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STRUCTURE—ACTIVITY RELATIONSHIP OF INSECTICIDAL STEROIDS.* III. $\Delta^{4,7}$-6-KETOSTEROIDS

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*The toxicity of steroids **1**-**11** for colorado beetle (*Leptinotarsa decemlineata *Say.) larvae was studied by contact-intestinal treatment. Active insect-growth regulators were found among the studied compounds.*

Key words: $\Delta^{4,7}$-6-ketosteroids, insecticidal activity.

We previously synthesized steroids **1-11** [1-3]. The majority of these compounds have a conjugated 4,7-dien-6-ketone as a common structural fragment. The remainder are closely related structurally. Certain of these compounds, namely $\Delta^{4,7}$-3,6-diketone **1a** and its 9α,14α-dihydroxy derivative **1b**, are identical to steroids that have been found in natural sources [6-11]. The remainder are structural analogs of natural ecdysteroids such as 4-dehydro-ecdysterone [4] or diaulusterols A and B [5]. The structural similarity of **1-11** to molting hormones and insect metamorphosis ecdysteroids makes these compounds interesting. We have found that certain ecdysteroids are highly toxic to insects that are agricultural pests [12]. Therefore, it seemed interesting to investigate the insecticidal activity of the synthesized steroids. We studied the toxicity of **1-11** for colorado beetle (*Leptinotarsa decemlineata* Say., Coleoptera) larvae, the most harmful potato pest in Belarus. This article is a continuation of previous research on the insecticidal activity for this insect of certain phytoecdysteroids [13] and 5α-hydroxy-Δ^7-ketosteroids [14].

The insecticidal activity of the $\Delta^{4,7}$-ketosteroids was determined by a contact-intestinal method for second-growth colorado beetle larvae. This method is most widely used in practice to combat this pest. The insects and their natural food, potato leaves, were sprayed with aqueous suspensions (0.01%) of the studied compounds containing surfactant OP-10.

Treated food was supplied for one day. Then, natural food without steroids was given. We used natural phytoecdysteroid 20-hydroxyecdysone (**12**) as a control. This has previously shown the greatest activity in this test [13]. Control larvae were treated analogously except that **1-12** were not included in their diet. The mortality of the larvae was calculated on the second, third, and fifth days after administration. Table 1 contains results for the effect of **1-12** on colorado beetle larvae. It was found that steroids ingested with food were toxic for a prolonged period and were lethal for larvae even five days after administration. Taking into account the dynamics of larval death, **1-11**, like the phytoecdysteroids that we studied earlier [13] and 5α-hydroxy-Δ^7-ketosteroids, most probably act through the same mechanism and can be considered insect-growth regulators.

The test results indicate that rather active insect-growth regulators are found among **1-11**. Thus, $\Delta^{4,7}$-3,6-diketosteroid **1a**, 2β,3β-diacetoxy-$\Delta^{4,7}$-6-ketosteroid **4b**, and 2β,3β,14α-trihydroxy-$\Delta^{4,7}$-6-ketosteroid **5** are slightly less toxic for colorado beetle larvae than the standard (**12**).

Several preliminary conclusions can be made about the importance of certain functional groups in the studied compounds for their high insecticidal activity. Thus, **1-11** were synthesized chemically from the corresponding sterols ergosterol, β-sitosterol, or cholesterol. The chemical transformations involved only the cyclic parts of the molecules. Therefore, they have the intact side chains of the corresponding starting sterols. In certain instances the structure of the side chains is very

*For Nos. I and II, see *Khim. Prir. Soedin.*, 460 and 462 (2001).

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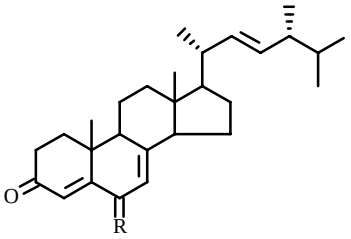
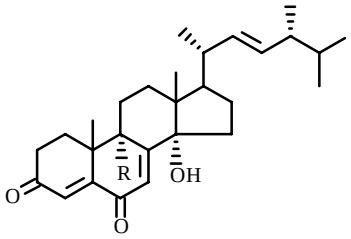
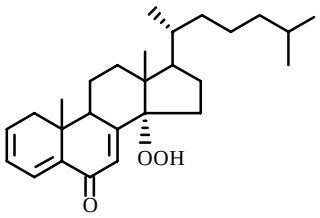
important for the insecticidal activity (Table 1). For example, **4a** and **4b** have the identical structure in the cyclic part of the molecule but differ in the side chains. Stigmastane derivative **4b** is much more active than cholestane derivative **4a**. On the other hand, 14α-hydroperoxy-$\Delta^{4,7}$-6-ketosteroids **6a** and **6b** exhibit identical overall toxicity for colorado beetle larvae although they are cholestane and stigmastane steroids, respectively. Nevertheless, the dynamics of insect death caused by these compounds differ slightly.



1a: R = O
1b: R = H_2



2a: R = H
2b: R = OH



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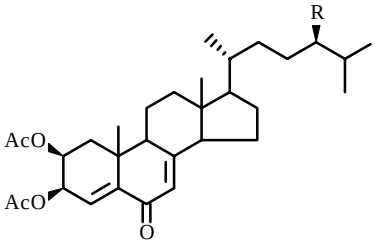
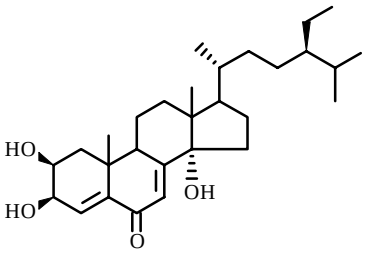


4a: R = H
4b: R = Et



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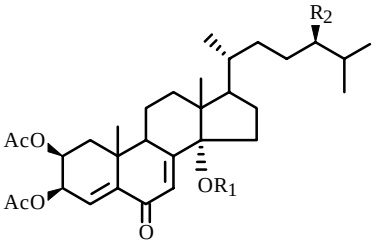
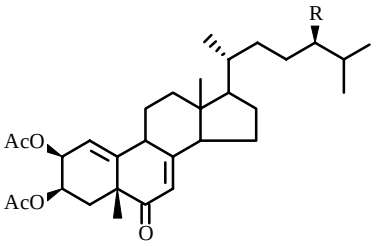
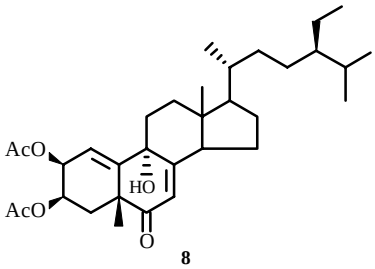


6a: R_1 = OH, R_2 = H
6b: R_1 = OH, R_2 = Et
6c: R_1 = Ac, R_2 = Et



7a: R = H
7b: R = Et



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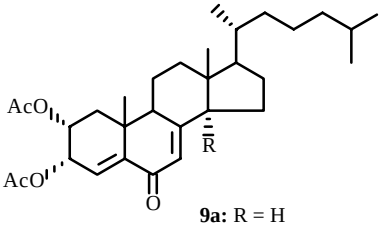
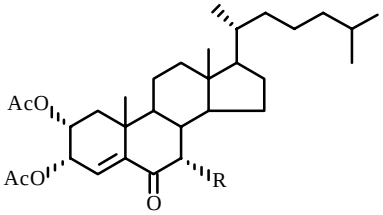
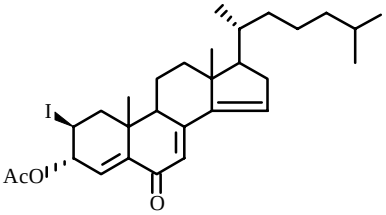


9a: R = H
9b: R = OOH



10a: R = H
10b: R = Br



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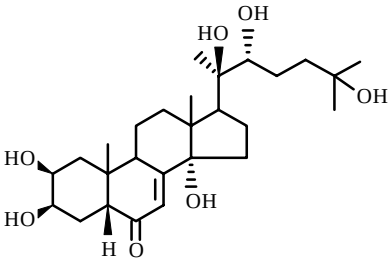

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A comparison of the activity of ergostanes $\Delta^{4,7}$-3,6-diketosteroid **1a** and $\Delta^{4,7}$-3-ketosteroid **1b** shows that the latter lacks a 6-ketone and is significantly less toxic. Comparison of the activities of **1a**, **2a**, and **2b** shows that introducing the additional 14α-hydroxy or 9α,14α-diol into **1a** has an analogous effect.

Compounds **7a-b** and **8** were isolated as side products during preparation of the $\Delta^{4,7}$-6-ketosteroids. These compounds are products of Westphalen—Lettre rearrangement, which is accompanied by migration of the angular methyl from C-10 to C-5. According to Table 1, **7a-b** and **8** are inactive as insecticides.

TABLE 1. Toxicity of **1-12** for Colorado Beetle Larvae

Compound		Larval death after days							
		1		3		5		Total	
		number	%	number	%	number	%	number	%
1a	(22E,24R)-Ergosta-4,7,22-trien-3,6-dione	0	0	3	10.0	8	26.7	11	36.7
1b	(22E,24R)-Ergosta-4,7,22-trien-3-one	0	0	1	3.3	5	16.7	6	20.0
2a	(22E,24R)-14α-Hydroxyergosta-4,7,22-trien-3,6-dione	0	0	0	0	1	3.3	1	3.3
2b	(22E,24R)-9α,14α-Dihydroxyergosta-4,7,22-trien-3,6-dione	0	0	0	0	3	10.0	3	10.0
3	14α-Hydroperoxycholesta-2,4,7-trien-6-one	0	0	2	6.7	2	6.7	4	13.3
4a	2β,3β-Diacetoxycholesta-4,7-dien-6-one	0	0	0	0	1	3.3	1	3.3
4b	(24R)-2β,3β-Diacetoxystigmasta-4,7-dien-6-one	0	0	3	10.0	10	33.3	13	43.3
5	(24R)-2β,3β,14α-Trihydroxystigmasta-4,7-dien-6-one	0	0	4	13.3	8	26.7	12	40.0
6a	2β,3β-Diacetoxy-14α-hydroperoxycholesta-4,7-dien-6-one	1	3.3	1	3.3	5	16.7	7	23.3
6b	(24R)-2β,3β-Diacetoxy-14α-hydroperoxystigmasta-4,7-dien-6-one	1	3.3	4	13.3	2	6.7	7	23.3
6c	(24R)-2β,3β,14α-Triacetoxystigmasta-4,7-dien-6-one	0	0	3	10	8	26.7	11	36.7
7a	2β,3β-Diacetoxy-5-methyl-19-nor-5β-cholesta-1(10),7-dien-6-one	0	0	0	0	0	0	0	0
7b	(24R)-2β,3β-Diacetoxy-5-methyl-19-nor-5β-stigmasta-1(10), 7-dien-6-one	0	0	0	0	5	16.7	5	16.7
8	(24R)-2β,3β-Diacetoxy-9α-hydroxy-5-methyl-19-nor-5β-stigmasta-1(10),7-dien-6-one	0	0	0	0	0	0	0	0
9a	2α,3α-Diacetoxycholesta-4,7-dien-6-one	0	0	0	0	0	0	0	0
9b	2α,3α-Diacetoxy-14α-hydroperoxycholesta-4,7-dien-6-one	0	0	1	3.3	4	13.3	5	16.7
10a	2α,3α-Diacetoxycholest-4-en-6-one	0	0	1	3.3	2	6.7	3	10.0
10b	2α,3α-Diacetoxy-7α-bromocholest-4-en-6-one	0	0	2	6.7	8	26.7	10	33.3
11	2β-Iodo-3α-acetoxycholesta-4,7,14-trien-6-one	0	0	0	0	0	0	0	0
12	20-Hydroxyecdysone	2	6.7	4	13.3	10	33.3	16	53.3
	Control	0	0	1	3.4	2	6.9	3	10.3

Number of larvae in experiment, 30; in control, 29.

Therefore, it can be concluded that the toxicity of ecdysteroids and their analogs to insects is sensitive to very small structural changes, especially those connected with rearrangement of the carbon skeleton.

EXPERIMENTAL

The syntheses of **1-11** have been reported [1-3]. Detailed conditions for determining their insecticidal activity for second-growth colorado beetle larvae have been published [14].

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REFERENCES

1. N. V. Kovganko and S. N. Sokolov, *Khim. Prir. Soedin.*, 354 (1999).

2. N. V. Kovganko and S. N. Sokolov, *Khim. Prir. Soedin.*, 244 (2000).
3. N. V. Kovganko and S. N. Sokolov, *Khim. Prir. Soedin.*, 388 (2001).
4. P. A. Searle and T. F. Molinski, *J. Nat. Prod.*, **58**, 264 (1995).
5. D. E. Williams, S. W. Ayer, and R. J. Andersen, *Can. J. Chem.*, **64**, 1527 (1986).
6. A. Malorni, L. Minale, and R. Riccio, *Nouv. J. Chim.*, **2**, 351 (1979).
7. M. Hirotani, I. Asaka, C. Ino, T. Furuya, and M. Shiro, *Phytochemistry*, **26**, 2797 (1987).
8. T. Nishitoba, H. Sato, K. Oda, and S. Sakamura, *Agric. Biol. Chem.*, **52**, 211 (1988).
9. N. Kawahara, S. Sekita, and M. Satake, *Phytochemistry*, **37**, 213 (1994).
10. N. Kawahara, S. Sekita, and M. Satake, *Phytochemistry*, **38**, 947 (1995).
11. N. V. Kovganko, *Khim. Prir. Soedin.*, 691 (1999).
12. A. A. Akhrem and N. V. Kovganko, *Ecdysteroids: Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1989).
13. R. M. Zolotarʹ, A. I. Bykhovets, and N. V. Kovganko, *Khim. Prir. Soedin.*, 460 (2001).
14. R. M. Zolotarʹ, A. I. Bykhovets, S. N. Sokolov, and N. V. Kovganko, *Khim. Prir. Soedin.*, 462 (2001).