Synthesis and Characterization of 3-butyryloxy-16-(β-naphthylmethylene)-5α-androstane-17-one

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Abstract. 3-Butyryloxy-16-(β-naphthylmethylene)-5α-androstane-17-one, a novel androstanone derivative, was synthesized by aldol condensation between epiandrosterone and β-naphthaldehyde and subsequent esterification reaction. The structure of the new product was characterized by ^{1}H NMR, ^{13}C NMR, IR, UV and mass spectra. The new compound may have potential application as anti-cancer agent and/or 5α-reductase inhibitor.

Introduction

Steroids play significant roles in medical and pharmaceutical systems and increasing interest has been stimulated in the study on synthesis and biological activity of steroidal derivatives such as estrogen, androstene, pregnane and progesterone. Steroids have showed various biological activities including anti-tumor and antiproliferative effects on cells [1-5] and anti-flu virus effects [6]. Very recently it was reported that some new steroids could also be used as potential antileukemic agents [7] and 5α-reductase inhibitors [8]. As a continuation of our ongoing study on steroids synthesis and biological evaluation [9-12], herein we present the synthesis and structural characterization of a new androstanone derivative as lead compound for potential anti-cancer agents and/or 5α-reductase inhibitors.

Experimental

The reagents and solvents were purchased from commercial sources and used as received. Melting points were determined on a WRS-2A capillary melting apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer. $CDCl_3$ was used as solvent and chemical shifts recorded were internally referenced to Me_4Si (0 ppm). IR spectra were obtained on a Thermo Electron Corporation Nicolet 380 FT-IR spectrophotometer. UV spectra were recorded on a U-3310 photospectrometer. Mass spectra were recorded on a Bruker APEX III-7.0 spectrometer using electron ionization (EI).

3-Hydroxy-16-(β-naphthylmethylene)-5α-androstane-17-one (2)

A mixture of epiandrosterone (**1**) (0.70 g, 2.5 mmol), β-naphthaldehyde (0.47 g, 3.0 mmol) and KOH (0.70 g, 12.5 mmol) in 20 mL of methanol was stirred at ambient temperature. White solid precipitated in 1 h and the mixture was further stirred for 5 h. The resulted precipitate was collected by filtration and washed with water. Recrystallization from ethyl acetate afforded **2** (0.70 g, 65%) as white powder. M.p.

225–226℃. R_f = 0.16 (petroleum ether/ethyl acetate, 4:1, v/v). IR (KBr) ν = 3515, 3419, 3054, 2922, 2854, 1712, 1621, 1455 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.92-7.84 (m, 3H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.61 (s, 1H), 7.55-7.51 (m, 2H), 3.65-3.60 (m, 1H), 3.05-2.93 (m, 1H), 2.56-2.52 (m, 1H), 1.99-1.92 (m, 2H), 1.86-1.83 (m, 1H), 1.78-1.69 (m, 3H), 1.63-1.60 (m, 2H), 1.52-1.49 (m, 1H), 1.48-1.46 (m, 1H), 1.46-1.33 (m, 6H), 1.21-1.17 (m, 1H), 1.06-1.03 (m, 1H), 1.01 (s, 3H), 0.90 (s, 3H), 0.81-0.75 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.76, 136.43, 133.42, 133.27, 133.10, 133.06, 130.77, 128.50, 128.25, 127.69, 127.06, 126.90, 126.54, 71.15, 54.59, 49.60, 47.62, 44.89, 38.11, 36.91, 35.77, 34.80, 31.74, 31.49, 31.18, 29.41, 28.45, 20.61, 14.56, 12.36. UV (CH_2Cl_2): 268, 278, 321 nm. EI-MS: *m/z* (%) 429.04 (M^++1, 100), 380.05 (76), 274.11 (89).

3-Butyryloxy-16-(β-naphthylmethylene)-5α-androstane-17-one (3)

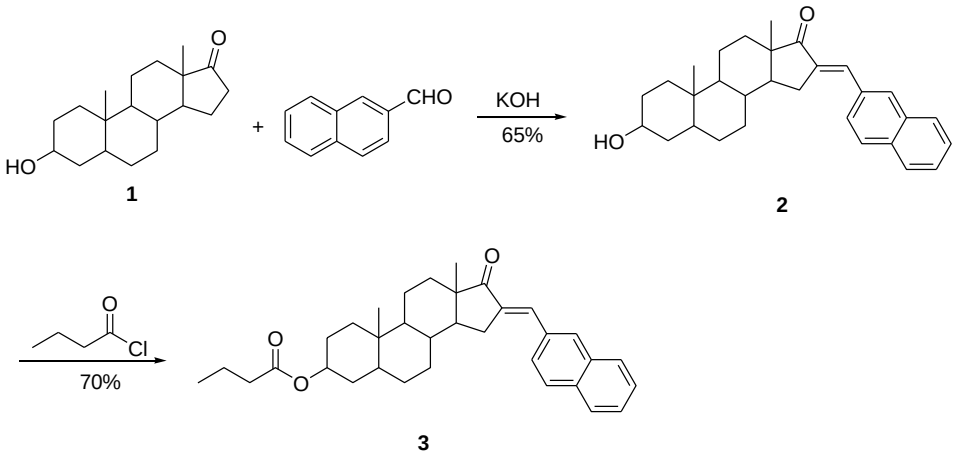
To a flask containing compound **2** (0.22 g, 0.51 mmol), anhydrous triethylamine (0.20 g, 2.0 mmol) and methylene dichloride (10 mL) was added butyryl chloride (66.1 mg, 0.62 mmol). The mixture was stirred at 0 ℃ for 6 h. After reaction the mixture was extracted with methylene dichloride (50 mL × 3). The combined extract was washed with water and dried over anhydrous sodium sulfate. After filtration the filtrate was concentrated by evaporation under reduced pressure. The residue was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (20:1, v/v) as eluent to afford **3** (0.216 g, 70%) as white powder. M.p. 158–162℃. R_f = 0.20 (petroleum ether/ethyl acetate, 20:1, v/v). IR (KBr) ν = 3431, 3051, 2990, 2857, 1721, 1623, 1454 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.92-7.84 (m, 3H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.61 (s, 1H), 7.57-7.51 (m, 2H), 4.75-4.71 (m, 1H), 3.04-2.97 (m, 1H), 2.60-2.49 (m, 1H), 2.27 (t, *J* = 7.4 Hz, 2H), 2.00-1.90 (m, 2H), 1.90-1.83 (m, 1H), 1.82-1.71 (m, 3H), 1.68-1.65 (m, 3H), 1.58 (s, 3H), 1.44-1.38 (m, 4H), 1.30-1.25 (m, 1H), 1.13-1.03 (m, 2H), 1.01 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.93 (s, 3H), 0.86-0.77 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.62, 173.19, 136.39, 133.43, 133.28, 133.25, 133.07, 130.74, 128.48, 128.23, 127.67, 127.03, 126.89, 126.51, 73.17, 54.48, 49.55, 47.58, 44.72, 36.68, 36.62, 35.79, 34.79, 34.03, 31.70, 31.10, 29.37, 28.32, 27.48, 20.56, 18.56, 14.54, 13.59, 12.25. UV (CH_2Cl_2): 246, 268, 278, 321 nm. EI-MS: *m/z* (%) 453.16 (53), 396.68 (70), 340.19 (38), 274.09 (100).

Results and Discussion

The new androstanone derivative **3** was synthesized from epiandrosterone as showed in Scheme 1. The aldol condensation between epiandrosterone and an aromatic aldehyde could be carried out in the presence of NaOH at 40℃ and the reaction was completed in 12 h [13]. We employed KOH to replace NaOH for the aldol condensation between epiandrosterone and β-naphthaldehyde. The reaction was completed at room temperature in 6 h and product **2** was obtained in 65% yield. Esterification of **2** was realized by using 1.2 equivalent of butyryl chloride in the presence of triethylamine with methylene dichloride as solvent. The reaction proceeded at 0℃ and the target compound was separated from the resulted mixture by extraction and column chromatography. Removal of two byproducts from the reaction mixture afforded the target product **3** in 70% yield.

The chemical structures of compound **2** and androstanone ester **3** were fully characterized by ^1H NMR, ^{13}C NMR, IR, UV and mass spectra. ^{13}C NMR spectrum of compound **2** and ^1H NMR spectrum of ester **3** were showed in Fig. 1 and Fig. 2, respectively. In Fig. 1 a signal corresponding to C on C=O group at 209.76 ppm was

visible. While one more signal at 173.19 ppm ascribed to C on C=O group in the ester moiety appeared in ^{13}C NMR spectrum of compound **3**. ^{1}H NMR spectrum of **2** showed two signals corresponding to H on methyl group at 1.01 (s, 3H) and 0.90 (s, 3H) ppm, respectively. While in ^{1}H NMR spectrum of **3** one more signal due to H on methyl group of the butyl moiety at 0.97 (t, J = 7.4 Hz, 3H) ppm together with a signal at 2.27 (t, J = 7.4 Hz, 2H) ppm which was assigned to H on the OCH_2 group were observed. All of the spectra demonstrated the designed chemical structure of compound **2** and **3**. Both compounds exhibited several UV absorption bands and the maximum absorption peak appeared at 278 nm due to the existence of a large π conjugated system comprising of the anthracene ring and the ketene moiety. The new androstanone ester **3** may have excellent anticancer activities against the human cancer cell lines such as SW480, A549, HepG2 and HeLa [13]. Investigation on the biological activity of **3** is now underway.



Scheme 1 Synthetic route to new androstanone derivative **3**

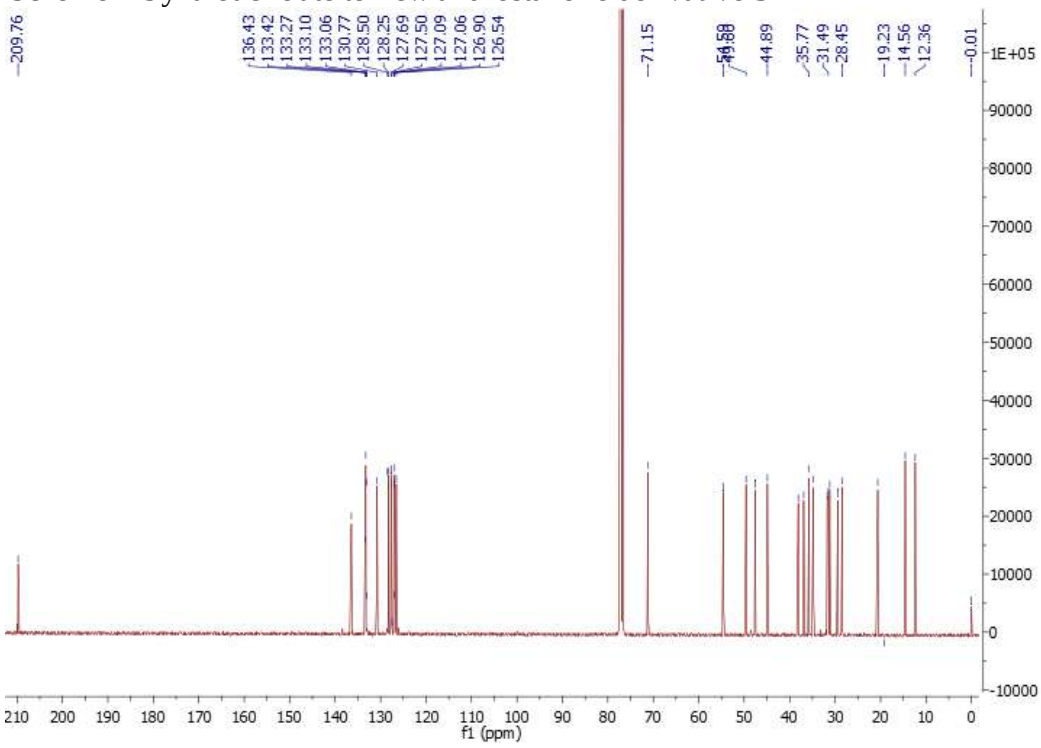


Figure 1 ^{13}C NMR spectrum of compound **2**

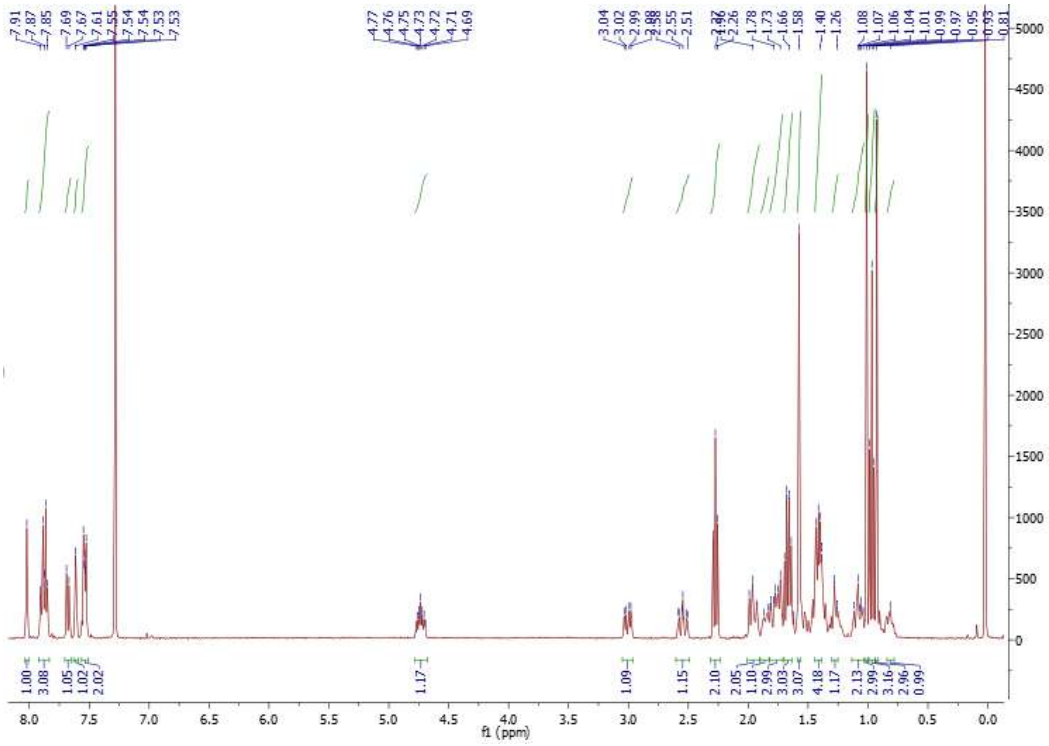


Figure 2 ^{1}H NMR spectrum of androstanone derivative **3**

Summary

In conclusions, a new androstanone derivative was synthesized for potential application as anti-cancer agent and/or 5α-reductase inhibitor by aldol condensation between epiandrosterone and β-naphthaldehyde and subsequent esterification. The structure of the new product was characterized by ^{1}H NMR, ^{13}C NMR, IR, UV and mass spectra. Investigation of the biological activity of the new compound is underway.

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