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Synthesis of steroidal derivatives containing substituted, fused and spiro pyrazolines

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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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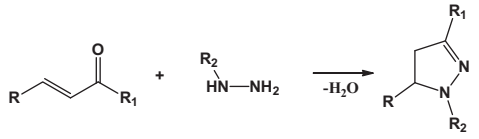


Scheme 1. General reaction for the formation of pyrazoline.

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:

0.59 (s, 3H, CH_3-18), 0.77 (s, 3H, CH_3-19), 1.99 (s, 3H, 3-Ac-CH_3), 2.35 (s, 3H, N-Ac-CH_3), 3.92 (m, 1H, H-16), 4.46 (d, 1H, $J_{5'-4'}$ = 12 Hz, H-5′), 4.66 (m, 1H, H-3), 7.37 (m, 2H, H-3″and 5″), 7.71 (m, 3H, H-2″, 4″and 6″). ^{13}C NMR ($CDCl_3$) δ: 12.1 (C-19), 12.4 (C-18), 21.0 (C-11), 21.4 (N-Ac-CH_3), 21.5 (C-15), 27.3 (C-2), 28.3 (C-6), 30.8 (C-15), 32.2 (C-7), 33.8 (4), 35.1 (C-8), 55.4 (C-10), 36.6 (C-1), 38.9 (C-12), 44.4 (C-5), 45.9 (C-13), 49.3 (C-16), 53.7 (C-9), 54.3 (C-14), 72.1 (C-5′), 73.5 (C-3), 126.8 (C- 2″ and 6″), 128.5 (C-4″), 129.8 (C-3″ and 5″), 131.0 (C-1″), 157.4 (C-17), 169.29 (N-Ac-CO), 170.6 (3-Ac-CO). HREI: (*m/z*) calcd for $C_{30}H_{42}N_2O_2$: 476.6502, found 477.3176.

2.3.4. N-Acetyl-20-hydrazonopregna-5,16-dien-3β-yl acetate ***11***

Yellow oil; yield 30%; $[\alpha]_D$ +0.51 (c 0.76, $CHCl_3$) IR $\bar{\nu}_{max}$: 2941, 1718, 1667 ^{1}H NMR ($CDCl_3$) δ: 0.98 (s, 3H, CH_3-18), 1.0 (s, 3H, CH_3-19), 1.90 (s, 3H, CH_3-21), 2.0 (s, 3H, 3-Ac-CH_3), 2.2 (s, 3H, N-Ac-CH_3), 4.60 (m,1H, H-3), 5.39 (d,1H, *J* = 4 Hz, H-6), 6.11 (dd, 1H, *J* = 4 Hz, *J* = 4 Hz, H-16), 8.56 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 12.3 (C-21), 15.8 (C-18), 19.1 (C-19), 20.8 (N-Ac-CH_3), 20.9 (C-11), 21.4 (3-Ac-CH_3), 27.6 (C-2), 30.1 (C-8), 31.4 (C-7), 31.6 (C-15), 35.4 (C-12), 36.6 (C-10), 36.8 (C-1), 38.0 (C-4), 46.6 (C-13), 50.2 (C-9), 56.8 (C-14), 73.8 (C-3), 122.1 (C-6), 133.8 (C-16), 140 (C-5), 145.5 (C-20), 153.3 (C-17), 170.5 (3-Ac-CO), 173.6 (N-CO). HREI: (*m/z*) calcd for $C_{25}H_{36}N_2O_3$: 412.2726, found 412.2813.

2.3.5. 51′-Acetyl-3′-methyl-16,17-dihydro[1,2]-diazolo[5′,4′:16,17]-androst-6-en-3β-yl acetate ***12***

White solid powder; yield 80% from **10** and 90% from **11**; mp. 170–173 °C; $[\alpha]_D$ +20.4° (*c* 0.1, $CHCl_3$) IR $\bar{\nu}_{max}$: 1734, 1660, 1645. ^{1}H NMR ($CDCl_3$) δ: 0.90 (s, 3H, CH_3-18), 1.0 (s, 3H, CH_3-19), 1.9 (s, 3H, CH_3-3″), 2.0 (s, 3H, 3-Ac-CH_3), 2.2 (s, 3H, N-Ac-CH_3), 3.0 (d,1H, $J_{17\text{-}16}$ = 12 Hz, H-17), 4.5 (m, 1H, H-3), 4.71 (dd, 1H, $J_{16\text{-}17}$ = 12 Hz, $J_{16\text{-}15}$ = 4 Hz, H-16), 5.34 (d, 1H, *J* = 4 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ,: 17.0 (C-3″), 19.1 (C-19), 20.8 (C-18), 20.8 (3-Ac-CH3), 21.3 (N-Ac-CH_3), 21.8 (C-11), 27.6 (C-1), 31.4 (C-7), 31.9 (C-8), 34.0 (C-15), 35.8 (C-12), 36.5 (C-10), 36.9 (C-4), 37.9 (C-12), 46.2 (C-13), 49.4 (C-9), 50.3 (C-14), 60.8 (C-16), 65.5 (C-17), 73.7 (C-3), 122.2 (C-6), 139.2 (C-5), 156.6 (C-3′), 168.3 (N-Ac-CO), 170.5 (3-Ac-CO). HREI: (*m/z*) calcd for $C_{25}H_{36}N_2O_3$: 412.2726, found 412.2722.

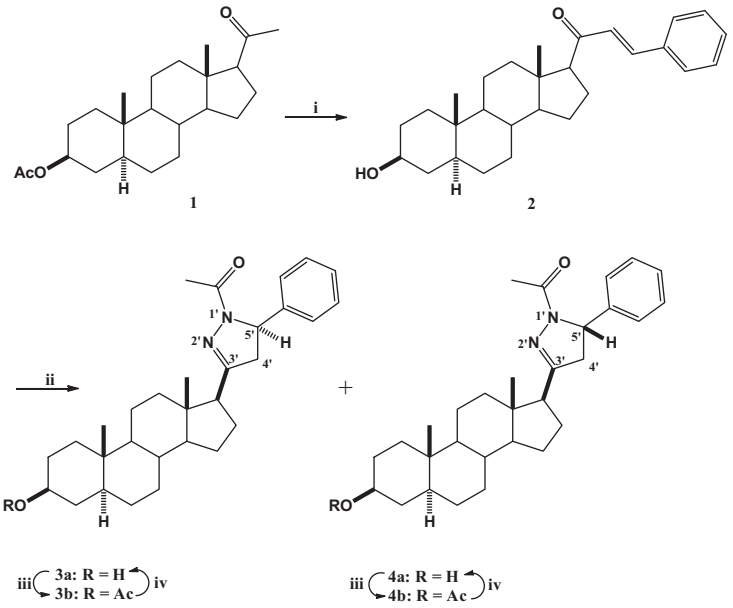
*2.3.6. 6(22)-[1′-acetyl-3′-methyl-4′(3″-acetoxy-2″-methyl)propyl]-16β,22-epoxy-23,24-dinorspiro[22.5′]-1H-pirazolinyl)-chol-6-en-3β-yl acetate (****16****)*

White solid powder; yield 70%; mp. 192–194 °C; $[\alpha]_D$ −16.3° (*c* 0.1, $CHCl_3$); IR $\bar{\nu}_{max}$: 2943, 1725, 1706, 1238. ^{1}H NMR ($CDCl_3$) δ: 0.93 (d, 1H, *J* = 4 Hz, CH_3-27) 0.95 (s, 3H, CH_3-18), 1.0 (s, 3H, CH_3-19), 1.2 (d, 1H, *J* = 8 Hz, H-21), 1.8 (s, 3H, N-Ac-CH_3), 2.05 (s, 3H, 26-Ac-CH_3), 2.0 (s, 3H, 3-Ac-CH_3), 2.1 (s, 3H, H-3″), 3.0 (m, 2H, H-20 and H-23), 3.8 (dd, 1H, *J* = 10 Hz, *J* = 4 Hz, H-26), 3.9 (dd, 1H, *J* = 10 Hz, *J* = 4 Hz, H-26), 4.58 (m, 1H, H-3), 4.8 (m, 1H, H-16), 5.3 (d, 1H, *J* = 4 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ: 10.4 (C-3″), 12.6 (C-18), 17.2 (C-27), 19.2 (C-19), 20.7 (C-11), 20.9 (26-Ac-CH_3), 21.3 (3-Ac-CH_3), 21.6 (C-21), 27.2 (C-24), 27.6 (C-2), 28.0 (C-20), 31.3 (C-8), 31.5 (C-7), 34.0 (C-25), 35.0 (C-15), 36.5 (C-10), 36.8 (C-1), 38.0 (C-4), 39.6 (C-12), 42.3 (C-13), 49.8 (C-9), 54.3 (C-14), 58.4 (C-17), 68.6 (C-26), 73.8 (C-3), 75.8 (C-16), 111.3 (C-22), 122.2 (C-6), 139.6 (C-5), 153.7 (C-3′), 170.1 (-N-CO), 170.5 (3-Ac-CO), 171.0 (26-Ac-CO). HREI: (*m/z*) calcd for $C_{25}H_{36}N_2O_3$: 596.3825, found 597.3885 $[M+1]^+$.

3. Results and discussion

The importance of different steroidal heterocycles is well known. In our attempt to increase the biological activity we decided to build up pyrazoline rings from steroidal α,β-unsaturated ketones and hydrazine. Three different types of compounds were synthesized as precursors of steroidal pyrazoline derivatives.

The first α,β-unsaturated ketone was synthesized through the aldolic condensation of pregnanolone acetate **1** with benzaldehyde under basic conditions to give the corresponding benzylidene derivative **2** (Scheme 2). It is important to mention that due to the basic conditions used, the first step in the reaction was the hydrolysis of the acetate on C-3 affording pregnanolone **1*a*** and then the condensation with the benzaldehyde took place yield a single product. The ^{1}H NMR spectrum of the steroidal benzyliden



Reagents and conditions: (i) EtOH, KOH, benzaldehyde. (ii) AcOH, hydrazine acetate, reflux. (iii) DMAP, acetic anhydride, rt. (iv) Methanol, KOH, rt.

Scheme 2. Synthesis of steroidal pyrazolines **3** and **4**.

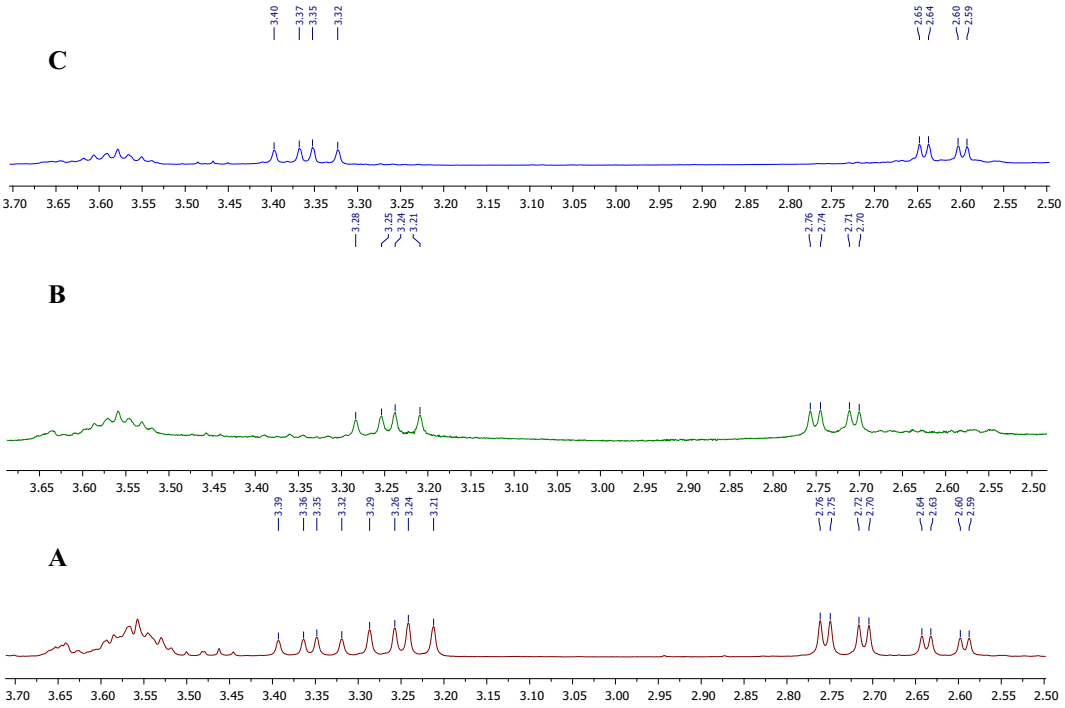


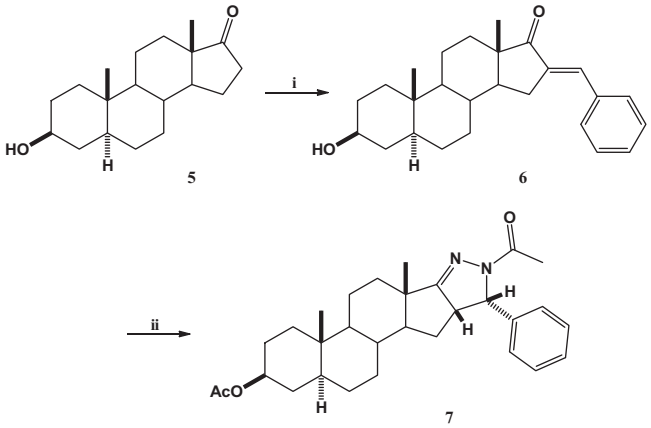
Fig. 1. ^{1}H NMR epimeric resolution of **3a** and **4a**.

2 showed two characteristic peaks around 6.75 and 7.55 ppm, which can be assigned to the coupling protons of the double bond at C-21 Their coupling constant of 16 Hz, indicates their *trans* relative position, therefore the configuration of the double bond is *E*. The signals corresponding to the phenyl group (δ = 7.56–7.52 ppm) and a multiplet at 3.55 ppm assigned to H-3 were also observed. In the ^{13}C NMR spectrum of **2**, it is observed at high frequencies, the signals of the phenyl group (δ = 128.5–135.0 ppm), the vinylic carbons C-21 and C-22 (δ = 126.7 and 141.3 ppm, respectively) and the carbonyl group at 200.4 ppm.

To obtain the steroidal pyrazoline derivative and study the stereochemistry of their formation, we treated (*E*)-21-benzyliden-3β-hydroxy-pregn-20-one **2** with hydrazine acetate in acetic acid into reflux during 3 h; the TLC indicated the presence of two products **3a** and **4a**, with a very close R_F, in a ratio 1:2 found by ^{1}H NMR spectroscopy. For a more effective separation, the product mixture was acetylated under standard conditions and the two products ***3b*** and ***4b*** were fully separated by column chromatography. After separation, 2D NMR showed that the minor product was the 5′*R* isomer (***3b***), while the major product was the epimer 5′*S* (***4b***). After separation of the acetylated pyrazoline products (***3b*** and ***4b***), they were hydrolyzed to yield the 3β-hydroxy derivatives (***3a*** and ***4a***).

The assignment of the stereochemistry of 5′ position was achieved by comparison with the steroidal pyrazoline analogue described by Ivanyi et al. [11]. In the ^{1}H NMR spectrum of the epimeric mixture (Fig. 1A) it is observed two signal groups at 3.39–3.21 and 2.76–2.59 corresponding to the pyrazoline ring protons H-4′, the major one (Fig. 1B) belonging to the 5′*S* diasteroisomer (**4a**) and the minor one (Fig. 1C) assigned to the pyrazoline 5′*R* (**3a**).

Pyrazolines **3a** and **4a** represent two examples of steroidal pyrazolines located on C-17; continuing with our study we decided to synthesize some compounds where the heterocycle is fused to C-16 and C-17. For this purpose we obtained the α,β-unsaturated ketone **6** in quantitative yields through the reaction of *trans*-androsterone **5** with benzaldehyde in the presence of potassium hydroxide in ethanol [17,18]. Compound **6** and hydrazine acetate



Reagents and conditions: (i) EtOH, KOH, benzaldehyde. (ii) AcOH, hydrazine acetate, reflux.

Scheme 3. Synthesis of pyrazoline steroidal derivative **7** from *trans*-androsterone.

were refluxed in acetic acid during 5 h to afford pyrazoline **7** as a single product (Scheme 3).

The most remarkable signals of the ^{1}H NMR spectrum of **7** are multiplets (7.36–7.69 ppm) corresponding to Ar–H, a doublet at 4.46 ppm assigned to H-5′ and a doublet of triplets signal at 3.92 ppm assigned to H-16 characteristic of pyrazoline heterocycles, four singlets of methyl protons at 2.35, 1.99, 0.53 and 0.76 ppm corresponding to the NC(O)CH_3, $COOCH_3$, CH_3-18 and CH_3-19, respectively. In the ^{13}C NMR spectrum the characteristic signals of pyrazoline heterocycle are observed at 157.4, 72.1 and 49.3 ppm corresponding to C-17, C-5′ and C-16, respectively.

The stereochemistry of the new stereogenic center C-16 of compound **7** was established considering the chemical shift of CH_3-18 in the ^{1}H NMR spectrum. According with the literature [19], when a substituent on C-6 is placed with β orientation, the signal of the CH_3-18 is deshielded, and it can be observed at 0.78 ppm, approximately. In the H^{1}-NMR spectrum of **7**, the signal of CH_3-18 is

Table 1
Calculated vicinal coupling constants $^{3}J_{16H\text{-}5H}$ (Hz) for the particular *Cis* configuration of **7** and their comparison with experimental vicinal coupling constants.

Methods			
Compound **7**	PM3	RHF/6-31+G(d)	B3LYP/6-31+G(d)
Energy (u.a.)	−0.1579	−1492.8676	−1502.5142
Dihedral angle	16.23	23.94	21.26
$^{3}J_{H16\text{-}H5'}^{calculated}$	–	13.33	10.61
$^{3}J_{H16\text{-}H5'}^{expimental}$	12.00		

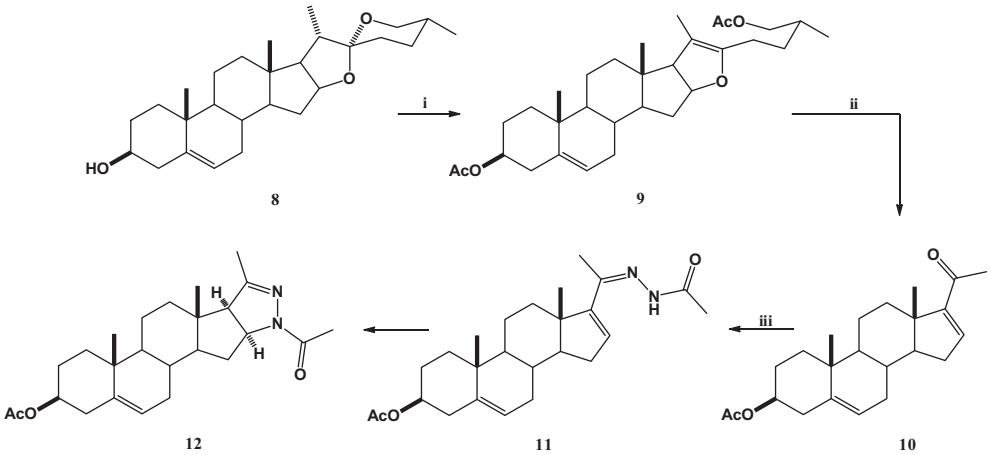
Table 2
Calculated vicinal coupling constants $^{3}J_{16H\text{-}17H}$ (Hz) for the particular *Cis* configuration of **12** and their comparison with experimental vicinal coupling constants.

Methods			
Compound **12**	PM3	RHF/6-31+G(d)	B3LYP/6-31+G(d)
Energy (u.a.)	−0.1975	−1301.1908	−1309.5671
Dihedral angle	3.83	13.14	12.85
$^{3}J_{H16\text{-}H17}^{calculated}$	–	17.38	11.36
$^{3}J_{H16\text{-}H17}^{expimental}$	12.00		

observed at 0.53 ppm, such value is similar to the chemical shift observed of some derivatives with the substituent on C-16 α-orientated [20,21]. Based on these observations we concluded that C-16 in compound **7** has an *S* configuration. Furthermore, in the analogue synthesized by Amr et al. [19] it was found that the value of the coupling constant of $J_{5',16\beta}$ = 1.2 Hz, corresponding to a dihedral angle close to 120°, evidencing the *trans* disposition of the protons H-5′ and H-16; nevertheless, the value of the coupling constant observed for compound **7** is $J_{5',16\beta}$ = 12 Hz, corresponding a dihedral angle close to 0°, suggesting that H-5′ is *β*-orientated (5′S). In addition, theoretical studies (PM3, RHF/6-31+G(d) and B3LYP/6-31G(d) level of theory) were carried out to calculate the J_{vic} values for the *cis* configuration of **7**, this values are near to experimental value (Table 1).

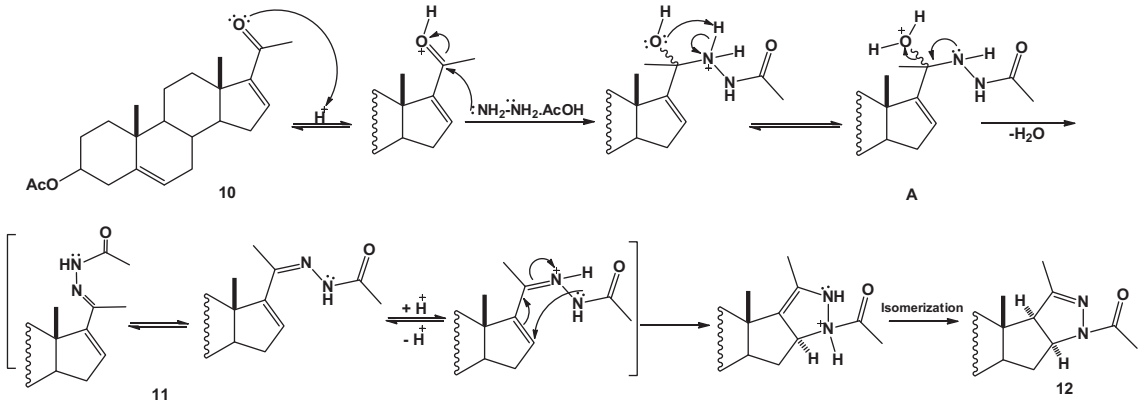
Additionally, we decided to use as starting material the α,β-unsaturated ketone **10** previously reported [22,23]; compound **10** was synthesized from commercial diosgenin **8** through an acetolysis reaction affording pseudodiosgenin **9** [24] followed by an oxidative cleavage of furan ring (Scheme 4). Fused pyrazoline **12** was obtained by refluxing ketone **10** with hydrazine acetate in AcOH during 5 h.

After one hour of reaction, the starting material was transformed completely, and two new products were visualized by TLC. The less polar was identified as the intermediate hydrazone **11** and the more polar corresponding to compound **12** (Scheme 4). The presence of the hydrazone is consistent with the widely accepted mechanism for the formation of pyrazolines from α,β-unsaturated ketone systems (Scheme 5) [11,25,26] involving the nucleophilic attack of the hydrazine nitrogen on the carbonyl

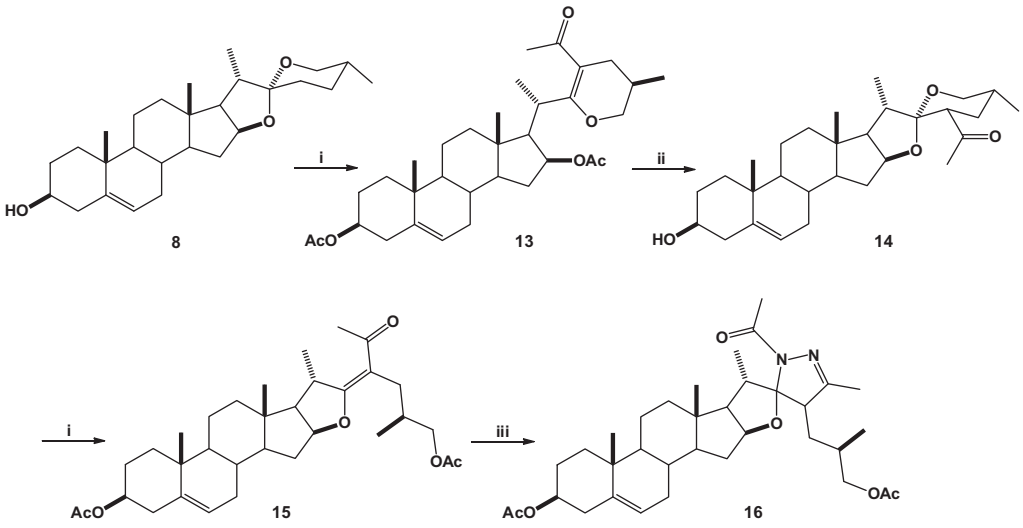


Reagents and conditions: (i) BF_3OEt_2, $AcOCOCF_3$, CH_2Cl_2. (ii) CrO_3, AcOH, CH_2Cl_2. (iii) AcOH, hydrazine acetate, reflux.

Scheme 4. Synthesis of **12** from 3β-acetoxy-5,16-pregnadien-20-one (**10**).



Scheme 5. Presumed mechanism for the formation of pyrazoline rings.



Reagents and conditions: (i) Ac_2O, BF_3OEt_2, rt. (ii) KOH (iii) AcOH, hydrazine acetate, reflux.

Scheme 6. Synthesis of steroidal pyrazoline **16**.

group of the enone affording intermediate **A**; then a elimination of a water molecule gave hydrazone **11**. Intramolecular Michael addition of the nitrogen to the double bond led the pyrazoline **12**.

In the ^{1}H NMR spectrum of derivative **11** it is observed one broad singlet around 8.56 ppm assigned to N-H proton, one doublet of double signals at 6.11 and a doublet at 5.39 ppm corresponding to the vinyl protons H-16 and H-6 respectively, three singlets at 2.39, 2.00 and 1.90 ppm corresponding to $NC(O)CH_3$, $COOCH_3$ and CH_3-21, respectively. In ^{13}C NMR spectrum the most significant change compared with compound **10**, is the disappearance of the signal at 196.6 ppm corresponding to the α,β-unsaturated ketone and the appearance of a signal at 145.5 ppm assigned to C-20; it also can be observed a disappearance of the vinylic C-16 and C-17 at 133.8 and 153.3 ppm, respectively.

The hydrazone **11** was treated with AcOH and hydrazine acetate at reflux during 3 h for complete transformation into pyrazoline **12**. The ^{1}H NMR showed an upfield shift of H-16 to 4.71 ppm and the appearance of a signal at 3.01 ppm corresponding to the new formed H-17. In the ^{13}C NMR two signals are observed of the pyrazoline ring C-17 and C-16 at 60.8 and 65.6 ppm, respectively.

The configuration of C-16 and C-17 of compound **12**, was established considering the chemical shift of CH_3-18 (0.90 ppm) in the ^{1}H NMR spectrum, which is deshielded due to the influence of the new pyrazoline ring place in β position [19,21]. The value of the coupling constant of $J_{16,17}$ = 12 Hz, confirms that both H-16 and H-17 are in *cis* position, concluding that the configuration of C-16 and C-17 is *R*, *S*, respectively. Also the theoretical studies to calculate the J_{vic} give the approximate value obtained experimentally (Table 2).

Most of the steroidal heterocycles found in the literature are located on the D ring; with the aim to generate a new group of steroidal pyrazolines, we studied the synthesis of α,β-unsaturated systems on the E ring. Our research group has developed in recent years new methodologies for the modification of the side chain of sapogenins [27–29]; since our intention was to transform the E ring, we decided to use the well known furostene derivative **15**, described by our research group [30]. The acetolysis reaction of diosgenin gave the 22,26-epoxycholest-22-ene derivative **13** as a major product, a basic hydrolysis of **13** afforded 23-acetyldiosgenin **14** which was treated with Ac_2O and $BF_3{\cdot}OEt_2$ to form the desired furostene **15** in quantitative yield [30] (Scheme 6). Treatment of compound **15** under the cycloaddition conditions already established gave the spiro-pyrazoline **16** (Scheme 6).

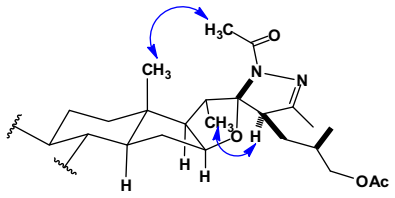


Fig. 2. NOESY correlations of the steroidal pyrazoline **16**.

In the ^{13}C NMR spectrum of compound **16** it can be observed a signal at 153.7 ppm characteristic of the C-3′ of the pyrazoline ring. Furthermore, C-5′, involved in the spiranic junction, resonates at 111.3 ppm, whereas the same carbon resonates at roughly 60 ppm for fused pyrazolines, so confirming the formation of a spiranic pyrazoline located on E ring.

The NOESY experiment of spiro compound **16** showed cross-peaks from NOE interactions between the protons of AcN-2′ of the pyrazoline ring and CH_3-18 (Fig. 2). These observations suggest that the configuration of the spiro C-22 is *R*. Moreover, the configuration of C-23 is proposed as *R* since it is observed a dipolar coupling between H-23 and CH_3-21 (Fig. 2).

In conclusion, we accomplished the synthesis of different steroidal pyrazolines, in good yields through the cycloaddition of different α,β-unsaturated ketones and hydrazine acetate as the 1,2-binucleofilic compound, under acidic conditions, containing the pyrazoline scaffold on the C-17, fused to D ring or forming a first spiranic junction at C-22.

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