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Regio- and stereodirected transformation of 20-hydroxyecdysone to 2-dehydro-3-*epi*-20-hydroxyecdysone under ozonization in pyridine

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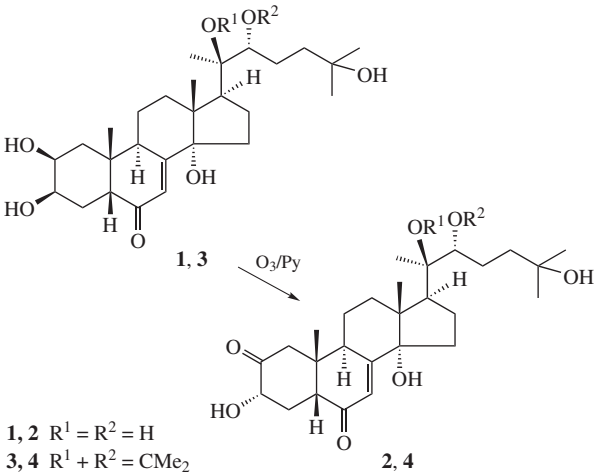
A minor ecdysteroid from *Froelichia floridana*, namely, 2-dehydro-3-*epi*-20-hydroxyecdysone has been obtained by the one stage ozonization of 20-hydroxyecdysone in pyridine.

The oxidation of 20-hydroxyecdysone **1** and its derivatives directed toward carbon atoms bearing hydroxy groups opens a way to the synthesis of natural ecdysteroids.[1] 3-Dehydro-20-hydroxyecdysone,[2] ecdysteroid isolated from *Calliphora erythrocephala*[3] and some other species of insects[4] were obtained under oxygen action catalysed by Pt. The synthesis of 2-dehydro-3-*epi*-20-hydroxyecdysone **2**, a minor ecdysteroid, which was isolated from the seeds of *Froelichia floridana*,[5] is complicated as a result of preferable cleavage of the C(20)–C(22) bond in **1** by the action of various oxidants to form poststerone. The oxidation of 20-hydroxyecdysone 20,22-acetonide or 2-acetate proceeds ambiguously. As a result, ecdysteroid **2** (amorphous product) has been obtained from **1** by a six stage procedure in 7% total yield.[6]

Here we report a new synthesis of ecdysteroid **2** (crystalline product) by the ozonization of 20-hydroxyecdysone **1** in pyridine† (Scheme 1). About 50% of compound **1** was found to convert into less polar compound **2** for 20 min by bubbling an O_3/O_2 mixture through the pyridine solution of **1**. Compound **2** was isolated by column chromatography on SiO_2 in 43% yield. According to TLC data, the reaction mixture together with compounds **1** and **2** contained small amounts of polar compounds.



Scheme 1

We found that conversion of compound **1** rises with ozonization duration. At the same time, one could observe an increase in the content of polar compounds, whereas the yield of compound **2** decreased. Compound **2** isolated under the same ozonization conditions gradually transformed into polar compounds. The attempts to obtain compound **2** by the ozonization of compound **1** in other solvents (CH_2Cl_2, $CHCl_3$, CCl_2FCClF_2, MeOH and AcOH) failed. Addition of **1** in saturated with ozone pyridine solution did not result in compound **2**.

The structure of compound **2** was proved by 1D and 2D NMR (^{1}H, ^{13}C) spectra (CD_3OD). Typical of ecdysteroids Δ^7-6-keto moiety remained invariable under ozonization that was confirmed by signals at 203.3, 167.1 and 122.1 ppm in ^{13}C NMR spectrum (it is well known chemical inertness to ozone of sterically hindered double bond with conjugated keto group in ecdysteroids[1,7]). The presence of a signal at 203.3 ppm and the appearance of additional signal at 210.9 ppm provided evidence for transformation of one from two secondary hydroxy groups into keto group. Essential position change of signals in ^{13}C NMR spectrum, which are referred to carbon atoms in ring A, indicates the keto group appearance in it as well. The correlation of two doublets of geminal protons H_2C^1 at 2.38 and 2.57 ppm (^2J 13.8 Hz) only with a signal of carbonyl group (210.9 ppm) shows a position of oxo group at C-2 (HMBC spectrum). The appearance of a 2-keto moiety gave a shift of signals of C^3 and proton at this carbon atom (the reference was made from HSQC spectrum analysis) into low field ($\Delta\delta_C = 7.4$, $\Delta\delta_H = 0.4$ ppm).

† The ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance-400 spectrometer at 400.13 and 100.62 MHz, respectively. IR spectra were recorded on a Specord 75I-R spectrometer in KBr pellets.

Through a solution of **1** (0.3 g, 0.63 mmol) in 3 ml of dry pyridine, an ozone–oxygen mixture (~20 °C, 70 ml min^{-1}, 20 min, 10 mmol O_3; productivity of ozonizer, 30 mmol O_3 per hour) was passed. The reaction mixture was blown by argon; the crude product was purified by column chromatography on SiO_2 ($CHCl_3$–MeOH, 20:1). Yield 0.13 g (43%) of compound **2** (R_f 0.59, Sorbfil, $CHCl_3$–MeOH, 3:1), 0.15 g (50%) of initial compound **1** (R_f 0.48).

For **2**: mp 136–138 °C, $[\alpha]_D^{20}$ +13.05 (*c* 2.18, $CHCl_3$). IR (KBr, ν/cm^{-1}): 3360, 2940, 1710, 1650. ^{1}H NMR (CD_3OD) δ: 0.87 (s, 3H, 18-Me), 1.07 (s, 3H, 19-Me), 1.18 (s, 3H, 21-Me), 1.18 (s, 3H, 26-Me), 1.18 (s, 3H, 27-Me), 1.29 and 1.66 (m, 2H, 23-H), 1.41 and 1.78 (m, 2H, 24-H), 1.58–1.96 (m, 4H, 15-H, 16-H), 1.73 and 1.97 (m, 2H, 11-H), 1.81 and 2.18 (m, 2H, 4-H), 1.84 and 2.09 (m, 2H, 12-H), 2.37 (m, 1H, 17-H), 2.38 and 2.57 (2d, 2H, 1-H, ^2J 13.8 Hz), 2.66 (dd, 1H, 5-H, *J* 13.5 and 4.0 Hz), 2.79 (m, 1H, 9-H), 3.31 (m, 1H, 22-H), 4.34 (dd, 1H, 3-H, *J* 11.9 and 7.3 Hz), 5.83 (s, 1H, 7-H). ^{13}C NMR (CD_3OD) δ: 18.14 (C-18), 21.06 (C-21), 21.18 (C-11), 21.45 (C-16), 21.60 (C-19), 27.46 (C-23), 29.16 (C-27), 29.77 (C-26), 31.86 (C-15), 32.41 (C-12), 36.36 (C-4), 36.94 (C-9), 42.48 (C-24), 44.06 (C-10), 48.84 (C-1), 50.64 (C-13), 50.70 (C-17), 56.38 (C-5), 71.47 (C-25), 75.23 (C-3), 78.08 (C-22), 78.63 (C-20), 85.09 (C-14), 122.07 (C-7), 167.05 (C-8), 203.27 (C-6), 210.98 (C-2).

The high coupling constant in a doublet of proton at C^3, which corresponds to the interaction with the axial proton of the H_2C^4 group, testifies about its axial β-orientation. The hydroxy group associated with the C^3 atom is α-oriented. The proton at the C^5 atom does not alter its β-orientation, and *J* 13.5 Hz confirms its axial position in ring A with an axial proton of H_2C^4 group. The ^{1}H and ^{13}C NMR spectra of compound **2** in C_5D_5N were identical to those reported earlier.[6]

The selective oxidation observed under ozonization of compound **1** depends on the direction of attack by ozone molecule of sterically less hindered (more accessible) axial H–C^2 bond to afford α-hydroxyhydrotrioxide. The latter, as reported,[8] was transformed into 2-oxo derivative, which in pyridine basic medium epimerized at the adjacent to keto group C^3 atom to give target compound **2**.[9]

The ozonization of 20-hydroxyecdysone 20,22-acetonide **3** in pyridine proceeded similarly to that of 20-hydroxyecdysone **1** leading to 2-dehydro-3-*epi*-20-hydroxyecdysone acetonide **4**‡ (Scheme 1), whereas 20-hydroxyecdysone 2,3:20,22-diacetonide did not enter into this reaction and returned without any change in structure. The structure of compound **4** was confirmed by ^{1}H and ^{13}C NMR data and high-resolution mass spectrometry.

Thus, we present a one-stage procedure, which allows us to synthesize 2-dehydro-3-*epi*-20-hydroxyecdysone, a minor ecdysteroid isolated recently from the seeds of *Froelichia floridana*, by ozonization of 20-hydroxyecdysone in pyridine.

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‡ The ^{1}H and ^{13}C NMR spectra were measured on a Bruker AM-300 spectrometer at 300.13 and 75.48 MHz, respectively. Mass spectra were measured on a MAT 95 high resolution spectrometer.

The ozonization of compound **3** (0.3 g, 0.58 mmol) and following treatment was carried out, as it was described for **1**, having received 0.13 g (42%) of compound **4** (R_f 0.59, Sorbfil, $CHCl_3$–MeOH, 5:1) and 0.14 g (50%) of compound **3** (R_f 0.55).

For **4**: mp 100–102 °C, $[\alpha]_D^{20}$ +24.2 (*c* 3.8, $CHCl_3$). IR (KBr, ν/cm^{-1}): 3400, 2950, 1705, 1650. ^{1}H NMR (CD_3OD) δ: 0.82 (s, 3H, 18-Me), 1.05 (s, 3H, 19-Me), 1.16 (s, 3H, 21-Me), 1.19 (s, 3H, 26-Me), 1.19 (s, 3H, 27-Me), 1.22–2.08 [m, 16H, $(CH_2)_8$], 1.30 and 1.37 (s, 2Me, Pr^iO_2), 2.29 (m, 1H, 17-H), 2.41 and 2.53 (2d, 2H, 1-H, *J* 13.8 Hz), 2.65 (dd, 1H, 5-H, *J* 13.8 and 3.1 Hz), 2.75 (m, 1H, 9-H), 3.66 (m, 1H, 22-H), 4.34 (m, 1H, 3-H), 5.82 (s, 1H, 7-H). ^{13}C NMR (CD_3OD) δ: 17.98 (C-18), 21.62 (C-11), 22.71 (C-16), 22.71 (C-21), 22.91 (C-19), 24.99 (C-23), 27.51 (C-26), 29.29 (C-27), 29.67 and 29.80 (20,22-Me_2C), 31.98 (C-15), 32.41 (C-12), 36.55 (C-4), 37.15 (C-9), 42.52 (C-24), 44.06 (C-10), 48.76 (C-1), 50.18 (C-13), 50.73 (C-17), 56.53 (C-5), 71.44 (C-25), 75.39 (C-3), 83.60 (C-22), 85.30 (C-20), 86.14 (C-14), 122.23 (C-7), 166.66 (C-8), 203.40 (C-6), 211.09 (C-2). MS, *m*/*z* (%): 500.3071 $[M - H_2O]^+$. Calc. for $C_{30}H_{44}O_6$: 500.3132.

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