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STRUCTURE-ACTIVITY RELATIONSHIP OF STEROIDAL INSECTICIDES. VIII. CHOLESTANE AND STIGMASTANE ECDYSTEROIDS AND THEIR STRUCTURAL ANALOGS

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Structural analogs of ecdysteroids were screened for insecticidal activity for larvae of the Colorado beetle Leptinotarsa decemlineata *using a contact-feed method. High activity was found for compounds **3**, **6b**, **7a**, and **9a**.*

Key words: insecticidal activity, ecdysteroids, structural analogs.

Several hormones control the most important metabolic processes of insects [1, 2]. In particular, these include ecdysteroids, which induce insect molting and metamorphosis at exceedingly low concentrations. Several insects are sensitive to the action of exogenic ecdysteroids. Addition of these compounds to food causes anomalous development of insects that have not adapted to them and leads to their death. The occurrence of such biological action enables ecdysteroids to be viewed as potential sources of controlling populations of agricultural insect pests.

We have previously [3-7] synthesized a series of compounds that are structural analogs of some natural ecdysteroids, in particular, the basic representative 20-hydroxyecdysone **1**. The present article contains results from a study of the insecticidal activity of these structural analogs of ecdysteroids on the Colorado beetle *Leptinotarsa decemlineata* Say. (Coleoptera). This is a continuation of our previous work [8-14] on the insecticidal activity of natural ecdysteroid insect hormones and their synthetic structural analogs. Our main goal was to identify the most active insecticides among the synthesized steroids **2-11**.

The toxicity of **2-11** was investigated using second-growth Colorado beetle larvae obtained from egg sacs collected under natural conditions in June 2002 and grown in the laboratory using the usual feed, potato leaves. Larvae and their feed were sprayed with 0.01% suspensions of the tested compounds in water containing the surfactant OP-10. Larvae were fed during the first day feed treated with the tested compounds. Then, the potato leaves were replaced with fresh ones. The number of dead larvae was counted after one, three, and five days. The standard was 20-hydroxyecdysone (**1**), as before [9-14]. This compound exhibited the greatest insecticidal activity for the larvae [8]. Then, the insecticidal activities of the most active compounds were studied at a concentration of 0.001%. Table 1 lists the results for Colorado beetle larvae.

Table 1 shows that the magnitude of the insecticidal activity of **1** should be examined. At a concentration of 0.01%, it caused 86.7% of treated insects to perish. The toxicity of **1** is more noticeable compared with the same property that was determined previously in 2001 for Colorado beetle larvae [8-14]. Such a difference can be understood if it is considered that we carried out the work in all instances with insects taken from the natural population. Apparently the resistance of the Colorado beetle to the action of **1** and related compounds under actual conditions depends on several factors, for example, meteorological conditions, which varied drastically in 2001 and 2002.

The ecdysteroids should have *cis*-A/B fusion and contain a sterol side chain with several hydroxy groups, e.g., $2\beta,3\beta$-diol and 14α-hydroxy-Δ^7-6-keto groups, in order to exhibit high insect-molting hormonal activity [5]. It can be seen that the synthesized steroids **2-11** have these features in their molecules. Therefore, we think that the structural similarity of **2-11** to the natural ecdysteroids may be a factor in their high insecticidal activity. Table 1 shows that not nearly all the studied compounds were toxic for the Colorado beetle. In particular, 22,25-dideoxyecdysone (**5**) exhibited no insecticidal activity.

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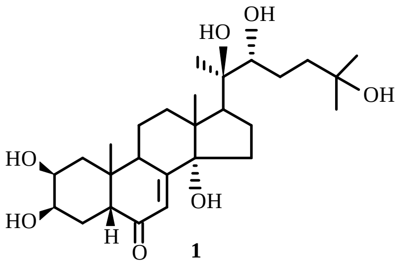
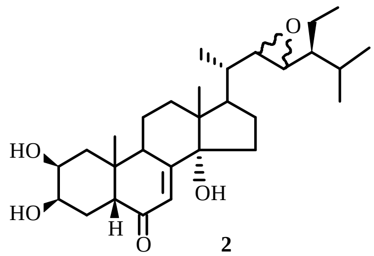
However, this compound was very active as an insect-molting hormone [15].



1



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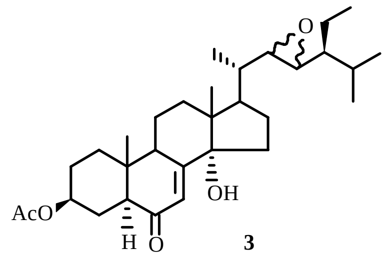
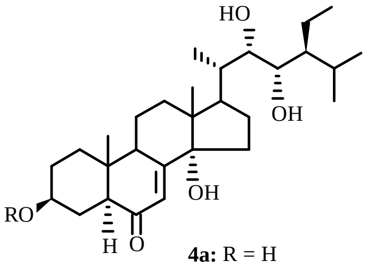
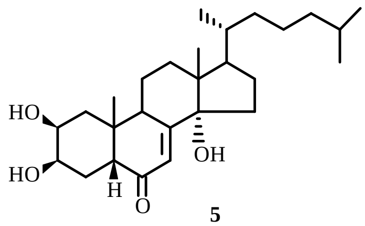


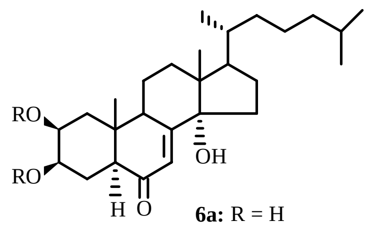
3



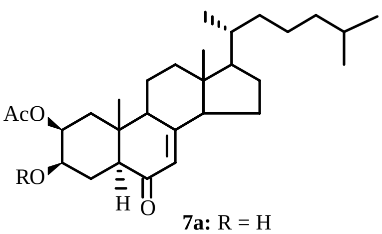
4a: R = H
b: R= Ac



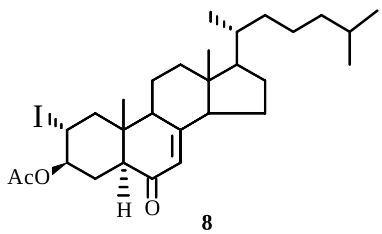
5



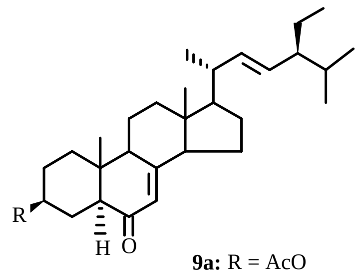
6a: R = H
b: R = Ac



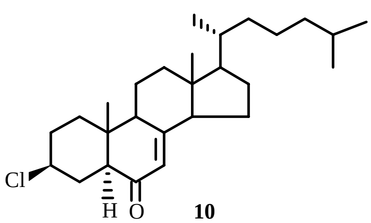
7a: R = H
b: R = Ac



8



9a: R = AcO
b: R = Cl



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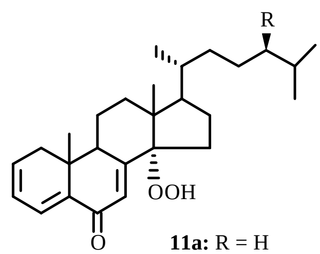

11a: R = H
b: R = Et

Compounds **3**, **6b**, **7a**, and **9a** showed the greatest toxicity for Colorado beetle larvae. These ecdysteroid analogs at a concentration of 0.01% were similar to **1** in insecticidal activity. However, their activity decreased significantly compared with **1** when the concentration was reduced an order of magnitude to 0.001%. These compounds have the Δ^7-6-keto group and *trans*-A/B fusion as common structural fragments. Their molecules also contain other functional groups typical of natural ecdysteroids. In our opinion, the fact that one or more hydroxy groups are present not as free groups but protected as acetates is important for the occurrence of insecticidal activity. The literature indicates [15] that this apparently leads to a significant decrease of hormonal activity and an increase of insecticidal activity owing to a slower metabolism of these compounds in the insect.

Of the four most active ecdysteroid analogs, **6b** and **7a** belong to the cholestane series. These compounds typically have the maximal toxicity even during the first day after administration. However, **3** and **9a**, which belong to the stigmastane series, have a slower insecticidal activity. Their maximal toxicity for Colorado beetle larvae is observed three days after administration.

Thus, these compounds indicate that the insecticidal activity of the synthesized ecdysteroid analogs is highly selective. This provides hope that specific insect-growth and -development regulators will be found among such compounds.

TABLE 1. Toxicity of Steroids **1-11** for Colorado Beetle Larvae

Compound	Concent-ration, %	No. of larvae	Larvae mortality after							
			1 day		3 days		5 days		Total	
			number	%	number	%	number	%	number	%
1. 20-Hydroxyecdysone	0.01	30	21	70.0	4	13.3	1	3.3	26	86.7
	0.001	30	8	26.7	4	13.3	4	13.3	16	53.3
2. (22*RS*,23*RS*,24*S*)-2β,3β,14α-Trihydroxy-22,23-epoxy-5α-stigmast-7-en-6-one	0.01	29	3	10	0	0	0	0	3	10
3. (22*RS*,23*RS*,24*S*)-3β-Acetoxy-14α-hydroxy-22,23-epoxy-5α-stigmasta-7,22-dien-6-one	0.01	30	0	0	18	60.0	5	16.7	23	76.7
	0.001	28	1	3.6	7	25.0	0	0	8	28.6
4a. (22*S*,23*S*,24*S*)-3β-Acetoxy-22,23-dihydroxy-5α-stigmast-7-en-6-one	0.01	30	0	0	5	16.7	0	0	5	16.7
4b. (22*S*,23*S*,24*S*)-3β,14α,22,23-Tetrahydroxy-5α-stigmast-7-en-6-one	0.01	30	3	10.0	4	13.3	0	0	7	23.3
5. 22,25-Dideoxyecdysone	0.01	30	0	0	0	0	0	0	0	0
6a. 5α-22,25-Dideoxyecdysone	0.01	30	0	0	0	0	0	0	0	0
6b. 2β,3β-Diacetoxy-14α-hydroxy-5α-cholest-7-en-6-one	0.01	28	19	67.9	6	21.4	0	0	25	89.3
	0.001	30	0	0	2	6.7	1	3.3	3	10.0
7a. 2β-Acetoxy-3β-hydroxy-5α-cholest-7-en-6-one	0.01	30	24	80.0	0	0	1	3.3	25	83.3
	0.001	30	0	0	1	3.3	1	3.3	2	6.7
7b. 2β,3β-Diacetoxy-5α-cholest-7-en-6-one	0.01	30	1	3.3	0	0	0	0	1	3.3
8. 2α-Iodo-3β-acetoxy-5α-cholest-7-en-6-one	0.01	30	1	3.3	0	0	0	0	1	3.3
9a. (22*E*,24*S*)-3β-Acetoxy-5α-stigmasta-7,22-dien-6-one	0.01	33	0	0	24	72.4	1	3.0	25	75.7
	0.001	32	3	9.4	3	9.4	3	9.4	9	28.1
9b. (22*E*,24*S*)-3β-Chloro-5α-stigmasta-7,22-dien-6-one	0.01	30	0	0	1	3.3	1	3.3	2	6.7
10. 3β-Chloro-5α-cholest-7-en-6-one	0.01	32	2	6.3	0	0	0	0	2	6.3
11a. 14α-Hydroperoxycholesta-2,4,7-trien-6-one	0.01	30	2	6.7	0	0	0	0	2	6.7
11b. (24*R*)-14α-Hydroperoxystigmasta-2,4,7-trien-6-one	0.01	30	0	0	0	0	0	0	0	0
Control		30	0	0	0	0	0	0	0	0

EXPERIMENTAL

The syntheses of **2-11** have been reported [3-7].

The experimental conditions for determining the insecticidal activity of **1-11** for second-growth Colorado beetle larvae have been published [8].

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