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Regio and Stereo Directional Oxidation of Ecdysteroids and Their 7,8-Dihydroanalogs with Ozone in Pyridine*

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Abstract—The oxidation of ecdysteroids and their 7,8-dihydroanalogs with ozone in pyridine occurred regio- and stereoselectively at the hydroxy groups of the A ring giving 2-dehydro-3α-hydroxy derivatives.

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The oxidation of ecdysteroids at the hydroxy groups of the A ring is a promising process for the synthesis of difficultly available ecdysteroids [2]. The catalytic oxidation of 20-hydroxyecdysone with oxygen over platinum furnished 3-dehydro-20-hydroxyecdysone [3] isolated from the plants of species *Calliphora erythrocephala* [4]. In oxidation of 20-hydroxyecdysone with sodium periodate and compounds of six-valent chromium the main reaction path was the cleavage of the C^{20}–C^{22} bond to form poststerone [5–8]. The selective oxidation of the hydroxy groups in the A ring was not achieved also after the protection of hydroxy groups in the side chain by their conversion into 20,22-acetonide [9]. As a result the synthesis of 2-dehydro-3-epi-20-hydroxyecdysone, minor ecdysteroid isolated from the seeds of the plant *Froelichia floridana* [10] was performed in 6 stages with an overall yield of 7% [9].

The spatially hindered Δ^7-bond conjugated with 6-oxo group in the ecdysteroids is well known to be chemically inert toward ozone [2, 11], but the secondary alcohol groups present in the ecdysteroids can add an ozone molecue at the C–H bond with the formation of α-hydroxyhydrotrioxide whose subsequent decomposition should lead to the corresponding ketone [12].

We investigated the possibility of oxidation of ecdysteroids and their 7,8-dihydroanalogs with ozone. The ozonation of 20-hydroxyecdysone 20,22-acetonide (**I**) in pyridine at room temperature at 50% conversion of the initial compound provided in ~40% yield 2-dehydro-3-epi-20-hydroxyecdysone 20,22-acetonide (**II**) (Scheme 1) [1].

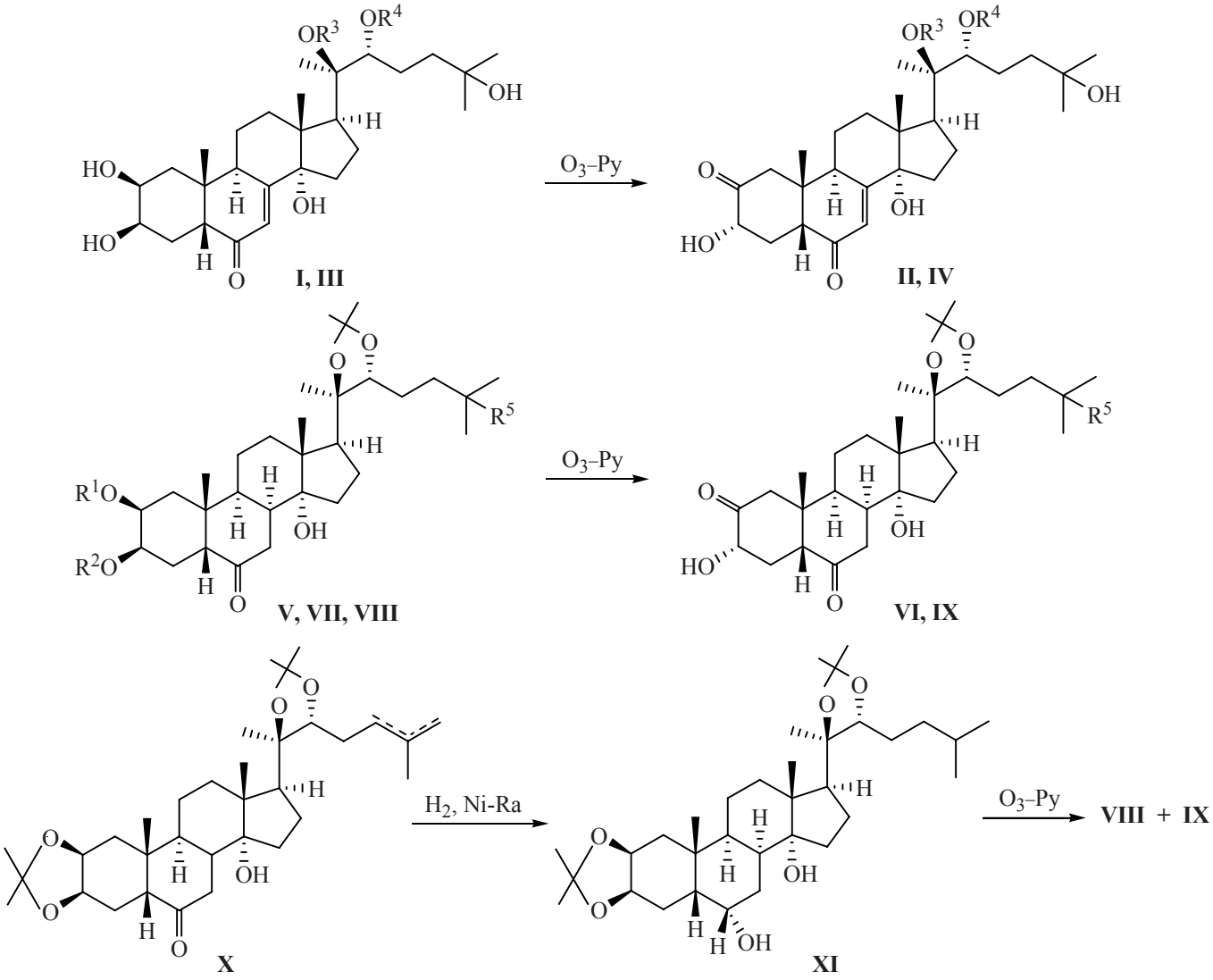
Under the same conditions, with the same yield and the same selectivity 2-dehydro-3-epi-20-hydroxyecdysone (**IV**) was obtained from 20-hydroxyecdysone (**III**) although the latter contained an additional secondary hydroxy group (in the side chain). Thus a one-stage synthesis procedure was discovered for phyto-ecdysteroid **IV** [1], which had been previously obtained from the same initial compound **III** by significantly more difficult method [9].

The structure of compounds **II** and **IV** synthesized was proved by 1D and 2D ^1H and ^{13}C NMR spectra [1]. The attempts to use at the ozonation of compounds **I** and **III** the other solvents instead of pyridine (CH_2Cl_2, $CHCl_3$, CCl_2FCClF_2, MeOH, AcOH) proved to be unsuccessful: at the prolonged ozonation we obtained only complex mixture of polar compounds. The performance of the ozonation in pyridine to higher conversion of the initial compound resulted not in the higher yield of the target product but in the formation of a mixture of more polar compounds.

The oxidation selectivity at ecdysteroids ozonation in pyridine apparently is caused by the formation of a complex of ozone with pyridine [13], less reactive than ozone proper. However since such complexes are unstable, no reaction of compounds **I** and **III** proceeds in pyridine preliminary saturated with ozone.

*For preliminary communication, see [1].

Scheme 1.



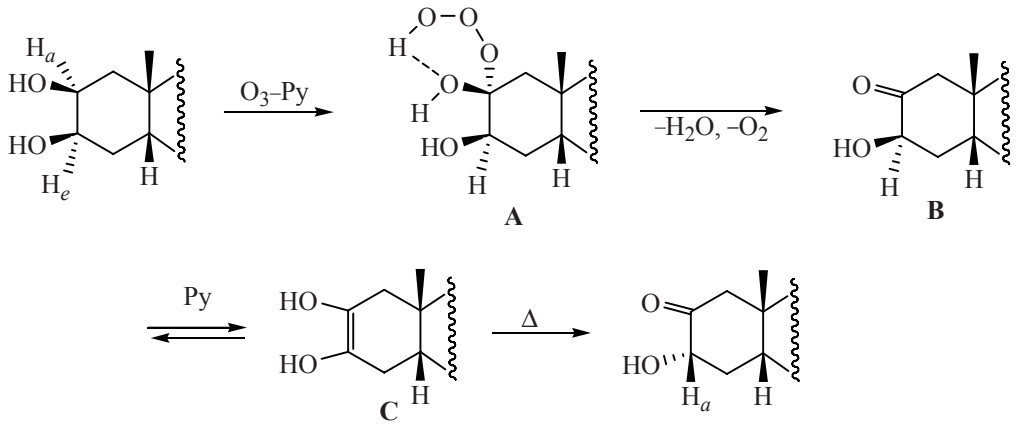
$R^1 = R^2 = H$ (**V**); $R^1 + R^2 = Me_2C$ (**VII, VIII**); $R^3 = R^4 = H$ (**III, IV**); $R^3 + R^4 = Me_2C$ (**I, II**); $R^5 = OH$ (**V–VII**), H (**VIII, IX**).

The prevalence of the reaction of compounds **I** or **III** at the hydroxy group attached to atom C^2 is due apparently to the attack on the spatially more accessible axial bond H–C^2 with the formation of α-hydroxyhydrotrioxide **A** stabilized by an intramolecular hydrogen bond. In its decomposition (with the elimination of a water molecule and a singlet oxygen) formed 2-oxo derivative **B** [12] that in the pyridine environment suffered epimerization [14] through endiol **C** [9] giving evidently the more stable 2-oxo-3α-hydroxy derivative with an equatorial hydroxy group at C^3 atom (Scheme 2).

The ozonation in pyridine of 7,8-dihydroanalog of 20-hydroxyecdysone 20,22-acetonide (**V**) proceeded similarly to the reaction of compound **I** and provided at the same conversion the same yield of 2-dehydro-3-epi-7,8-dihydro-20-hydroxyecdysone 20,22-acetonide (**VI**) whose structure was proved by ^1H and ^{13}C NMR spectra analogous to the spectra of compound **II** published in [1].

Unlike 20-hydroxyecdysone 2,3:20,22-diacetonide that did not enter into the studied reaction [1], its 7,8-dihydroanalog **VII** at the ozonation in pyridine was cleanly converted into the same compound **VI** obtained from 20,22-acetonide **V**. Analogously the ozonation of 7,8α-dihydroponasterone 2,3:20,22-diacetonide **A** (**VIII**) resulted in 2-dehydro-3-epi-7,8α-dihydroponasterone

Scheme 2.



20,22-acetonide A (**IX**) in 60% yield. The changes in the spectral characteristics of compound **IX** with respect to the spectra of initial compound **VIII** were the same as in 2-dehydro-3-epi derivatives [1].

As seen, in diacetonides of 7,8-dihydroanalogs **VII** and **VIII** the bond H–C^2 in 2,3-isopropylidenedioxide rings is more available for ozone attack than in 20-hydroxyecdysone diacetonide [1].

7,8-Dihydroanalog **VIII** was synthesized in two stages from the previously obtained [15] mixture of diacetonides of Δ24(25)- and Δ25(26)-7,8α-dihydroecdysones **X**. The hydrogenation of compounds **X** over Raney nickel gave 6-dihydroponasterone diacetonide **A XI**. Its ozonation in pyridine provided a mixture of compounds **VIII** and **IX** separated by colum chromatography. The ^{13}C NMR spectrum of compound **VIII** is close to the spectrum of the 7,8α-dihydro-20-hydroxyecdysone diacetonide, and the ^{13}C NMR spectrum of compound **XI** resembles the spectrum of the corresponding 25-hydroxy compound [15]. The exclusion form the signals of atoms C^{25}, C^{26}, and C^{27} that occupy the position similar to the corresponding signals in the spectrum of ponasterone diacetonide A [16].

EXPERIMENTAL

^1H and ^{13}C NMR spectra were registered on a spectrometer Bruker Avance-400 (operating frequencies 400.13, 100.62 MHz respectively) in $CDCl_3$. Homo- and heteronuclear experiments DEPT-135°, COSY, HSQC, and HMBC were performed on the spectrometer Bruker Avance-400, internal reference TMS. Melting points were measured on a Boetius heating block. The specific optical rotation was recorded on a polarimeter Perkin Elmer-141. TLC was carried out on plates with SiO_2 (Silufol), spots were visualized with vanillin solution in ethanol acidified with sulfuric acid.

The synthesis and characteristics of compounds **II** and **IV** are described in [1], of compounds **V, VII, X**, in [15].

(20*R*,22*R*)-2-Oxo-3α,14α,25-trihydroxy-20,22-(isopropylidenedioxy)-5β,8α-cholestan-6-one (2-dehydro-3-epi-7,8α-dihydro-20-hydroxyecdysone 20,22-acetonide) (VI). *a*. Through a solution of 0.05 g (0.096 mmol) of compound **V** in 2 ml of anhydrous pyridine at 0°C while stirring was passed a flow of ozone-oxygen mixture for 15 min at a rate 70 mmol/min (10 mmol of O_3, ozonizer output 30 mmol of O_3 per hour). The reaction mixture was flushed with argon and evaporated, The residue was subjected to column chromatography (20 g of SiO_2, eluent $CHCl_3$). We obtained 0.015 g (30%) of initial compound **V** (R_f 0.6, $CHCl_3$–MeOH, 5 : 1) and 0.022 g (44%) of compound **VI**, R_f 0.28 ($CHCl_3$–MeOH, 10 : 1), mp 114–116°C, $[\alpha]_D^{20}$ +11.7° (*c* 1.11, $CHCl_3$). ^1H NMR spectrum, δ, ppm: 1.07 s (3H, H_3C^{18}), 1.15 s (3H, H_3C^{19}), 1.15 s (3H, H_3C^{21}), 1.24 s (3H, H_3C^{26}), 1.24 s (3H, H_3C^{27}), 1.32 s and 1.42 s (6H, Me_2C), 1.48–1.84 m (15H, CH, CH_2), 1.56 m (2H, H_2C^4), 1.84 m (2H, H_2C^1), 2.05 d (1H, $H^{7\alpha}$, *J* 13.4 Hz), 2.71 br.s (1H, H^5, $W_{1/2}$ 8.4 Hz), 2.83 d (1H, $H^{7\beta}$, *J* 13.4 Hz), 3.68 m (1H, H^{22}), 4.46 m (1H, H^3). ^{13}C NMR spectrum, δ, ppm: 18.47 (C^{18}), 18.66 (C^{11}), 21.09 (C^{16}), 21.43 (C^{21}), 23.58 (C^{23}), 26.75 (C^{19}), 28.97 (C^{27}), 29.20 (Me_2CO_2), 29.61 (C^{26}), 30.35 (C^4), 31.52 (C^{15}), 33.09 (C^{12}), 41.19 (C^7), 41.38 (C^{24}), 41.59 (C^9), 44.08 (C^8), 46.35 (C^{10}), 47.03 (C^{13}), 48.39 (C^1), 49.88 (C^5), 49.93 (C^{17}), 70.33 (C^{25}), 71.77 (C^3), 81.91 (C^{22}), 84.45 (C^{14}), 84.71 (C^{20}), 107.60 (20,22-Me_2CO_2), 210.30 (C^2), 211.10 (C^6).

b. The ozonation of 0.07 g (0.13 mmol) of compound **VII** in 3 ml of anhydrous pyridine by passing the mixture O_3/O_2 (10 mmol O_3) was carried out for 15 min. The reaction mixture was worked up as described for procedure *a*. We obtained 0.02 g (28%) of initial compound **VII**, R_f 0.63 ($CHCl_3$–MeOH, 10:1) and 0.03 g (46%) of compound **VI** identical according to ^1H and ^{13}C NMR spectra to compound obtained by procedure *a*.

(20*R*,22*R*)-14α-Hydroxy-2β,3β:20,22-bis-(isopropylidenedioxy)-5β,8α-cholestan-6-one (7,8α-dihydroponasterone A 2,3:20,22-diacetonide) (VIII) and (20*R*,22*R*)-2-oxo-3α,14α-dihydroxy-20,22-(isopropylidenedioxy)-5β,8α-cholestan-6-one (2-dehydro-3-epi-7,8α-dihydroponasterone A 20,22-acetonide) (IX). The ozonation of 0.08 g (0.15 mmol) of compound **XI** in 3 ml of anhydrous pyridine by passing the mixture O_3/O_2 (10 mmol O_3) was carried out for 7 min. The reaction mixture was worked up as described for procedure *a*. We obtained 0.05 g (63%) of compound **VIII**, R_f 0.56 ($CHCl_3$–MeOH, 40:1) and 0.012 g of compound **IX**, R_f 0.59 ($CHCl_3$–MeOH, 20:1).

Compound VIII. mp 249–250°C, $[\alpha]_D^{20}$ +17.24° (*C* 2.4, $CHCl_3$). ^1H NMR spectrum, δ, ppm: 0.88 s (3H, H_3C^{26}), 0.89 s (3H, H_3C^{27}), 1.12 s (3H, H_3C^{18}), 1.12 s (3H, H_3C^{19}), 1.29 s, 1.30 s, 1.41 s, 1.51 s (12H, Me_2C), 1.39 s (3H, H_3C^{21}), 1.55–2.06 m (18H, CH, CH_2), 2.15 d.d (1H, $H^{7\alpha}$, *J* 13.6 Hz), 2.18 m (1H, H^{17}), 2.36 m (1H, H^8), 2.58 br.s (1H, H^5, $W_{1/2}$ 11.6 Hz), 2.72 m (1H, $H^{7\beta}$, *J* 13.6 Hz), 3.63 m (1H, H^{22}), 4.21 t (1H, H^3, *J* 22.4 Hz), 4.51 m (1H, H^2, $W_{1/2}$ 13.5 Hz). ^{13}C NMR spectrum, δ, ppm: 18.15 (C^{11}), 18.55 (C^{18}), 21.14 (C^{16}), 21.43 (C^{21}), 22.47 and 22.55 (2,3-Me_2CO_2), 25.43 (C^{23}), 26.77 (C^{19}), 26.01 and 26.77 (20,22-Me_2CO_2), 26.82 (C^4), 28.25 (C^{27}), 28.52 (C^{26}), 29.05 (C^{25}), 31.51 (C^{15}), 33.31 (C^{12}), 34.38 (C^{24}), 36.41 (C^1), 39.60 (C^{10}), 41.36 (C^7), 41.70 (C^9), 43.86 (C^8), 46.92 (C^{13}), 49.96 (C^{17}), 50.60 (C^5), 70.90 (C^3), 73.56 (C^2), 81.59 (C^{22}), 84.19 (C^{20}), 85.15 (C^{14}), 106.70 (20,22-Me_2CO_2), 107.70 (2,3-Me_2CO_2), 212.02 (C^6).

Compound IX. mp 115°C, $[\alpha]_D^{20}$ +9.42° (*C* 0.76, $CHCl_3$). ^1H NMR spectrum, δ, ppm: 0.90 s (3H, H_3C^{26}), 0.91 s (3H, H_3C^{27}), 1.08 s (3H, H_3C^{18}), 1.13 s (3H, H_3C^{19}), 1.15 sC (3H, H_3C^{21}), 1.32 s and 1.42 s (6H, Me_2C), 1.57 m (2H, H_2C^4), 1.61 m (2H, H_2C^{23}), 1.85 m (2H, H_2C^1), 1.52–2.50 m (13H, CH, CH_2), 2.19 m (1H, $H^{7\alpha}$, *J* 14.4 Hz), 2.38 d.t (1H, H^8, ^2J 13, ^3J 4.0 Hz), 2.73 br.s (1H, H^5, $W_{1/2}$ 8.4 Hz), 2.88 m (1H, $H^{7\beta}$, *J* 14.4 Hz), 3.64 m (1H, H^{22}), 4.47 m (1H, H^3). ^{13}C NMR spectrum, δ, ppm: 18.61 (C^{18}), 18.87 (C^{11}), 18.88 (C^{19}), 21.07 (C^{16}), 21.45 (C^{21}), 22.47 (C^{27}), 22.57 (C^{26}), 25.32 (C^{25}), 26.81 (C^{23}), 28.26 and 29.05 (20,22-Me_2CO_2), 30.35 (C^4), 31.60 (C^{15}), 33.10 (C^{12}), 36.44 (C^{24}), 41.64 (C^9), 44.13 (C^8), 46.39 (C^{10}), 46.91 (C^{13}), 48.38 (C^1), 49.93 (C^5), 49.93 (C^{17}), 71.77 (C^3), 81.61 (C^{22}), 84.12 (C^{14}), 84.86 (C^{20}), 106.78 (20,22-Me_2CO_2), 210.32 (C^2), 211.62 (C^6).

Compound **IX** was also obtained by ozonation of compound **VIII** in 2 ml of anhydrous pyridine by bubbling the mixture O_3/O_2 (10 mmol O_3) for 5 min. The reaction mixture was worked up as described for procedure *a*. We obtained 0.019 g (27%) of initial compound **VIII** and 0.027 g (43%) of compound **IX**.

(20*R*,22*R*)-6α,14α-Dihydroxy-2β,3β:20,22-bis-(isopropylidenedioxy)-5β,8α-cholestane (XI). Through a dispersion of 0.2 g (0.37 mmol) of a mixture of alkenes **X** and 2 g of Raney nickel in 5 ml of ethanol was passed hydrogen at room temperature (~24 h, the completion of the reaction was monitored by TLC), then the catalyst was filtered off, the filtrate was evaporated, and the residue was subjected to chromatography on a column packed with 25 g of SiO_2 (eluent $CHCl_3$–MeOH, 40:1). Yield 0.08 g (42%), R_f 0.45 ($CHCl_3$–MeOH, 30:1), mp 206–208°C, $[\alpha]_D^{20}$ +21.8 (*C* 9.8, $CHCl_3$). ^1H NMR spectrum, δ, ppm: 0.82 s (3H, H_3C^{26}), 0.84 s (3H, H_3C^{27}), 0.98 s (3H, H_3C^{18}), 1.01 s (3H, H_3C^{21}), 1.14 s (3H, H_3C^{19}), 1.25 s, 1.35 s, 1.45 s (12H, Me_2C), 1.49–1.93 m (21H, CH, CH_2), 2.12 m (1H, H^{17}, $W_{1/2}$ 6 Hz), 2.30 m (1H, H^8, $W_{1/2}$ 28 Hz), 3.58 m (1H, H^{22}, $W_{1/2}$ 15 Hz), 3.93 br.s (1H, H^6, $W_{1/2}$ 8 Hz), 4.28 m (1H, H^2, $W_{1/2}$ 10 Hz), 4.56 m (1H, H^3, $W_{1/2}$ 15 Hz). ^{13}C NMR spectrum, δ, ppm: 18.23 (C^{11}), 18.64 (C^{18}), 21.56 (C^{16}), 21.60 (C^{21}), 22.66 (C^{27}), 22.71 (C^{26}), 26.06 and 28.79 (2,3-Me_2CO_2), 26.97 (C^{23}), 26.97 and 29.25 (20,22-Me_2CO_2), 27.42 (C^{19}), 28.42 (C^{25}), 31.20 (C^{15}), 31.40 (C^4), 33.63 (C^{10}), 33.79 (C^7), 34.31 (C^{12}), 36.61 (C^1), 36.78 (C^{24}), 36.97 (C^8), 41.52 (C^5), 42.64 (C^9), 46.85 (C^{13}), 50.14 (C^{17}), 72.23 (C^6), 72.84 (C^3), 74.87 (C^2), 81.82 (C^{22}), 84.60 (C^{14}), 86.42 (C^{20}), 106.74 (20,22-Me_2CO_2), 107.22 (2,3-Me_2CO_2).

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