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Synthesis of 20-Hydroxyecdysone Oxime, Its Diacetonide, and Their 14,15-Anhydro Derivatives

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Abstract—20-Hydroxyecdysone oxime, its diacetonide, and the corresponding 14,15-anhydro derivatives were synthesized. Conditions were found for the preparation of the *Z*- and *E*-oximes, and their characteristic ^{1}H and ^{13}C NMR parameters were determined.

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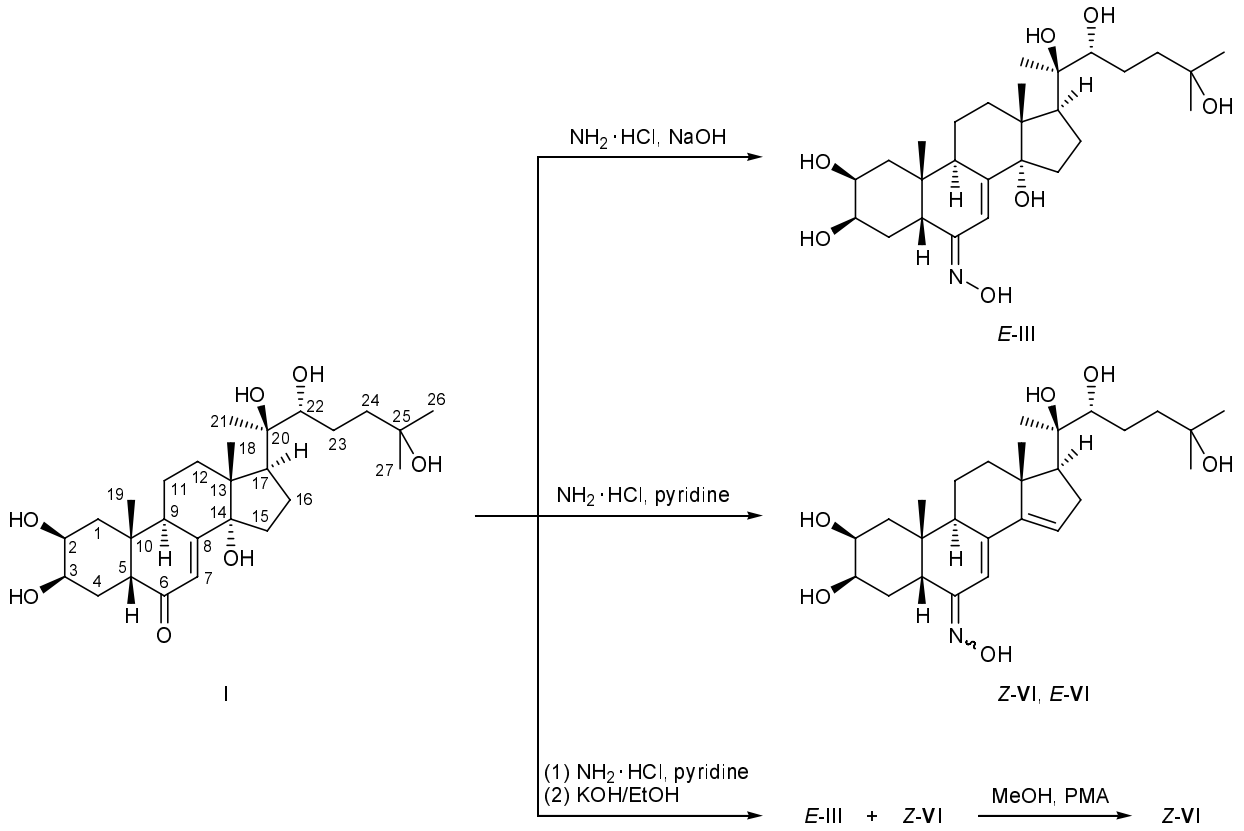
Steroid oximes recently isolated from *Cynachyrella alloclada* and *C. apion* marine sponges, as well as their synthetic structural analogs, turned out to efficiently inhibit aromatase which is the key enzyme responsible for the biosynthesis of estrogens. These compounds also attract strong attention as potential antitumor agents [1–3]. 20-Hydroxyecdysone oxime and its derivatives were not reported. It was noted only that derivatives of 20-hydroxyecdysone at the carbonyl group exhibit a stronger antitumor activity as compared to the parent compound [4].

With a view to obtain potential biologically active oximes in the series of ecdysteroids we performed reactions of 20-hydroxyecdysone (**I**) and its 2,3:20,22-di-*O*-isopropylidene derivative **II** with hydroxylamine hydrochloride in aqueous alcohol in the presence of sodium hydroxide. In the reaction with compound **I** we isolated the corresponding stereochemically pure oxime *E*-**III** (Scheme 1). Its structure follows from the ^{1}H and ^{13}C NMR data. The ^{1}H NMR spectrum of *E*-**III** (CD_3OD) contained a doublet at δ 6.56 ppm (^4J = 2.1 Hz) from the 7-H proton. The C^5 signal appeared in the ^{13}C NMR spectrum of *E*-**III** at δ_C 42.6 ppm. The α-CH group in the *anti* position with respect to the oxime hydroxy group resonates in the same region [5]. Insofar as the group on C^5 is senior to that on C^7, the isolated oxime should be assigned to the *E* series.

Diacetonide **II** is poorly soluble in aqueous alcohol; therefore, it was treated with hydroxylamine in pyridine. As a result, we obtained an equimolar mixture of oxime *E*-**IV** and 14,15-anhydro derivative *Z*-**V** (Scheme 2). The signal at δ_C 42.9 ppm in the ^{13}C NMR spectrum of *E*-**IV** (C_5D_5N) corresponds to *anti* orientation of the 5-H proton with respect to the N–OH group, i.e., this compound has *E* configuration. The structure of oxime *Z*-**V** follows from its mass spectrum and ^{1}H and ^{13}C NMR data. The *m*/*z* value of the molecular ion peak in the mass spectrum of *Z*-**V** is lower by 18 units than that of $[M]^+$ in the spectrum of *E*-**IV**, which indicates loss of water molecule. The ^{1}H NMR spectrum of *Z*-**V** ($CDCl_3$) contains an additional vinyl proton signal at δ 5.75 ppm (apart from the 7-H signal at δ 6.21 ppm). Its ^{13}C NMR spectrum lacked signals at δ_C 85.0 and 29.3 ppm (C^{14} and C^{15} in *E*-**IV**), but signals from sp^2-hybridized carbon atoms appeared at δ 139.5 (C^{14}) and 116.7 ppm (C^{15}). The C^5 signal is located at δ 37.9 ppm, which corresponds to *syn* orientation of C^5H with respect to the oxime hydroxy group [5], in keeping with *Z* configuration of this compound.

The oximation of ecdysteroid **I** in pyridine gave only a mixture of *Z*- and *E*-isomeric 14,15-anhydro-6-oximes *Z*-**VI** and *E*-**VI** (Scheme 1). The signal at δ_C 38.2 ppm in the ^{13}C NMR spectrum (C_5D_5N) of oximes *Z*/*E*-**VI** belongs to C^5H in the *syn* position with respect to N–OH, i.e., to *Z*-**VI**, while that located at δ_C 49.5 ppm belongs to C^5H in the *anti* position, i.e., to *E*-**VI**. The 7-H and 15-H protons of *Z*-**VI** resonate in the ^{1}H NMR spectrum (C_5D_5N) at δ 6.67 and 5.85 ppm, respectively, and the corresponding signals of *E*-**VI** appear at δ 7.43 and 5.89 ppm, respectively. The ratio of isomeric oximes *Z*-**VI** and *E*-**VI** was estimated at

Scheme 1.



~1 : 2.5 from the relative intensities of the 7-H or 15-H signals.

Taking into account that the oximation of compound **I** in aqueous alcohol in the presence of alkali was not accompanied by elimination of water molecule, we presumed that addition of alkali to the reaction mixture obtained after oximation in pyridine would prevent anhydridization. However, from compound **I** we obtained a mixture of oximes *E*-**III** and *Z*-**VI**, the latter prevailing (*E*-**III** : *Z*-**VI** ≈ 1 : 2.5, as determined from the relative intensities of the 7-H signals at δ 6.56 and 6.17 ppm; CD_3OD). By treatment of mixture *E*-**III**/*Z*-**VI** with methanol in the presence of phosphomolybdic acid (PMA) we obtained pure isomer *Z*-**VI**.

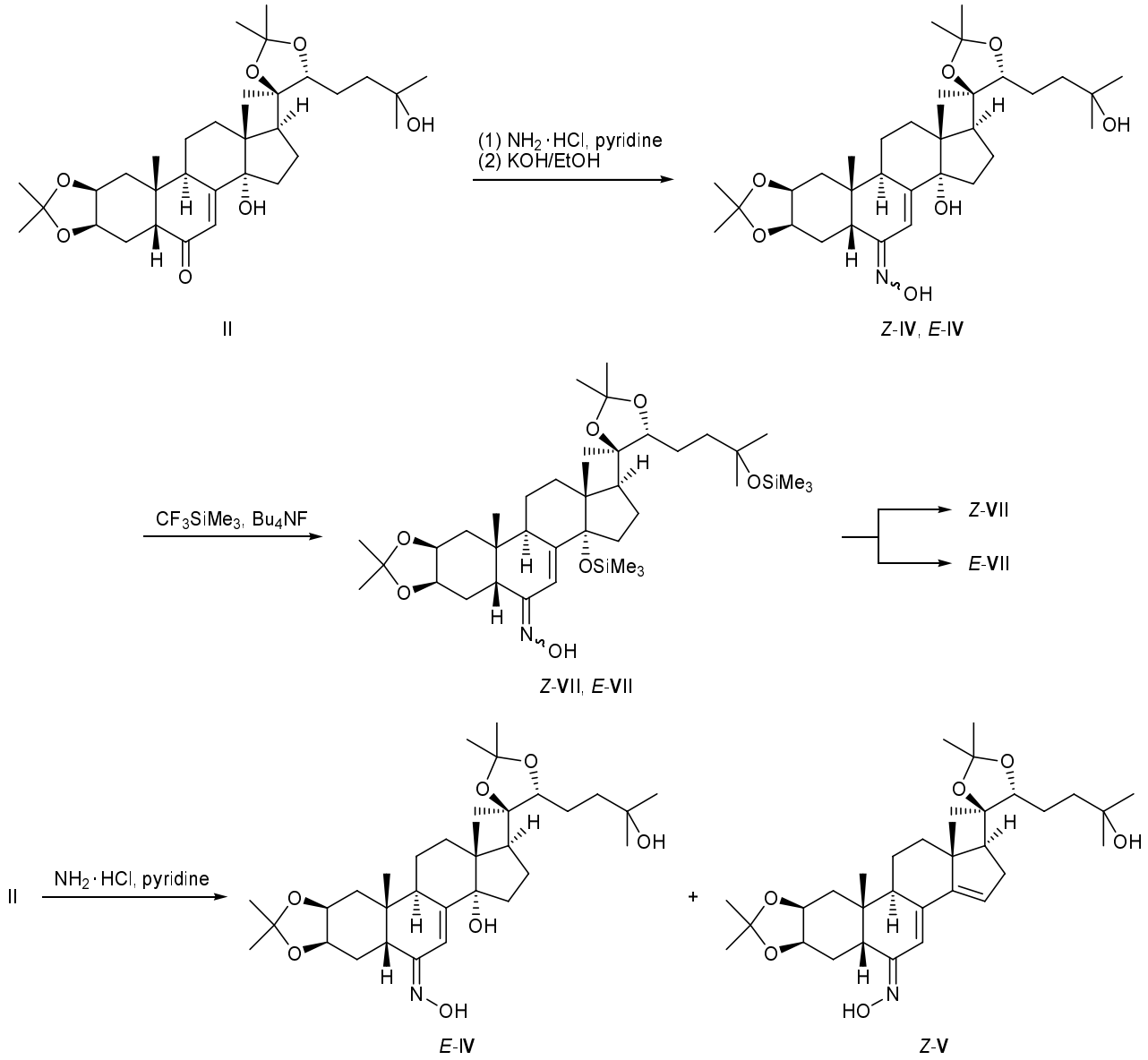
No anhydridization occurred when the reaction mixture obtained after oximation of diacetonide **II** was treated with alcoholic alkali, and we isolated an equimolar mixture of oximes *Z*-**IV** and *E*-**IV** (according to the relative intensities of the 7-H signals at δ 5.95 and 6.61 ppm; solvent $CDCl_3$). The signal at δ_C 36.4 ppm in the ^{13}C NMR spectrum ($CDCl_3$) was assigned to C^5 in *Z*-**IV** (*syn* orientation with respect to N–OH), and that at δ_C 42.1 ppm, to C^5 in *E*-**IV** (*anti* orientation). We can conclude that isomeric diacetonide oximes are more resistant to 14,15-anhydridization than 20-hydroxyecdysone oximes. Differences in the reactivity of 20-hydroxyecdysone and its derivatives were noted by us previously [6, 7].

Following the procedure reported in [8], oxime mixture *Z*-**IV**/*E*-**IV** was converted into a mixture of isomeric bis(trimethylsilyl) ethers *Z*-**VII** and *E*-**VII** (Scheme 2) and the latter were separated by column chromatography on silica gel (we failed to separate oximes *Z*-**IV** and *E*-**IV** in such a way). The ^1H NMR spectra of oximes *Z*-**VII** and *E*-**VII** each contained two singlets with equal intensities at δ 0.06/0.11 and 0.08/0.11 ppm, respectively, indicating the presence of two Me_3SiO groups. The corresponding carbon signals appeared in the ^{13}C NMR spectra of *Z*-**VII** and *E*-**VII** at δ_C 1.9 and 2.6 ppm. Etherification of the hydroxy groups on C^{14} and C^{25} led to downfield shift of the C^{14} and C^{25} signals by 3–4 ppm (cf. [8, 9]).

EXPERIMENTAL

The IR spectra were recorded on a Specord 75IR spectrometer. The UV spectra were measured on a Specord M-40 spectrophotometer from solutions in

Scheme 2.



methanol and chloroform. The ^1H and ^{13}C NMR spectra were obtained on a Bruker AM-300 instrument at 300.13 and 75 MHz, respectively, using CD_3OD, C_5D_5N, or $CDCl_3$ as solvent and tetramethylsilane as internal reference. Signals in the ^{13}C NMR spectra were assigned using *J*-modulation technique. The mass spectra (electron impact, 70 eV), including high-resolution mass spectra, were recorded on a Finnigan MAT-8200 mass spectrometer. The melting points were determined on a Boetius melting point apparatus. The specific optical rotations (deg ml g^{-1} dm^{-1}) were measured on a Perkin–Elmer 141 polarimeter; the concentrations are given in g/100 ml. Thin-layer chromatography was performed on silica gel plates (Silufol); spots were developed by spraying with a solution of vanillin in ethanol acidified with sulfuric acid. Silica gel 5–40 μm (Chemapol, Czechia) was used for column chromatography.

20-Hydroxyecdysone (6*E*)-oxime (*E*-III). 20-Hydroxyecdysone (**I**) (mp 246°C, prepared according to [10]), 4.0 g (8.3 mmol), was dissolved in 50 ml of freshly distilled ethanol, 4.5 g (65 mmol) of hydroxylamine hydrochloride was added, and the mixture was neutralized with 20% aqueous sodium hydroxide and heated for 14 days at the boiling point. The mixture was then concentrated to a volume of 10 ml and applied to a column charged with 10 g of silica gel, the column was eluted with ethanol, the eluate was evap-

orated on a rotary evaporator, the residue was extracted with ethyl acetate (3×100 ml), the extract was evaporated, and the residue was subjected to column chromatography on silica gel (160 g) using chloroform–methanol (5:1) as eluent. We isolated 2.2 g (53%) of oxime ***E*-III** (R_f 0.22; $CHCl_3$–MeOH, 3:1) and 1.5 g (38%) of initial compound **I** (R_f 0.45; $CHCl_3$–MeOH, 3:1; mp 244–245°C; the ^1H NMR spectrum was identical to that of an authentic sample).

Oxime ***E*-III**. mp 186–188°C, $[\alpha]_D^{18}$ = +6.0° (*c* = 9.38, MeOH). IR spectrum (Nujol), ν, cm^{-1}: 3650–3050, 1610, 1450, 1380, 1050, 1020, 950. UV spectrum (MeOH): λ_{max} 243 nm (ε = 11244). ^1H NMR spectrum, δ, ppm: in CD_3OD: 0.85 s (3H, $C^{19}H_3$), 0.88 s (3H, $C^{18}H_3$), 1.18 s (6H, $C^{26}H_3$, $C^{27}H_3$), 1.28 s (3H, $C^{21}H_3$), 2.94 m (1H, 9-H, $W_{1/2}$ = 25 Hz), 3.34 m (1H, 22-H, $W_{1/2}$ = 25 Hz), 3.84 m (1H, 2-H, $W_{1/2}$ = 24 Hz), 3.92 br.s (1H, 3-H, $W_{1/2}$ = 9 Hz), 6.56 d (1H, 7-H, ^4J = 2.1 Hz); in C_5D_5N: 1.04 s (3H, $C^{19}H_3$), 1.23 s (3H, $C^{18}H_3$), 1.36 s (6H, $C^{26}H_3$, $C^{27}H_3$), 1.57 s (3H, $C^{21}H_3$), 2.99 t (1H, 17-H, *J* = 8.1 Hz), 3.16 d.d (1H, 5-H, ^3J = 3.5, 13.0 Hz), 3.46 m (1H, 9-H, $W_{1/2}$ = 22 Hz), 3.86 m (1H, 22-H, $W_{1/2}$ = 17 Hz), 4.22 br.s (1H, 3-H, $W_{1/2}$ = 9 Hz), 4.25 br.s (1H, 2-H, $W_{1/2}$ = 15 Hz), 7.32 br.s (1H, 7-H, $W_{1/2}$ = 3 Hz). ^{13}C NMR spectrum (CD_3OD), δ_C, ppm: 18.3 q (C^{18}), 21.1 q (C^{19}), 21.6 t (C^{11}), 21.7 t (C^{16}), 24.7 q (C^{21}), 27.3 t (C^{23}), 29.1 q (C^{26}), 29.7 q (C^{27}), 32.0 t (C^{15}), 32.8 t (C^4), 34.8 t (C^{12}), 35.4 d (C^9), 37.9 s (C^{10}), 38.0 t (C^1), 42.4 t (C^{24}), 42.6 d (C^5), 48.7 s (C^{13}), 50.6 d (C^{17}), 68.8 d (C^3), 69.4 d (C^2), 71.4 s (C^{25}), 78.1 s (C^{20}), 78.4 d (C^{22}), 86.1 s (C^{14}), 110.5 d (C^7), 153.5 s (C^8), 157.5 s (C^6). Mass spectrum, *m*/*z* (I_{rel}, %): 463 (6), 462 (5), 461 (15), 360 (26), 344 (22), 343 (23), 342 (17), 326 (20), 316 (18), 315 (12), 310 (11), 300 (14), 298 (11), 266 (12), 264 (10), 212 (11), 144 (10), 143 (14), 99 (26), 91 (11), 83 (13), 81 (37), 71 (14), 69 (22), 59 (26), 57 (20), 55 (38), 43 (100). Found, %: C 65.47; H 9.01; N 2.78. $C_{27}H_{45}NO_7$. Calculated, %: C 65.43; H 9.15; N 2.83.

2,3:20,22-Di-*O*-isopropylidene-20-hydroxyecdysone (6*E*)-oxime (*E*-IV) and 14,15-anhydro-2,3:20,22-di-*O*-isopropylidene-20-hydroxyecdysone (6*Z*)-oxime (*Z*-V). Diacetonide **II** (mp 234–235°C, prepared according to [11]), 2.07 g (3.7 mmol), was dissolved in 20 ml of freshly distilled pyridine, 2.07 g (30 mmol) of hydroxylamine hydrochloride was added under stirring, and the mixture was heated for 3 days at 70°C and evaporated on a rotary evaporator. Water, 100 ml, was added to the residue, the mixture was extracted with ethyl acetate (3×100 ml), the extract was dried over Na_2SO_4 and evaporated, and the residue was subjected to column chromatography on silica gel (60 g) using chloroform–methanol (30:1) as eluent to isolate 0.91 g (43%) of oxime ***E*-IV** containing less than 5% of the *Z* isomer (R_f 0.37; $CHCl_3$–MeOH, 10:1) and 0.88 g (42%) of ***Z*-V** containing less than 5% of the *E* isomer (R_f 0.50; $CHCl_3$–MeOH, 10:1).

Oxime ***E*-IV**. mp 166–168°C, $[\alpha]_D^{18}$ = –20.4° (*c* = 2.21, $CHCl_3$). IR spectrum (CCl_4), ν, cm^{-1}: 3600–3100, 1720, 1620, 1450, 1370, 1240, 1200, 1160, 1050, 960. UV spectrum ($CHCl_3$): λ_{max} 243 nm (ε = 12811). ^1H NMR spectrum, δ, ppm: in C_5D_5N: 0.92 s (3H, $C^{18}H_3$); 0.98 s (3H, $C^{19}H_3$); 1.31 s (9H, $C^{21}H_3$, $C^{26}H_3$, $C^{27}H_3$); 1.39 s, 1.41 s, 1.49 s, and 1.53 s (3H each, Me_2C); 2.96 m (1H, 9-H, $W_{1/2}$ = 25 Hz); 3.87 m (1H, 22-H, $W_{1/2}$ = 19 Hz); 4.03 m (1H, 3-H, $W_{1/2}$ = 10 Hz); 4.11 m (1H, 2-H, $W_{1/2}$ = 27 Hz); 7.17 br.s (1H, 7-H, $W_{1/2}$ = 7 Hz); in $CDCl_3$: 0.79 s (3H, $C^{18}H_3$); 0.86 s (3H, $C^{19}H_3$); 1.16 s (3H, $C^{21}H_3$); 1.25 s and 1.27 s (3H each, $C^{26}H_3$, $C^{27}H_3$); 1.33 s, 1.42 s, 1.50 s, and 1.54 s (3H each, Me_2C); 2.34 d.d (1H, 5-H, ^3J = 4.5, 13.0 Hz); 2.63 m (1H, 9-H, $W_{1/2}$ = 30 Hz); 3.67 m (1H, 22-H, $W_{1/2}$ = 16 Hz); 4.20 m (1H, 2-H, $W_{1/2}$ = 22 Hz); 4.25 m (1H, 3-H, $W_{1/2}$ = 15 Hz); 6.61 d (1H, 7-H, ^4J = 2.0 Hz). ^{13}C NMR spectrum, δ_C, ppm: in C_5D_5N: 17.4 q (C^{18}), 20.7 t (C^{11}), 22.1 t (C^{16}), 22.3 q (C^{21}), 24.05 t (C^{23}), 24.12 q (C^{19}), 26.7 q (C^{26}), 27.1 q (C^{27}), 28.9 q and 29.7 q (2,3-**Me**$_2$C), 29.3 t (C^{15}), 29.3 q and 29.9 q (20,22-**Me**$_2$C), 30.2 t (C^{12}), 31.7 t (C^4), 34.4 d (C^9), 36.7 s (C^{10}), 39.1 t (C^1), 42.0 t (C^{24}), 42.9 d (C^5), 47.7 s (C^{13}), 49.8 d (C^{17}), 69.2 s (C^{25}), 72.3 d (C^2), 72.8 d (C^3), 82.3 d (C^{22}), 84.5 s (C^{20}), 85.0 s (C^{14}), 106.7 s (20,22-Me_2**C**), 107.7 s (2,3-Me_2**C**), 109.8 d (C^7), 151.1 s (C^8), 154.6 s (C^6); in $CDCl_3$: 17.2 q (C^{18}), 19.7 t (C^{11}), 21.0 t (C^{16}), 20.2 q (C^{21}), 23.5 t (C^{23}), 23.6 q (C^{19}), 26.5 q (C^{26}), 26.8 q (C^{27}), 28.6 q and 28.9 q (2,3-**Me**$_2$C), 28.8 q and 29.3 q (20,22-**Me**$_2$C), 29.5 t (C^{12}), 31.2 t (C^4), 31.9 t (C^{15}), 34.1 d (C^9), 36.5 s (C^{10}), 38.1 t (C^1), 41.4 t (C^{24}), 42.1 d (C^5), 47.2 s (C^{13}), 49.1 d (C^{17}), 70.4 s (C^{25}), 72.1 d (C^2), 72.2 d (C^3), 82.0 d (C^{22}), 84.6 s (C^{20}), 85.6 s (C^{14}), 106.9 s (20,22-Me_2**C**), 108.0 s (2,3-Me_2**C**), 109.5 d (C^7), 151.5 s (C^8), 155.6 s (C^6). High-resolution mass spectrum: *m*/*z* 575.38360 $[M]^+$. $C_{33}H_{53}NO_7$. Calculated: *M* 575.38218. Mass spectrum, *m*/*z* (I_{rel}, %): 575 (2), 558 (9), 542 (15), 482 (14), 464 (16), 417 (19), 408 (11), 400 (22), 397 (13), 384 (19), 383 (27), 366 (12), 355 (38), 354 (100), 338 (17), 296 (26), 281 (11), 279 (23), 245 (12), 142 (32), 124 (29), 108 (15), 106 (10), 104 (11), 101 (41), 98 (35), 96 (14), 94 (12), 92 (13), 84 (12), 82 (14), 80 (29), 70 (12), 68 (25), 58 (84), 43 (71).

Oxime ***Z*-V**. mp 148–150°C, $[\alpha]_D^{18}$ = –220.8° (*c* = 3.32, $CHCl_3$). IR spectrum (Nujol), ν, cm^{-1}: 3720, 3600–3100, 1720, 1610, 1450, 1380, 1250, 1200, 1170, 1050, 960. UV spectrum ($CHCl_3$), λ_{max}, nm (ε): 285 (19172), 210 (8854). ^1H NMR spectrum, δ, ppm: in C_5D_5N: 0.94 s (3H, $C^{19}H_3$); 1.19 s (3H, $C^{18}H_3$); 1.29 s (3H, $C^{21}H_3$); 1.42 s (6H, $C^{26}H_3$, $C^{27}H_3$); 1.39 s, 1.44 s, 1.51 s, and 1.63 s (3H each, Me_2C); 3.83 d.d (1H, 5-H, ^3J = 4.5, 13.0 Hz); 3.94 m (1H, 22-H, $W_{1/2}$ = 14 Hz); 4.37 m (1H, 2-H, $W_{1/2}$ = 19 Hz); 4.42 m (1H, 3-H, $W_{1/2}$ = 12 Hz); 5.80 br.s (1H, 15-H, $W_{1/2}$ = 6 Hz); 6.69 d (1H, 7-H, ^4J = 1.9 Hz); in $CDCl_3$: 0.85 s (3H, $C^{19}H_3$); 1.00 s (3H, $C^{18}H_3$); 1.18 s (3H, $C^{21}H_3$); 1.21 s (6H, $C^{26}H_3$, $C^{27}H_3$); 1.28 s, 1.32 s, 1.40 s, and 1.51 s (3H each, Me_2C); 2.54 m (1H, 9-H, $W_{1/2}$ = 23 Hz); 3.27 d.d (1H, 5-H, ^3J = 4.5, 13.0 Hz); 3.72 m (1H, 22-H, $W_{1/2}$ = 20 Hz); 4.11 m (1H, 2-H, $W_{1/2}$ = 27 Hz); 4.20 br.s (1H, 3-H, $W_{1/2}$ = 14 Hz); 5.75 br.s (1H, 15-H, $W_{1/2}$ = 7 Hz); 6.21 br.s (1H, 7-H, $W_{1/2}$ = 6 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 18.8 q (C^{18}), 20.6 t (C^{11}), 21.2 q (C^{19}), 23.4 q (C^{21}), 23.6 t (C^{23}), 25.9 t (C^{16}), 26.4 q (C^{26}), 26.8 q (C^{27}), 28.6 q and 29.2 q (2,3-**Me**$_2$C), 28.9 q and 29.3 q (20,22-**Me**$_2$C), 31.2 t (C^4), 35.9 s (C^{10}), 36.3 d (C^9), 36.4 t (C^{12}), 37.9 d (C^5), 39.8 t (C^1), 41.2 t (C^{24}), 47.2 s (C^{13}), 57.5 d (C^{17}), 70.4 s (C^{25}), 72.1 d (C^3), 72.2 d (C^2), 81.7 d (C^{22}), 83.5 s (C^{20}), 106.9 s (20,22-Me_2**C**), 108.1 s (2,3-Me_2**C**), 116.7 d (C^{15}), 123.1 d (C^7), 139.5 s (C^{14}), 149.5 s (C^8), 159.1 s (C^6). High-resolution mass spectrum: *m*/*z* 557.37240 $[M]^+$. $C_{33}H_{51}NO_6$. Calculated: *M* 557.37161. Mass spectrum, *m*/*z* (I_{rel}, %): 557 (2), 543 (3), 542 (7), 482 (12), 464 (18), 400 (12), 397 (10), 384 (16), 383 (25), 366 (12), 356 (59), 355 (100), 338 (20), 298 (13), 297 (27), 280 (22), 143 (16), 125 (12), 101 (18), 98 (31), 85 (12), 83 (17), 81 (20), 68 (18), 59 (77), 43 (72).

14,15-Anhydro-20-hydroxyecdysone (6*Z*)- and (6*E*)-oximes *Z*-VI and *E*-VI. Hydroxylamine hydrochloride, 1.0 g (14.4 mmol), was added under stirring to a solution of 1.0 g (2.1 mmol) of 20-hydroxyecdysone (**I**) in 25 ml of freshly distilled pyridine, and the mixture was heated for 3 days at 70°C and evaporated on a rotary evaporator. Water, 50 ml, was added to the residue, the mixture was extracted with ethyl acetate (3 × 50 ml), the extract was dried over Na_2SO_4 and evaporated, and the residue was subjected to column chromatography on silica gel (50 g) using chloroform–methanol (7:1) as eluent. Yield of oxime mixture *Z*-**VI**/*E*-**VI** 0.9 g (87%, ratio 1:2.5), R_f 0.22 ($CHCl_3$–MeOH, 3:1). Mass spectrum, *m*/*z* (I_{rel}, %): 462 (6), 461 (20), 428 (10), 424 (14), 360 (25), 344 (29), 343 (36), 342 (21), 326 (29), 318 (13), 316 (35), 315 (22), 310 (15), 300 (19), 299 (12), 298 (17), 284 (10), 266 (15), 224 (12), 212 (12), 210 (11), 198 (11), 171 (10), 170 (11), 143 (14), 129 (11), 105 (10), 99 (32), 91 (11), 83 (13), 81 (39), 71 (14), 69 (24), 59 (26), 55 (38), 45 (17), 44 (26), 43 (100).

14,15-Anhydro-20-hydroxyecdysone (6*Z*)-oxime (*Z*-VI). Hydroxylamine hydrochloride, 2.0 g (28.8 mmol), was added under stirring to a solution of 2.0 g (4.2 mmol) of 20-hydroxyecdysone (**I**) in 20 ml of freshly distilled pyridine, and the mixture was heated for 3 days at 70°C. The mixture was then cooled to 0°C, a solution of 1.6 g (28.8 mmol) of potassium hydroxide in 16 ml of anhydrous ethanol was added under stirring, and the mixture was evaporated on a rotary evaporator. Water, 100 ml, was added to the residue, the mixture was extracted with ethyl acetate (3 × 100 ml), and the extract was dried over Na_2SO_4 and evaporated. The residue was 2.0 g of a mixture of oximes *E*-**III** and *Z*-**VI** at a ratio of ~1:2.5; it was mixed with 50 ml of anhydrous methanol, 2.0 mg of phosphomolybdic acid was added, and the mixture was stirred for 1 h, concentrated to 1/2 of the initial volume, diluted with 20 ml of distilled water, treated with 20 ml of a 2% solution of $NaHCO_3$, and extracted with ethyl acetate (3 × 100 ml). The extracts were combined and evaporated on a rotary evaporator to isolate 1.7 g (86%) of oxime *Z*-**VI**, mp 166–168°C, $[\alpha]_D^{18}$ = –197.4° (*c* = 0.27, MeOH). IR spectrum (Nujol), ν, cm^{-1}: 3600, 2900, 1640, 1450, 1370, 1060, 960. UV spectrum (MeOH), λ_{max}, nm (ε): 212 (9263), 283 (15906). ^1H NMR spectrum, δ, ppm: in CD_3OD: 0.87 s (3H, $C^{19}H_3$), 1.08 s (3H, $C^{18}H_3$), 1.18 s and 1.20 s (3H each, $C^{26}H_3$, $C^{27}H_3$), 1.23 s (3H, $C^{21}H_3$), 3.31 m (1H, 22-H, $W_{1/2}$ = 21 Hz), 3.75 m (1H, 2-H, $W_{1/2}$ = 25 Hz), 3.91 br.s (1H, 3-H, $W_{1/2}$ = 9 Hz), 5.78 br.s (1H, 15-H, $W_{1/2}$ = 5 Hz), 6.17 d (1H, 7-H, ^4J = 2.0 Hz); in C_5D_5N: 1.00 s (3H, $C^{19}H_3$), 1.35 s (3H, $C^{18}H_3$), 1.40 s and 1.41 s (3H each, $C^{26}H_3$, $C^{27}H_3$), 1.51 s (3H, $C^{21}H_3$), 2.86 m (1H, 9-H, $W_{1/2}$ = 30 Hz), 2.99 t (1H, 17-H, ^3J = 8.1 Hz), 3.79 m (1H, 22-H, $W_{1/2}$ = 16 Hz), 4.13 d.d (1H, 5-H, ^3J = 3.0, 12.0 Hz), 4.25 m (1H, 2-H, $W_{1/2}$ = 23 Hz), 4.48 m (1H, 3-H, $W_{1/2}$ = 12 Hz), 5.85 br.s (1H, 15-H, $W_{1/2}$ = 7 Hz), 6.67 br.s (1H, 7-H, $W_{1/2}$ = 6 Hz). ^{13}C NMR spectrum, δ_C, ppm: in CD_3OD: 19.6 q (C^{18}), 20.4 q (C^{19}), 21.8 t (C^{11}), 24.3 q (C^{21}), 27.3 t (C^{23}), 28.8 q (C^{26}), 29.8 q (C^{27}), 31.3 t (C^4), 31.3 t (C^{16}), 36.7 d (C^9), 37.3 s (C^{10}), 37.8 t (C^{12}), 39.1 d (C^5), 41.5 t (C^1), 42.1 t (C^{24}), 48.5 s (C^{13}), 58.8 d (C^{17}), 68.5 d (C^3), 69.3 d (C^2), 71.2 s (C^{25}), 77.2 s (C^{20}), 78.4 d (C^{22}), 117.4 d (C^{15}), 124.0 d

(C^7), 141.8 s (C^{14}), 151.2 s (C^8), 160.6 s (C^6); in C_5D_5N: 19.6 q (C^{18}), 20.5 q (C^{19}), 21.1 t (C^{11}), 24.2 q (C^{21}), 26.9 t (C^{16}), 29.5 q (C^{26}), 30.3 q (C^{27}), 30.8 t (C^{23}), 31.3 t (C^4), 36.2 d (C^9), 36.6 s (C^{10}), 37.3 t (C^{12}), 38.2 d (C^5), 40.5 t (C^1), 42.3 t (C^{24}), 47.7 s (C^{13}), 58.1 d (C^{17}), 68.0 d (C^3), 68.9 d (C^2), 69.5 s (C^{25}), 76.0 s (C^{20}), 77.4 d (C^{22}), 118.0 d (C^{15}), 122.7 d (C^7), 139.3 s (C^{14}), 150.6 s (C^8), 158.9 s (C^6). Found, %: C 67.67; H 9.01; N 3.07. $C_{27}H_{43}NO_6$. Calculated, %: C 67.90; H 9.07; N 2.93.

2,3:20,22-Di-*O*-isopropylidene-14,25-*O*-bis(trimethylsilyl)-20-hydroxyecdysone (6*Z*)- and (6*E*)-oximes *Z*-VII and *E*-VII. Hydroxylamine hydrochloride, 0.2 g (2.87 mmol), was added under stirring to a solution of 0.2 g (0.36 mmol) of diacetonide **II** in 2 ml of freshly distilled pyridine, and the mixture was heated for 3 days at 70°C. The mixture was cooled to 0°C, a solution of 0.16 g (2.87 mmol) of potassium hydroxide in 1.6 ml of anhydrous ethanol was added, and the mixture was evaporated on a rotary evaporator. Water, 20 ml, was added to the residue, the mixture was extracted with ethyl acetate (3×30 ml), and the extract was dried over Na_2SO_4 and evaporated to obtain 0.18 g of oxime mixture *Z*-**IV**/*E*-**IV** (1:1). The oxime mixture was treated at 0°C with a solution of 0.42 g (3 mmol) of trimethyl(trifluoromethyl)silane in 3 ml of anhydrous tetrahydrofuran, 0.8 mol % of tetrabutylammonium fluoride was added, and the reaction was complete in 3 min (TLC). The mixture was evaporated on a rotary evaporator, and the residue was subjected to column chromatography on silica gel (5 g) using chloroform as eluent. We isolated 0.13 g (50%) of oxime *Z*-**VII** (containing less than 5% of the *E* isomer, according to the ^{1}H NMR data), R_f 0.73 ($CHCl_3$–MeOH, 10:1) and 0.07 g (44%) of oxime *E*-**VII** (containing less than 5% of the *Z* isomer), R_f 0.61 ($CHCl_3$–MeOH, 10:1).

Oxime *Z*-**VII**. mp 109–111°C, $[\alpha]_D^{18}$ = –286.5° (*c* = 6.2, $CHCl_3$). IR spectrum (KBr), ν, cm^{-1}: 3400, 1615, 1445, 1370, 1245, 1050, 835. UV spectrum (MeOH): λ_{max} 242 nm (ε = 10812). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.06 s and 0.11 s (9H each, Me_3Si); 0.73 s (3H, $C^{18}H_3$); 0.89 s (3H, $C^{19}H_3$); 1.15 s (3H, $C^{21}H_3$); 1.23 s and 1.25 s (3H each, $C^{26}H_3$, $C^{27}H_3$); 1.32 s, 1.36 s, 1.41 s, and 1.55 s (3H each, Me_2C); 2.53 m (1H, 9-H, $W_{1/2}$ = 16 Hz); 3.31 d.d (1H, 5-H, ^3J = 4.5, 13.0 Hz); 3.64 m (1H, 22-H, $W_{1/2}$ = 13 Hz); 4.17 m (1H, 2-H, $W_{1/2}$ = 24 Hz); 4.26 br.s (1H, 3-H, $W_{1/2}$ = 8 Hz); 5.88 d (1H, 7-H, ^4J = 2.0 Hz). ^{13}C NMR spectrum, δ_C, ppm ($CDCl_3$): 1.9 q and 2.6 q (2$SiMe_3$), 16.4 q (C^{18}), 20.3 t (C^{11}), 21.6 t (C^{16}), 21.9 q (C^{21}), 23.7 t (C^{23}), 24.0 q (C^{19}), 25.9 t (C^{12}), 26.5 q (C^{26}), 26.9 q (C^{27}), 28.7 q and 29.4 q (2,3-**Me**$_2$C), 29.0 q and 30.2 q (20,22-**Me**$_2$C), 30.1 t (C^4), 31.1 t (C^{15}), 33.3 d (C^9), 35.8 s (C^{10}), 36.3 d (C^5), 38.0 t (C^1), 42.1 t (C^{24}), 48.6 s (C^{13}), 49.5 d (C^{17}), 72.2 d (C^2), 72.3 d (C^3), 73.5 s (C^{25}), 81.8 d (C^{22}), 84.4 s (C^{20}), 88.0 s (C^{14}), 106.5 s (20,22-Me_2**C**), 108.2 s (2,3-Me_2**C**), 117.3 d (C^7), 148.5 s (C^8), 159.4 s (C^6). Found, %: C 64.78; H 9.77; N 2.08. $C_{39}H_{69}NO_7Si_2$. Calculated, %: C 65.05; H 9.66; N 1.94.

Oxime *E*-**VII**. mp 110°C, $[\alpha]_D^{18}$ = +22.6° (*c* = 9.7, $CHCl_3$). IR spectrum (KBr), ν, cm^{-1}: 3400, 1615, 1445, 1370, 1245, 1050, 835. UV spectrum (MeOH): λ_{max} 242 nm (ε = 11038). ^{1}H NMR spectrum, δ, ppm: in C_5D_5N: 0.21 s (18H, Me_3Si); 0.89 s (3H, $C^{18}H_3$); 0.93 s (3H, $C^{19}H_3$); 1.28 s (9H, $C^{21}H_3$, $C^{26}H_3$, $C^{27}H_3$); 1.42 s, 1.46 s, 1.52 s, and 1.61 s (3H each, Me_2C); 2.68 m (1H, 9-H, $W_{1/2}$ = 20 Hz); 2.79 d.d (1H, 5-H, ^3J = 4.5, 13.0 Hz); 3.84 m (1H, 22-H, $W_{1/2}$ = 16 Hz); 4.37 m (1H, 2-H, $W_{1/2}$ = 27 Hz); 4.44 br.s (1H, 3-H, $W_{1/2}$ = 14 Hz); 7.14 s (1H, 7-H); in $CDCl_3$: 0.08 s and 0.11 s (9H each, Me_3Si); 0.74 s (3H, $C^{18}H_3$); 0.87 s (3H, $C^{19}H_3$); 1.14 s (3H, $C^{21}H_3$); 1.23 s and 1.24 s (3H each, $C^{26}H_3$, $C^{27}H_3$); 1.33 s, 1.35 s, 1.41 s, 1.51 s (3H each, Me_2C); 2.35 d.d (1H, 5-H, ^3J = 4.5, 13.0 Hz); 2.51 m (1H, 9-H, $W_{1/2}$ = 9 Hz); 3.64 m (1H, 22-H, $W_{1/2}$ = 15 Hz); 4.16 m (1H, 2-H, $W_{1/2}$ = 26 Hz); 4.25 br.s (1H, 3-H, $W_{1/2}$ = 7 Hz); 6.56 s (1H, 7-H). ^{13}C NMR spectrum, δ_C, ppm ($CDCl_3$): 1.9 q and 2.6 q ($SiMe_3$), 16.5 q (C^{18}), 20.3 t (C^{16}), 21.6 t (C^{11}), 21.9 q (C^{21}), 23.7 t (C^{23}), 23.9 q (C^{19}), 26.5 q (C^{26}), 26.8 q (C^{27}), 28.6 q and 29.4 q (2,3-**Me**$_2$C), 28.7 t (C^{12}), 29.0 q and 30.2 q (20,22-**Me**$_2$C), 30.0 t (C^4), 31.3 t (C^{15}), 34.6 d (C^9), 36.6 s (C^{10}), 38.2 t (C^1), 42.1 t (C^{24}), 42.2 d (C^5), 49.0 s (C^{13}), 49.5 d (C^{17}), 72.3 d (C^2), 72.3 d (C^3), 73.5 s (C^{25}), 81.8 d (C^{22}), 84.4 s (C^{20}), 88.4 s (C^{14}), 106.5 s (20,22-Me_2**C**), 108.1 s (2,3-Me_2**C**), 109.8 d (C^7), 151.4 s (C^8), 155.4 s (C^6). Found, %: C 64.93; H 9.56; N 2.02. $C_{39}H_{69}NO_7Si_2$. Calculated, %: C 65.05; H 9.66; N 1.94.

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