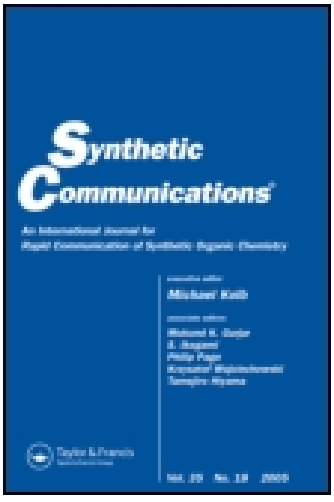

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Preparation of 3β-Acetoxy-17a-Oxo-Androst-5-Ene-7,17-Dione, a Biologically Active Impurity Isolated from the Production of 3β-Acetoxyandrost-5-Ene-7,17-Dione

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Abstract: An impurity isolated during the kilo-lab preparation of 3β-acetoxyandrost-5-ene-7,17-dione was identified as 3β-acetoxy-17a-oxo-androst-5-ene-7,17-dione. This novel D-ring secosteroid was postulated to be a Baeyer–Villiger oxidation by-product of the preparation reaction. A genuine sample was prepared by the selective peracid-mediated Baeyer–Villiger reaction on 3β-acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA acetate), confirming the novel structure.

Keywords: 3β-Acetoxyandrost-5-ene-7,17-dione, allylic oxidation, Baeyer–Villiger oxidation, lactone, 7-oxo-DHEA acetate

INTRODUCTION

The conversion of 3β-acetoxyandrost-5-ene-17-one [dehydroepiandrosterone (DHEA) acetate], **2**, to 3β-acetoxyandrost-5-ene-7,17-dione [7-oxo-DHEA acetate†], **3**, has been reported as early as 1949 in the chemical literature.[1] Most of these preparations utilize a transition-metal-catalyzed allylic

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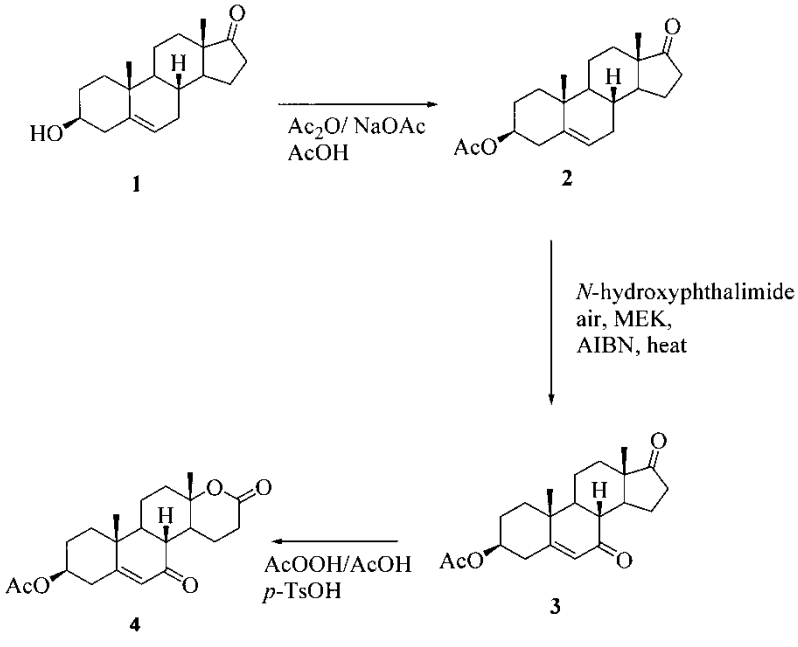
†Trade name: 7-Keto™, developed and distributed by Humanetics Corporation, Chanhassen, MN 55317.

oxidation protocol. Since the early 1990s, there have been several reports of a free-radical-mediated process for the oxidation of acidic or allylic alkyl groups,[2–4] which avoids the downstream problems posed by the transition-metal-mediated oxidations.

While developing this oxidation method for the commercial production of **3**, we observed a minor by-product in the crude product, which was slightly more polar than **3** by TLC and HPLC analysis. The level of this impurity varied 3–7% by area under the curve HPLC analysis. After recrystallization from methanol, this impurity was routinely reduced to $<1\%$, but it was never totally removed from the purified **3**. The recrystallization mother liquors were found to be enriched with this impurity to the level of $\sim 20\%$ by HPLC analysis.

The recrystallization mother liquor concentrates were purified by silica-gel column chromatography (eluted with 3:2 hexanes/ethyl acetate) to provide a sample of this impurity as a white powder. Preliminary mass spectral analysis indicated that the impurity had an $M+16$ ($M^+ + H = 361$), in comparison to the molecular weight for **3** ($M^+ + H = 345$). The combustion analysis was correct for the molecular formula of $C_{21}H_{28}O_5$ (**3** + one oxygen atom). There were no major changes observed in the proton NMR spectrum of this impurity as compared to **3**.



Scheme 1.

There was a significant difference in the carbon NMR spectrum with the disappearance of the D-ring ketone carbonyl carbon at δ 219 ppm and the appearance of a lactone carbonyl carbon at δ 171 ppm. The initial biological activity of this impurity has been reported by Lardy's group,[5] but their report does not describe where or how the sample was obtained.

In the presence of a peracid, the Baeyer–Villiger oxidation is a synthetically useful process for the conversion of cyclic and acyclic ketones into lactones and esters, respectively. The regioselectivity and stereospecificity of this reaction have been reviewed.[6–8] There are reports of the rearrangement of a steroid's D-ring ketone, but none of them contain an additional carbonyl or α,β-unsaturated carbonyl group in the B-ring. To selectively rearrange the D-ring ketone in the presence of the α,β-unsaturated ketone in the B-ring, without any further rearrangements or epoxidation, would be a worthwhile synthetic transformation.

To confirm that the impurity formed during the conversion of **2** to **3** was indeed a Baeyer–Villiger oxidation product, we directly synthesized compound **4** (Scheme 1). The synthesis of lactone **4** was accomplished using the method of Hassall,[6] which involved oxidizing a compound similar to **3** with toluenesulfonic acid and peracetic acid in acetic acid. Following an aqueous workup and purification by column chromatography, this product was confirmed to be more polar than **3** by TLC and HPLC analyses and coeluted with the impurity isolated from the free-radical-mediated conversion of **2** to **3**. Solid **4** prepared by this new route was characterized by ^{1}H NMR, ^{13}C NMR, IR (KBr), DI MS, TLC, HPLC, melting point, and elemental analysis and found to be identical with the impurity isolated from the production process.

In summary, the careful investigations of the oxidation to prepare 3β-acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA acetate), **3**, under free radical conditions, resulted in the identification and characterization of the novel lactone **4**. A sample of **4** was prepared directly from **3** by utilizing a mild variation of the Baeyer–Villiger oxidation, thus completing the structure proof.

EXPERIMENTAL

The reactions were performed under a dry nitrogen atmosphere. Reagents and solvents were obtained from commercial sources and used as received. Proton magnetic resonance spectra were obtained on a Bruker AMX-500 MHz NMR, using either tetramethylsilane or chloroform as an internal reference. Carbon magnetic resonance spectra were obtained on a Bruker AMX-300 at 75 MHz using $CDCl_3$ as the solvent. Infrared spectra were obtained as KBr pellets on a Perkin Elmer Spectrum 1000 Infrared Spectrophotometer. Mass spectrum analysis was performed on a Shimadzu QP-5000 GC/MS (CI mass

spectrometry). Melting points were obtained on a Thomas Hoover Capillary Melting Point Apparatus. Elemental analysis was obtained from Quantitative Technologies, Inc. HPLC data were obtained on a Perkin Elmer chromatogram equipped with a Phenomenex Hypersil 5 C18 (150 × 4.6 mm) column at a detector-wavelength setting of 205 nm.

Preparation of 3β-Acetoxyandrost-5-Ene-17-One (DHEA Acetate) (2)

To a vigorously stirred slurry of 3β-hydroxyandrost-5-ene-17-one (**1**, 4995 g, 17.3 mol) in glacial acetic acid (15.2 L) was added acetic anhydride (3.3 L), followed by sodium acetate (1700 g, 20.7 mol, 1.2 equiv). The resulting slurry was heated to 65°C for 18 h; TLC analysis (silica-gel plate eluted with a 1:1.5 ethyl acetate/hexanes mixture) indicated that the starting material had been consumed. The reaction mixture was cooled to 50°C and water (36 L) was added over 15–20 min. The resulting slurry was cooled to ambient temperature and stirred for 4 h. The solids were collected by vacuum filtration, washed with water (2 × 10 L), and air-dried for 12 h. The solids were dissolved in ethanol (44 L) and heated to 70°C. The solution was clarified through a steam-heated line filter and allowed to cool to ambient temperature with moderate agitation. The product crystallized at about 50°C. The solid product was collected by vacuum filtration, washed with ethanol (2 × 4 L), and air-dried for 4 h. The solids were transferred to glass-drying trays and vacuum-dried at 60°C for 24 h to afford **2** as a white solid (4322 g, 75%). A second crop was isolated by concentrating the combined mother and wash liquors to about 10 L and allowing the slurry to age for 24 h. These solids were vacuum-dried at 60°C to afford additional **2** as a white solid (1084 g, 19%), making the combined yield of **2** 94% (5406 g).

Mp 172–175°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm = 5.40 (d, 1H), 4.68–4.55 (m, 1H), 2.52–2.40 (m, 1H), 2.40–2.30 (m, 3H), 2.18–2.05 (m, 2H), 2.05 (s, 3H), 1.99–1.75 (m, 3H), 1.70–1.10 (m, 7H), 1.05 (s, 3H), 0.95 ppm (3H, s). DI MS (m/z): 331 $[M + H]^+$. Anal. calcd. for $C_{21}H_{30}O_3$: C, 69.98; H, 7.83. Found: C, 69.77; H, 7.70.

Preparation of 3β-Acetoxyandrost-5-ene-7,17-dione (3)

A stirred mixture of *N*-hydroxyphthalimide (1996 g, 12.2 mol) and 2-butanone (26 L) was heated to 80°C and then 3β-acetoxyandrost-5-ene-17-one (DHEA acetate) (**2**, 4000 g, 12.1 mol) was added in one portion. After the solids dissolved, the solution was allowed to return to 80°C; 2,2′-azobisisobutyronitrile (AIBN, 450 g, 2.7 mol) was added in one portion. Oxygen was bubbled into the solution for 2 h, and the reaction was analyzed by TLC (silica-gel plate eluted with a 1:1.5 ethyl acetate/hexanes mixture) to ensure the

oxidation of **2** had begun. The reaction was continued for an additional 6 h and then tested for completion by TLC. An additional portion of AIBN (100 g) was added. The reaction continued for 12 h and was tested for completion by TLC. The oxygen flow to the stirred reaction mixture was replaced with nitrogen and the heat was turned off. Once cooled, most of the 2-butanone (22 L) was removed by vacuum concentration and replaced with methylene chloride (13 L). The resulting slurry was stirred at ambient temperature for 24 h and vacuum-clarified through a table-top funnel lined with celite filter aid. The reaction flask and filter cake (recovered *N*-hydroxyphthalimide) were rinsed with methylene chloride (5 L) and this rinse was added to the clarified reaction mixture.

To this stirred solution was added pyridine (1.2 L) followed by acetic anhydride (0.6 L); the mixture was stirred for 3 h. The reaction mixture was concentrated under reduced pressure to dryness and the residue was redissolved in methyl *t*-butyl ether (25 L). The resulting solution was washed with 2 N hydrochloric acid (2 × 8 L), water (2 × 4 L), saturated aqueous sodium bicarbonate solution (4 × 8 L), water (2 × 4 L), and saturated sodium chloride solution (2 × 4 L). The organic solution was concentrated under reduced pressure to about 7 L volume and cooled to 0°C for 4 h while the product crystallized. The solids were collected by vacuum filtration, rinsed with cold methyl *t*-butyl ether, and vacuum-dried for 24 h to afford 1850 g of crude **3** as a white solid.

A stirred suspension of crude **3** (1800 g) in methanol (25 L) was heated to reflux until the solids dissolved. This stirred solution was treated with a mixture of bentonite clay (200 g of Clarion 470) and decolorizing carbon (200 g of Darco G-60) and was clarified through a celite-coated filter. The flask and filter cake were washed with hot methanol (4 × 1 L). The filtrate was allowed to cool to ambient temperature and was stirred for 18 h. The solid was collected by vacuum filtration, rinsed with cold methanol, and dried under reduced pressure at 60°C for 24 h to afford a 23% yield (951.6 g) of purified **3**. The combined mother/wash liquors were concentrated under reduced pressure to about 10 L and the remaining solution was allowed to cool for 12 h. The resulting solids were collected and dried under reduced pressure at 60°C for 24 h to afford an additional 6% yield (251 g) of purified **3**. The combined yield of recrystallized **3** was 29% of a white solid.

Mp 186–192°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm = 5.75 (s, 1H), 4.75–4.65 (m, 1H), 2.88–2.75 (m, 1H), 2.65–2.40 (m, 3H), 2.15 (dd, 1H, J = 12, 12 Hz), 2.05 (3H, s), 2.04–1.95 (dd, 1H, J = 12, 11 Hz), 1.88–1.55 (m, 7H), 1.35–1.20 (m, 6H), and 0.90 ppm (s, 3H). ^{13}C NMR ($CDCl_3$): 220.2, 200.8, 170.3, 164.5, 126.6, 82.1, 72.1, 50.1, 47.9, 45.9, 44.5, 38.6, 37.9, 36.1, 35.7, 30.8, 27.5, 24.3, 21.3, 20.7, 17.5, and 16.8 ppm; IR (KBr): 2954, 1741, 1673, 1629, 1457, 1379, 1298, 1235, 1180, 1031, 903, 871, 607, and 533 cm^{-1}. DI MS (m/z) 345 $[M+H]^+$. Anal. calcd. for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.33; H, 8.07.

Preparation of 3β-Acetoxy-17a-Oxo-Androst-5-Ene-7,17-Dione (4)

To a vigorously stirred solution of 3β-acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA acetate) (**3**, 35.0 g, 0.10 mol) in acetic acid (700 mL) was added *p*-toluenesulfonic acid monohydrate (*p*-TsOH, 0.70 g), followed by commercial peracetic acid solution (32 wt% in glacial acetic acid, 49.43 g, 0.65 mol, 6.5 equiv); the mixture was stirred for 18 h at ambient temperature. The solution was slowly diluted with water (2.5 L) and the slurry was stirred for 15 min at ambient temperature. The resulting solids were collected by filtration, washed with water (3 × 50 mL), and then dried at ambient temperature under reduced pressure to remove residual solvents. The white solid was further purified by column chromatography (silica-gel column eluted using a gradient system: 1:4:5 ethyl acetate/hexanes/methylene chloride to 2:3:5 ethyl acetate/hexanes/methylene chloride to 3:2:5 ethyl acetate/hexanes/methylene chloride). Recrystallization of the solid isolated from the combined fractions of purified **4**, from a methanol/diisopropyl ether mixture provided **4** (21.9 g, 67%) as a white solid.

Mp 267–271°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm = 5.75 (s, 1H), 4.71 (m, 1H), 2.84 (m, 1H), 2.72–2.56 (m, 3H), 2.47 (dd, 1H, $J = 12$, 2 Hz), 2.16 (dd, 1H, $J = 12$, 11 Hz), 2.05 (3H, s), 1.99–1.63 (7H, m), 1.49–1.46 (3H, m), 1.32 (3H, s), 1.29 (dd, 1H, $J = 11$, 4 Hz), 1.21 ppm (3H, s). ^{13}C NMR ($CDCl_3$): 199.4, 171.3, 170.1, 163.5, 126.2, 82.1, 71.9, 48.8, 46.3, 40.3, 38.8, 37.8, 37.5, 35.8, 28.9, 27.1, 21.5, 21.2, 20.7, and 16.8. IR (KBr): 2968, 1731, 1669, 1461, 1386, 1243, 1090, 1029, and 965 cm^{-1}. DI MS (m/z): 361 $[M + H]^+$. Anal. calcd. for $C_{21}H_{28}O_5$: C, 69.98; H, 7.83. Found: C, 69.77; H, 7.70. R_f 0.43 (silica-gel plate eluted with 5:3:2 methylene chloride/ethyl acetate/hexanes).

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