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SHORT COMMUNICATIONS

Oxidation of Estra-1,3,5(10)-triene-3,11α,17β-triol Triacetate with Ceric Ammonium Nitrate

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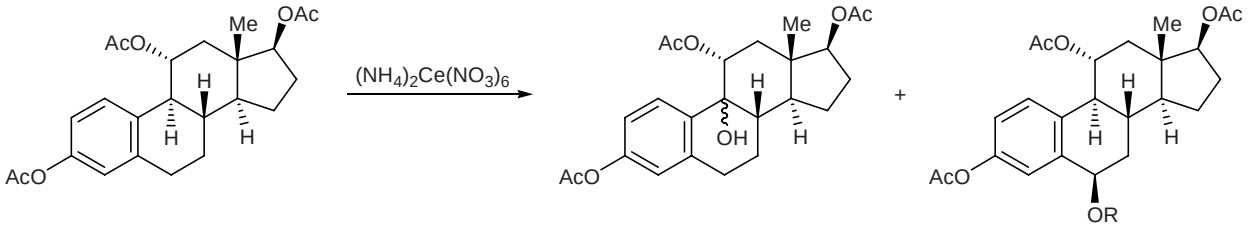
Reaction of ceric ammonium nitrate with steroid derivatives having aromatic A ring leads to oxidation of the benzylic C^9 atom and oxidative nitration at the homobenzylic position (C^{11}) [1]. Isologous 8β-estra-1,3,5(10)-trienes are oxidized with ceric ammonium nitrate at the other benzylic position (C^6) [2, 3]. This reaction direction gives rise to 6-oxo estrogens which have found application in the synthesis of haptenes for radioimmunological assay of estrogenic hormones in biological media [4]. Introduction of an additional oxygen-containing functionality into steroid molecule enhances its hydrophilicity, and amphiphilic steroids containing both hydrophilic and hydrophobic fragments exhibit a broad spectrum of biological activity, including antitumor effect [5].

The present communication reports on the oxidation with ceric ammonium nitrate of 11α-hydroxyestradiol [estra-1,3,5(10)-triene-3,11α,17β-triol] triacetate (**I**) which has recently became accessible [6, 7]. We believed that the substituent in position *11* should hinder oxidation of the neighboring C^9 atom, so that the reaction will occur at the B ring. Treatment of triacetate **I** with 5.8 equiv of ceric ammonium nitrate in acetic acid resulted in the formation of a mixture of four compounds, the chromatographic mobility of three of which was comparable with that of the initial steroid (R_f 0.78, 0.74, 0.52); the fourth product was considerably more polar (R_f 0.38). According to the HPLC data, the fractions of these compounds were 33, 37, 18, and 12%, respectively. The ^{1}H NMR data showed a relation between the least and the most polar products. The first of these contained an additional secondary acetoxy group, and the second, a hydroxy group; the latter was readily converted into the former under standard acetylation conditions. The Jones oxidation [8] product of the alcohol showed in the UV spectra absorption typical of 6-oxo estrogens [9] and was assigned structure **IV**, while its acetylation product was assumed to be tetraacetate **V**. The β-orientation of the substituent on C^6 followed from the corresponding ^{1}H–^{1}H coupling constants for the 6-H proton (4.8 and 2 Hz), which indicated its equatorial position [10].

The remaining two products were identified as epimeric alcohols **II** and **III** (9α-OH and 9β-OH, respectively) on the basis of their ^{1}H NMR and mass



I **II** (9α), **III** (9β) **IV**, **V**

IV, R = H; **V**, R = Ac.

spectra. The mass spectra of these compounds contained a fragment ion peak with *m*/*z* 204 (I_{rel} 5%) which is characteristic of 9-hydroxyestratrienes [11]. Their stereochemical assignment was made taking into account downfield position of the angular methyl group signal in the ^{1}H NMR spectra of 9β-steroids [12]. Unlike analogous oxidation of classical estrogens, where the fraction of the 9β-epimer was 20–25% [13], the ratio of epimers **II** and **III** formed in the reaction under study is characterized by increased fraction of the 9β-epimer.

The ratio of the oxidation products at C^9 and C^6 was 55:45 (HPLC data for the product mixture) or 63:37 (after isolation). Thus, among two possible directions of benzylic oxidation of **I** with ceric ammonium nitrate, the oxidation at C^9 predominates over the reaction at C^6 despite greater steric hindrances in the former case; this means that thermodynamic factor in this reaction is more important than kinetic.

Oxidation of estra-1,3,5(10)-triene-3,11α,17β-triol triacetate (I) with ceric ammonium nitrate. A solution of 5.4 g (9.85 mmol) of ceric ammonium nitrate in 5 ml of water was added to a solution of 700 mg (1.69 mmol) of compound **I** in 37 ml of acetic acid. The orange solution was stirred for 6 h at room temperature and was poured into 100 ml of a 1:1 chloroform–water mixture. The aqueous phase was additionally extracted with chloroform, and the extracts were combined with the organic phase, washed in succession with a 10% solution of $NaHCO_3$ and a saturated solution of NaCl, and dried over $MgSO_4$. The solvent was evaporated, and the residue was subjected to chromatography on 30 g of silica gel using methylene chloride–hexane–acetone (20:4:1) as eluent to isolate (in the order of elution) 193 mg (24%) of tetraacetate **V**, 237 mg (32.5%) of 9α-epimer **II**, 136 mg (18%) of 9β-epimer **III**, and 44 mg (6%) of alcohol **IV**.

9α-Hydroxyestra-1,3,5(10)-triene-3,11α,17β-triyl triacetate (II). Yield 192 mg, mp 204–207°C, R_f 0.46, relative retention time (RRT, HPLC) 7.2, $[\alpha]_D = -61°$. UV spectrum, λ_{max}, nm (log ε): 265 (2.78), 273 (2.69). IR spectrum, ν, cm^{-1}: 3520 (OH), 1755, 1725, 1200, 1245, 1270, 1490. ^{1}H NMR spectrum, δ, ppm: 0.88 s (3H, 18-CH_3); 2.02 s, 2.13 s, and 2.26 s (3H each, OAc); 4.7 t; 5.4 d.d (1H, 11-H, *J* = 5, 8 Hz). Mass spectrum, *m*/*z* (I_{rel}, %): 430 $[M]^+$ (13), 378 [*M* – AcOH] (44), 328 [378 – CH_2CO] (53), 204 (100).

9β-Hydroxyestra-1,3,5(10)-triene-3,11α,17β-triyl triacetate (III). Yield 104 mg, oily substance, R_f 0.34, RRT 5.2, $[\alpha]_D = +0.1°$. IR spectrum, ν, cm^{-1}: 3450; 1725, 1750, 1760, 1200, 1225, 1235; 1605, 1490 (C=C_{arom}). ^{1}H NMR spectrum, δ, ppm: 1.08 s (3H, 18-CH_3); 2.0 s, 2.17 s, and 2.27 s (3H each, OAc); 4.5 t; 5.4 d.d; 6.8–6.86 m; 8.1 d. Mass spectrum, *m*/*z* (I_{rel}, %): 430 (24), 378 (100), 204 (86).

6β-Hydroxyestra-1,3,5(10)-triene-3,11α,17β-triyl triacetate (IV). Yield 32 mg, oily substance, R_f 0.25, RRT 4.8. ^{1}H NMR spectrum, δ, ppm: 0.87 s, 2.03 s, 2.05 s, and 2.27 s (3H each); 4.6 m (2H, 6-H, 17-H); 6.9 d.d (1H, 2-H, *J* = 2, 9 Hz); 7.08 d (1H, 1-H, *J* = 9 Hz); 7.16 d.d (1H, 4-H, *J* = 2, 9 Hz).

A 10-mg portion of compound **IV** was acetylated with 0.1 ml of a 1:1 mixture of acetic anhydride and pyridine at 20°C (reaction time 16 h). After appropriate treatment, we isolated 10 mg of a product which was identical to compound **V** in the TLC and HPLC data. A solution of 5 mg of the triacetate fraction in acetone was treated at 0°C with a solution of chromic acid over a period of 5 min; as a result, 4 mg of the corresponding triacetoxy ketone was isolated. UV spectrum, λ_{max}, nm: 256, 327.

Estra-1,3,5(10)-triene-3,6β,11α,17β-tetrayl tetraacetate (V). Yield 144 mg (after additional purification). Oily substance, R_f 0.51, RRT 9.4, $[\alpha]_D = -77°$. UV spectrum, λ_{max}, nm (log ε): 267 (2.76), 275 (2.72). IR spectrum, ν, cm^{-1}: 1720–1730, 1760–1770, 1200, 1225–1250 (OAc); 1490 (C=C_{arom}). ^{1}H NMR spectrum, δ, ppm: 0.88 s (3H, 18-CH_3), 2.03 s and 2.07 s (3H each, 11-OAc, 17-OAc), 2.05 s (3H, 6-OAc), 2.27 s (3H, 3-OAc), 4.7 t (1H, 17-H, *J* = 7.6 Hz), 5.35 t.d (1H, 11-H, *J* = 5, 10 Hz), 5.94 t.d (1H, 6-H, *J* = 4.8, 2 Hz), 6.91–6.96 m (2H, 2-H, 4-H), 7.02 d (1H, 1-H, *J* = 9 Hz). Found, %: C 65.76; H 6.58. $C_{26}H_{32}O_8$. Calculated, %: C 66.08; H 6.83.

The melting point was determined on a Boetius hot stage. The optical rotations were measured on a Polamat polarimeter from solutions in chloroform (*c* = 0.7–0.9). The UV spectra were recorded on a Specord UV-Vis spectrophotometer from solutions in ethanol. The IR spectra were obtained in KBr on a Specord 75 IR instrument. The ^{1}H NMR spectra were measured on a Bruker DPX-400 spectrometer using $CDCl_3$ as solvent and TMS as internal reference. The mass spectra (electron impact, 20 eV) were run on a Kratos MS-30 instrument. High-performance liquid chromatography was performed on a Millikhrom-1 liquid chromatograph at λ 280 nm using a 80×2-mm Silasorb C_{18} column (eluent acetonitrile–water, 3:2). Silufol-254 plates were used for thin-layer chromatography (ethyl

acetate–hexane, 3:7). The products were separated by column chromatography on silica gel (40–100 μm, Czechia).

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