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Insight into reduction of obacunone, and their ester derivatives as insecticidal agents against *Mythimna separata* Walker

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ABSTRACT

Here we have prepared a series of ester compounds of obacunone, a naturally occurring limonoid, isolated from plants such as *Citrus* and *Dictamnus angustifolius*. Their insecticidal activity was evaluated at 1 mg/mL against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker), a typical lepidopteran pest. When obacunone reacted with $NaBH_4$, the ratio of two reduction products, C7α-hydroxyobacunone (**2**) and C7β-hydroxyobacunone (**3**), was related to the reaction mixing solvents. C7α-Propionyloxybacunone (**4b**) and C7β-(*n*)heptanoyloxybacunone (**5g**) exhibited the more promising insecticidal activity than their precursor obacunone and toosendanin.

Recently, more eco-friendly pesticides for use in integrated pest management (IPM) is required. Nowadays, the discovery of new pesticidal agents from plant secondary metabolites, or by using them as the lead compounds for further structural modification, has received much research attention.[1–9] Obacunone (**1**, Fig. 1), is isolated as an naturally occurring limonoid from many species of plants such as *Citrus* and its hybrids,[10] and *Dictamnus angustifolius*.[11] Besides exhibiting some medicinal activities such as antiproliferative,[12] anticancer,[13] antimalarial,[14] and antioxidant activities,[15] compound **1** also showed insecticidal activities such as the moult inhibiting activity against mosquito *Culex quinquefasciatus*,[16] and the antifeedant activity against *Spodoptera frugiperda*.[17] As part of an ongoing search for new potential natural-product-based insecticidal agents to control the lepidopteran pests,[18–21] herein we synthesized a series of ester compounds of obacunone by modification of its B ring. Their insecticidal activity was evaluated against the oriental armyworm (*Mythimna separata* Walker), an important and typical lepidopteran pest.

As described in Table 1, first, reduction of **1** with $NaBH_4$ in the presence of different solvents such as CH_3OH, THF, CH_2Cl_2, and CH_3CN, was investigated. Interestingly, we found that the ratio of two reduction isomers, C7α-hydroxyobacunone (**2**) and C7β-hydroxyobacunone (**3**), was related with the reaction mixing solvents. For example, when a solution of $NaBH_4$ (1.0 mmol) in CH_3OH was added dropwise to a solution of **1** (0.5 mmol) in CH_3OH at −5 °C, after reaction for 1 h, the ratio of **2** and **3** was 1/3.8 (entry 1); when a solution of $NaBH_4$ (1.0 mmol) in THF, CH_3OH, CH_3CN or CH_2Cl_2 was added dropwise to a solution of **1** (0.5 mmol) in CH_3OH or CH_3CN at −5 °C, respectively, after reaction for 1–2 h, the ratio of **2** and **3** was between 1/2.87 and 1/2.63 (entries 2–6). When a solution of $NaBH_4$ (1.0 mmol) in CH_3OH was added dropwise to a solution of **1** (0.5 mmol) in CH_2Cl_2 at −5 °C, after reaction for 2 h,

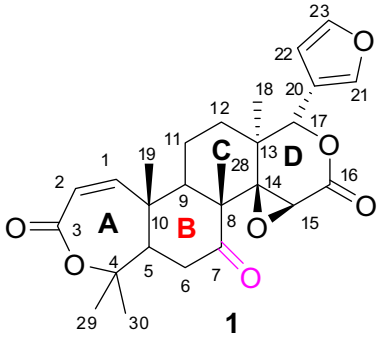

Figure 1. Chemical structure of obacunone (**1**).

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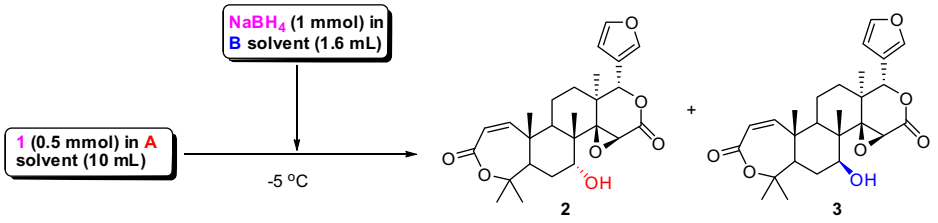
Table 1
Investigation of reduction of **1** with $NaBH_4$ in the presence of different solvents



Entry	A	B	Reaction time (h)	Yield (%)		Ratio of **2**/**3**
				2	**3**	
1	CH_3OH	CH_3OH	1	20	76	1:3.8
2	CH_3OH	THF[a]	1	23	66	1:2.87
3	CH_3CN	CH_3OH	1.5	25	71	1:2.84
4	CH_3OH	CH_3CN	1	19	51	1:2.68
5	CH_3OH	CH_2Cl_2	1	22	58	1:2.64
6	CH_3CN	THF	2	24	63	1:2.63
7	CH_2Cl_2	CH_3OH	2	34	60	1:1.76
8	THF	CH_3OH	1	36	37	1:1.03
9	THF	THF	2.5	47	26	1.81:1
10	THF	CH_3CN	2	43	32	1.34:1
11	THF	CH_2Cl_2	2	46	44	1.05:1
12	CH_2Cl_2	THF	10	0	0	/

[a] THF: Tetrahydrofuran.

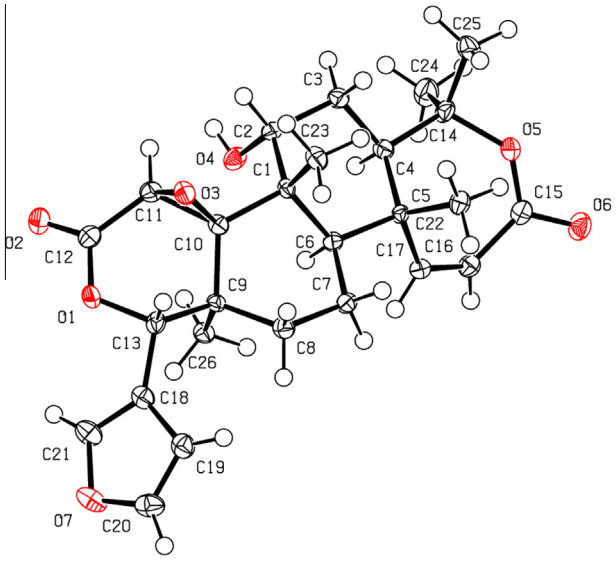


Figure 2. X-ray crystal structure of C7α-hydroxyobacunone (**2**).

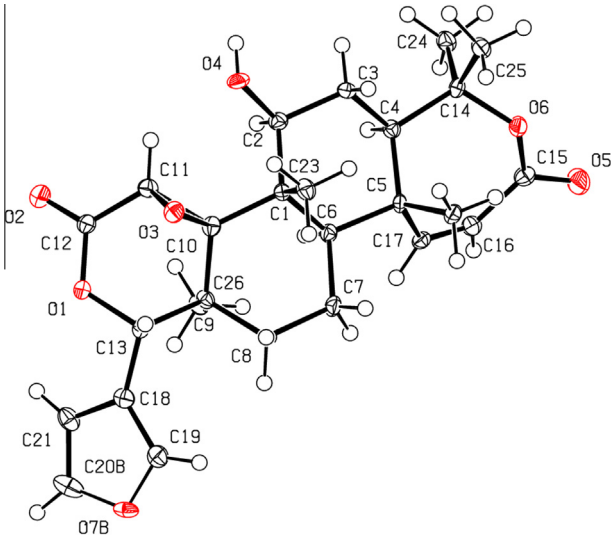
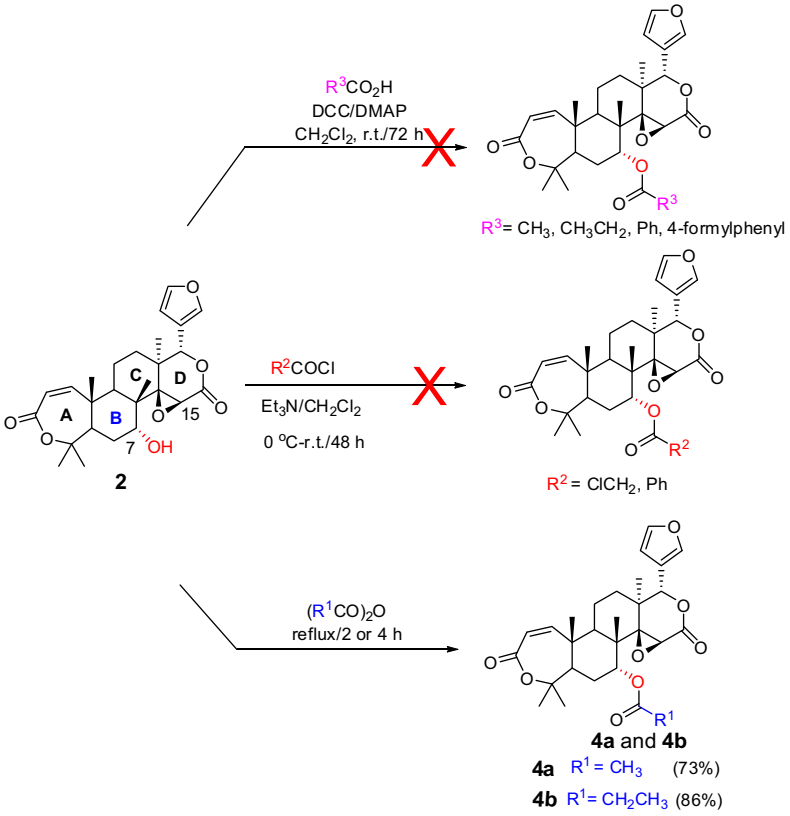


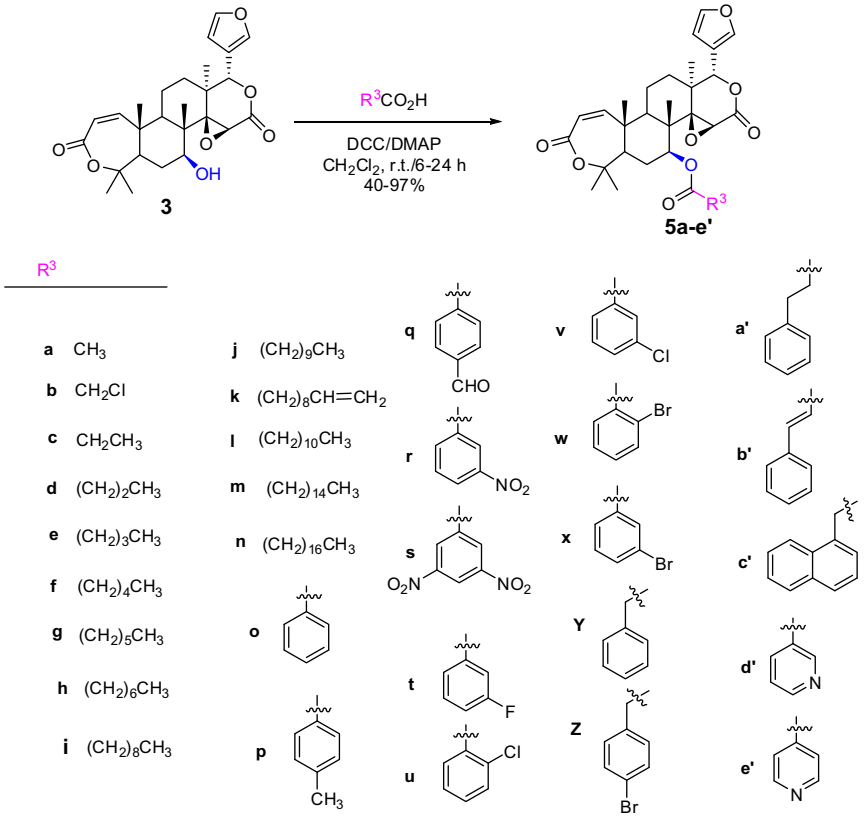
Figure 3. X-ray crystal structure of C7β-hydroxyobacunone (**3**).

the ratio of **2** and **3** was 1/1.76 (entry 7). However, when a solution of $NaBH_4$ (1.0 mmol) in CH_3OH or CH_2Cl_2 was added dropwise to a solution of **1** (0.5 mmol) in THF at −5 °C, after reaction for 1 or 2 h, the ratio of **2** and **3** was nearly 1 (entries 8 and 11). If a solution of $NaBH_4$ (1.0 mmol) in CH_3CN was added dropwise to a solution of **1** (0.5 mmol) in THF at −5 °C, after reaction for 2 h, the ratio of **2** and **3** was 1.34/1 (entry 9). Moreover, when a solution of $NaBH_4$ (1.0 mmol) in THF was added dropwise to a solution of **1** (0.5 mmol) in THF at −5 °C, after reaction for 2.5 h, the ratio of **2** and **3** was increased to 1.81/1 (entry 10). But when a solution of $NaBH_4$ (1.0 mmol) in THF was added dropwise to a solution of **1** (0.5 mmol) in CH_2Cl_2 at −5 °C, even if the reaction time was prolonged to 10 h, **2** and **3** were not obtained (entry 12). The steric structures of **2** and **3** were further determined by X-ray crystallography as shown in Figures 2 and 3.[22] It demonstrated that the C7-hydroxy groups of **2** and **3** were present in α and β configuration, respectively.

Second, **2** or **3** reacted with different carboxylic acids in the presence of DCC and DMAP. It is noteworthy that the reaction activity of **2** or **3** with different carboxylic acids in the presence of DCC and DMAP was associated with the stereo configuration of their hydroxy groups at the C-7 position. For example, as shown in Schemes 1 and 2 containing 7α-hydroxy group did not reacted with carboxylic acids such as acetic acid, propanoic acid, benzoic acid, and 4-formylbenzoic acid in the presence of DCC and DMAP, even if the reaction time was prolonged to 72 h. Moreover, when **2** reacted with benzoyl chloride or chloroacetyl chloride for 48 h, no target compounds were obtained. Only when **2** reacted with acetic or propionic anhydride under reflux, the corresponding esters **4a**



Scheme 1. Investigation of synthesis of C7α-ester derivatives of obacunone.



Scheme 2. Synthesis of C7β-ester derivatives of obacunone (**5a–e′**).

and **4b** were afford, respectively. It may be due to the steric hindrance between the hydrogen atom at the C-15α position and the hydroxy group at the C-7α position of **2**. On the contrary, when **3** reacted with different carboxylic acids in the presence of DCC and DMAP, the title products **5a**–**e**′ were smoothly obtained in 40–97% yields (Scheme 2). The structures of all target compounds were well characterized by ^{1}H NMR, HRMS, optical rotation, IR, and mp (see Supporting information). The steric configuration of **5q** was further confirmed by X-ray crystallography (Fig. 4).[22] It suggested that the (4-formyl)benzoyloxy group at the C-7 position of **5q** adopted β configuration.

As shown in Table 2, the insecticidal activity of compounds **1**–**3**, **4a**, **4b**, and **5a**–**e**′ against the pre-third-instar larvae of *M. separata* in vivo was tested by the leaf-dipping method at a concentration of 1 mg/mL.[23] Toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*, was used as the positive control at 1 mg/mL. Leaves treated with acetone alone were used as a blank control group. It was found that the obacunone derivatives exhibited the delayed insecticidal activity against *M. separata*. For example, the corrected mortality rates of **4b** against *M. separata* after 10 and 20 days were 3.3% and 13.3%, respectively; whereas after 35 days it was sharply increased to 57.1%, which was more than 17-fold of that after 10 days. In addition, the symptoms of *M. separata* treated by the obacunone derivatives were observed in the same way as our previous reports.[18–21] For instance, in the treated groups, many larvae generally died with the slim and wrinkled bodies during the stage of larva (Fig. S1, see Supporting information); a majority of larvae molted to malformed pupae or died during the pupation period (Fig. S2, see Supporting information); some malformed moths were with imperfect wings during the stage of adult emergence (Fig. S3, see Supporting information). Among all derivatives, compounds **4b**, **5g** and **5l** showed the more potential insecticidal activity than their precursor **1** and toosendanin (a positive control). For example, the final mortality rates of **4b**, **5g** and **5l** were 57.1%, 57.1% and 50%, respectively. Meanwhile, the preliminary relationship between the structures of the obacunone derivatives and their insecticidal activity was also analysed: (1) The configuration of the hydroxy group at the C-7 position of **1** was important for the insecticidal activity. For instance, the final mortality rate of **2** (containing C7α-hydroxy) was 32.1%, whereas the final mortality rate of **3** (containing C7β-hydroxy) was 46.4%. (2) To C7α-alkylacyloxy derivatives **4a** and **4b**, introduction of the propionyloxy group at the C-7α position of **1** could lead to the more promising compound **4b** (e.g., the final mortality rates: 57.1% for **4b** versus 39.3% for **4a**). (3) To C7β-acyloxy series (**5a**–**e**′), in general, the insecticidal activity of C7β-alkylacyloxy derivatives (**5a**–**n**) was more potent than that of C7β-arylacyloxy ones (**5o**–**e**′). (4) To C7β-alkylacyloxy series **5a**–**n**, the proper length of their side chain at the C-7β position to the insecticidal activity was very necessary. Especially introduction of the (*n*)heptanoyloxy or (*n*)dodecanoyloxy group at the C-7β position of **1** resulted in the more potential compounds **5g** and **5l** than toosendanin. However, to C7β-arylacyloxy series **5o**–**e**′, whether introduction of the electron-withdrawing or electron-donating groups on the phenyl ring, or heterocyclic rings at the C-7β position of **1** did not lead to the potential compounds, and their final mortality rates were even less than their precursors (e.g., **1** and **3**).

In conclusion, we have prepared a series of ester compounds of obacunone modified in the B ring and tested their insecticidal activity against the pre-third-instar larvae of *M. separata* in vivo. When obacunone was reduced by $NaBH_4$, the ratio of products **2** and **3** was related to the reaction mixing solvents. The key steric structures of three compounds **2**, **3**, and **5q** were determined by single-crystal X-ray diffraction. Especially **4b** and **5g** exhibited the more promising insecticidal activity than their precursor obacunone and toosendanin. It will pave the way for further design

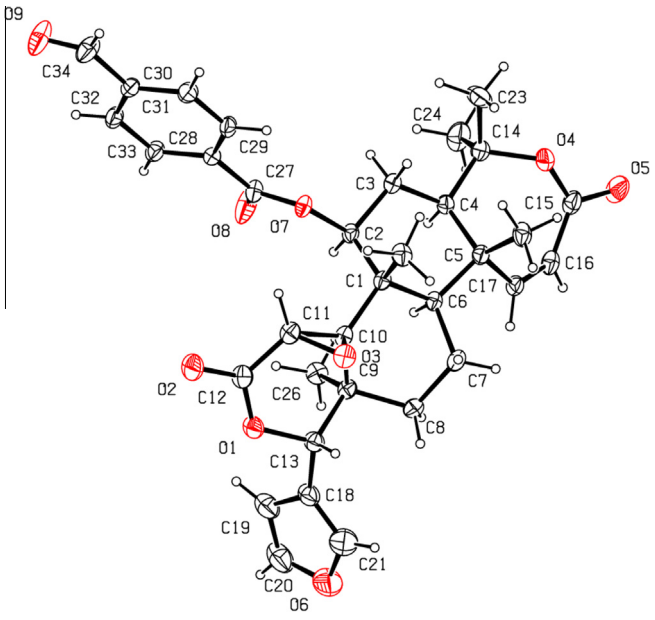


Figure 4. X-ray crystal structure of C7β-(4-formyl)benzoyloxybacunone (**5q**).

Table 2
Insecticidal activity of ester compounds of obacunone (**4a**, **4b**, and **5a**–**e**′) against *M. separata* on leaves treated with a concentration of 1 mg/mL

Compd	Corrected mortality rate (%)		
	10 days	20 days	35 days
1	6.7 ± 6.7	20.0 ± 5.8	42.9 ± 3.3
2	0 ± 0	16.7 ± 3.3	32.1 ± 3.3
3	10.0 ± 5.8	30.0 ± 5.8	46.4 ± 5.8
4a	6.7 ± 6.7	13.3 ± 3.3	39.3 ± 3.3
4b	3.3 ± 3.3	13.3 ± 3.3	57.1 ± 0
5a	20.0 ± 0	26.7 ± 3.3	46.4 ± 5.8
5b	0 ± 0	23.3 ± 6.7	42.9 ± 3.3
5c	13.3 ± 3.3	20.0 ± 0	39.3 ± 3.3
5d	16.7 ± 6.7	23.3 ± 6.7	42.9 ± 3.3
5e	10.0 ± 5.8	13.3 ± 6.7	39.3 ± 3.3
5f	10.0 ± 5.8	16.7 ± 3.3	21.4 ± 3.3
5g	0 ± 0	30.0 ± 0	57.1 ± 0
5h	3.3 ± 3.3	13.3 ± 3.3	39.3 ± 6.7
5i	10.0 ± 0	16.7 ± 3.3	17.9 ± 3.3
5j	6.7 ± 6.7	20.0 ± 0	25.0 ± 0
5k	0 ± 0	26.7 ± 3.3	35.7 ± 0
5l	3.3 ± 3.3	20.0 ± 0	50.0 ± 3.3
5m	0 ± 0	33.3 ± 6.7	46.4 ± 5.8
5n	0 ± 0	20.0 ± 0	35.7 ± 0
5o	13.3 ± 3.3	16.7 ± 3.3	25.0 ± 0
5p	0 ± 0	6.7 ± 3.3	28.6 ± 3.3
5q	6.7 ± 6.7	13.3 ± 3.3	28.6 ± 3.3
5r	0 ± 0	20.0 ± 5.8	25.0 ± 5.8
5s	10.0 ± 5.8	16.7 ± 3.3	28.6 ± 3.3
5t	6.7 ± 6.7	10.0 ± 0	28.6 ± 3.3
5u	13.3 ± 6.7	23.3 ± 3.3	28.6 ± 3.3
5v	10.0 ± 5.8	13.3 ± 6.7	28.6 ± 3.3
5w	10.0 ± 0	13.3 ± 3.3	39.3 ± 3.3
5x	0 ± 0	10.0 ± 0	21.4 ± 3.3
5y	6.7 ± 3.3	13.3 ± 6.7	28.6 ± 3.3
5z	6.7 ± 3.3	13.3 ± 6.7	28.6 ± 3.3
5a′	3.3 ± 3.3	26.7 ± 3.3	39.3 ± 3.3
5b′	6.7 ± 6.7	10.0 ± 5.8	17.9 ± 3.3
5c′	0 ± 0	10.0 ± 5.8	25.0 ± 0
5d′	3.3 ± 3.3	10.0 ± 5.8	17.9 ± 3.3
5e′	6.7 ± 3.3	20.0 ± 5.8	32.1 ± 6.7
Toosendanin	3.3 ± 3.3	23.3 ± 3.3	46.4 ± 5.8
Blank control	0 ± 0	0 ± 0	6.7 ± 3.3

and chemical modifications of obacunone as botanical insecticidal agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.027.

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21. Xu, H.; Wang, Q. T.; Guo, Y. *Chem. Eur. J.* **2011**, *17*, 8299.
22. Crystallographic data (excluding structure factors) for the structures of **2**, **3**, and **5q** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 965981, 970433, and 965982, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.
23. *Biological assay*: The insecticidal activity of compounds **4a**, **4b**, and **5a**–**e**′ against the pre-third-instar larvae of *Mythimna separata* was assessed by leaf-dipping method. Toosendanin, a commercial insecticide isolated from *Melia azedarach*, was used as a positive control and supplied by Research & Development Center of Biorational Pesticide, Northwest A&F University, Shaanxi province, China. For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of all the above tested compounds, and toosendanin were prepared at the concentration of 1 mg/mL. Fresh wheat leaves were dipped into the corresponding solution for 3 s, then taken out, and dried in a room. Leaves treated with acetone alone were used as a blank control group. Several treated leaves were kept in each dish, where every 10 larvae were raised. If the treated leaves were consumed, additional treated leaves were added to the dish. After 48 h, untreated fresh leaves were added to all dishes until adult emergence. The experiment was carried out at 25 ± 2 °C and on 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds against the pre-third-instar larvae of *M. separata* was calculated by the following formula:

$$\text{corrected mortality rate } (\%) = (T - C) \times 100/(100\% - C)$$

where T is the mortality rate in the treated group expressed as a percentage and C is the mortality rate in the untreated group expressed as a percentage.