hydrogen atoms)	compound			
	9 ^a	10 ^a	11 ^a	12 ^a
1 (1H)	1.67–1.71 (m)	1.62–1.66 (m)	1.66–1.70 (m)	1.60–1.66 (m)
2 (1H)	4.92 (dt, $J_{2,3} = 2.5$ Hz, $J_{2,3} = 2.5$ Hz, $J_{1,2} = 4.5$ Hz)	4.57 (dt, $J_{2,3} = 10.1$ Hz, $J_{2,3} = 10.1$ Hz, $J_{1,2} = 4.4$ Hz)	4.92 (dt, $J_{2,3} = 2.6$ Hz, $J_{2,3} = 2.6$ Hz, $J_{1,2} = 4.5$ Hz)	4.57 (dt, $J_{2,3} = 10.1$ Hz, $J_{2,3} = 10.1$ Hz, $J_{1,2} = 4.5$ Hz)
3 (2H)	1.35–1.44 (m), 1.88–1.94 (m)	1.26–1.34 (m), 1.97–2.02 (m)	1.34–1.46 (m), 1.90–1.96 (m)	1.25–1.33 (m), 1.96–2.01 (m)
4 (2H)	1.45–1.54 (m)	1.08–1.11 (m), 1.56–1.61 (m)	1.44–1.56 (m)	1.06–1.12 (m), 1.56–1.61 (m)
5 (2H)	1.19–1.27 (m), 1.65–1.75 (m)	1.27–1.34 (m), 1.65–1.73 (m)	1.20–1.28 (m), 1.64–1.75 (m)	1.25–1.33 (m), 1.65–1.72 (m)
6 (2H)	1.40–1.53 (m)	0.94–0.98 (m), 1.65–1.73 (m)	1.39–1.52 (m)	0.92–0.99 (m), 1.65–1.72 (m)
7a,b (2H)	2.39 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 8.0$ Hz) 2.56 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 6.9$ Hz)	2.20 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 9.2$ Hz) 2.84 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 3.8$ Hz)	2.38 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7a} = 8.0$ Hz) 2.55 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7b} = 6.9$ Hz)	2.18 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 9.2$ Hz) 2.84 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 3.8$ Hz)
9 (2H)	7.00 (m)	7.03 (m)	6.99 (m)	7.01 (m)
10 (2H)	6.80 (m)	6.81 (m)	6.83 (m)	6.84 (m)
12 (2H)	4.46 (dq, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,14} = 1.4$ Hz)	4.47 (dq, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,14} = 1.4$ Hz)	5.16 (dq, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,14} = 1.8$ Hz)	5.17 (dq, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,18} = 0.8$ Hz, $J_{12,14} = 1.8$ Hz)
14 (1H)	6.06 m ($5 \times J = 1.5$ Hz)	6.06 m ($5 \times J = 1.5$ Hz)	5.81 m ($5 \times J = 1.6$ Hz)	5.81 m ($5 \times J = 1.6$ Hz)
16 (2H)	4.17 (q, $J_{16,17} = 7.1$ Hz)	4.18 (q, $J_{16,17} = 7.1$ Hz)	4.17 (q, $J_{16,17} = 7.1$ Hz)	4.18 (q, $J_{16,17} = 7.1$ Hz)
17 (3H)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)
18 (3H)	2.19 (dt, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{12,18} = 1.4$ Hz)	2.20 (dt, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{12,18} = 1.4$ Hz)	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{12,18} = 1.5$ Hz)	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{12,18} = 1.5$ Hz)
2' (2H)	2.35 (t, $J_{2',3'} = 7.5$ Hz)	2.26 (t, $J_{2',3'} = 7.3$ Hz)	2.35 (t, $J_{2',3'} = 7.3$ Hz)	2.26 (t, $J_{2',3'} = 7.4$ Hz)
3' (2H)	1.72 (m, $5 \times J = 7.4$ Hz)	1.67 (m, $5 \times J = 7.4$ Hz)	1.72 (m, $5 \times J = 7.4$ Hz)	1.67 (m, $5 \times J = 7.4$ Hz)
4' (3H)	1.00 (t, $J_{3',4'} = 7.5$ Hz)	0.96 (t, $J_{3',4'} = 7.5$ Hz)	1.00 (t, $J_{3',4'} = 7.5$ Hz)	0.96 (t, $J_{3',4'} = 7.4$ Hz)

^a ^1H NMR signal (type of multiplicity; coupling constants, if applicable).

Table 4. ¹H NMR Spectra of the Compounds 13–16

carbon atom number (numbers of hydrogen atoms)	compound			
	13 ^a	14 ^a	15 ^a	16 ^a
1 (1H)	1.65–1.73 (m)	1.61–1.66 (m)	1.63–1.73 (m)	1.60–1.65 (m)
2 (1H)	4.94 (dt, $J_{2,3} = 2.6$ Hz, $J_{2,3} = 2.6$ Hz, $J_{1,2} = 4.5$ Hz)	4.58 (dt, $J_{2,3} = 10.0$ Hz, $J_{2,3} = 10.0$ Hz, $J_{1,2} = 4.4$ Hz)	4.94 (dt, $J_{2,3} = 2.5$ Hz, $J_{2,3} = 2.5$ Hz, $J_{1,2} = 4.6$ Hz)	4.58 (dt, $J_{2,3} = 10.0$ Hz, $J_{2,3} = 10.0$ Hz, $J_{1,2} = 4.4$ Hz)
3 (2H)	1.20–1.39 (m), 1.86–1.94 (m)	1.25–1.34 (m), 1.97–2.03 (m)	1.19–1.38 (m), 1.86–1.94 (m)	1.25–1.34 (m), 1.96–2.03 (m)
4 (2H)	1.40–1.53 (m)	1.07–1.12 (m), 1.55–1.61 (m)	1.41–1.53 (m)	1.07–1.12 (m), 1.54–1.62 (m)
5 (2H)	1.20–1.39 (m), 1.65–1.73 (m)	1.25–1.34 (m), 1.66–1.74 (m)	1.19–1.38 (m), 1.63–1.73 (m)	1.25–1.34 (m), 1.63–1.75 (m)
6 (2H)	1.40–1.53 (m)	0.94–0.97 (m), 1.63–1.74 (m)	1.38–1.56 (m)	0.92–0.97 (m), 1.63–1.75 (m)
7a,b (2H)	2.39 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7a} = 8.0$ Hz) 2.58 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7b} = 6.8$ Hz)	2.19 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7a} = 9.3$ Hz) 2.87 (dd, $J_{7a,7b} = 13.7$ Hz, $J_{1,7b} = 3.6$ Hz)	2.38 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7a} = 8.1$ Hz) 2.57 (dd, $J_{7a,7b} = 13.6$ Hz, $J_{1,7b} = 6.7$ Hz)	2.18 (dd, $J_{7a,7b} = 13.5$ Hz, $J_{1,7a} = 9.6$ Hz) 2.87 (dd, $J_{7a,7b} = 13.5$ Hz, $J_{1,7b} = 3.5$ Hz)
9 (2H)	7.00 (m)	7.03 (m)	6.99 (m)	7.01 (m)
10 (2H)	6.80 (m)	6.80 (m)	6.82 (m)	6.83 (m)
12 (2H)	4.46 (dq, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,14} = 1.4$ Hz)	4.46 (dq, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,18} = 0.7$ Hz, $J_{12,14} = 1.4$ Hz)	5.16 (dq, $J_{12,18} = 0.9$ Hz, $J_{12,18} = 0.9$ Hz, $J_{12,18} = 0.9$ Hz, $J_{12,14} = 1.8$ Hz)	5.16 (dq, $J_{12,18} = 0.9$ Hz, $J_{12,18} = 0.9$ Hz, $J_{12,18} = 0.9$ Hz, $J_{12,14} = 1.8$ Hz)
14 (1H)	6.06 (m, $5 \times J = 1.5$ Hz)	6.06 (m, $5 \times J = 1.5$ Hz)	5.81 (m, $5 \times J = 1.6$ Hz)	5.81 (m, $5 \times J = 1.6$ Hz)
16 (2H)	4.17 (q, $J_{16,17} = 7.1$ Hz)	4.17 (q, $J_{16,17} = 7.1$ Hz)	4.17 (q, $J_{16,17} = 7.1$ Hz)	4.17 (q, $J_{16,17} = 7.1$ Hz)
17 (3H)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)	1.29 (t, $J_{16,17} = 7.1$ Hz)
18 (3H)	2.19 (dt, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{12,18} = 1.4$ Hz)	2.19 (dt, $J_{14,18} = 0.6$ Hz, $J_{14,18} = 0.6$ Hz, $J_{12,18} = 1.4$ Hz)	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{12,18} = 1.5$ Hz)	2.02 (dt, $J_{14,18} = 0.8$ Hz, $J_{14,18} = 0.8$ Hz, $J_{12,18} = 1.5$ Hz)
2' (1H)	5.76 (m, $6 \times J = 1.4$ Hz)	5.69 (m, $6 \times J = 1.3$ Hz)	5.76 (m, $6 \times J = 1.3$ Hz)	5.71 (m, $6 \times J = 1.3$ Hz)
4' (3H)	1.93 (d, $J = 1.4$ Hz)	1.90 (d, $J = 1.4$ Hz)	1.93 (d, $J = 1.4$ Hz)	1.90 (d, $J = 1.4$ Hz)
5' (3H)	2.20 (d, $J = 1.4$ Hz)	2.19 (d, $J = 1.3$ Hz)	2.20 (d, $J = 1.3$ Hz)	2.19 (d, $J = 1.3$ Hz)

^a¹H NMR signal (type of multiplicity; coupling constants, if applicable).

eggs, which can be presented in the form of a statistical evaluation of the hatchability and can be demonstrated in a form of a histological analysis of ovaries and eggs (10, 12, 13).

The application of juvenogens is an environmentally safe approach to the insect pest management. This class of compounds allows us to better understand the relationship between the chemical structure and biological activity.

The objectives of the present investigation were to synthesize the designed series of juvenogens, to subject the compounds to the biological screening tests on blowfly (*N. bullata*), and to evaluate the results of the screening tests.

MATERIALS AND METHODS

General. The NMR data were recorded on either a Bruker AVANCE 400 MHz equipped with a 5 mm z-gradient BBI probe with ATM module (working frequency of 400.13 MHz) or a Bruker AVANCE 500 MHz equipped with a 5 mm z-gradient BBI probe with ATM module (working frequency of 500.13 MHz for ¹H and 125.76 MHz for ¹³C). The samples were dissolved in deuteriochloroform using tetramethylsilane ($\delta = 0.0$ ppm for ¹H) and a central line of a signal of the solvent ($\delta = 77.0$ ppm for ¹³C) as internal references. Signals in ¹³C NMR were assigned to the corresponding carbon atoms by the heteronuclear multiple-quantum coherence (HMQC) measurement on a Bruker AVANCE 500 MHz instrument. Multiplicity is described by the following abbreviations: s for singlet, d for doublet, dd for doublets, t for triplets, q for quartet, and m for multiplet. Mass spectra (MS) (FAB⁺) were recorded on a VG analytical 70-250 SE mass spectrometer, ZAB-EQ (BEQQ configuration) at 70 eV. IR spectra were recorded in chloroform on a Bruker IFS-55 instrument in a 0.108 mm cell. Preparative column chromatography

was performed on a silica gel type 60 (particle size of 0.04–0.063 mm, Fluka, Switzerland). Thin-layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel 60 (Merck, Germany) using the petroleum ether/diethyl ether (pe/e) mixtures identified below, as mobile phases.

Isomeric Ethyl 4-{4-[(2-Hydroxycyclohexyl)methyl]phenoxy}-3-methylbut-2-enoates. The synthetic procedure for the preparation of the juvenoids 1–4 has already been described (11). However, their ¹H and ¹³C nuclear magnetic resonance (NMR) spectra are presented here (Table 1), because those mentioned earlier (11) are already 30 years old. The compounds are described by the following number system: 1 for (*E*, *cis*), 2 for (*E*, *trans*), 3 for (*Z*, *cis*), and 4 for (*Z*, *trans*).

Hexadecanoyl- (6–8), Butanoyl- (9–12), 3-Methylbut-2-enoyl (13–16) Esters of the Isomeric Alcohols 1–4. The alcohols 1–4 were dissolved in benzene under stirring; then pyridine was added; and the mixture was cooled in an external ice–water bath. The acyl chloride (3 equiv) was added dropwise by a syringe within 10 min into the reaction mixture under vigorous stirring. The reaction mixture was stirred at room temperature overnight. To quench the reaction, the mixture was poured onto the mixture of ice (25 mL) with hydrochloric acid (the amount of hydrochloric acid was reflecting the used amount of pyridine), the organic layer was extracted with diethyl ether (3 × 40 mL), and the extract was dried over anhydrous sodium sulfate. The crude product was purified by column chromatography using a mobile-phase light petroleum ether/diethyl ether with a gradient of 7:1–3:1. The ¹H and ¹³C NMR data are presented in Tables 2–5. Additional analytical data and yields are summarized below.

Compound 5: 154 mg, 98% yield, TLC (3:1 pe/e). $R_F = 0.60$. IR (cm^{-1}) 2929 vs, 2855 s, 1728 s, 1666 m, 1511 s, 1448 m, 1327 m, 1224 s, 1147 s, 1176 m, 981 w, 892 w, 864 w, 561 vw, 550 vw. FAB–MS m/z (%): 594 (35), 593

Table 5. ^{13}C NMR Spectra of the Compounds 5–16

carbon atom number (multiplicity)	compound											
	5	6	7	8	9	10	11	12	13	14	15	16
1 (d)	42.52	43.85	42.52	43.90	42.52	43.84	42.50	43.89	71.08	75.69	71.19	75.75
2 (d)	71.71	76.47	71.86	76.53	71.72	76.48	71.86	76.53	42.59	44.06	42.58	44.10
3 (t)	29.96	31.87	29.96	31.87	29.97	31.87	29.96	31.88	27.05	29.90	27.01	29.90
4 (t)	20.84	24.52	20.87	24.53	20.84	24.51	20.86	24.53	25.07	25.13	25.06	25.14
5 (t)	25.05	25.08	25.04	25.08	25.05	25.07	25.02	25.08	20.91	24.59	20.94	24.60
6 (t)	26.98	29.90	26.93	29.89	26.99	29.91	26.93	29.90	30.02	32.03	30.02	32.04
7 (t)	37.76	37.84	37.75	37.83	37.77	37.84	37.74	37.83	37.75	37.78	37.73	37.79
8 (s)	133.24	133.03	132.79	132.59	133.24	133.03	132.78	132.59	133.39	133.16	132.94	132.72
9 (d)	129.98	130.15	129.93	130.10	129.98	130.14	129.93	130.10	130.02	130.19	129.98	130.14
10 (d)	114.57	114.46	114.32	114.23	114.58	114.47	114.32	114.24	114.54	114.43	114.29	114.20
11 (s)	156.48	156.42	156.76	156.72	156.48	156.42	156.76	156.72	156.43	156.39	156.46	156.42
12 (t)	71.76	71.70	67.20	67.20	71.77	71.74	67.19	67.19	71.71	71.71	67.20	67.20
13 (s)	152.99	152.97	156.41	156.39	153.00	152.98	156.41	156.38	153.03	153.02	156.72	156.69
14 (d)	115.61	115.62	117.31	117.34	115.62	115.62	117.32	117.36	115.59	115.61	117.30	117.33
15 (s)	166.55	166.54	165.92	165.39	166.56	166.53	165.93	165.93	166.56	166.55	165.32	165.93
16 (t)	59.81	59.82	59.96	59.97	59.82	59.82	59.97	59.97	59.80	59.81	59.96	59.97
17 (q)	14.27	14.28	14.27	14.27	14.28	14.27	14.27	41.27	14.28	14.28	14.27	14.27
18 (q)	15.67	15.68	21.34	21.33	15.68	15.67	21.34	21.33	15.67	15.68	21.34	21.33
1' (s)	173.37	173.59	173.36	173.59	173.18	173.39	173.17	173.40	166.31	166.45	166.45	166.45
2' (t), (d) ^a	34.68	34.73	34.86	34.74	36.78	36.60	36.77	36.61	116.71	116.46	116.73	116.49
3' (t), (s) ^a	25.27	25.14	25.27	25.14	18.74	18.60	18.73	18.60	155.88	156.31	156.82	156.28
4' (q)					13.80	13.71	13.79	13.71	27.43	27.41	27.42	27.75
5' (q)									20.30	20.20	20.30	20.20
4'–13' (t)	29.26–29.68	29.20–29.69	29.26–29.68	29.19–29.68								
14' (t)	31.91	31.91	31.91	31.92								
15' (t)	22.68	22.68	22.68	22.68								
16' (q)	14.11	14.11	14.11	14.11								

^a Multiplicity of the carbon atom signal for the compounds 13–16.

(100), 525 (1), 588 (1), 337 (1), 315 (2), 269 (3). Anal. Calcd for $\text{C}_{36}\text{H}_{58}\text{O}_5$ (570.85): C, 75.75; H, 10.24; O, 14.01. Found: C, 75.60; H, 10.20.

Compound 6: 131 mg, 85% yield, TLC (3:1 pe/e). R_F = 0.63. IR (cm^{-1}) 2928 vs, 2856 s, 1726 s, 1665 m, 1511 s, 1448 m, 1327 m, 1224 s, 1148 s, 1176 m, 1002 w, br, 876 w, 865 w, 566 vw, 555 vw. FAB–MS m/z (%): 594 (34), 593 (100), 592 (1), 525 (1), 337 (2), 315 (2), 269 (3), 187 (1). Anal. Calcd for $\text{C}_{36}\text{H}_{58}\text{O}_5$ (570.85): C, 75.75; H, 10.24; O, 14.01. Found: C, 75.68; H, 10.18.

Compound 7: 97 mg, 56% yield, TLC (3:1 pe/e). R_F = 0.81. IR (cm^{-1}) 2929 vs, 2855 s, 1716 s, 1731 s, 1648 w, 1512 s, 1447 m, 1340 w, 1211 s, br, 1151 s, 1176 s, 1002 w, br, sh, 877 w, 855 w, 566 vw, 555 vw. FAB–MS m/z (%): 594 (34), 593 (100). Anal. Calcd for $\text{C}_{36}\text{H}_{58}\text{O}_5$ (570.85): C, 75.75; H, 10.24; O, 14.01. Found: C, 75.63; H, 10.33.

Compound 8: 134 mg, 87% yield, TLC (3:1 pe/e). R_F = 0.84. IR (cm^{-1}) 2928 vs, 2856 s, 1716 s, 1730 s, 1648 w, 1511 s, 1446 m, 1340 w, 1210 s, br, 1151 s, 1176 s, 1002 w, br, sh, 877 w, 855 w, 566 vw, 555 vw. FAB–MS m/z (%): 594 (34), 593 (100). Anal. Calcd for $\text{C}_{36}\text{H}_{58}\text{O}_5$ (570.85): C, 75.75; H, 10.24; O, 14.01. Found: C, 75.51; H, 10.25.

Compound 9: 141 mg, 86% yield, TLC (3:1 pe/e). R_F = 0.77. IR (cm^{-1}) 2936 s, 2875 m, 1728 vs, 1716 s, 1666 m, 1511 s, 1448 m, 1327 m, 1224 vs, 1147 s, 1258 m, sh, 1185 s, sh, 1095 m, 980 w, 865 w, 561 vw, 550 vw. FAB–MS m/z (%): 402 (10), 315 (38), 269 (14), 233 (58), 187 (41), 159 (21), 127 (34), 119 (24), 107 (65), 99 (100), 71 (74), 55 (22). Anal. Calcd for $\text{C}_{24}\text{H}_{34}\text{O}_5$ (402.53): C, 71.61; H, 8.51; O, 19.87. Found: C, 71.40; H, 8.42.

Compound 10: 142 mg, 92% yield, TLC (3:1 pe/e). R_F = 0.81. IR (cm^{-1}) 2937 s, 2860 s, 1727 vs, 1665 m, 1511 vs, 1448 m, 1327 m, 1224 vs, 1148 vs, 1259 m, sh, 1182 s, 1089 m, 999 m, 865 w, 565 vw, 553 vw. FAB–MS m/z (%): 441 (9), 426 (15), 425 (38), 332 (4), 317 (10), 316 (50), 288 (100), 287 (28), 249 (14). Anal. Calcd for $\text{C}_{24}\text{H}_{34}\text{O}_5$ (402.53): C, 71.61; H, 8.51; O, 19.87. Found: C, 71.45; H, 8.42.

Compound 11: 179 mg, 93% yield, TLC (3:1 pe/e). R_F = 0.59. IR (cm^{-1}) 2936 s, 2858 m, 1716 s, 1731 vs, 1648 m, 1511 vs, 1446 m, 1340 w, 1210 s, 1151 vs, 1259 m, sh, 1095 m, 980 w, 852 w, 561 vw, 550 vw. FAB–MS m/z (%): 402 (3), 356 (4), 269 (12), 187 (15), 127 (100), 107 (35), 99 (69), 71 (23). Anal. Calcd for $\text{C}_{24}\text{H}_{34}\text{O}_5$ (402.53): C, 71.61; H, 8.51; O, 19.87. Found: C, 71.53; H, 8.46.

Compound 12: 131 mg, 87% yield, TLC (3:1 pe/e). R_F = 0.62. IR (cm^{-1}) 2937 s, 2860 m, 1716 s, 1731 s, 1649 m, 1511 vs, 1446 m, 1340 w, 1209 s, 1151 vs, 1260 m, sh, 1185 s, sh, 1090 w, 999 m, 855 w, 566 vw, 553 vw. FAB–MS m/z (%): 426 (22), 425 (100), 420 (11), 403 (5), 357 (4), 269 (4), 187 (2). Anal. Calcd for $\text{C}_{24}\text{H}_{34}\text{O}_5$ (402.53): C, 71.61; H, 8.51; O, 19.87. Found: C, 71.58; H, 8.45.

Compound 13: 139 mg, 89% yield, TLC (3:1 pe/e). R_F = 0.75. IR (cm^{-1}) 1707 s, 1702 s, sh, 1664 m, sh, 1653 m, 1511 s, 1447 m, 1328 m, 1351 m, 1232 vs, 1154 vs, 1143 s, sh, 990 w, 863 w, 854 w. FAB–MS m/z (%): 438 (24), 437 (100), 315 (12). Anal. Calcd for $\text{C}_{25}\text{H}_{34}\text{O}_5$ (414.54): C, 72.44; H, 8.27; O, 19.30. Found: C, 71.34; H, 8.20.

Compound 14: 146 mg, 94% yield, TLC (3:1 pe/e). R_F = 0.80. IR (cm^{-1}) 1708 s, 1702 s, sh, 1662 m, sh, 1649 m, sh, 1511 s, 1447 m, 1328 m, 1353 m, 1231 vs, 1153 vs, 1002 m, 863 w, sh, 856 m. FAB–MS m/z (%): 438 (24), 437 (100), 315 (13), 269 (8), 233 (8), 187 (4). Anal. Calcd for $\text{C}_{25}\text{H}_{34}\text{O}_5$ (414.54): C, 72.44; H, 8.27; O, 19.30. Found: C, 72.28; H, 8.21.

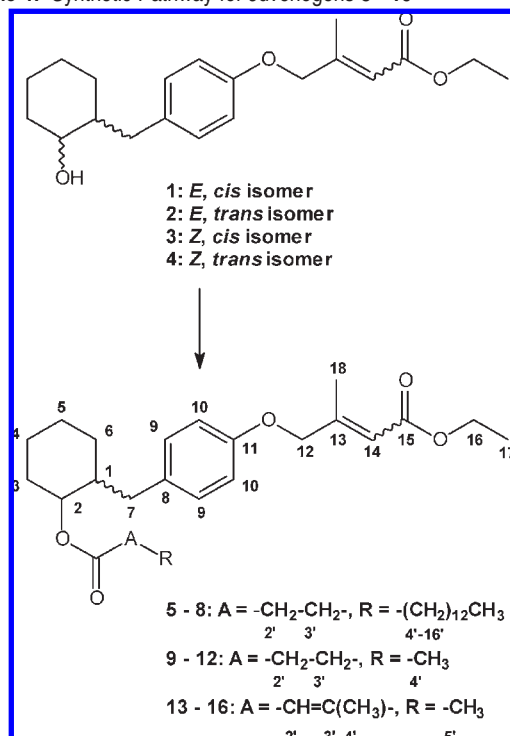
Compound 15: 108 mg, 69% yield, TLC (3:1 pe/e). R_F = 0.85. IR (cm^{-1}) 1702 s, br, 1651 m, 1511 s, 1447 m, 1341 w, 1351 w, 1232 vs, 1154 vs, 1143 s, sh, 990 w, 853 m. FAB–MS m/z (%): 438 (24), 437 (100), 411 (4), 337 (5), 269 (5). Anal. Calcd for $\text{C}_{25}\text{H}_{34}\text{O}_5$ (414.54): C, 72.44; H, 8.27; O, 19.30. Found: C, 72.30; H, 8.12.

Compound 16: 133 mg, 85% yield, TLC (3:1 pe/e). R_F = 0.87. IR (cm^{-1}) 1701 vs, br, 1647 s, 1511 s, 1446 s, 1342 m, 1353 m, 1233 vs, 1154 vs, 1003 m, 854 m. FAB–MS m/z (%): 438 (23), 437 (100), 337 (6), 269 (4). Anal. Calcd for $\text{C}_{25}\text{H}_{34}\text{O}_5$ (414.54): C, 72.44; H, 8.27; O, 19.30. Found: C, 72.39; H, 8.22.

Experimental Insect. *Neobellieria (Sarcophaga) bullata* Parker. *N. bullata* was used as the experimental insect. A standard strain of *N. bullata* was obtained from the University of Illinois. Larvae were bred on calf's liver.

Biological Tests. *Screening Test with N. bullata.* The solutions of the compounds 5–16 in acetone (1 mg mL^{-1}) were 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;

Scheme 1. Synthetic Pathway for Juvenogens 5–16

Table 6. Results of the Biological Screening Tests of the Juvenogens 5–16 on *N. bullata*

compound	hatchability (%) ^a	ED ₅₀ (μg individual ⁻¹)	EC ₅₀ (μg μL ⁻¹)
methoprene	>90	>9	>1.8
5	60	6	1.2
6	50	5	1.0
7	>90	>9	>1.8
8	>90	>9	>1.8
9	60	6	1.2
10	60	6	1.2
11	70	7	1.4
12	70	7	1.4
13	>90	>9	>1.8
14	20	2	0.4
15 ^b	40	4	0.8
16	60	6	1.2

^a A 90% hatchability corresponded with the hatchability in the reference experiment, which means that the compounds displayed no biological activity.

^b Compound 15 displayed a low toxic effect besides the effect on hatchability.

after day 4, they were offered beef liver, which later also served as an oviposition medium. A control experiment was performed simultaneously. The only difference between the screening test experiment and the control one was the presence or absence of the tested compound (5–16). The females were dissected at regular intervals (6, 9, 12, 15, and 21 days after application), and the morphology of their ovaries was studied. The shape of eggs, yolk deposition in the first and second egg chambers, 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 in the uterus where a larva was developed and those where development had not taken place.

RESULTS AND DISCUSSION

A total of 12 new juvenogens 5–16 (three series), derived from the racemic juvenoids 1–4 were synthesized, identified, and tested on the blowfly *N. bullata* for their influence on reproduction (Scheme 1). The synthetic protocol has been designed to be as simple as possible, already taking into account the economy of the

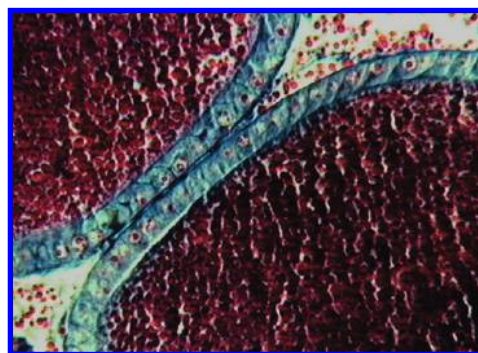
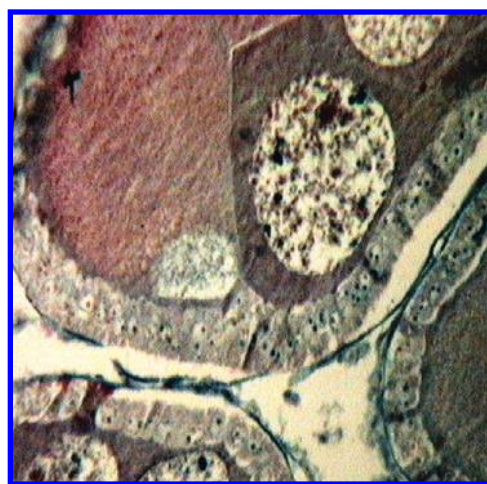
Figure 1. Normal development of ovaries of *N. bullata* (a reference picture).

Figure 2. Division of nucleoli in the nucleus of follicular cells of the second egg chamber. The first stadium of the effect of juvenogens.

synthesis of these compounds in the case of a possible production for practical applications. The development of the first batch of eggs was not morphologically affected, but the hatchability of the eggs was lowered. Significant differences within/among juvenogen groups were observed. To compare, methoprene decreased larval hatching to 90%, a value in the range of hatching rates for untreated females (85–95%). Important results were gained after application of juvenogens with 3-methylbut-2-enoic acid moiety. While compound 13 showed no effect, application of the juvenogen 14 led to a decreasing hatchability of 20%, 15 was even toxic for the treated insect, and 16 displayed only a mild effect (60% hatchability). From the group of hexadecanoic acid derivatives, only compound 6 displayed considerable ovidical activity, 50% hatchability. The effect of the juvenogens 9–12 was lower (60–70% hatchability). Table 6 summarizes the data of the biological testing of the compounds 5–16 in comparison to methoprene (4, 6), often used as a reference juvenoid. Besides presenting the values of hatchability (in percentages), the effective dose (ED₅₀) and effective concentration (EC₅₀) were calculated. ED₅₀ shows the amount of the tested compound applied on the tested insect species on average that resulted in 50% reduction of hatchability. EC₅₀ shows the concentration of a solution of the tested compound, the application of which in the given volume (5 μL per individual) resulted in 50% reduction of hatchability. It follows from Table 6 that the fatty acid used affected substantially the biological activity of the studied juvenogens. The most remarkable effect of the fatty acyl was observed with the juvenogens 13–16. Generally, it was possible to conclude that only the EC₅₀ values not exceeding 1 μg μL⁻¹ indicated the potential of the

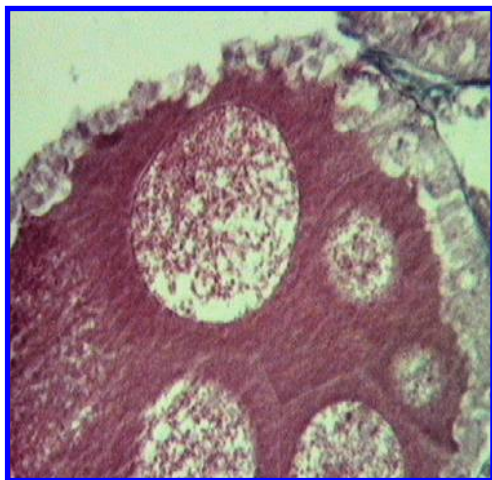


Figure 3. Nucleoli of the follicular epithelium cells divide, and the cell layer adjoining the oocyte is thickened.

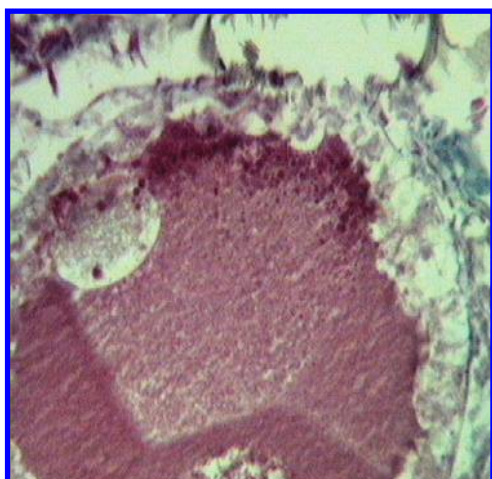


Figure 4. Proliferation of the follicular cells into the region of the oocyte.

juvenogen (**6**, **14**, and **15**) for practical applications. However, the histological studies resulted in specific observations (cf. below).

Histological observation of the ovaries revealed pathological changes in all structures of the egg chamber. Several histological pictures of ovaries are presented in **Figures 1–4**. Histological changes in the development of the second egg chamber are manifested by disruption of the structure of follicular cells in a low number of ovarioles, often about 10–20%, in the case of compounds **6**, **14**, and **16** even more, 25–65%. They start as a division of nucleoli in the nucleus of follicular cells (**Figure 2**) and subsequent proliferation of the nuclei of follicular cells. Seldom proliferation of follicular cells follows. Such formation does not invade the whole space of oocytes or trophocytes [as seen in

previous tests with related juvenogens (**10**, **12**, **13**)] but stops at a comparatively early stage (**Figures 3** and **4**). However, such an ovariole does not produce viable egg, which decreases offspring production.

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LITERATURE CITED

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