Chemistry of Natural Compounds, Vol. 38, No. 2, 2002

STRUCTURE-ACTIVITY RELATIONSHIP FOR INSECTICIDAL STEROIDS. VI. 5,6-DISUBSTITUTED β-SITOSTEROLS

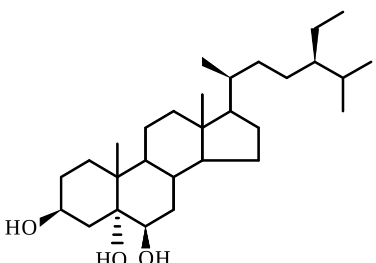
R. M. Zolotar′, A. I. Bykhovets, Zh. N. Kashkan, Yu. G. Chernov, and N. V. Kovganko

UDC 547.92:632.936:634.1

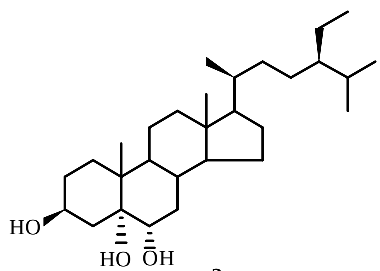
*The toxicity of steroids **1-14** for Colorado beetle (*Leptinotarsa decemlineata *Say.) larvae was studied by a contact-intestinal method. The most active are* 3β,5α-*dihydroxy-6-ketone* ***4a*** *and* 5α-*hydroxy-3,6-diketone* ***8a***.

Key words: 5,6-disubstituted β-sitosterols, insecticidal activity.

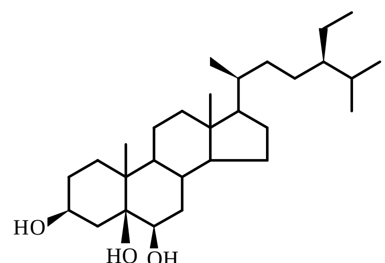
We previously synthesized several steroids containing hydroxy-, keto-, or hydroxyimino groups on C-5 and C-6 by reactions at the 5(6)-double bond of β-sitosterol [1-6]. Certain of the synthesized steroids are structurally identical to corresponding natural compounds isolated from various sources. These include 3β,5α,6β-triol **1**, 3β,5α-dihydroxy-6-ketone **4a**, 3,6-diketone **10**, Δ^4-3,6-diketone **11a**, and its 6-monooxime **11b**. This article reports results from a study of the insecticidal activity of these and structurally related compounds for Colorado beetle (*Leptinotarsa decemlineata* Say., Coleoptera) larvae. Our principal goal was to find the most active insecticides among **1-14**. This is a continuation of research that we previously started [7-11] on the insecticidal activity of natural ecdysteroid insect hormones and structurally analogous synthetic compounds.



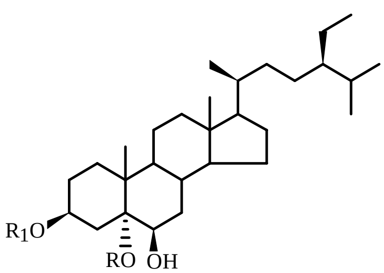
1



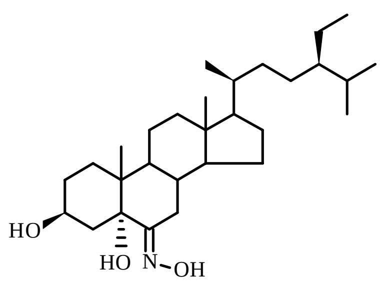
2



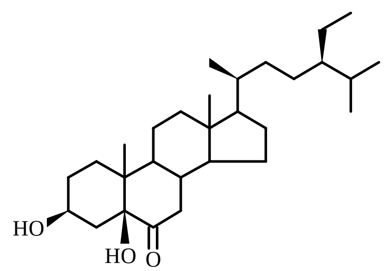
3



4a: R = R_1 = H
b: R = H, R_1 = Ac
c: R = R_1 = Ac

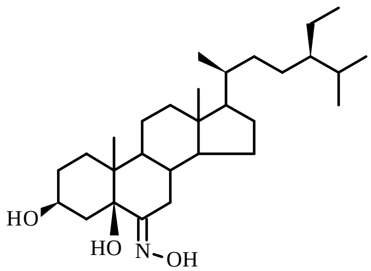


5

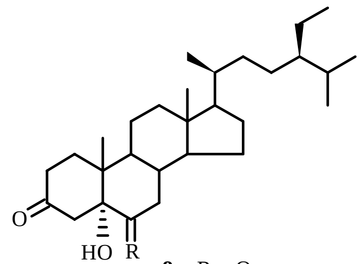


6

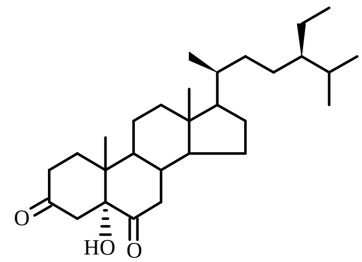
Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Belarus, Minsk, ul. Akad. Kuprevicha, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 140-142, March-April, 2002. Original article submitted March 28, 2002.



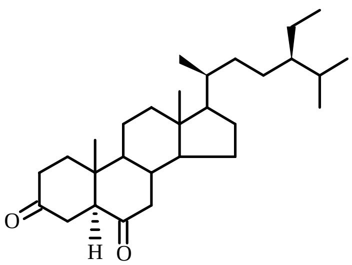
7



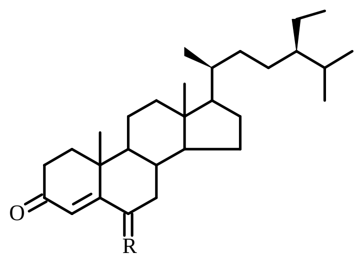
8a: R = O
b: R = NOH



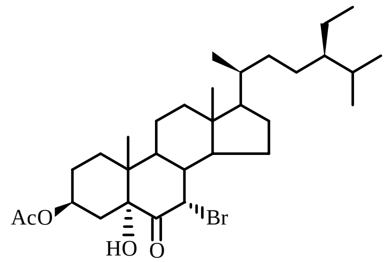
9



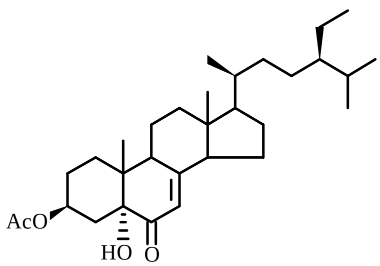
10



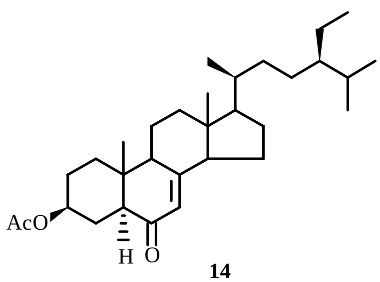
11a: R = O
b: R = NOH



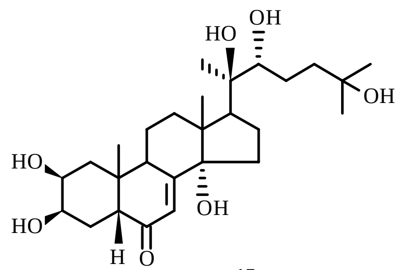
12



13



14



15

We selected Colorado beetle as the test specimens, like in previous work [7-11], because this insect is the most harmful potato pest in Belarus. The insecticidal activity of **1-14** was determined using a contact-intestinal method of administration to second-growth larvae. This method is used to combat this pest under field conditions. Colorado beetle larvae are fed their usual food, potato leaves, sprayed with suspensions (0.01%) of the studied compounds in water with surfactant OP-10. Larvae are fed treated food for one day. Then, they receive natural food without steroids. The standard was 20-hydroxyecdysone (**15**), as noted previously [7], the most active of the natural phytoecdysteroids in this test.

Control larvae were treated analogously except that their diet did not contain steroids **1-15**. Mortality of the larvae was calculated on the second, third, and fifth days after administration. Table 1 contains results for **1-15** on Colorado beetle larvae. The overall toxic effect of the studied compounds on larvae can be determined. The mortality dynamics of the insects caused by these compounds can be evaluated.

It has been found that the studied steroids differ markedly in the effect on the larvae. They include active insecticides and inactive compounds. The most active are 3*β*,5*α*-dihydroxy-6-ketone **4a** and 5*α*-hydroxy-3,6-diketone **8a**. These compounds are practically as toxic as 20-hydroxyecdysone. Steroids **8b**, **9**, and **11a**, in turn, also exhibit a noticeable insecticidal effect. However, it is less significant than that for **4a** and **8a**. Still less active were **2**, **4b**, **5**, **7**, and **11b**. The remaining studied compounds possess no noticeable insecticidal activity for Colorado beetle larvae.

The data in Table 1 lead to several conclusions about the effect of certain functional groups in the studied steroids on the magnitude of the insecticidal effect. First, it should be noticed that 3*β*,5*α*,6*β*-triol **1** is inactive. Triols **2** and **3**, which are isomeric with it, possess insignificant insecticidal activity. The 6-ketosteroids are structurally similar to these compounds and are highly active. Therefore, replacing the 6-hydroxy by 6-ketone leads to a significant increase of the insecticidal activity of these compounds. Comparing the data for 6-ketosteroids and their corresponding oximes shows that the oximes are less active. A comparison of the toxicity of 3*β*,5*α*-dihydroxy-6-ketone **4a**, 3*β*,5*β*-dihydroxy-6-ketone **6**, 3,6-diketone **10**, and Δ^4-3,6-diketone **11a** leads to the conclusion that the 5*α*-hydroxyl is important for imparting significant insecticidal activity in this series of steroids. On the other hand, a comparison of the activities for **4a-c**, **12**, and **13** shows that the hydroxyls on C-3 and C-5 should be free and not acetylated in order to elicit high activity.

TABLE 1. Toxicity of **1-15** for Colorado Beetle Larvae

Compound	Larvae mortality after days							
	1		3		5		Total	
	number	%	number	%	number	%	number	%
1. (24R)-Stigmastan-3β,5α,6β-triol	0	0	0	0	0	0	0	0
2. (24R)-Stigmastan-3β,5α,6α-triol	1	3.3	1	3.3	0	0	2	6.7
3. (24R)-Stigmastan-3β,5β,6β-triol	0	0	0	0	1	3.3	1	3.3
4a. (24R)-3β,5α-Dihydroxystigmastan-6-one	0	0	2	6.7	14	46.7	16	53.3
4b. (24R)-3β-Acetoxy-5α-hydroxystigmastan-6-one	0	0	0	0	2	6.7	2	6.7
4c. (24R)-3β,5α-Diacetoxystigmastan-6-one	0	0	0	0	0	0	0	0
5. (24R)-3β,5α-Dihydroxystigmastan-6-one oxime	0	0	0	0	2	6.7	2	6.7
6. (24R)-3β,5β-Dihydroxystigmastan-6-one	0	0	0	0	2	6.7	2	6.7
7. (24R)-3β,5β-Dihydroxystigmastan-6-one oxime	0	0	0	0	2	6.7	2	6.7
8a. (24R)-5α-Hydroxystigmastan-3,6-dione	0	0	5	16.7	10	33.3	15	50.0
8b. (24R)-5α-Hydroxystigmastan-3,6-dione 6-oxime	0	0	1	3.3	2	6.7	3	10.0
9. (24R)-5β-Hydroxystigmastan-3,6-dione	0	0	1	3.3	2	6.7	3	10.0
10. (24R)-5α-Stigmastan-3,6-dione	0	0	0	0	0	0	0	0
11a. (24R)-Stigmast-4-en-3,6-dione	0	0	0	0	3	10.0	3	10.0
11b. (24R)-Stigmast-4-en-3,6-dione 6-oxime	0	0	0	0	2	6.7	2	6.7
12. (24R)-3β-Acetoxy-5α-hydroxy-7α-bromostigmastan-6-one	0	0	0	0	0	0	0	0
13. (24R)-3β-Acetoxy-5α-hydroxystigmast-7-en-6-one	0	0	0	0	0	0	0	0
14. (24R)-3β-Acetoxy-5α-stigmastan-6-one	0	0	0	0	0	0	0	0
15. 20-Hydroxyecdysone	2	6.7	4	13.3	10	33.3	16	53.3
Control	0	0	0	0	0	0	0	0

Number of larvae, 30.

Table 1 indicates that the studied compounds do not have an acute toxic effect on Colorado beetle larve, as is characteristic of neuro-active insecticides such as pyrethroids or neonicotinoids. Death does not come immediately from **1-15** but during a prolonged period. The mortality dynamics for **1-15** in general are the same as for administration of phytoecdysteroids [7] or structurally similar compounds [8-11]. This is probably due to the same mechanism of action for these compounds.

In conclusion, it should be noted that certain hormonal or antihormonal insecticides have the same prolonged lethal effects on insects. These include juvenoids, chitin biosynthesis inhibitors, or 1,2-diacylhydrazine ecdysteroid agonists.

EXPERIMENTAL

Melting points were determined on a Kofler block. ^{1}H NMR spectra in $CDCl_3$ were obtained on a Bruker AC-200 NMR spectrometer at working frequency 200 MHz. Chemical shifts are given relative to TMS internal standard.

The syntheses of **1**, **2**, and **4-14** were previously reported [1-6].

(24R)-Stigmastan-3β,5β,6β-triol 3. A mixture of **6** (0.50 g, obtained by the literature method [3]) and $NaBH_4$ (0.03 g) in methanol (20 mL) was stirred at room temperature for 30 min, treated with $NaBH_4$ (0.03 g), left for 30 min, evaporated in vacuum to half the volume, diluted with aqueous NaOH (5%, 50 mL), and extracted with ethylacetate (3×25 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness in vacuum. The solid was chromatographed over a silica-gel column with elution by a hexane:ethylacetate (3:1) mixture to give **3** (0.30 g, 60%), mp 147-149°C (dichloroethane).

^{1}H NMR spectrum (δ, ppm, J/Hz): 0.67 (3H, s, 18-Me), 0.82 (3H, d, J = 6.0, 26-Me), 0.83 (3H, d, J = 6.0, 27-Me), 0.85

(3H, t, J = 6.0, 29-Me), 0.91 (3H, d, J = 6.0, 21-Me), 1.08 (3H, s, 19-Me), 3.02 (3H, br.m, OH), 3.57 (1H, m, W/2 = 7, H-6α), 4.14 (1H, m, W/2 = 8, H-3α).

Details of experimental conditions for determining the insecticidal activity of **1-15** for second-growth Colorado beetle larvae have been published [8].

REFERENCES

1. A. A. Akhrem, Zh. N. Kashkan, and N. V. Kovganko, *Dokl. Akad. Nauk SSSR*, **305**, 618 (1989).
2. N. V. Kovganko and Zh. N. Kashkan, *Khim. Prir. Soedin.*, 771 (1990).
3. N. V. Kovganko and Zh. N. Kashkan, *Khim. Prir. Soedin.*, 718 (1993).
4. N. V. Kovganko and Yu. G. Chernov, *Khim. Prir. Soedin.*, 149 (2000).
5. N. V. Kovganko and Yu. G. Chernov, *Khim. Prir. Soedin.*, 218 (2001).
6. N. V. Kovganko and Yu. G. Chernov, *Khim. Prir. Soedin.*, 297 (2001).
7. R. M. Zolotar′, A. I. Bykhovets, and N. V. Kovganko, *Khim. Prir. Soedin.*, 460 (2001).
8. R. M. Zolotar′, A. I. Bykhovets, S. N. Sokolov, and N. V. Kovganko, *Khim. Prir. Soedin.*, 462 (2001).
9. R. M. Zolotar′, A. I. Bykhovets, S. N. Sokolov, and N. V. Kovganko, *Khim. Prir. Soedin.*, 54 (2002).
10. R. M. Zolotar′, A. I. Bykhovets, S. N. Sokolov, and N. V. Kovganko, *Khim. Prir. Soedin.*, 57 (2002).
11. R. M. Zolotar′, A. I. Bykhovets, S. K. Ananich, and N. V. Kovganko, *Khim. Prir. Soedin.*, 137 (2002).