Steroids 87 (2014) 86–92

Contents lists available at ScienceDirect

Steroids

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

Synthesis of steroidal derivatives containing substituted, fused and spiro pyrazolines

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ARTICLE INFO

Article history:
Received 26 January 2014
Received in revised form 3 May 2014
Accepted 25 May 2014
Available online 11 June 2014

Keywords:
Pyrazoline
Unsaturated ketones
Hydrazine acetate
Cycloaddition
Hydrazone

ABSTRACT

An efficient and facile synthesis of fused, substituted and spiro pyrazoline steroid derivatives through a cycloaddition reaction of different α,β-unsaturated ketones with hydrazine acetate in acetic acid is reported. Depending on the starting material, the ring closure reaction provided a mixture of two steroidal pyrazoline epimers that were separated and studied by NMR techniques. In one case it was possible to isolate and characterize the hydrazone derivative as the reaction intermediate, which confirms the mechanism proposed in the literature [11,25,26].

1. Introduction

Several types of steroids fused to heterocycles have been carefully studied since the 1960s and their chemistry has been progressing subsequently; based on these results, it was possible to establish the structure–activity relationship between the steroidal structure and their physiological properties [1,2]. In recent years, the efforts have been undertaken towards the rational modification of steroid molecules involving the incorporation of a heteroatom, such as N or O. This kind of compounds has shown many different biological activities including anti-microbial, anti-inflammatory, hypotensive, hypocholesterolemic and diuretic activities [3–6]. Pyrazoline derivatives are electron rich nitrogen heterocycles which show interesting pharmacological properties such as analgesic, antipyretic, antirheumatic [7,8], anti-inflammatory [9], antidiabetic [10], anticancer [11–14] and antimicrobial activities [15].

Herein we report a very facile and high yielding synthesis of steroidal pyrazolines located on D and E rings, starting from 20-keto pregnane, *trans*-androsterone and diosgenin through a condensation reaction between α,β-unsaturated carbonyl compounds and 1,2-binucleophilic compounds such as hydrazine (Scheme 1) [16].

2. Experimental methods

2.1. General methods

NMR spectra 1D and 2D ^{1}H and ^{13}C (DEPT, COSY, NOESY, HSQC, HMBC) were recorded on a VARIAN Mercury spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). Chemical shifts are stated in ppm (δ), and are referred to the ^{1}H signal ($\delta = 7.24$) or to the central ^{13}C triplet signal ($\delta = 77.0$) for $CDCl_3$. Coupling constants (J) are quoted in Hz. IR spectra were acquired on a Nicolet FT-IR 380 spectrophotometer (ν_{max}, cm^{-1}). FAB-MS, EI-MS and high resolution mass (HRMS) spectra were recorded on a Jeol-JMS-700 MS Station spectrometer. Optical rotations were determined on a Perkin Elmer 241 polarimeter at room temperature using chloroform solutions. Melting points were measured on a Mel-Temp apparatus and were not corrected. Analytical TLC was performed on silica gel ALUGRAM® SIL G/UV254 plates and chromatographic columns were carried out on silica gel Davisil™ grade 633 (200–425 mesh).

2.2. General procedure for the preparation of steroidal benzylidene derivatives

A mixture of **1** or **5** (1 mmol) and benzaldehyde (1 mmol) was dissolved in ethanol (5 mL) and an alcoholic solution of potassium hydroxide (10%) was added drop wise. The mixture was stirred at room temperature. After completion of the reaction, the precipi-

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http://dx.doi.org/10.1016/j.steroids.2014.05.013
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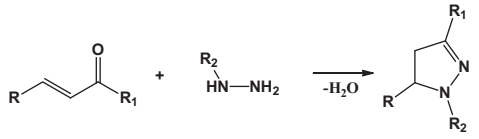

Scheme 1. General reaction for the formation of pyrazoline.

tated solid was filtered and washed with ethanol (10 mL) to afford the product **2** or **6** as solid powder.

*2.2.1. (21E)-21-Benzyliden-3β-hydroxypregn-20-one **2***

Colorless solid powder; yield 80%; mp. 110–111 °C; $[\alpha]_D$ +37.6° (*c* 1, $CHCl_3$); IR $\bar{\nu}_{max}$: 3411, 2926, 1660, 1607. ^{1}H NMR ($CDCl_3$) δ: 0.61 (s, 3H, CH_3-18), 0.79 (s, 3H, CH_3-19), 2.82 (t, 1H, *J* = 8.5 Hz, H-17), 3.55 (m, 1H, H-3), 6.75 (d, 1H, *J* = 16.0 Hz, H-21), 7.37 (overlapping multiplets, 3H, H-3′, H-4′, H-5′), 7.55 (overlapping multiplets, 3H, H-2′, H-6′, H-22). ^{13}C NMR ($CDCl_3$) δ: 12.5 (C-18), 13.9 (C-19), 21.5 (C-11), 22.9 (C-15), 24.8 (C-6), 28.8 (C-16), 31.6 (C-2), 32.3 (C-7), 35.7 (C-10), 35.8 (C-1), 37.2 (C-8), 38.3 (C-4), 39.6 (C-12), 45.0 (C-5), 45.5 (C-13), 54.5 (C-9), 57.1 (C-14), 62.4 (C-17), 71.20 (C-3), 126.7 (C-21), 128.5 (C-2′,6′), 129.1 (C-3′,5′), 130.4 (C-4′), 135.0 (C-1′), 141.3 (C-22), 200.4 (C-20). HREI: (*m*/*z*) calcd for $C_{28}H_{38}O_2$: 406.2872, found 406.2881.

*2.2.2. (16E)-16-Benzyliden-3β-hydroxyl-5α-androstan-17-one **6***

Colorless solid powder; yield 98%; mp. 163–165 °C. ^{1}H NMR ($CDCl_3$) δ: 0.86 (s, 3H, CH_3-19), 0.95 (s, 3H, CH_3-18), 2.40 (m, 1H, H-15*a*), 2.86 (dd, 1H, H-15*b*), 3.59 (m, 1H, H-3), 7.37 (overlapping multiplets, 4H, H-1′, H-4′, H-5′, H-6′), 7.53 (overlapping multiplets, 2H, H-3′, H-7′). ^{13}C NMR ($CDCl_3$) δ: 12.3 (C-19), 14.4 (C-18), 20.5 (C-11), 28.3 (C-6), 29.3 (C-15), 31.0 (C-7), 31.3 (C-12), 31.6 (C-2), 34.6 (C-4), 35.7 (C-1), 36.8 (C-10), 37.9 (C-8), 44.7 (C-5), 47.5 (C-13), 49.4 (C-14), 54.4 (C-9), 71.1 (C-3), 128.6 (C-4′ and C-6′), 129.1 (C-5′), 130.2 (C-3′ and C-7′), 132.9 (C-1), 135.5 (C-2′), 136.1 (C-16), 210.0 (C-17). The spectroscopy data of the compound **6** was compared with that reported in the literature [17].

2.3. General process for the preparation of steroidal pyrazoline derivatives

Compounds **2**, **6** or **10** (2.4 mmol) were dissolved in 10 ml of acetic acid and then hydrazine acetate (4.8 mmol) was added. The mixture was refluxed and monitored by TLC until complete disappearance of starting material (∼3 h). The product was precipitated by pouring the reaction mass into excessive amounts of ice-cold water. The formed precipitate was filtered, washed with water, and dried to afford the products **3a**, **4a**, **7**, **12** and **16**.

*2.3.1. (5′S)- and (5′R)-17β-(1′-Acetyl-5′-phenyl-1H-pyrazolin-3′-yl)-5α-androstan-3β-yl acetate (**3b** and **4b**)*

The mixture of epimers ***3a*** and ***4a*** was dissolved in CH_2Cl_2 (5 ml), subsequently acetic anhydride (5 ml) and catalytic amounts of DMAP were added, the solution was stirred at room temperature during 15 min. The mixture was then diluted with water and the organic phase was extracted with CH_2Cl_2 (50 mL). The residue was washed with saturated solution of $NaHCO_3$ (3 × 30 mL), and water (40 mL). The resulting organic phase was dried over Na_2SO_4 and concentrated to dryness under vacuum. The acetylated crude product was purified by column chromatography on silica gel (hexane/EtOAc 8:2), the product **3b** was eluted first and then **4b**. The product **3b** was obtained as a white solid powder; yield 25%; mp. 184–186 °C; $[\alpha]_D$ +69.3° (*c* 1.8, $CHCl_3$); IR $\bar{\nu}_{max}$: 2928, 2858, 1727, 1653. ^{1}H NMR ($CDCl_3$) δ: 0.62 (s, 3H, CH_3-18), 0.83 (s, 3H, CH_3-19), 2.02 (s, 3H, 3-Ac-CH_3), 2.31 (s, 3H, N-Ac-CH_3), 2.62 (dd, 1H, *J* = 20 Hz, *J* = 4 Hz, H-4′), 3.36 (dd, 1H, *J* = 20 Hz, *J* = 12 Hz, H-4′), 4.68 (m, 1H, H-3), 5.40 (1H, dd, *J* = 12 Hz, *J* = 4 Hz, H-5′), 7.15 (d, 2H, *J* = 8 Hz, H-2″ and H-6″), 7.22 (t, 1H, *J* = 8 Hz, H-4″), 7.30 (t, 2H, *J* = 8 Hz, H-3″and H-5″). ^{13}C NMR ($CDCl_3$) δ: 12.2 (C-19), 13.5 (C-18), 20.9 (3-Ac-CH_3), 21.4 (N-Ac-CH_3), 21.8 (C-11), 24.9 (C-15), 27.3 (C-2), 28.4 (C-6), 29.6 (C-16), 31.8 (C-7), 33.9 (C-4), 35.4 (C-8), 35.5 (C-10), 36.7 (C-1), 38.4 (C-12), 44.1 (C-13), 44.5 (C-5), 45.9 (C-4′), 51.9 (C-17), 54.1 (C-9), 56.0 (C-14), 59.0 (C-5′), 73.5 (C-3), 125.2 (C- 2″ and 6″), 127.3 (C-4″), 128.7 (C-3″ and 5″), 141.9 (C-1″), 159.5 (C-3′), 168.4 (N-Ac-CO), 170.7 (3-Ac-CO). HRMS-FAB (*m*/*z*): calcd for $C_{32}H_{44}N_2O_3$: 504.3352, found 505.3365 $[M+1]^+$. The product ***4b*** was obtained as a white solid powder; yield 50%; mp. 149–152; $[\alpha]_D$ −17.7° (*c* 1 $CHCl_3$); IR $\bar{\nu}_{max}$: 2928, 2858, 1727, 1653. ^{1}H NMR ($CDCl_3$) δ: 0.63 (s, 3H, CH_3-18), 0.80 (s, 3H, CH_3-19), 2.01 (s, 3H, 3-Ac-CH_3), 2.31 (s, 3H, N-Ac-CH_3), 2.74 (dd, 1H, *J* = 16 Hz, *J* = 4 Hz, H-4′), 3.26 (dd, 1H, *J* = 16 Hz, *J* = 12 Hz, H-4′), 4.67 (m, 1H, H-3), 5.40 (1H, dd, *J* = 12 Hz, *J* = 4 Hz, H-5′), 7.15 (d, 2H, *J* = 8 Hz, H-2″ and 6″), 7.23 (t, 1H, *J* = 8 Hz, H-4″), 7.31 (t, 2H, *J* = 8 Hz, H-3″ and 5″). ^{13}C NMR ($CDCl_3$) δ: 12.1 (C-19), 13.6 (C-18), 21.0 (3-Ac-CH_3), 21.4 (N-Ac-CH_3), 21.8 (C-11), 24.2 (C-15), 24.5 (C-2), 27.3 (C-6), 28.4 (C-16), 29.6 (C-7), 31.8 (C-4), 33.9 (C-8), 35.6 (C-10), 36.7 (C-1), 38.6 (C-12), 44.1 (C-13), 44.5 (C-5), 46.2 (C-4′), 51.7 (C-17), 54.1 (C-9), 56.1 (C-14), 59.0 (C-5′), 73.6 (C-3), 125.2 (C- 2″ and 6″), 127.3 (C-4″), 128.8 (C-3″ and 5″), 142.2 (C-1″), 159.3 (C-3′), 168.5 (N-Ac-CO), 170.7 (3-Ac-CO). HRMS-FAB (*m*/*z*): calcd for $C_{32}H_{44}N_2O_3$: 504.3352, found 505.3365 $[M+1]^+$.

*2.3.2. (5′R)- and (5′S)-17β-(1′-Acetyl-5′-phenyl-1H-pyrazolin-3′-yl)-5α-androstan-3β-ol (**3a** and **4a**)*

The individual compounds **3b** or **4b** (100 mg) were dissolved in a solution (10%) of KOH in methanol (15 ml). The mixture was allowed to stand at room temperature, and the progress of the reaction was monitored by TLC. After completion of the transformation, the reaction mixture was diluted with water. The resulting precipitate was filtered off, washed with water and dried under vacuum to afford **3a** as a white solid powder; yield 85%; mp. 239–241 °C; $[\alpha]_D$ +0.57° (*c* 0.1, CHCl3). IR $\bar{\nu}_{max}$: 3408, 2920, 2855, 1648. ^{1}H NMR ($CDCl_3$) δ: 0.63 (s, 3H, CH_3-18), 0.81 (s, 3H, CH_3-19), 2.31 (s, 3H, N-Ac-CH_3), 2.66 (dd, 1H, J_{gem} = 20 Hz, $J_{4'a\text{-}5'}$ = 4 Hz H-4′), 3.36 (dd, 1H, J_{gem} = 20 Hz, $J_{4'b\text{-}5'}$ = 12 Hz, H-4′), 3.59 (m, 1H, H-3), 5.40 (1H, dd, $J_{5'\text{-}4'b}$ = 12 Hz, $J_{5'\text{-}4'a}$ = 4 Hz, H-5′), 7.14 (d, 2H, *J* = 8 Hz, H-2″ and 6″), 7.22 (t, 1H, *J* = 8 Hz, H-4″), 7.30 (t, 2H, *J* = 8 Hz, H-3″ and 5″). ^{13}C NMR ($CDCl_3$) δ: 12.3 (C-19), 13.3 (C-18), 21.0 (C-11), 21.8 (N-Ac-CH_3), 24.3 (C-15), 24.4 (C-6), 28.5 (C-16), 29.6 (C-2), 31.4 (C-7), 31.9 (C-1), 35.5 (C-10), 35.6 (C-8), 36.9 (C-4), 38.0 (C-12), 38.5 (C-13), 44.8 (C-5), 45.8 (C-4′), 51.9 (C-17), 54.2 (C-9), 56.1 (C-14), 59.0 (C-5′), 71.2 (C-3), 125.2 (C- 2″ and 6″), 127.3 (C-4″), 128.7 (C-3″ and 5″), 141.9 (C-1″), 159.5 (C-3′), 168.4 (N-Ac-CO). HRMS-FAB (*m*/*z*): calcd for $C_{32}H_{44}N_2O_3$: 462.3246, found 462.3219. ***4a***: white solid powder; yield 90%; mp. 149–152 °C; IR $\bar{\nu}_{max}$: 3411, 2921, 2854, 1644. ^{1}H NMR ($CDCl_3$) δ: 0.63 (s, 3H, CH_3-18), 0.78 (s, 3H, CH_3-19), 2.31 (s, 3H, N-Ac-CH_3), 2.71 (dd, 1H, J_{gem} = 16 Hz, $J_{4'a\text{-}5'}$ = 4 Hz, H-4′), 3.26 (dd, 1H, J_{gem} = 15.6 Hz, $J_{4'b\text{-}5'}$ = 12 Hz, H-4′), 3.54 (m, 1H, H-3), 5.40 (1H, dd, $J_{4'b\text{-}5'}$ = 12 Hz, $J_{4'a\text{-}5'}$ = 4 Hz, H-5′), 7.15 (d, 2H, *J* = 8 Hz, H-2″ and 6″), 7.23 (t, 1H, *J* = 8 Hz, H-4″), 7.31 (t, 2H, *J* = 8 Hz, H-3″ and 5″). ^{13}C NMR (δ, ppm, $CDCl_3$): 12.2 (C-19), 13.5 (C-18), 21.0 (C-11), 21.8 (N-Ac-CH_3), 24.2 (C-15), 24.4 (C-6), 28.5 (C-16), 29.6 (C-2), 31.3 (C-7), 31.9 (C-1), 35.4 (C-10), 35.5 (C-8), 36.9 (C-4), 37.9 (C-12), 38.6 (C-13), 44.7 (C-5), 46.1 (C-4′), 51.7 (C-17), 54.2 (C-9), 56.1 (C-14), 59.0 (C-5′), 71.1 (C-3), 125.2 (C- 2″ and 6″), 127.3 (C-4″), 128.7 (C-3″ and 5″), 142.2 (C-1″), 159.4 (C-3′), 168.6 (N-Ac-CO). HREI: (*m*/*z*) calcd for $C_{30}H_{42}N_2O_2$: 462.3246, found 462.3219.

*2.3.3. 31′-Acetyl-5′-phenyl-4′,5′-dihydro[1,2]-diazolo[4′,3′:16,17]-5α-androstan-3β-yl acetate **7***

White solid powder; yield 70%; mp. 145–148 °C; [α]D −1.48° (*c* 0.1, CHCl3); IR $\bar{\nu}_{max}$: 2929, 2856, 1727, 1652. ^{1}H NMR ($CDCl_3$) δ: