

## SYNTHESIS OF SILENOSTERONE, AN INSECT-MOLTING HORMONE

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UDC 547.926:591.147

*Silenosterone is synthesized in five steps: acetylation of 2-desoxy- $\alpha$ -ecdysone (1), saponification of 2-desoxy- $\alpha$ -ecdysone-3,22-diacetate (2) to the 22-monoacetate (3), and oxidation of the latter to a ketone (4) and its base hydrolysis (5). The structures of the compounds are confirmed by PMR, IR spectroscopy, and mass spectrometry.*

Ecdysteroids are naturally occurring compounds that exhibit various types of biological activity. They possess anabolic, hypoglycemic, hepatoprotective, adaptogenic, and tonic activities [1]. Ecdysteroids are found in the free state in plant sources. Conjugates of ecdysteroids in addition to the free compounds have recently been observed in plant sources. These include acetic, benzoic, and sulfate esters and isopropylidene derivatives. Silenosterone (5), an ecdysteroid with a ketone at C-3 [2], is found for the first time in the aerial organs of *Silene praemixta*. The physiological functions of ecdysteroids until now has not been explained in detail. It can be assumed that they play a significant role in plant biosynthetic processes.

3-Dehydroecdysteroids are known to be metabolites of animal origin [3, 4]. Ecdysteroids with a ketone at C-3 are found only in *Calliphora vicina*. For example, 3-dehydro- $\alpha$ -ecdysone and 3-dehydroecdysterone are described only as metabolites of the principal insect hormones, ecdysterone and  $\alpha$ -ecdysone, in insect homogenates [3-5]. Later, silenosterone, which can also be called 3-dehydro-2-desoxyecdysone [6], was isolated from *Locusta migratoria* [6]. Silenosterone might also be a metabolite of the principal phytoecdysteroids, especially because it is found in comparatively small quantities.

Therefore, the synthesis of monoesters and 3-ketones of ecdysteroids holds promise for creating new types of biologically active compounds based on them.

Chemical transformation of available phytoecdysteroids is the simplest and cheapest method for preparing rarely encountered natural ecdysteroids. One of the available natural ecdysteroids is 2-desoxy- $\alpha$ -ecdysone (1). Owing to its structural features, we acetylated compound 1 with acetic anhydride in pyridine to produce 2-desoxy- $\alpha$ -ecdysone-3,22-diacetate (2) (Scheme 1) [2].

Saponification of diacetate 2 produced 2-desoxy- $\alpha$ -ecdysone-22-monoacetate (3). The IR spectrum of monoacetate 3 contains absorption bands characteristic of esters. The PMR spectrum of 3 contains a three-proton singlet at 1.95 ppm, indicating that one acetyl group was retained in molecule 3. In fact, the PMR spectra of compounds 1 and 3 differ substantially only for the resonance of the proton on C-22.

The signal for this proton in the spectrum of 3 is shifted to downfield by 1.14 ppm and resonates as a multiplet at 5.14 ppm. The data are consistent with acetate esterification of the hydroxyl on C-22. The mass spectrum of 3 shows key fragments with  $m/z$  331, 314, 303, 285, and 284. This confirms the hypothesis and also indicates that ecdysteroid 3 contains two hydroxyls in the steroid portion and is similar to 2-desoxy- $\alpha$ -ecdysone [7]. Oxidation of 3 by  $\text{CrO}_3$  at room temperature gave 3-keto-22-acetyl-2-desoxy- $\alpha$ -ecdysone (4). The IR spectrum of substance 4 exhibits an absorption band at  $1710\text{ cm}^{-1}$ , characteristic of an additional nonconjugated carbonyl in a six-membered ring.

Comparison of the molecular ions of the 22-monoacetate (3,  $M^+$  490) and the 3-keto-22-monoacetate (4,  $M^+$  488) showed that the molecular mass of 4 is 2 amu less than that of compound 3. The side chain fragments the same in both compounds. This is confirmed by the identical chemical shifts in the PMR of these compounds for the methyl protons on C-21, C-26, and C-27 (Table 1).

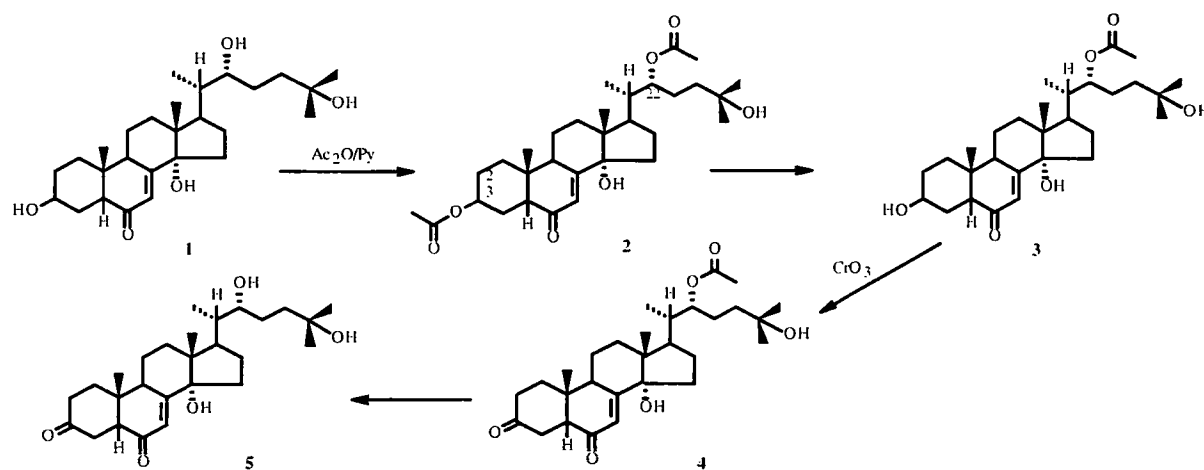
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TABLE 1. Chemical Shifts of Protons in 2-Desoxy- $\alpha$ -ecdysone (**1**) and its Diacetate (**2**), 2-Desoxy- $\alpha$ -ecdysone-22-monoacetate (**3**), 3-Keto-2-desoxy- $\alpha$ -ecdysone-22-monoacetate (**4**), and Silenosterone (**5**) ( $\delta$ ,  $C_5D_5N$ ,  $0 = HMDS$ )

Compound	Proton position								
	H-3	H-22	H-7	H-9	CH <sub>3</sub> -18	CH <sub>3</sub> -19	CH <sub>3</sub> -21	CH <sub>3</sub> -26/27	OAc
<b>1</b>	4.00	4.00	6.13	3.42	0.61	0.95	1.17	1.26	-
<b>2</b>	4.82	5.19	6.03	3.20	0.60	0.85	1.00	1.20	1.85;1.95
<b>3</b>	3.93	5.14	5.59	3.30	0.59	0.76	1.01	1.22	1.95
<b>4</b>	-	5.13	5.94	-	0.60	0.89	0.99	1.21	1.94
<b>5</b>	-	3.90	5.96	3.45	0.62	0.94	1.16	1.27	-

**Note.** Signals of the CH<sub>3</sub>-21 group are doublets; of the other methyl groups, singlets. Proton H-7 appears as a broad singlet; remaining protons, as broad multiplets.



Scheme 1

Comparison of the mass numbers of the principal fragments from the steroid nucleus of compound **3** ( $m/z$  332, 314, 284, 234) and of molecule **4** ( $m/z$  330, 312, 282, 232) indicates that differences in the structures of compounds **3** and **4** are caused by factors associated with the tetracyclic skeleton. The ions of compound **4** are 2 amu less than the corresponding fragments of compound **3**. This difference indicates that the C-3 hydroxyl in compound **3** was oxidized to a ketone. These data are also confirmed by comparing the PMR spectra of compounds **3** and **4**. The spectrum of compound **4** lacks a signal for the C-3 proton (Table 1).

The 3-keto-22-monoacetate (**4**) was hydrolyzed by base. The reaction products contained compound **5**, which was identified as silenosterone by spectral data and physicochemical constants [2].

## EXPERIMENTAL

TLC was performed on Silufol plates. A KSK 100/160  $\mu m$  column was used for column chromatography.

Mass spectra were recorded on MX-1310 and MX-1303 instruments at 50 V ionization potential and 100-140°C; IR

spectra, on a UR-20 spectrophotometer in KBr; PMR, on a JNM-4H-100/100 MHz ( $C_5D_5N$ ,  $\delta$ , 0 = HMDS) instrument.

**2-Desoxy- $\alpha$ -ecdysone-3,22-diacetate (2).** 2-Desoxy- $\alpha$ -ecdysone (**1**, 7 g, obtained from *S. praemixta* [2]) in pyridine (142 ml) was acetylated by acetic anhydride (61 ml) at room temperature for 24 h. The reaction mixture was treated with water and left overnight in a refrigerator. A crystalline compound was produced. The crystals were filtered off and chromatographed on a silica-gel column. The column was eluted with  $CDCl_3$  to give diacetate **2**, 6.14 g,  $C_{31}H_{48}O_7$ , mp 245-246°C (methanol—water),  $[\alpha]_D^{24} = +39.0 \pm 3^\circ$  (c 0.25, methanol). IR spectrum (KBr,  $\nu$ ,  $cm^{-1}$ ): 3460 (OH), 1740, 1250 (ester), 1660 (7-en-6-ketone) [2].

**2-Desoxy- $\alpha$ -ecdysone-22-acetate (3).** Compound **2** (1.71 g) was dissolved in methanolic KOH (0.5%, 48 ml). The reaction mixture was left for 6 h, then neutralized with acetic acid, and diluted with water. The precipitate was filtered off and chromatographed on a silica-gel column. Elution with  $CHCl_3$ — $CH_3OH$  (100:1) gave compound **3**, 1.25 g,  $C_{29}H_{46}O_6$ , mp 158-160°C (recrystallized from aqueous  $CH_3OH$ ),  $[\alpha]_D^{22} = +51.3 \pm 2^\circ$  (c 0.20, methanol). IR spectrum (KBr,  $\nu$ ,  $cm^{-1}$ ): 3445 (OH), 1730, 1255 (ester), 1665 (7-en-6-ketone).

Mass spectrum,  $m/z$  (%): 490 ( $M^+$ , 6), 472 (12), 462 (12), 444 (27), 412 (22), 397 (41), 394 (26), 384 (47), 379 (16), 332 (12), 331 (14), 314 (14), 303 (4), 302 (14), 285 (46), 284 (100), 235 (94), 234 (85), 99 (46), 81 (50).

For the PMR spectrum, see Table 1.

**3-Keto-2-desoxy- $\alpha$ -ecdysone-22-acetate (4).** Compound **3** (500 mg) in acetone (30 ml) was treated with water (2 drops) and  $CrO_3$  (275 mg). The mixture was stirred, left at room temperature for 40 min, and poured into water. The product was extracted with ethylacetate. The ethylacetate fraction was chromatographed on a silica-gel column. Elution with  $CHCl_3$ — $CH_3OH$  (50:1) isolated compound **4**, 120 mg,  $C_{29}H_{44}O_6$ , mp 210-211°C ( $CH_3OH$ —hexane),  $[\alpha]_D^{27} = +43.5 \pm 2^\circ$  (c 0.16,  $CH_3OH$ ). IR spectrum (KBr,  $\nu$ ,  $cm^{-1}$ ): 3480 (OH), 1735, 1250 (ester), 1710 (C=O at C-3), 1670 (7-en-6-ketone).

Mass spectrum,  $m/z$  (%): 488 ( $M^+$ , 1), 470 (7), 458 (5), 452 (4), 442 (5), 428 (0.5), 410 (16), 395 (35), 392 (20), 377 (13), 330 (2), 329 (4), 312 (10), 311 (5), 300 (15), 283 (45), 282 (57), 281 (30), 233 (100), 232 (89), 99 (25), 81 (20).

For the PMR spectrum, see Table 1.

**3-Keto-2-desoxy- $\alpha$ -ecdysone (silenosterone) (5).** Compound **4** (80 mg) was dissolved in methanolic KOH (2.5 ml, 0.5%). The reaction mixture stood for one day, was neutralized, diluted with water, and extracted with ethylacetate. The solvent was removed. The solid was chromatographed on a silica-gel column. Elution with  $CHCl_3$ — $CH_3OH$  (50:1) isolated compound **5**, 35 mg,  $C_{27}H_{42}O_5$ , mp 114-115°C ( $CHCl_3$ —benzene),  $[\alpha]_D^{25} = +86.5 \pm 2^\circ$  (c 0.35,  $CH_3OH$ ). IR spectrum (KBr,  $\nu$ ,  $cm^{-1}$ ): 3425 (OH), 1710 (C=O), 1660 (7-en-6-ketone).

Mass spectrum,  $m/z$  (%): 446 ( $M^+$ , 9), 428 (98), 413 (40), 410 (50), 395 (98), 377 (8), 359 (40), 341 (50), 330 (95), 312 (96), 297 (50), 283 (30), 232 (35), 99 (100), 81 (98).

For the PMR spectrum, see Table 1.

## ACKNOWLEDGEMENT

This work was supported by INTAS Grant No. 96-1291.

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