STRUCTURE—ACTIVITY RELATIONSHIP OF INSECTICIDAL STEROIDS. IV. 3β-CHLOROSUBSTITUTED DERIVATIVES OF CHOLESTEROL AND β-SITOSTEROL

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*The toxicity of 3β-chlorosteroids **1-8** for colorado beetle (*Leptinotarsa decemlineata *Say.) larvae was studied by a contact-intestinal method. The most active insect growth and development regulators of the studied compounds are 3β-chlorocholest-5-ene (**1a**), 3β-chloro-5α,6β-diol 6-formate (**3b**), 3β-chloro-5α-hydroxy-6-ketone (**4a**), and 3β-chloro-7α-bromo-Δ^4-6-ketone (**8**).*

Key words: 3β-chloro derivatives of cholesterol and β-sitosterol, insecticidal activity.

In contrast with plants, higher animals, and man, insects cannot biosynthesize sterols and must obtain them from food [1, 2]. Sterols are necessary to insects primarily to facilitate structural organization of cell membranes and to biosynthesize molting hormones and metamorphosis ecdysteroids [3]. Such a critical dependence on food sources of sterols can be used to combat against dangerous agricultural pests. In particular, certain simple synthetic sterol derivatives are active insecticides for colorado beetle [3-7]. These compounds are prepared via chemical transformation of cholesterol or β-sitosterol by replacing the sterol 3β-hydroxy by chlorine with subsequent introduction of oxygen-containing functional groups in rings A and B of the corresponding 3β-chloro derivatives. We continued the search for active insecticides among **1-8** [8-10], which belong to this class of compounds.

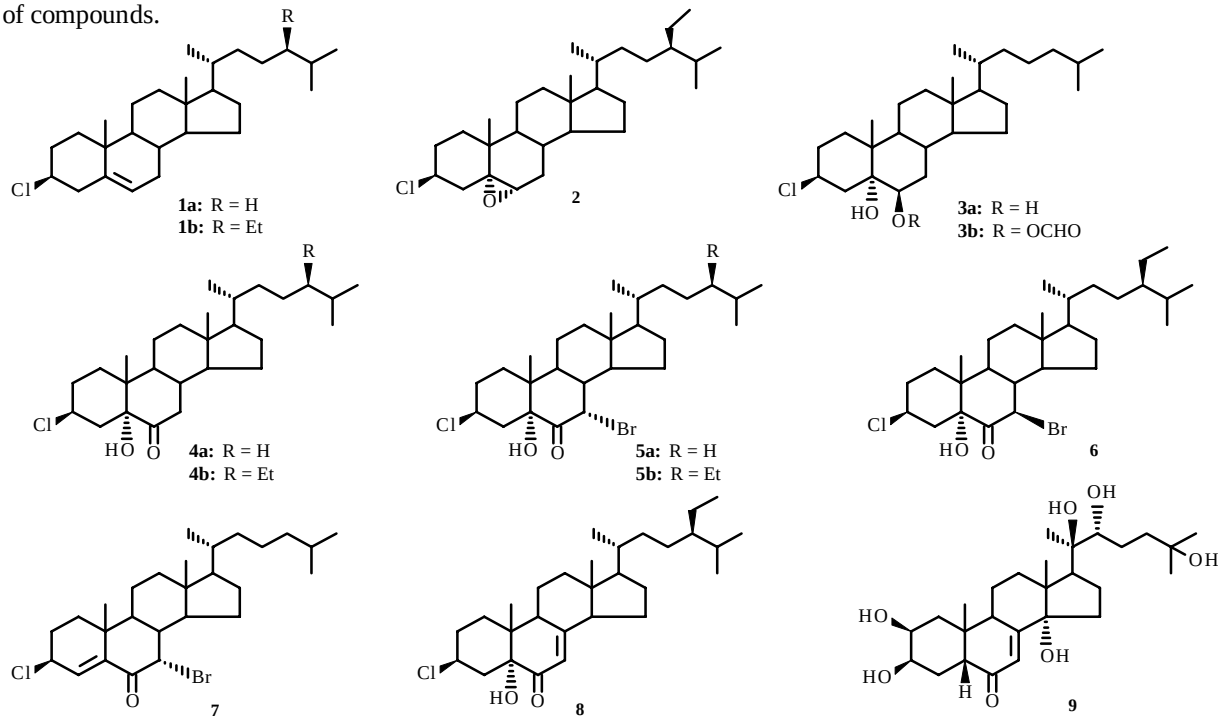

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TABLE 1. Toxicity of **1-9** for Colorado Beetle Larvae

Compound		Larval death after days							
		1		3		5		Total	
		number	%	number	%	number	%	number	%
1a	3β-Chlorocholest-5-ene	0	0	5	16.7	7	23.3	12	4.0
1b	(24R)-3β-Chlorostigmast-5-ene	0	0	0	0	4	13.3	4	13.3
2	(24R)-3β-Chloro-5,6α-epoxy-5α-stigmastane	0	0	2	6.7	5	16.7	7	23.3
3a	3β-Chloro-5α-cholestan-5,6β-diol	0	0	0	0	2	6.7	2	6.7
3b	3β-Chloro-5α-cholestan-5,6β-diol 6-formate	0	0	2	6.7	15	50.0	17	56.7
4a	3β-Chloro-5-hydroxy-5α-cholestan-6-one	1	3.3	2	6.7	10	33.3	13	43.3
4b	(24R)-3β-Chloro-5-hydroxy-5α-stigmastan-6-one	1	3.3	3	10.0	4	13.3	8	26.7
5a	3β-Chloro-7α-bromo-5-hydroxy-5α-cholestan-6-one	0	0	2	6.7	3	10.0	5	16.7
5b	(24R)-3β-Chloro-7α-bromo-5-hydroxy-5α-stigmastan-6-one	0	0	3	10.0	3	10.0	6	20.0
6	(24R)-3β-Chloro-7β-bromo-5-hydroxy-5α-stigmastan-6-one	0	0	0	0	6	20.0	6	20.0
7	3β-Chloro-7α-bromocholest-4-en-6-one	0	0	4	13.3	9	30.0	13	43.3
8	(24R)-3β-Chloro-5-hydroxy-5α-stigmast-7-en-6-one	0	0	1	3.3	3	10.0	4	13.3
9	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.

We used colorado beetle (*Leptinotarsa decemlineata* Say., Coleoptera) larvae, like in previous investigations [11-13]. They were chosen because they are very dangerous potato pests in Belarus.

The insecticidal activity of **1-8** was determined by a contact-intestinal method on second-growth colorado beetle larvae. This method is most widely used in practice to combat against these pests. 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 the natural phytoecdysteroid 20-hydroxyecdysone **9** as a control. This exhibits the greatest activity in this test [11]. Control larvae were treated analogously except that steroids **1-9** were not included in their diet. Larval mortality was calculated on the second, third, and fifth days after administration. Table 1 contains results for **1-9** on colorado beetle larvae.

It has been found that steroids absorbed with food have a cumulative effect, remain toxic for a prolonged period, and are fatal to larvae on the fifth day after administration. The dynamics of larval death due to 3β-chlorosteroids **1-8** are in general the same as for previously administered phytoecdysteroids [11] and their structural analogs [12, 13]. This is most probably caused by the structural similarities and identical mechanisms of action.

In general, the studied compounds differ markedly in biological activity. 3β-Chlorocholest-5-ene (**1a**), 3β-chloro-5α,6β-diol 6-formate (**3b**), 3β-chloro-5α-hydroxy-6-ketone (**4a**), and 3β-chloro-7α-bromo-Δ^4-6-ketone (**8**) are most toxic for colorado beetle larvae. Compound **3b** exhibits the same activity as 20-hydroxyecdysone **9**. Because 3β-chloro-5α,6β-diol (**3a**) is slightly active, the significant toxicity of **3b** must be due to the presence of the formyloxy group in it. Introducing this functionality into steroids is usually facile and can be used in the future to synthesize very active compounds in this series.

The most active of the studied compounds have the cholestane structure. Therefore, the activities of **1a** and **b** should be compared with those of **4a** and **b**. Compounds synthesized from cholesterol exhibit high insecticidal activity for colorado beetle larvae compared with analogous derivatives of β-sitosterol. Apparently this is due to the fact that β-sitosterol is the principal sterol of this insect [14]. Therefore, we presume that β-sitosterol derivatives are metabolized by colorado beetle larvae and rapidly detoxified.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded in KBr pellets on a UR-20 instrument

at 700-3600 cm^{-1}. ^1H NMR spectra in $CDCl_3$ solutions were obtained on a Bruker AC-200 NMR spectrometer at working frequency 200 MHz. Chemical shifts are given relative to TMS internal standard.

Syntheses of **1**-**3a** and **4**-**8** were previously described [1-3].

3β-Chloro-5α-cholestan-5α,6β-diol 6-Formate (3b). A solution of **1a** (15.72 g) in THF (350 mL) and formic acid (80 mL) was stirred and treated with hydrogen peroxide (40 mL, 30%). After 4.5 h the bath temperature was adjusted to 35-36°C. The solution was stirred for another 2 h and cooled. After 19 h the mixture was heated at 35°C for 1.5 h, evaporated in vacuum to two thirds the volume, treated with water (200 mL), and extracted with $CHCl_3$ (5×60 mL). The extracts were washed with water (50 mL) and $NaHCO_3$ solution (5%, 2×40 mL) and then water again (50 mL) and evaporated in vacuum. The solid was dissolved in acetone (250 mL), stirred, and treated with chromic acid (40 m L, 8 N). After 20 min the excess of oxidant was neutralized. Propan-2-ol (35 mL) was added. The reaction mixture was filtered through a layer of Al_2O_3. The filtrate was evaporated in vacuum. The solid was dissolved in ethylacetate (200 mL) and dried over $MgSO_4$. The desiccant was removed. The solvent was evaporated in vacuum. The solid was chromatographed over a column of silica gel with elution by cyclohexane:dichloroethane of increasing polarity (from 5:1 to pure dichloroethane).

Fraction 1: **3b** (2.74 g, 15%), mp 155-158°C (ethanol—ethylacetate). IR spectrum (ν, cm^{-1}): 3460 (OH), 1715 (C=O), 1220 (C–O). ^1H NMR spectrum (δ, ppm, J/Hz): 0.67 (3H, s, 18-Me), 0.86 (6H, d, J = 6.5, 26-Me, 27-Me), 0.90 (3H, d, J = 6, 21-Me), 1.17 (3H, s, 19-Me), 4.32 (1H, m, W/2 = 24, H-3α), 4.81 (1H, m, W/2 = 6, H-6α), 8.10 (1H, s, 6β-HCOO).

Fraction 2: **4a** (5.01 g, 30%), mp 182-183°C (ethanol—ethylacetate), lit. [15] mp 180-186°C (acetone-petroleum ether). IR spectrum (ν, cm^{-1}): 1715 (C=O). ^1H NMR spectrum (δ, ppm, J/Hz): 0.65 (3H, s, 18-Me), 0.83 (3H, s, 19-Me), 0.86 (6H, d, J = 6.5, 26-Me, 27-Me), 0.91 (3H, d, J = 6,21-Me), 2.70 (1H, t, J = 12.5, H-7α), 4.21 (1H, m, W/2 = 21, H-3α).

Fraction 3: **3a** (2.05 g, 12%), mp 126-128°C (ethanol—ethylacetate), lit. [15] mp 99-102°C (hexane). IR spectrum (KBr, ν, cm^{-1}): 3440, 3585 (OH). ^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 0.86 (6H, d, J = 6.5, 26-Me, 27-Me), 0.90 (3H, d, J = 6, 21-Me), 1.21 (3H, s, 19-Me), 2.46 (1H, t, J = 12.5, H-4β), 3.53 (1H, br.s, W/2 = 9, H-6α), 4.36 (1H, m, W/2 = 24, H-3α).

Experimental details for determining insecticidal activity of **1**-**9** for second-growth colorado beetle larvae have been published [12].

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