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Protective effects of semi-synthetic ecdysteroids in the Colorado potato beetle adult under chronic and acute toxic stress

Протекторные эффекты полусинтетических экдистероидов при хроническом и остром токсическом стрессе у имаго колорадского жука

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Ключевые слова: экдистероиды, 20-гидроксиэкдизон, 9α-гидрокси-5α-экдистероиды, *Leptinotarsa decemlineata,* протекторный эффект.

Abstract. Semi-synthetic ecdysteroids synthesized from 20-hydroxyecdysone isolated from *Serratula coronata* were tested on adult Colorado potato beetles. Four out of the ten compounds stimulated the viability of the insects that developed on insecticide-pretreated *Solanum tuberosum* plants. Pretreatment of the beetle adults with the ecdysteroids promoted selective mitigation of the toxic effect of fipronil and bensultap on the insects.

Резюме. Полусинтетические экдистероиды, синтезированные на основе 20-гидроксиэкдизона, выделенного из *Serratula coronata* испытаны на имаго колорадского жука. Для развившихся на обработанных химическими инсектицидами растениях *Solanum tuberosum* насекомых 4 из 10 соединений продемонстрировали стимулирующий жизнеспособность эффект. Предварительная обработка экдистероидами имаго способствовала селективному снижению токсического эффекта действия фипронила и бенсултапа.

The Colorado potato beetle *Leptinotarsa decemlineata* Say (Chrysomelidae, Coleoptera) is known as the most dangerous, widely encountered potato pest; therefore, it is used most often as a model to assess the activity of insecticides — toxic compounds that target, most often, the fine structures of insect neuroendocrine system: synapses, receptors, and membrane channels. Currently, the most intense search for the structure-insecticidal activity relationships is carried out among compounds that are regarded not so much as toxicants, but rather as disturbers of growth and development processes: these are either analogs or antagonists of insect hormones.

The development of the interdisciplinary knowledge of the mechanisms of interaction between exogenous agents and biological targets served as the impetus for rational search for the potential environmentally safe regulators of insect population. Quite promising in this respect are ecdysteroids, which are insect and arthropod hormones, important regulators of reproduction, diapause, molting, and metamorphosis in the preimaginal stage of development [Dinan, Lafont, 2006; Gaertner et al., 2012]. Studies addressing the effect of ecdysteroids or their analogs on the Colorado potato beetle life cycle are virtually missing as yet. There are only data on the toxicity of some ecdysteroids and 5α-hydroxy-Δ^7-6 ketosteroids [Zolotar et al., 2001; Hormann et al., 2002; Smagghe et al., 2002] towards the Colorado potato beetle larvae; according to these data, the presence of *cis*-2,3-dihydroxyl moiety is very important for the molecule to exhibit insecticidal activity.

A significant and characteristic structural unit of most of active ecdysteroids contributing to the ecdysone activity of the natural molecule is the γ-hydroxy-α,β-enone group [Bergamasco, Horn, 1980]. The chemical lability of this group under alkaline conditions enables a number of specific transformations giving new ecdysteroids and their analogs [Savchenko et al., 2008, 2009, 2010]. Previously, 9α-hydroxy-5α-ecdysteroids containing a bis(γ-9,14-dihydroxy-α,β-enone) moiety were synthesized by auto-oxidation of ecdysteroid substrates: 20-hydroxyecdysone (1-3) and its 25-anhydro derivative 10 in an alkaline medium (NaOH-MeOH); the prepared compounds exhibited stress-protective activity on the *Musca domestica* model [Savchenko et al., 2015]. The objective of this study is to establish the relationship between the biological effects and structural features of semi-synthetic ecdysteroids upon the action on adult *L. decemlineata* under toxic stress induced by chemical toxicants.

Materials and Methods

Semi-synthetic ecdysteroids 2-10 (Fig. 1) were prepared via targeted transformations of 20-hydroxyecdysone (20-E, 1), a phytoecdysteroid isolated from the *Serratula coronata* plant [Savchenko et al., 2014].

The stress-protective action under toxic stress was assessed on summer-generation adult insects collected from potato plots and pretreated with chemical insecticides (Regent, Actara, Zeppelin, Prestige) in the doses recommended by the manufacturer in the period of wide occurrence of II and III instars larvae [Mardanshin et al., 2012]. Since the development took place on insecticide-treated plants, surviving insects had experienced chronic toxic stress. The control was obtained by collecting the beetle adults from plants growing at insecticide-untreated plots. The protective effect of 20Å and its derivatives was assessed for the beetle adults. The adults were kept under laboratory conditions on fresh food. The effect of ecdysteroids under chronic toxic stress was estimated by topically treating the beetle adults with ethanol solutions ($2\cdot10^{-4}$ M, 1 μL per insect). The acute toxic stress model comprised treating the adults with insecticides. To elucidate the protective effect of the prepared ecdysteroids under acute toxic stress, the beetle adults were topically pretreated with ecdysteroids in the same doses as in the chronic stress experiments. After 24 hours, some of the pretreated insects were treated with chemical insecticides either topically with fipronil (a phenylpyrazole, the active ingredient of the Regent insecticide, http://www.pesticidy.ru), 1 μL per insect, or by contact intestinal method, with the nereistoxin bensultap (http://www.pesticidy.ru) in concentrations equal to LC20 (the lethal concentration for 20 % of sensitive insects). The mortality was determined on the 3rd day and after 30 days. Each experiment was repeated twice for 30 insects each.

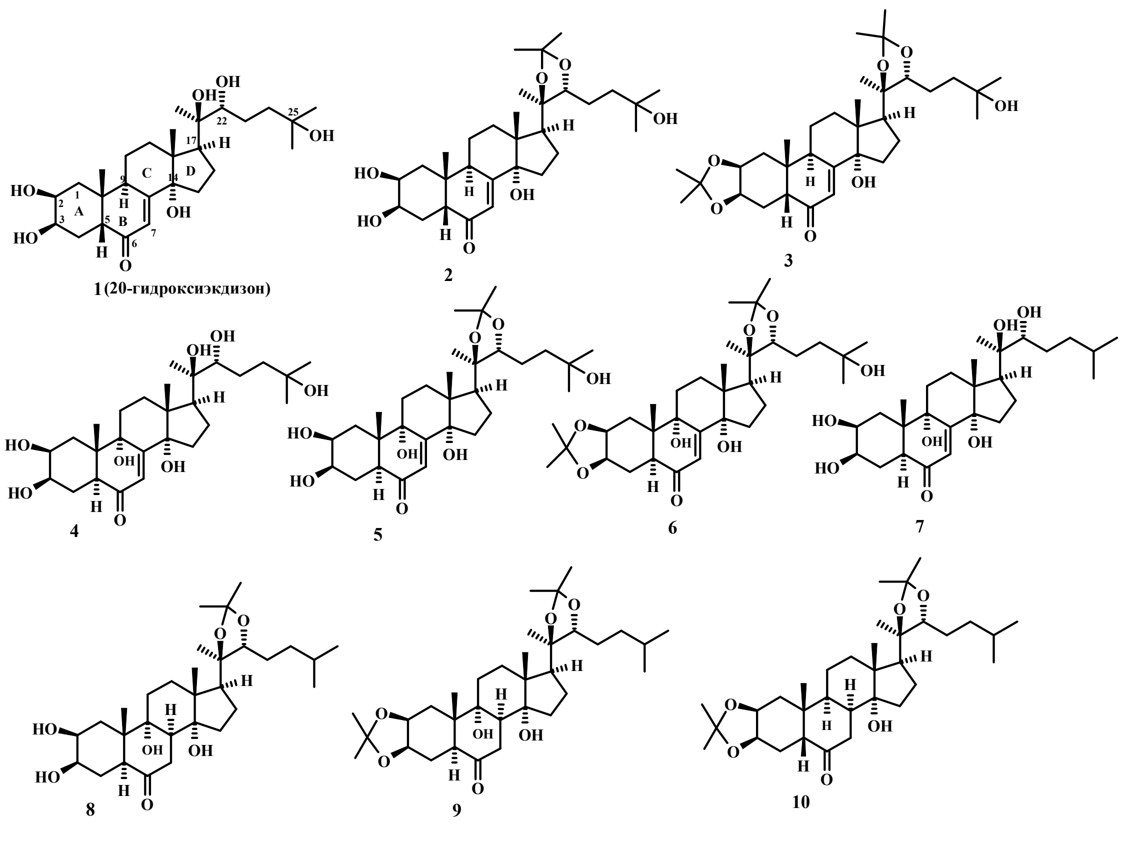

Fig. 1. Structure of 20-hydroxyecdysone (20E) and its semi-synthetic ecdysteroids 2-10.
Рис. 1. Структура 20-гидроксиэкдизона (20E) и его полусинтетических экдистероидов 2-10.

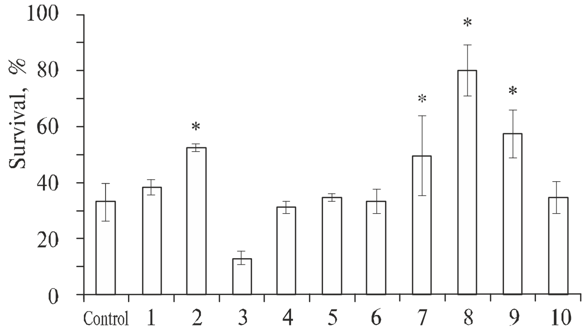

Fig. 2. Effect of modified ecdysteroids on the viability of Colorado potato beetle adults under chronic toxic stress experienced at the larval stage. * — statistically significant ($p \leq 0.05$) increase in the number of insects surviving after 30 days in comparison with the control experiment.

Рис. 2. Влияние модифицированных экдистероидов на жизнеспособность имаго колорадского жука при хроническом токсическом стрессе, перенесённом на стадии личинки. * — статистически значимое ($p \leq 0.05$) повышение числа выживших через 30 суток особей по сравнению с контрольным вариантом.

Results and Discussion

The influence of structural and configuration details of the steroid core and the side chain of the modified matrix of the initial 20E molecule (compounds 2-10) was monitored by measuring the viability of Colorado potato beetle adults under chronic toxic stress. The use of low doses of ecdysteroid substrates for insects that were under the action of insecticide throughout the larval development did not have an adverse effect on their development. Indeed, within 30 days after treatment with 20E, the viability of beetle adults somewhat increased; a statistically significant increase was observed for the use of compounds 2 and 7–9 (Figs 1, 2).

The appearance of the 9α-hydroxy function in the modified ecdysteroid structures (compounds 4-9) accompanied by the change (from *cis*- to *trans*-) of the fusion of rings A and B in the steroid core gives rise to various chronic intoxication consequences in the model insect, which were missing for 20E. Compounds 4–7 lead to an insignificant decrease in the viability of the beetle adults, whereas compounds 7–9 induce a pronounced stress-protective effect (Fig. 2).

The action of ecdysteroids on the model insect under acute toxic stress is evidently selective with respect to chemical insecticides (fipronil and bensultap) (Fig. 3). Without pretreatment with ecdysteroids, the mortality reached 23–27 % as soon as by the 3rd day. In the variants with fipronil, 20E derivatives 7 and 10 considerably reduced the mortality of beetle adults, whereas for bensultap, this beneficial effect was observed for 20E diacetonide 3 and for 9-hydroxylated derivatives 5, 7–9.

The subsequent study of the viability of Colorado potato beetle adults after the past acute toxic stress (after 30 days) revealed the viability stimulation induced by the test ecdysteroid substrates. The most pronounced decrease in the consequences of the past intoxication in the fipronil experiments was found for 20E and its 25-anhydro-20,22-acetonide 10, the percentage of surviving insects being more than 50 %. The beneficial effect observed on the 3rd day in the bensultap experiments (Fig. 4) was also retained after 30 days for the same compounds.

It is known that steroids, in particular 20E, are bound to the allosteric site of the gamma-aminobutyric acid (GABA-A) receptor, which is also a target for fipronil

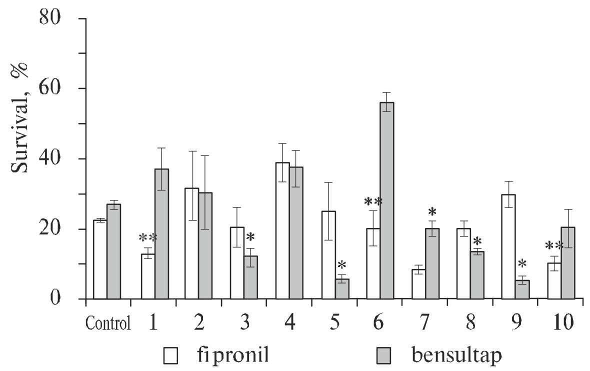

Fig. 3. Decrease in the mortality of Colorado potato beetle adults under acute toxic stress induced by modified ecdysteroids. * — the statistically significant decrease in the mortality with respect to the control experiment for bensultap; ** — the same for fipronil ($p \leq 0.05$).

Рис. 3. Снижение смертности имаго колорадского жука при остром токсическом стрессе под влиянием модифицированных экдистероидов. * — статистически значимое снижение смертности по сравнению с контрольным вариантом для бенсултапа; ** — для фипронила ($p \leq 0,05$)

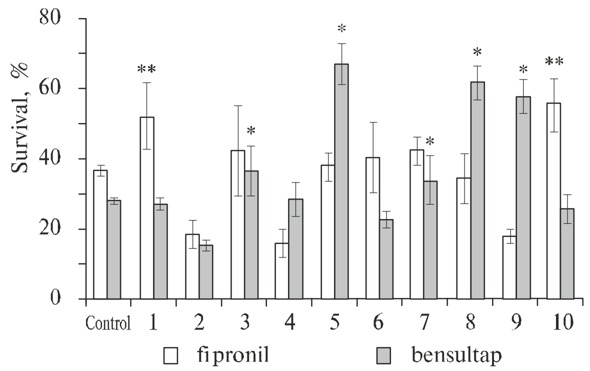

Fig. 4. Effect of modified ecdysteroids on the viability of Colorado potato beetle adults 30 days after the toxic stress. * — the statistically significant increase in the survival rate with respect to the control experiment for bensultap; ** — the same for fipronil ($p \leq 0.05$).

Рис. 4. Влияние модифицированных экдистероидов на жизнеспособность имаго колорадского жука через 30 суток после перенесённого токсического стресса. * — статистически значимое повышение выживания по сравнению с контрольным вариантом для бенсултапа; ** — для фипронила ($p \leq 0,05$)

[Tsujiyama et al., 2014]. Bensultap targets entirely different synapses and receptors, which accounts for the lack of correlation between the stimulating effects of ecdysteroids after the use of these two insecticides. The identified selective effects of semi-synthetic ecdysteroids on the viability of adult *Leptinotarsa decemlineata* Say after the past insecticide-induced toxic stress can be attributed to the direct participation of ecdysone signaling mediated by agonistic or antagonistic activity of the test compounds in the protective mechanisms of the insect [Dinan, Hormann, 2005; Ogura et al., 2005; Gaertner et al., 2012].

Conclusion

The performed experiments demonstrated that the structural and configuration changes of the steroid core and side chain of natural molecules (the number and configuration of hydroxyl groups, a modified ã-hydroxy-á,â-enone moiety) can serve as a selective tool in the regulation of insect viability.

Acknowledgments

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