Synthesis and structure of 4′,4′-dimethyl[16α,17α]spiropentanopregn-4-ene-3,20-dione*

I. S. Levina, L. E. Kulikova, E. V. Shulishov, I. P. Klimenko, A. V. Kamernitskii, and Yu. V. Tomilov

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (495) 135 6390. E-mail: tom@ioc.ac.ru

4′,4′-Dimethyl[16α,17α]spiropentanopregn-4-ene-3,20-dione was synthesized. The addition of diazo-2,2-dimethylcyclopropane generated from *N*-(2,2-dimethylcyclopropyl)-*N*-nitrosourea to 16,17-didehydropregnenolone acetate occurs regio- and stereospecifically to give 3β-acetoxy-1′,1′-dimethyl-20-oxopregn-5-ene-[16α,17α;7′,6′]-4′,5′-diazaspiro[2.4]-hept-4′-ene in high yield. Its thermolysis affords a spiropentane-containing steroid, which is transformed into the target diketone. The *anti* position of the *gem*-dimethyl group in the fused spiropentane fragment is evident from the X-ray diffraction study of the final product.

Key words: steroids, progesterone, pentaranes, receptor, fused cyclopropanes, 1,3-dipolar cycloaddition, X-ray diffraction analysis.

The possibility of using progestins (analogs of the natural hormone progesterone) obtainable by modifications of the natural steroid skeleton in contraception, hormone replacement and antitumor therapy, and treatment of various gynecological diseases depends substantially on the ability of these compounds to interact with the progesterone receptor (PR) and transfer this effect to the competent DNA region. The details of this interaction were revealed by X-ray diffraction study of a complex of the ligand-binding domain of PR with progesterone.[1] It appeared that the 3-keto group of the progesterone molecule forms (with involvement of the water molecule) two hydrogen bonds with amino acid residues of the receptor protein, whereas the 20-keto group is apparently not involved in hydrogen bonding. In the α region of the ring D of the molecule, the ligand-binding domain of PR contains a large cavity, which allows docking of rather bulky substituents with retention of high binding activity.[2] These observations are confirmed by studies of a series of progesterone analogs fused at positions 16α,17α with an additional C_3—C_6 carbocycle D′ (pregna-D′-pentaranes), which we have synthesized earlier.[3,4] Many of these compounds proved to be highly active progestins. In particular, 16α,17α-cyclopropanoprogesterone (D′$_3$-pentarane) is characterized by strong binding to PR and high progestagenic activity *in vivo*, which is higher than that of both progesterone and its full antagonist, *viz.*, 16α,17α-cyclohexanoprogesterone (D′$_6$-pentarane), as well as of closest homologs of the latter.[4] However, investigation of the relative affinity of steroid-receptor complexes of both D′$_6$- and D′$_3$-pentaranes with the competent DNA region demonstrated, first, that the affinity for progesterone, D′$_6$-pentarane, and closest homologs change in parallel with each other and, second, this is not the case for D′$_3$-pentarane.[5] This fact can be explained only assuming that steroid—receptor complexes of D′$_6$- and D′$_3$-pentaranes with the same receptor adopt different conformations. To test this assumption, we started the synthesis of substituted 16α,17α-cyclopropanoprogesterones and investigation of their interactions with PR and the competent DNA region.

The design of new modified progestins that contain bulky cycloalkane substituents in positions 16α,17α and can form more tight hydrophobic contacts with the cavity of the ligand-binding domain of PR in the vicinity of the ring D, and their docking to this domain would allow one to determine the limits of the bulk of the substituent in positions 16α,17α of the steroid skeleton of such analogs of progesterone, which would be characterized by the minimal affinity for PR.

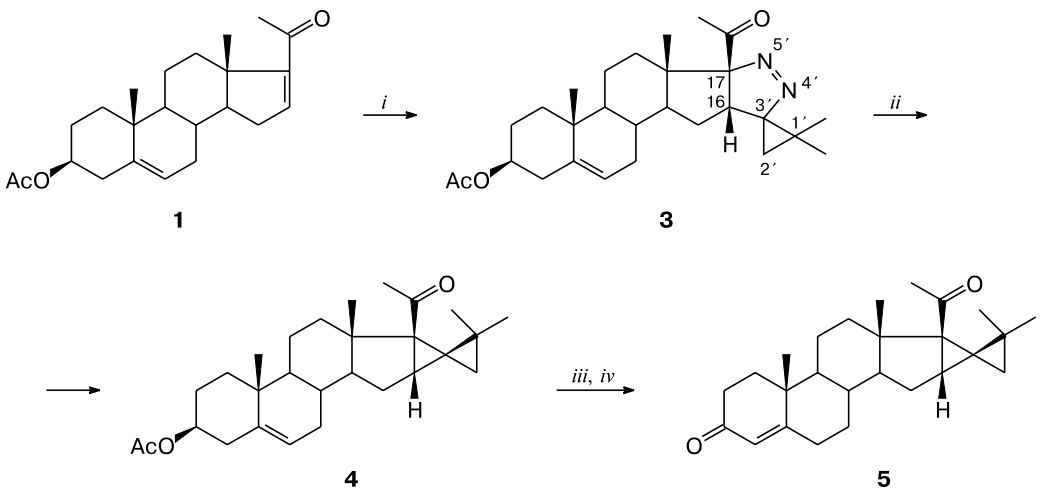
The aim of the present study was to synthesize an analog of progesterone containing the dimethylspiropentane fragment in positions 16α,17α. This compounds was synthesized in four steps starting from 16,17-didehydropregnenolone acetate (**1**) according to a scheme analogous to that used for the introduction of the unsubstituted spiropentane fragment.[6] Reactive diazo-2,2-dimethylcyclopropane was generated by decomposition of *N*-(2,2-dimethylcyclopropyl)-*N*-nitrosourea (**2**)[7] with $K_2CO_3 \cdot 2H_2O$ (the molar ratio **1** : **2** = 1 : 2) in CH_2Cl_2 at 5 °C. It appeared that diazo-2,2-dimethylcyclopropane

* Dedicated to Academician O. M. Nefedov on the occasion of his 75th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2040—2042, November, 2006.

1066-5285/06/5511-2119

Scheme 1



Reagents and conditions: *i*. **2**, $K_2CO_3 \cdot 2H_2O$, CH_2Cl_2, 5 °C; *ii*. 180—200 °C, 20 Torr; *iii*. KOH—MeOH; *iv*. $Al(OPr^i)_3$, cyclohexanone, PhMe, refluxing for 2.5 h.

generated under these conditions undergoes regio- and stereospecific addition to the conjugated double bond in **1** to form spirocyclopropane-containing pyrazoline **3** in a yield of up to 75% (Scheme 1). The use of potassium carbonate instead of methanolic KOH or MeONa leads to an increase in the reaction time, while retaining the 3β-acetoxy group of the steroid, which is essential for dediazotization. Pyrolysis of pyrazoline **3** was performed in a melt at 180—200 °C and at a pressure of 20 Torr. Under these conditions, decomposition of pyrazoline occurs with a high degree of conversion (>95%) and is accompanied by partial resinification of the reaction mixture. The resulting spiropentane-containing steroid **4** was isolated by silica gel column chromatography and additionally purified by recrystallization (the yield was ~55%). According to the data from ^1H and ^{13}C NMR spectroscopy, the resulting product is an individual isomer. Saponification of the 3β-acetoxy group of compound **4** and the Oppenauer oxidation of the resulting 3β-hydroxy derivative afforded the target compound **5** containing the *gem*-dimethylspiropentane fragment in positions 16α,17α of the steroid in ~80% yield. The structures of the reaction products were confirmed by elemental analysis, mass spectrometry, and ^1H and ^{13}C NMR spectroscopy (see Experimental). The *anti* position of the *gem*-dimethyl group in the fused spiropentane fragment of the steroid molecule is evident from the results of X-ray diffraction study of compound **5** (Fig. 1).

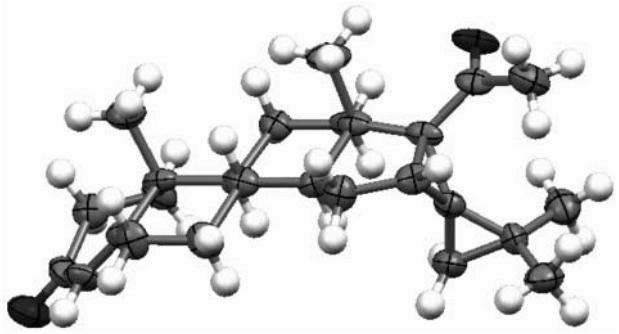


Fig. 1. Overall view of molecule **5**.

Experimental

The ^1H and ^{13}C NMR spectra were recorded on Bruker AM-300 (300.13 MHz for ^1H and 75.5 MHz for ^{13}C) and Bruker DRX-500 (500.13 MHz for ^1H and 125.3 MHz for ^{13}C) instruments in $CDCl_3$ containing 0.05% $SiMe_4$ as the internal standard or with the use of signals of the solvent as the standards (δ_H 7.27 and δ_C 77.1). The COSY, NOESY, and HSQC experiments were carried out on a Bruker DRX-500 spectrometer. The mass spectra were obtained on a Finnigan LCQ instrument (electrospray ionization (ESI)) for solutions in CH_3CN and a Finnigan MAT INCOS-50 instrument (EI, 70 eV, direct inlet, 100 °C). Elemental analysis was performed on a PerkinElmer 2400 Series II C, H, N Elemental Analyzer. The melting points were determined on a Boetius hot-stage microscope. *N*-(2,2-Dimethylcyclopropyl)-*N*-nitrosourea (**2**) was synthesized according to a procedure described earlier.[8] In experiments, air-dried potassium carbonate corresponding to the approximate composition $K_2CO_3 \cdot 2H_2O$ was used. Analytical thin-layer chromatography was performed on Silicagel 60 F_{254} plates (Merck) in hexane—acetone and hexane—diethyl ether systems. Compounds were visualized with the use of a 1% cerium sulfate solution in 10% aqueous H_2SO_4 followed by heating. Preparative separations were carried out by column chromatography on silica gel (Kieselgel 60, 0.063—0.100 mm, Merck); the compound—sorbent ratio was 1 : 40. The specific rotations were

measured on a PU-07 polarimeter (Russia) in $CHCl_3$ at 27 °C. Single-crystal X-ray diffraction study of compound **5** was performed on an automated Bruker 1K SMART CCD diffractometer (Mo-Kα radiation). The crystals are monoclinic, at 120 K a = 12.936(3) Å, b = 6.7990(19) Å, c = 25.081(7) Å, V = 2205.5(11) Å^3, Z = 4, d_{calc} = 1.146 g cm^{-3}, space group $P2_1$. All calculations were carried out with the use of the SHELXTL PLUS program package.[9] The atomic coordinates and complete crystallographic data were deposited with the Cambridge Structural Database.

16,17-Didehydropregnenolone acetate was purchased from Sigma.

3β-Acetoxy-1′,1′-dimethyl-20-oxopregn-5-ene-[16α,17α;7′,6′]-4′,5′-diazaspiro[2.4]hept-4′-ene (3). Potassium carbonate dihydrate (7.50 g, ~43 mmol) and *N*-(2,2-dimethylcyclopropyl)-*N*-nitrosourea (**2**) (2.10 g, 13.4 mmol) were added in three portions with vigorous stirring at 1 h intervals to a solution of 16,17-didehydropregnenolone acetate (**1**) (2.39 g, 6.7 mmol) in CH_2Cl_2 (20 mL) cooled to 5 °C. The reaction mixture was stirred for 4 h and then filtered. The precipitate was washed with CH_2Cl_2, the filtrate was concentrated *in vacuo*, the residue was dissolved in acetone, and the product was precipitated with light petroleum. Compound **3** was obtained in a yield of 2.27 g (75%) and additionally purified by recrystallization from a 5 : 1 acetone—light petroleum mixture, m.p. 149—151 °C, $[\alpha]_D$ +11 (c 1.07). Found (%): C, 74.25; H, 9.01; N 6.30. $C_{28}H_{40}N_2O_3$. Calculated (%): C, 74.30; H, 8.91; N, 6.19. MS (ESI), m/z: 475.3 $[M + Na]^+$, 415.2 $[M + Na - OAc]^+$. ^{1}H NMR, δ: 0.83 and 1.35 (both s, 3 H each, 2 Me at C(1′)); 0.93 and 1.87 (both d, 1 H each, $H_2C(2')$, J = 5.4 Hz); 1.02 and 1.08 (both s, 3 H each, Me(18), Me(19)); 2.03 (s, 3 H, AcO); 2.46 (s, 3 H, MeCO); 2.77 (d, 1 H, H(16), J = 8.2 Hz); 4.58 (m, 1 H, H(3)); 5.33 (d, 1 H, H(6), J = 5.0 Hz). ^{13}C NMR, δ: 15.73 (Me at C(1′)); 19.29 (C(18)); 21.43, 21.44 (Me at C(1′) and AcO); 23.53 (C(19)); 26.60 (C(2′)); 28.49 (C(1′)); 29.71 (C(21)); 34.55 (C(16)); 73.76 (C(3)); 83.93 (C(3′)); 118.03 (C(17)); 121.92 (C(6)); 139.73 (C(5)); 170.44 (COO); 202.89 (C=O).

3β-Acetoxy-4′,4′-dimethyl[16α,17α]spiropentanopregn-5-en-20-one (4). Pyrazoline **3** (1.99 g, 4.4 mmol) was heated *in vacuo* (20 Torr). Melting of compound **3** was observed at approximately 150 °C. At 180—200 °C, compound **3** foamed due to gas evolution, which ceased after 30—35 min. The reaction mixture was cooled and the reaction products were separated from the resinous residue by dissolution in CH_2Cl_2. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution with a light petroleum—acetone mixture (99 : 1) → (97 : 3) afforded compound **4** in a yield of 1.05 g (55%) and the starting pyrazoline **3** in a yield of 0.02 g. After recrystallization from a hexane—diethyl ether mixture, product **4** was obtained in a yield of 0.81 g, m.p. 155—156 °C, $[\alpha]_D$ +103 (c 1.07). MS (ESI), m/z: 447.3 $[M + Na]^+$. ^{1}H NMR, δ: 0.60 and 0.79 (both d, 1 H each, $H_2C(5')$, J = 4.5 Hz); 0.92 (s, 3 H, Me(18)); 1.01 and 1.03 (both s, 3 H each, 2 Me at C(4′)); 1.13 (s, 3 H, Me(19)); 1.96 (s, 3 H, Me(21)); 2.03 (s, 3 H, AcO); 4.58 (m, 1 H, H(3)); 5.36 (d, 1 H, H(6), J = 5.6 Hz). ^{13}C NMR, δ: 16.41 (C(4′)); 16.49 (C(18)); 17.51 (C(5′)); 19.31, 22.81 (2 Me at C(4′)); 21.48 (AcO); 23.21 (C(19)); 28.01 (C(21)); 35.47 (C(3′)); 36.84 (C(10)); 41.87 (C(17)); 51.05 (C(13)); 73.96 (C(3)); 122.22 (C(6)); 140.09 (C(5)); 170.55 (COO); 208.66 (C=O).

4′,4′-Dimethyl[16α,17α]spiropentanopregn-4-ene-3,20-dione (5). A solution of KOH (61 mg, 1.1 mmol) in water (0.5 mL) was added to a solution of acetate **4** (0.20 g, 0.47 mmol) in MeOH (15 mL). The reaction mixture was stirred at 20 °C for 3.5 h, neutralized with 10% HCl, and poured into ice water (100 mL). The colorless precipitate that formed was filtered off. After drying, the chromatographically homogeneous 3β-hydroxy derivative was obtained (0.163 g, 90%), and this compound was subjected to the Oppenauer oxidation by refluxing with $Al(Pr^iO)_3$ (0.20 g) and cyclohexanone (3 mL) in toluene (30 mL) for 2.5 h. The reaction mixture was acidified with dilute AcOH. The organic layer was separated, washed with water until the washings became neutral, dried with anhydrous $MgSO_4$, and concentrated *in vacuo*. The resulting oily residue was chromatographed on a silica gel column in a light petroleum—acetone system, (98 : 2) → (94 : 6). Diketone **5** was obtained in a yield of 0.13 g (80%), m.p. 169—171 °C (from a diethyl ether—hexane mixture), $[\alpha]_D$ +103 (c 1.07). Found (%): C, 82.04; H, 9.85. $C_{26}H_{36}O_2$. Calculated (%): C, 82.05; H, 9.53. MS (EI), m/z (I_{rel} (%)): 380 $[M]^+$ (35), 365 $[M - Me]^+$ (40), 43 (100). ^{1}H NMR, δ: 0.59 and 0.77 (both d, 1 H each, $H_2C(5')$, J = 4.5 Hz); 0.95 (s, 3 H, Me(18)); 1.01 and 1.13 (both s, 3 H each, Me at C(4′)); 1.19 (s, 3 H, Me(19)); 1.96 (s, 3 H, Me(21)); 5.72 (dd, 1 H, H(4), J = 1.5 Hz, J = 1.0 Hz). ^{13}C NMR, δ: 16.47 (C(4′)); 16.51 (C(18)); 17.32 (C(19)); 17.43 (C(5′)); 22.79, 23.19 (2 Me at C(4′)); 27.94 (C(21)); 35.24 (C(3′)); 38.78 (C(10)); 41.98 (C(17)); 50.77 (C(13)); 123.89 (C(4)); 171.27 (C(5)); 199.61 (C(3)); 208.55 (C=O).

We thank Yu. A. Strelenko (N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences) for recording ^{1}H and ^{13}C NMR spectra on a Bruker DRX-500 spectrometer.

This study was financially supported by the Russian Foundation for Basic Research (Project No. 05-03-33163).

References

1. S. P. Williams and P. B. Sigler, *Nature*, 1998, **393**, 392.
2. K. P. Madauss, S. Deng, R. J. H. Austin, M. H. Lambert, I. McLay, J. Pritchard, S. A. Short, E. L. Stewert, I. J. Uings, and Sh. P. Williams, *J. Med. Chem.*, 2004, **47**, 3381.
3. A. V. Kamernitskii and I. S. Levina, *Bioorg. Khim.*, 2005, **31**, 105 [*Russ. J. Bioorg. Chem.*, 2005, **31**, 115 (Engl. Transl.)].
4. A. V. Kamernitskii and I. S. Levina, *Bioorg. Khim.*, 2005, **31**, 199 [*Russ. J. Bioorg. Chem.*, 2005, **31**, 227 (Engl. Transl.)].
5. T. A. Shchelkunova, P. M. Rubtsov, I. S. Levina, A. V. Kamernitsky, and A. N. Smirnov, *Steroids*, 2002, **67**, 323.
6. P. Bladon and D. R. Rae, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2240.
7. I. P. Klimenko, Yu. V. Tomilov, and O. M. Nefedov, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 226 [*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 236].
8. Yu. V. Tomilov, E. V. Shulishov, I. P. Klimenko, and O. M. Nefedov, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 2698 [*Russ. Chem. Bull.*, 1996, **45**, 2557 (Engl. Transl.)].
9. G. M. Sheldrick, SHELXTL-97 V5.10, 1997, Bruker AXS Inc., Madison, WI-53719, USA.

Received July 14, 2006;
in revised form October 20, 2006