SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5β-HYDROXY ANALOGS OF α-ECDYSONE

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ABSTRACT

Two 5β-hydroxy analogs of α-ecdysone - 2β,3β,5β,14α-tetrahydroxy-5β-cholest-7-en-6-one and 3β,5β,14α-trihydroxy-5β-cholest-7-en-6-one were synthesized and their mass spectra obtained. The biological activity of these two compounds was compared with that of the most active synthetic ecdysone analog previously tested in insects, and the presence of the 5β-hydroxyl group generally enhanced the inhibitive activity.

In previous studies (1, 2) we showed that when synthetic compounds containing structural features similar to those of the insect molting hormone, α-ecdysone, were ingested they inhibited growth, development and reproduction in several species of insects. More recently, we reported on the synthesis of a number of analogs of α-ecdysone and the relationship of structure to biological activity in insects (3, 4). Our continued interest in this area and the report that a 5β,20ξ-dihydroxyecdysone (polypodine B) isolated from a fern, Polypodium vulgare L., was as active or more active in the Calliphora assay than synthetic α-ecdysone (5, 6) prompted us to synthesize analogs of α-ecdysone that contain a 5β-hydroxy group, namely, 2β,3β,5β,14α-tetrahydroxy-5β-cholest-7-en-6-one (VIIIa) and 3β,5β,14α-trihydroxy-5β-

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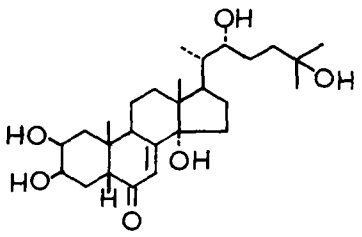
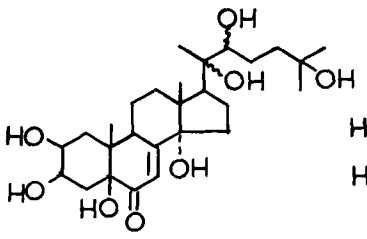
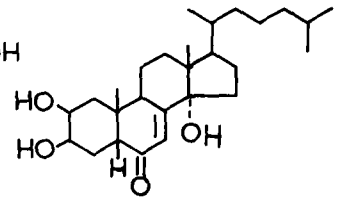


α-Ecdysone



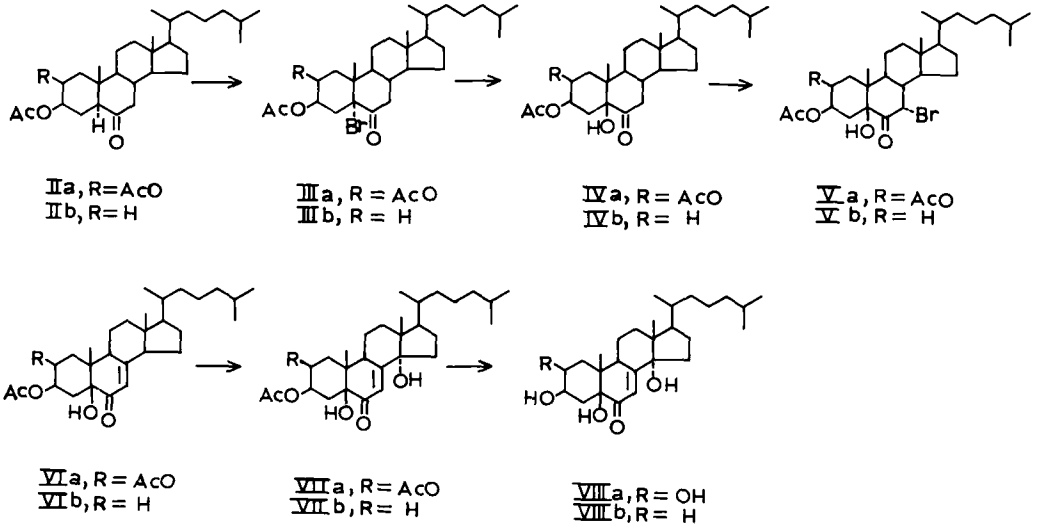
5β,20ξ-Dihydroxyecdysone



I

cholest-7-en-6-one (VIIIb). This paper reports on the synthesis and biological activity of these two compounds, and compares this biological activity with the activity of 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one (I). Compound I is the most active synthetic ecdysone analog previously tested and, generally, a far more potent inhibitor of growth, metamorphosis, and reproduction than either of the two major insect ecdysones, (α-ecdysone and 20-hydroxyecdysone) or than the phytoecdysones tested (ponasterone A, inokosterone and cyasterone) (4).

Since 5α-hydroxy-6-keto steroids are not readily isomerized to 5β-hydroxy-6-keto steroids, the 5β-hydroxyl group was introduced by using the method of Rowland (7). Thus, the 5α-bromide IIIa was obtained in quantitative yield by brominating the diacetoxy-ketone IIa in tetrahydrofuran at 5°. The reaction of IIIa with 5% ethanolic-potassium hydroxide solution, followed by acetylation (7) gave the



5β-hydroxyl ketone IVa in 48% yield. A thin-layer chromatographic (TLC) analysis of IVa showed that it migrated faster on the plate than its corresponding 5α-isomer. Bromination of IVa in a solution of acetic acid and hydrogen bromide yielded Va, and debromination with lithium carbonate in dimethyl formamide gave the 2β,3β-diacetoxy-5β-hydroxy-5β-cholest-7-en-6-one (VIa), λ max 250 mμ in methanol, ε11,450. Treatment of this diacetoxy Δ^7-6-keto compound with selenium dioxide (8) gave compound VIIa in 58% yield. The saponification of VIIa with 0.5% potassium bicarbonate in 90% methanol at 50° for 30 min gave the desired 2β,3β,5β,14α-tetrahydroxy-5β-cholest-7-en-6-one (VIIIa).

We also synthesized the 3β,5β,14α-trihydroxy-5β-cholest-7-en-6-one (VIIIb) via a similar sequence of reactions; the various intermediates

of this series are reported in the Experimental. This synthesis of VIIIb has permitted us to examine the biological effectiveness in an insect of an ecdysone analog that lacked the 2β-hydroxyl substituent.

Interestingly, in the NMR spectrum, the C-19 methyl protons in compound VIIIa resonated 0.09 ppm further down field than from those of compound I (3). Also, compound VIIIa, being a hydroxylated derivative of I would be expected by TLC analysis on silica gel plate to have a lower R_f value than I, but, in fact it has a higher R_f value than I in the solvent developing system of chloroform-ethanol (10:1). These same differences in the NMR spectrum and R_f value have been observed for cyasterone and sengosterone and again these two steroids differ structurally by only the presence of the 5β-hydroxyl group in the latter. Other pairs of ecdysteroids, namely, 20-hydroxyecdysone and polypodine B, and pterosterone and ponasterone C (9), have demonstrated similar behavior on TLC.

Compounds VIIIa and VIIIb were tested for molting hormone activity and inhibitive effects on insect growth, metamorphosis and reproduction by previously described methods (4). The percentage inhibition obtained, compared with compound I, is shown in Table 1.

In the house fly (Musca domestica L.) molting hormone assay, compound VIIIa was as active as compound I, but compound VIIIb was only about 1/10 as active. Compound VIIIa was a more potent inhibitor of larval or nymphal development than I in the confused flour beetle (Tribolium confusum Jaquelin du Val), the yellow fever mosquito (Aedes aegypti L.), and the German cockroach (Blattella germanica L.) but it was somewhat less active in the house fly larvae. The yellow fever mosquito larva was particularly susceptible to VIIIa: as little

Table 1. Effect of Synthetic Ecdysone Analogs on Development and Reproduction in Several Insects.

Insect Test System	Concentration in Diet or Medium	Percentage Inhibition* Compound I	VIIIa	VIIIb
House Fly				
Larval Development	(ppm)			
	150	100	95	32
	75	96	75	0
	15	60	14	-
Ovarian Development	(%)			
	0.50	100	17	0
	0.10	70	0	-
	0.05	23	-	-
Reproduction	0.10	88	32	0
Confused Flour Beetle				
Larval Development	(%)			
	0.50	100	100	100
	0.05	32	56	0
Reproduction	1.00	98	100**	100
	0.50	70	99**	79
	0.25	40	98	54
	0.10	0	82	0
Yellow Fever Mosquito				
Larval Development	(ppm)			
	1.0	100	100	100
	0.1	100	100	100
	0.01	15	100	20
	0.001	0	0	0
German Cockroach				
Nymphal Development	(%)			
	0.5	100	100	100
	0.05	100	100	0
	0.005	0	35	-

* Percentage inhibition based on the following criteria (4): larval or nymphal development tests - number of insects developing to normal adults compared with controls; house fly ovarian development test - number of flies showing severe inhibition of ovarian development (terminal oocytes less than 0.3 mm); house fly reproduction test - number of viable eggs produced compared with controls during continuous feeding on treated diet for 12 days (eggs collected on 6th and 12th days); confused flour beetle reproduction test - average number of progeny produced for 4 one-week egging periods following feeding on treated diet for 10 days.

** Toxic

as 0.01 ppm completely inhibited development and when intermediate concentrations between 0.001 and 0.01 ppm were tested, 0.005 ppm was found to inhibit development in 85% of the mosquito larvae. Also, its effectiveness with older larvae was equally as impressive. At concentrations of 0.01 to 0.1 ppm it completely inhibited development in third- and fourth-instar mosquito larvae.

The presence of a 5β-hydroxyl group in VIIIa, compared to I, brought about quite different responses in the reproductive tests: in the house fly it almost completely eliminated the inhibitive effect upon ovarian development and greatly reduced its effectiveness in the reproductive test; in the confused flour beetle reproductive test VIIIa was nearly 10 times as active as I.

Compound VIIIb, which differs from VIIIa only in the absence of the 2β-hydroxyl group, was considerably less active than VIIIa in all the test systems. However, this compound was at least as active as compound I in inhibiting the reproduction of the confused flour beetle and the larval development of the yellow fever mosquito.

In sum, the 5β-hydroxyl generally enhanced the inhibitive activity of VIIIa in test systems involving immature insects, except for the house fly, there it was somewhat less active than I. It is thus, the most potent synthetic ecdysone analog tested to date. However, the effect of the hydroxyl group on the reproduction of the house fly and the confused flour beetle was opposite: in the fly it severely decreased the activity; with the beetle it produced a near 10-fold increase in activity. The absence of a 2β-hydroxyl from VIIIb generally caused it to be less active than VIIIa in all test systems except in the mosquito

larval test and the confused flour beetle reproduction test. In these two tests the presence of the 5β-hydroxyl group sufficiently compensated for the loss of the 2β-hydroxyl so as to result in VIIIb still being as active as compound I.

EXPERIMENTAL

Melting points were taken on Kofler block (10) and are corrected. Rotations were determined at 23° in about 1% solutions in chloroform unless otherwise stated. Infrared spectra were obtained with a Perkin-Elmer Model 221 prism grating spectrophotometer, and ultraviolet spectra were taken in methanol, hexane, or cyclohexane with a Bausch and Lomb spectrophotometer 505. NMR spectra were recorded at 60 Mc with a Varian A-60A NMR spectrometer by using deuterated chloroform or pyridine as the solvent and TMS as an internal NMR standard. The mass spectra were measured by using a LKB model 9000 gas chromatograph mass spectrometer (LKB Produkter AB, Stockholm); the samples were introduced directly into the ionization chamber. The ionization energy was 70 ev.

2β,3β-Dihydroxy-5α-bromo-5α-cholestan-6-one 2,3-diacetate (IIIa) - To a stirring solution of 20g of 2β,3β-dihydroxy-5α-cholestan-6-one 2,3-diacetate (IIa) prepared as previously reported (3), 200 ml of dry tetrahydrofuran and 1 ml of 32% hydrogen bromide in acetic at 5° was added dropwise 6.6 g of bromine in 45 ml of acetic acid over a 15-min period. The reaction mixture was kept at 5° for an additional 5 min and was then poured into ice and water; the precipitate was collected, air dried, and then dried in dessicator overnight to give 22 g of crude IIIa. A 1-g sample recrystallized from hexane gave 450 mg of needles, m.p. 172-175°. Recrystallization twice from hexane gave analytically pure IIIa, m.p. 186-188°, α_D -107°.

Anal. Calcd. for $C_{31}H_{49}O_5Br$: C, 64.01; H, 8.49. Found: C, 63.70; H, 8.19.

3β-Hydroxy-5α-bromo-5α-cholestan-6-one 3-acetate (IIIb) - From the bromination of 20 g of IIb we obtained 23.2 g of crude IIIb; crystallization of a 0.5-g sample from hexane gave 365 mg of IIIb as needles, m.p. 165-167° with dec, α_D -136°. Lit. (11) m.p. 162°, α_D -133°.

2β,3β,5β-Trihydroxy-5β-cholestan-6-one 2,3-diacetate (IVa) - A suspension of 21 g of the crude 6-keto-5α-bromoacetate (IIIa) in 500 ml of a 5% solution of potassium hydroxide in 95% ethanol was stirred magnetically at room temperature for 5 hr (7). During the first hour, the steroid dissolved, the solution became orange-colored and potassium bromide salt precipitated from solution. The solution was diluted with ice water and extracted with chloroform. The chloroform extract was washed with water, dried over sodium sulfate, and concentrated to dryness in vacuum. The residue was reacetylated overnight at 65° with

90 ml of pyridine and 30 ml of acetic anhydride. The solution was then diluted with water, and the residue was collected, dried, and recrystallized from hexane to give 9.0 g of IVa as rods, m.p. 155-156°, α_D -32°, νmax in CS_2 3460 cm^{-1} (hydroxyl), 1735 (acetate), 1705 cm^{-1} (ketone), NMR, δ 0.62 (18-H), 0.97 (19-H), 2.10 and 2.14 (diacetate). The δ values for the C-18 and C-19 methyl resonances of the corresponding 5α-isomer of IVa were 0.67 and 1.2 respectively.

Anal. Calcd. for $C_{31}H_{50}O_6$: C, 71.77, H, 9.72. Found: C, 71.79; H, 9.77.

3β,5β-Dihydroxy-5β-cholestan-6-one 3-acetate (IVb) - From 21 g of IIIb, we obtained, after crystallization from an acetone-methanol mixture containing a little water, 10.4 g of IVb, m.p. 142-144°, α_D -23°, νmax in CS_2 3465 cm^{-1} (hydroxyl), 1735 (acetate), and 1705 cm^{-1} (ketone) Lit. (7) m.p. 142.5-144.5°, α_D -22°.

2β,3β,5β-Trihydroxy-7α-bromo-5β-cholestan-6-one 2,3-diacetate (Va) - To 8.4 g of IVa in 85 ml of acetic acid at 40° that contained 1 ml of 32% hydrogen bromide in acetic acid, was added dropwise 2.65 g of bromine in 45 ml of acetic acid over a 25-min period. The temperature was maintained at 40° for 1 hr, and the solution was cooled and poured into ice and water. The precipitate was collected, air dried, and then dried overnight in a dessicator to give 9.5 g of Va. A 200-mg sample recrystallized twice from hexane gave 100 mg of the bromo-acetate (Va) as rods, m.p. 131-133°, α_D -9°, νmax in CS_2 3485 cm^{-1} (hydroxyl), 1735 (acetate), and 1700 cm^{-1} (ketone).

Anal. Calcd. for $C_{31}H_{49}O_6Br$: C, 62.30; H, 8.26. Found: C, 61.93; H, 8.21.

3β,5β-Dihydroxy-7α-bromo-5β-cholestan-6-one 3-acetate (Vb) - The bromination of 10 g of IVb as in the preparation of Va gave 11.4 g of crude Vb. A recrystallization of a 1-g sample twice from hexane gave 600 mg of Vb as needles, m.p. 137-138°, α_D +9°, νmax in CS_2 3500 cm^{-1} (hydroxyl), 1735 (acetate), 1700 cm^{-1} (ketone).

Anal. Calcd. for $C_{29}H_{47}O_4Br$: C, 64.55; H, 8.78. Found: C, 64.37; H, 8.99.

2β,3β,5β-Trihydroxy-5β-cholest-7-en-6-one 2,3-diacetate (VIa) - A mixture of 6.5 g of crude Va, 65 ml of dimethylformamide, and 6.5 g of lithium carbonate was rapidly raised to reflux temperature and refluxed for 1.5 hr. After the lithium carbonate had been removed by filtration, the solution was cooled and diluted with ice water, and the precipitate was collected and dried. The crude mixture (5.6 g) was chromatographed over 60 g of hexane-benzene (1:1)-washed Unisil, and the following 100-ml fractions were collected: 1-3, hexane-benzene (1:1); 4-15, benzene-ether (98:2); and 16-18, benzene-ether (95:5). The fractions were monitored by TLC analysis. Fractions 8-16 were combined and recrystallized from hexane to give 3.2 g of VIa, m.p.

169-171°, αD +32° λ max in methanol 250 mμ, ε11,450, νmax in CS_2 3460 cm^{-1} (hydroxyl), 1735 (acetate), 1670 (ketone), and 1620 cm^{-1} (double bond).

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.20; H, 9.39.

3β,5β-Dihydroxy-5β-cholest-7-en-6-one 3-acetate (VIb) - The debromination of 7.5 g of crude Vb yielded, after chromatography and recrystallization from hexane, 3.6 g of VIb, m.p. 104-106°, αD +88°, λ max in methanol 244 mμ, ε12,400, νmax in CS_2 3465 cm^{-1} (hydroxyl), 1735 (acetate), 1670 (ketone), and 1620 cm^{-1} (double bond).

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.66; H, 9.94

2β,3β,5β,14α-Tetrahydroxy-5β-cholest-7-en-6-one 2,3-diacetate (VIIa) - To 3.0 g of VIa in 90 ml of dry dioxane at 80° was added in one portion 3.0 g of selenium dioxide (8). The reaction mixture was kept at 80-85° for 1 hr and then filtered. [A reaction time of 30 min as in the preparation of I (4) resulted in a 50% recovery of VIa] The filtrate was cooled and diluted with ice and water, and the crude precipitate was collected, dried, and chromatographed over 30 g of benzene-washed Unisil. The following 100-ml fractions were collected: 1, benzene; 2-5, benzene-ether (95:5); and 6-10, benzene-ether (80:20). Recrystallization of fraction 6-9 from ether-hexane gave 2 g of VIIa as spears, m.p. 192-194°, αD +100°, λ max 244 mμ, ε 10,450, νmax in Nujol 3425 cm^{-1} (hydroxyl), 1740, 1720 (acetate), 1660 (ketone), and 1618 cm^{-1} (double bond).

Anal. Calcd for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 70.15; H, 9.21

3β,5β,14α-Trihydroxy-5β-cholest-7-en-6-one 3-acetate (VIIb) - The oxidation of 3.0 g of VIb with selenium dioxide yielded after chromatography and recrystallization from hexane, 1.8 g of IIb, m.p. 168-170°, αD +104°, λ max in methanol 244 mμ, ε10,650, νmax in CS_2 3470 cm^{-1} (hydroxyl), 1735 (acetate), 1675 (ketone), and 1625 cm^{-1} (double bond) (12).

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.12; H, 9.59.

2β,3β,5β,14α-Tetrahydroxy-5β-cholest-7-en-6-one (VIIIa) - To 2 g of VIIa in 180 ml of methanol at 50°, was added 1 g of potassium bicarbonate in 20 ml of water. The reaction mixture was kept at 50° for 30 min, and the solution was reduced to about half its original volume in vacuum, and water was added to complete the precipitation of the compound. The precipitate was collected, dried, and recrystallized twice from ethyl acetate to give as elongated needles 1.4 g of VIIIa, m.p. 238-240°, αD +124°, λ max in methanol 244 mμ ε10,000, νmax in Nujol 3380 cm^{-1} (hydroxyl), 1685 (ketone), and 1638 cm^{-1}

(double bond), NMR, δ 0.75 (18-H), 1.17 (19-H), mass spectrum (m/e (rel. intensity)) (M^+ 448)(3), 430(73), 420(43), 415(23), 412(34), 404(42), 402(29), 397(19), 387(26), 384(12), 369(32), 360(31), 343(100), 317(16), 315(23), 314(17), 299(17), 289(8), 288(8), 286(9), 266(14), 265(39), 263(27), 247(23), 245(14), 230(28), 203(45), 189(23), 177(93), 175(29), 173(30), 163(30), 153(33), 137(67), 121(32), 107(27), 105(33), 97(31), 91(36), 81(43), 69(90), 55(100), with metastables at m/e 395, 320, 89 and 77.

The δ values for the C-18 and C-19 methyl resonances of the corresponding 5α-isomer of VIIIa were 0.79 and 1.62, respectively.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.31; H, 9.85. Found: C, 72.38; H, 9.84.

3β,5β,14α-Trihydroxy-5β-cholest-7-en-6-one (VIIIb) - The saponification of 1.7 g of VIIb in methanol-water (9:1) containing 0.5% potassium bicarbonate at reflux temperature for 30 min gave, after recrystallization from acetone-hexane, 1.35 g of VIIIb, m.p. 176-178°, α_D +145°, λ max in methanol 244 mμ, ϵ 10,400, νmax in Nujol 3425 cm^{-1} (hydroxyl), 1675 (ketone), and 1620 cm^{-1} (double bond), NMR δ, 0.76 (18-H), 1.13 (19-H), mass spectrum (m/e (rel. intensity)) (M^+ 432(4), 414(14), 404(79), 399(15), 386(54), 371(22), 368(16), 353(14), 343(63), 332(20), 315(25), 301(9), 286(12), 249(64), 231(28), 213(16), 201(18), 177(37), 173(27), 147(39), 139(100), 137(56), 133(25), 129(24), 121(34), 110(37), 105(32), 95(36), 81(46), 69(59), 57(63), 55(87), with metastables at m/e 369, 351, 214 and 89.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25. Found: C, 75.09; H, 10.40.

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12. We have isolated at this step what appears to be, by infrared analysis, a mixture of an α,β-unsaturated ketone and an isolated ketone; however, by TLC analysis the mixture showed only one component and upon saponification gave only VIIIb that exhibited in the infrared only α,β-unsaturated carbonyl and also showed only one spot by TLC analysis.