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STRUCTURAL DEPENDENCE OF INSECTICIDAL ACTIVITY OF 5α-HYDROXY-Δ^7-6-KETOSTEROIDS

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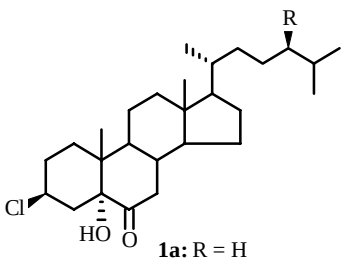
The toxicity of 5α-hydroxy-Δ^7-6-ketosteroids for colorado beetle Leptinotarsa decemlineata *Say. larvae was studied by topical and GI administration.*

Key words: *Leptinotarsa decemlineata*, 5α-hydroxy-Δ^7-6-ketosteroids, insecticidal activity.

Steroids with a 5α-hydroxyl and Δ^7-6-ketone are known to be important intermediates in the synthesis of insect ecdysteroid hormones [1]. We used 5α-hydroxy-6-ketosteroids to synthesize various structural analogs of ecdysteroids [2-6]. The main goal was to prepare new compounds that are structurally close to natural ecdysteroids and to use them as a basis to develop a new generation of ecologically safe insecticides. Also, it was interesting to study the insecticidal properties of not only the end products but also certain intermediates, in particular, 5α-hydroxy-Δ^7-6-ketosteroids. Considering that some natural ecdysteroids contain the same ring B structure, this is even more important. Therefore, it should be noted that the 5α-hydroxy-Δ^7-6-ketone moiety occurs in the structure of 5α-20-dihydroxyecdysone and its 3-glucoside isolated from plants [7].

We now present data for the insecticidal activity of previously synthesized 5α-hydroxy-Δ^7-6-ketosteroids **1**-**10** on colorado beetle (*Leptinotarsa decemlineata* Say., Coleoptera) larvae. The toxicity of these compounds was determined by topical and GI administration by spraying larvae and their food, potato leaves, with 0.01% solutions. Insects were fed treated feed for only one day, after which it was replaced by fresh feed free of steroids. The natural phytoecdysteroid 20-hydroxyecdysone (**11**) was used as the standard. It had previously exhibited toxicity for colorado beetle larvae in analogous experiments [8]. Table 1 contains results of the biological tests. It can be seen that larvae died shortly after treatment with the steroids. It should be noted that the situation was analogous for natural phytoecdysteroids [8]. For this reason, the compounds studied by us and natural phytoecdysteroids can be considered as insect growth regulators according to their biological profile.

The studied compounds **1**-**10** include both highly active and inactive substances. However, **1**-**10** are inferior to 20-hydroxyecdysone with respect to insecticidal activity for colorado beetle larvae. Table 1 provides a basis for several conclusions about the importance of one functional group or another for high insecticidal activity in 5α-hydroxy-Δ^7-ketosteroid derivatives. The practically total absence of toxicity of **4**-**6** for larvae is interesting. Their molecules contain a 2(3)-double bond and lack functional groups on C-2 and C-3. Therefore, functional groups at these positions are important for insecticidal activity.



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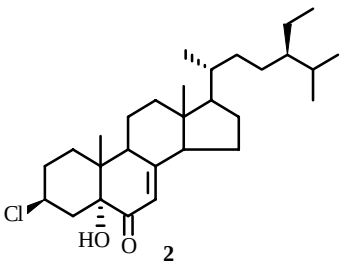
1b: R = Et



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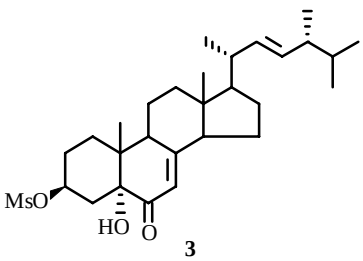

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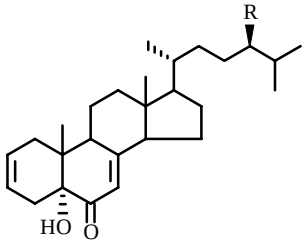
TABLE 1. Toxicity of Steroids for Colorado Beetle Larvae

Compound	Larvae, ea.	Larvae death, days							
		1		3		5		Total	
		number	%	number	%	number	%	number	%
1a. 3β-Chloro-5-hydroxy-5α-cholestan-6-one	30	1	3.3	2	6.7	10	33.3	13	43.3
1b. (24R)-3β-Chloro-5-hydroxy-5α-stigmastan-6-one	30	1	3.3	3	10.0	4	13.3	8	26.7
2. (24R)-3β-Chloro-5-hydroxy-5α-stigmast-7-en-6-one	30	0	0	1	3.3	3	10.0	4	13.3
3. (22E,24R)-3β-Mesyloxy-5-hydroxy-5α-ergosta-7,22-dien-6-one	30	2	6.7	3	10.0	3	10.0	8	26.7
4a. 5-Hydroxy-5α-cholesta-2,7-dien-6-one	30	0	0	0	0	1	3.3	1	3.3
4b. (24R)-5-Hydroxy-5α-stigmasta-2,7-dien-6-one	30	0	0	0	0	2	6.7	2	6.7
5. (22E,24R)-5-Hydroxy-5α-ergosta-2,7,22-trien-6-one	30	0	0	0	0	0	0	0	0
6. 5-Hydroxy-14α-hydroperoxy-5α-cholesta-2,7-dien-6-one	30	0	0	0	0	3	10.0	3	10.0
7a. 2β,3β-Diacetoxy-5-hydroxy-5α-cholest-7-en-6-one	30	1	3.3	3	10.0	5	16.7	9	30.0
7b. (24R)-2β,3β-Diacetoxy-5-hydroxy-5α-stigmast-7-en-6-one	30	0	0	1	3.3	3	10.0	4	13.3
8. 3β-Acetoxy-2β,5-dihydroxy-5α-cholest-7-en-6-one	30	0	0	0	0	3	10.0	3	10.0
9. 2α,3α-Diacetoxy-5-hydroxy-5α-cholest-7-en-6-one	30	0	0	2	6.7	8	26.7	10	33.3
10. (24R)-2α,5-Epoxy-3α-acetoxy-5α-stigmast-7-en-6-one	30	0	0	0	0	4	13.3	4	13.3
11. 20-Hydroxyecdysone	30	2	6.7	4	13.3	10	33.3	16	53.3
Control	29	0	0	1	3.4	2	6.9	3	10.3



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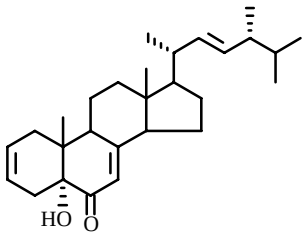

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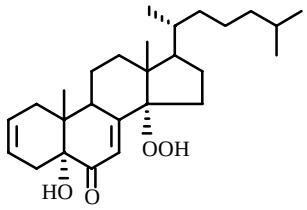

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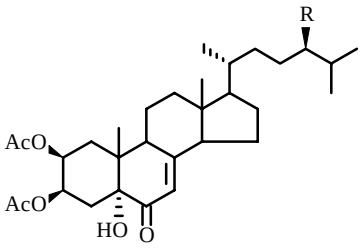

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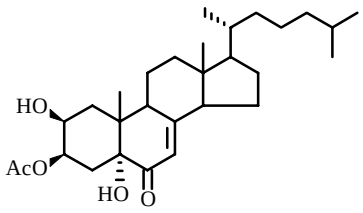

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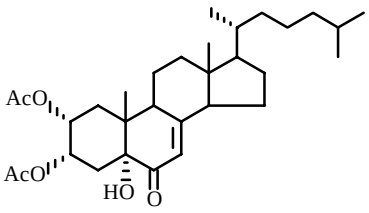

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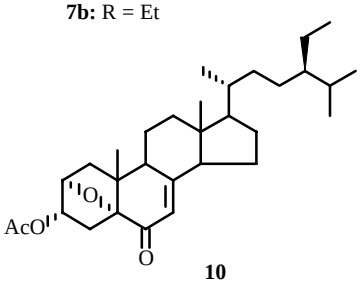

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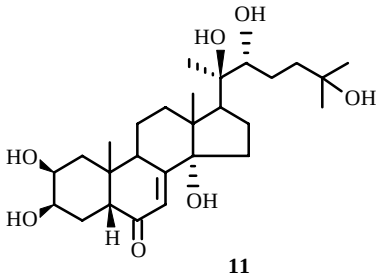

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Furthermore, Table 1 shows that cholestane steroids under otherwise equal conditions are more active than analogous stigmastane derivatives. Comparison of the activities of **7a** and **9** shows that the stereochemistry of the *cis*-2,3-diacetoxy moiety in this steroid series has no particular significance for insecticidal activity. However, replacing one acetoxyl on C-2 or C-3 by hydroxyl, as occurs for 2β-hydroxy-3β-acetoxy derivative **8**, significantly reduces the insecticidal activity. The analogous effect

occurs if the hydroxyls on C-2 and C-5 are combined into a tetrahydrofuran ring, like for **10**. Comparison of the activities of stigmastane derivatives **2** and **7b** indicates that a 3β-Cl is approximately equivalent to a 2,3-diacetoxy moiety. However, an unexpected conclusion arises if the insecticidal activity of 3β-chlorostigmastanes **1b** and **2** are compared. Introducing into 5α-hydroxy-6-ketosteroids an additional Δ^7-bond significantly decreases (at least two times) the insecticidal activity. Naturally such a trend cannot be predicted beforehand without performing special experiments. It is even more unexpected that the Δ^7-bond is a necessary structural feature of natural ecdysteroids. Natural ecdysteroids without a Δ^7-bond, for example, cheilantones A and B, are known to be inactive as insect molting hormones [9]. Our results unambiguously indicate that no strong correlation exists between the biological activity of ecdysteroids or their synthetic analogs as insect hormones and insecticides. This makes it promising to search for active insecticides among very simple sterol derivatives with only some structural elements characteristic of natural ecdysteroids.

EXPERIMENTAL

The studied steroids **1**-**10** were synthesized as described earlier [2-6]. Working solutions were prepared by placing steroid (1 mg) in a volumetric tube, adding acetone or ethanol (0.5 mL), and adjusting the total volume to 10 mL with distilled water containing surfactant OP-10 (1 drop per 1 L water).

Larvae of colorado beetle (*Leptinotarsa decemlineata* Say.) were obtained from egg sacs collected in the field. Two-day larvae were placed on potato leaves in Petri dishes and treated by spraying. Each dish contained 10 larvae. Experiments were repeated three times. The control was distilled water containing acetone or ethanol (0.5 mL/10 mL water) and surfactant OP-10 (1 drop per 1 L water). Each Petri dish received 0.5 mL of working solution. The concentration of the studied compounds was 0.01%.

Larvae were fed treated feed for one day. After one day, these potato leaves were replaced by fresh untreated ones. Then, these were replaced by fresh ones as necessary. Mortality of larvae was calculated on the second, third, and fifth day after treatment.

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