Steroids 115 (2016) 75–79

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of 17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-trien-3-one: A long-term metandienone metabolite

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ARTICLE INFO

Article history:
Received 23 June 2016
Received in revised form 11 August 2016
Accepted 14 August 2016
Available online 21 August 2016

Keywords:
Metandienone
Metabolism
Wagner-Meerwein rearrangement

ABSTRACT

The goal of this work was a good-yielding chemical synthesis of a metandienone metabolite which is of interest in doping analysis. 20βOH-NorMD (IUPAC: 17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-triene-3-one) has been identified as a long-term urinary metabolite which can be detected and attributed to metandienone up to almost 3 weeks after exposure. The chemical synthesis of its epimer 20αOH-NorMD has been described before, as was an enzymatic synthesis of 20βOH-NorMD, but no chemical synthesis was published.

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1. Introduction

Metandienone (**1**) is a synthetic anabolic androgenic steroid (AAS) that was first synthesized in 1955 [1] and is still being used by professional athletes today. It is one of the highly abused synthetic AAS together with Nandrolone and Stanozolol judging from adverse analytical findings (AAF) from 2004 to 2007 [2]. It is also reportedly used by amateur athletes [3]. Since the discovery of the first metabolite in 1963 [4] the metabolism has been extensively investigated [5–9].

As reported by Schänzer et al. in 1992 the 17-sulfate conjugate (**1a**) of metandienone can epimerize and/or rearrange (Wagner-Meerwein-Rearrangement) to give primarily NorMD (17,17-dimethyl-18-norandrosta-1,4,13-trien-3-one) (**1b**). This intermediate is further hydroxylated by Cytochrome P450 enzymes CYP21 and CYP3A4 to 20βOH-NorMD (**1c**) (Scheme 1) [10]. Hydroxylation can also take place at C-16 (CYP21) and on the 17α-methyl group (CYP3A4) giving the epimer [11]. Both 17-epimers have identical detection limits. Additionally it was reported that the metabolite is also formed by C-18 hydroxylation *via* CYP11B2 and subsequent rearrangement [12].

Compound **1c** (IUPAC: 17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-triene-3-one, also called "nightwatch" and "20βOH-NorMD") has been identified as a long-term urinary metabolite which can be detected and attributed to metandienone up to 19 days after administration of a single dose [13]. This constitutes a significantly longer window of detection than with metandienone metabolites used before. When screening routines using this new metabolite were implemented in 2006 the number of AAF rose from 15 to 68 for metandienone (450% increase) [14].

In 2011 a small quantity (10 mg) of this metabolite was synthesized from NorMD using recombinant strains of the fission yeast *Schizosaccharomyces pombe* expressing CYP21 [11]. The epimer of this metabolite: 17α-hydroxymethyl-17β-methyl-18-norandrosta-1,4,13-triene-3-one has been reportedly synthesized in 5 steps with an overall yield of about 0.1% [12]. Thus far there is no report on the chemical synthesis of this compound. We hereby report the first synthesis of this metabolite which is needed as a reference material for metandienone abuse in antidoping laboratories around the world.

2. Experimental

Dehydroepiandrosterone acetate and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone were purchased from FluoroChem. MSTFA was supplied by Machery & Nagel (Düren, Germany). Silica gel (0–63 μm, 60 Å) for chromatography was from Merck. Titanium tetrachloride, anhydrous dimethylformamide and anhydrous dimethylsulfoxide were from Acros Organics. Sodium sulfate was from VWR. All other non-specified chemicals were from Sigma Aldrich. Anhydrous methylene chloride, diethyl ether, toluene and 1,4-dioxane were retrieved from an Innovative Technologies PureSolv system. Anhydrous tetrahydrofuran was pre-dried using

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http://dx.doi.org/10.1016/j.steroids.2016.08.013
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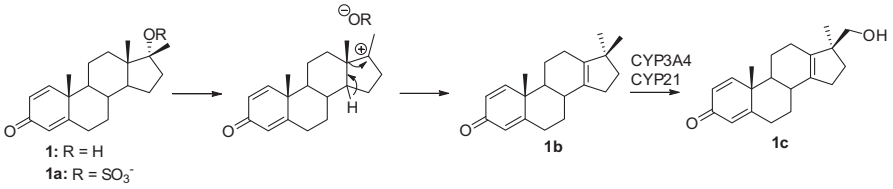

Scheme 1. Suspected *in vivo* formation of the metabolite.

an Innovative Technologies PureSolv system, refluxed over sodium/benzophenone and freshly distilled.

NMR spectra were recorded on a Bruker AC400. IR spectra were recorded on a Perkin Elmer Spectrum 65. TLC-analysis was performed with precoated aluminium-backed plates (Silica gel 60 F_{254}, Merck). Compounds were visualized by submerging in an acidic phosphomolybdic acid/cerium sulfate solution and heating. Melting points were determined with a Kofler hot-stage apparatus.

Preparative HPLC was carried out on a Reveleris® Prep system by Grace using a Luna® 10 μm, C18 (TMS endcapping) 100 Å, LC Column (250 × 21.2 mm).

GC-EI-MS analyses were performed using a Thermo Trace GC coupled to a Thermo Trace MS instrument (Thermo Quest, Austin, USA) equipped with a Restek RTX-5 ms GC column (length 15 m, inner diameter 0.25 mm, film thickness 0.25 μm). The GC oven temperature program started at 150 °C was increased at 20°/min to 320 °C using Helium as carrier gas 100 kPa, constant pressure). The injector temperature was set to 270 °C, the interface temperature to 280 °C and the ion source temperature to 250 °C. Ionization was accomplished using EI (70 eV), and full scan analysis (*m*/*z* 60–600) was employed at 2 scans/s. A volume of 1 μL of a derivatization mixture consisting of 5 μg of the target product in 200 μl of a mixture of MSTFA/ammonium iodide/ethanethiol-TMS 1000:2:6 (v/w/v) was injected in split mode (1/40) into the GC–MS system.

High resolution/high accuracy mass spectra were recorded on a Thermo Q Exactive Focus mass spectrometer (Thermo Scientific, Austin, Texas) by direct injection of a 10 μg/ml solution of the target product in acetonitrile.

The mass spectrometer was operated in electrospray ionization mode at a spray voltage of 4 kV at 400 °C. The instrument was calibrated using the manufacturers calibration mixture allowing or mass accuracies <3 ppm. Full scan mass spectra were recorded in profile mode at a resolution of 70.000 at a scan range of *m*/*z* 100–350.

2.1. 3β-Hydroxy-13α-methylandrost-5-en-17-one acetate (**2**)

A solution of dehydroepiandrosterone acetate (10 g, 30.26 mmol) in acetic acid (125 mL) and 1,2-phenylenediamine (5.43 g 50.2 mmol) was refluxed for 24 h. The color changed from light brown to dark green in about 2 h and then proceeded to turn very dark with blue complexion which disappeared after cooling the reaction. The solution was then flooded with 150 mL deionised water upon which a beige precipitate formed. This was extracted with 150 mL ethyl acetate and the organic phase washed ten times with small portions of water and four times with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and the solvent evaporated *in vacuo*. The crude product was recrystallized from diisopropylether, giving 6 g of product, and the mother liquors concentrated and separated on a 180 g silica gel column with 5/1 v/v petroleum ether/diethyl ether as eluent to give 1.16 g starting material and in total 8.2 g (82%) of the title compound **2** as white solid, m.p. 141–143 °C.

TLC: R_f: (petroleum ether/diethyl ether 3/1 v/v) 0.55. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.33 (1H, tt, *J* = 2.56), 4.53 (1H, h, *J* = 5.4), 2.18–2.38 (4H, m), 2.0–2.19 (3H, m), 1.96 (3H, s), 1.68–1.87 (3H, m), 1.45–1.63 (3H, m) 1.15 (1H, td, *J* = 13.44, 3.61), 0.98–1.1 (2H, m), 0.93 (3H, s), 0.92 (1H, m), 0.80 (3H, s), 0.8 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 222.09, 170.43, 139.19, 121.88, 73.72, 50.98, 50.02, 47.86, 37.86, 36.81, 36.61, 34.12, 34.05, 33.03, 31.56, 27.56, 25.12, 22.91, 22.04, 21.39, 19.05. IR [cm^{-1}]: 2935, 1731, 1235, 1024. $[\alpha]_D^{20}$ = −154.3 (c 0.78, dichloromethane).

2.2. 3β-Hydroxy-13α-methylandrost-5-en-17-one (**3**)

A solution of **2** (1.15 g, 3.48 mmol) and potassium carbonate (1 g, 7.24 mmol) in methanol (50 mL) was refluxed for an hour and TLC showed full conversion. The reaction mixture was diluted with deionised water and extracted with dichloromethane. The pooled extracts were washed with brine, dried over Na_2SO_4 and evaporated to give 982 mg (98%) of the title compound as white crystals, m.p. 180–181 °C.

TLC: R_f: (petroleum ether/ethyl acetate 5/1 v/v) 0.24. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.35 (1H, tt, *J* = 5.10, 2.55), 3.50 (1H, h, *J* = 5.28), 2.25–2.41 (3H, m), 2.03–2.24 (4H, m), 1.71–1.91 (4H, m), 1.41–1.67 (4H, m), 1.19 (1H, td, *J* = 13.56, 4.04), 1.0–1.14 (2H, m), 0.97 (3H, s), 0.94 (1H, td, *J* = 11.94, 2.1), 0.85 (1H, td, *J* = 12.9, 3.55), 0.83 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 222.50, 140.44, 121.03, 71.74, 51.18, 50.18, 48.07, 42.12, 36.98, 36.85, 34.29, 34.21, 33.17, 31.73, 31.57, 25.26, 23.09, 22.17, 19.25. IR [cm^{-1}]: 3456, 2932, 1722. $[\alpha]_D^{20}$ = −167.4 (c 0.81, dichloromethane).

2.3. 3β-Hydroxy-13α-methylandrost-5-en-17-one pivalate (**4**)

To a solution of **3** (1.63 g, 5.64 mmol) in pyridine (10 mL) there was added trimethylacetyl chloride (815 mg, 6.77 mmol) dropwise at 0 °C. The reaction was stirred for 3 h. The solvent was evaporated *in vacuo* and the residue dissolved in dichloromethane and saturated $NaHCO_3$ solution. After phase separation the aqueous layer was extracted three times with small portions of dichloromethane, the pooled organic phases washed with deionised water and brine and dried over Na_2SO_4 to give 1.94 g (92%) as off-white solid, m.p. 167–170 °C.

TLC: R_f: (petroleum ether /ethyl acetate 7/1 v/v) 0.55. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.38 (1H, tt, *J* = 2.58), 4.56 (1H, h, *J* = 5.42), 2.04–2.42 (7H, m), 1.74–1.93 (3H, m), 1.49–1.7 (5H, m), 1.21 (1H, td, *J* = 13.37, 3.59), 1.17 (9H, s), 1.04–1.13 (1H, m), 0.98 (3H, s), 0.98 (1H, td, *J* = 11.54, 2.73), 0.87 (1H, td, *J* = 13.29, 3.53), 0.85 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 222.45, 178.17, 139.49, 121.86, 73.48, 51.15, 50.20, 47.99, 38.75, 37.89, 36.97, 36.74, 34.30, 34.20, 33.18, 31.70, 27.58, 27.28, 25.26, 23.07, 22.16, 19.23. IR [cm-1]: 2957, 1732, 1717, 1480, 1284, 1161. $[\alpha]_D^{20}$ = −131 (c 0.96, dichloromethane).

2.4. 17-Methylene-13α-methylandrost-5-en-3β-ol pivalate (**5**)

Nysted reagent suspension (30.6 g, 20 wt%, 13.42 mmol) in THF (20 mL) was stirred in a Schlenk flask at 0 °C and $TiCl_4$ (8.05 mmol, 8 mL, 1 M solution) in dichloromethane was added dropwise. The white milky suspension turned yellow and then light brown over

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