SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME 17a-SUBSTITUTED HOMOLACTONES OF ANDROST-5-ENE DERIVATIVES

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Some new 17a-homolactones were prepared from 3β-hydroxy-16-(hydroxyimino)androst-5-en-17-one (**1**) as a starting compound, which was transformed first to the corresponding 17α-phenyl and 17α-benzyl derivatives **2** and **3**. The structure of compound **3** was confirmed by X-ray structure analysis. Beckmann fragmentation of compounds **2** and **3** yielded 16,17-seco-cyano ketones **4**–**7**, whose reduction with $NaBH_4$ gave 16,17-seco-cyano alcohols **8**–**11**, whereby the structure of compound **7** was established by X-ray structural analysis. Compounds **8**–**11** served as the starting compounds for obtaining lactones **12** and **13** in a reaction with potassium hydroxide in ethylene glycol. One-pot procedures were also developed for preparing 17a-homolactones **12**, **13** and **16** from the hydroxyimino alcohols **2**, **3** and **14**. Compounds **12** and **13** showed an inhibitory activity against the enzyme aromatase (63 and 59%, respectively).

Keywords: Steroids; D-homoandrostene derivatives; Steroidal lactones; Aromatase inhibitors; X-ray analysis; Oximes; D-ring cleavage.

Compounds that inhibit enzyme aromatase have potential applications in the treatment of advanced estrogen-dependent tumors such as breast cancer, endometrial cancer, prostatic hyperplasia, and prostate cancer[1,2].

Potent aromatase inhibitors are mainly A- and B-modified steroids. In our previous papers[3–5] we reported the synthesis of several groups of D-modified steroids as aromatase inhibitors. Kinetic analysis for anti-aromatase activity showed that certain 16,17-seco ketones and 17a-homolactones expressed a high inhibition (IC_{50} values were 0.42–16.84 μM for

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D-seco ketones and 0.25–0.70 μM for D-homolactones). Inhibition potency for 3β-hydroxy-17aα-methyl-17-oxa-17a-homoandrost-5-en-16-one (IC_{50} 0.25 μM) was the highest compared with the other 17a-lactones[5], and it was twice lower in comparison with the activity of aminoglutethimide[5] (IC_{50} 0.14 μM).

In view of the previously established fact that the presence of 17aα-methyl group in the 17a-homolactone system in the androstene series had a favorable effect on anti-aromatase activity[4], in this work we synthesized the corresponding 17aα-phenyl- and 17aα-benzyl-17a-homolactones of androst-5-ene with the aim to examine the effect of the phenyl and benzyl groups on the anti-aromatase activity of these compounds.

EXPERIMENTAL

Melting points were determined using a Büchi SMP 20 apparatus and are uncorrected. IR spectra (wavenumbers in cm^{-1}) were recorded in KBr pellets or as film on a NEXUS 670 SP–IR spectrometer. NMR spectra were taken on a Bruker AC 250 spectrometer operating at 250 (^{1}H) and 62.9 (^{13}C) MHz with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (*J*) are given in Hz. All reagents used were of analytical grade. All solutions were dried with anhydrous Na_2SO_4.

17-Benzyl-3β-hydroxy-17-oxo-16,17-secoandrost-5-ene-16-nitrile (**6**)

17α-Benzyl-16-(hydroxyimino)androst-5-ene-3β,17β-diol (**3**; 0.50 g, 1.22 mmol) was dissolved in ethanol (75 ml), a concentrated HCl–ethanol mixture (1:1, 15 ml) was added, and then an aqueous solution of titanium(III) chloride (15%; 4.5 ml, 4.8 mmol). The reaction mixture was refluxed for 10 min. Then the reaction mixture was poured into water (200 ml), neutralized with saturated solution of $NaHCO_3$ and extracted with dichloromethane (4 × 100 ml). The extract was dried and the solvent evaporated, and crude product **6** (0.38 g) was obtained. Chromatographic purification on a silica gel column (40 g, hexane–EtOAc 9:1) gave compound **6** (0.345 g, 72%) in the form of light-yellow oil.

General Procedure for Preparation of Diols **8**–**11** from **4**–**7**

Compounds **4**–**7** (0.8 mmol) were dissolved in methanol (10 ml), sodium borohydride (61 mg, 1.6 mmol) was added, and the reaction mixture was refluxed for 10 min (compounds **4** and **5**), or 30 min (compound **6**), or for 45 min (compound **7**). After cooling, the reaction mixture was poured into ice water (10 ml). The separated precipitates of crude **8**–**11** were collected and dried. The crude products were purified by column chromatography on silica gel (CH_2Cl_2–methanol 100:1 for **8**, **10** and **11** and CH_2Cl_2 for **9**).

(17R)-3β,17-Dihydroxy-17-phenyl-16,17-secoandrost-5-ene-16-nitrile (**8**). White crystals (75%, m.p. 187 °C after crystallization from MeOH). IR: 3700–3150, 2240, 1080, 1060, 1020, 710. ^{1}H NMR (DMSO-d_6): 0.76 s, 3 H (H-18); 0.89 s, 3 H (H-19); 3.26 m, 1 H (H-3); 4.55 d, 1 H, *J* = 3.9 (H-17); 4.62 d, 1 H, *J* = 4.6 (OH-3); 5.30 m, 1 H (H-6); 5.34 d, 1 H, *J* = 3.9 (OH-17); 7.20–7.30 m, 5 H (Ph). ^{13}C NMR (DMSO-d_6): 14.8, 17.0 (C-18); 19.0 (C-19); 19.2, 30.7, 31.3, 31.7, 32.4, 36.3, 36.5, 41.7, 41.8, 48.6, 69.9 (C-3); 76.8 (C-17); 119.8 (CN); 120.7, 126.6

(C-4′, Ph); 127.0 and 128.3 (C-2′, C-3′, C-5′, C-6′, Ph); 140.9 (C-1′, Ph); 142.4 (C-5). For $C_{25}H_{33}NO_2 \cdot 0.5H_2O$ (388.5) calculated: 77.28% C, 8.82% H; found: 77.47% C, 8.51% H.

(17R)-3β-Acetoxy-17-hydroxy-17-phenyl-16,17-secoandrost-5-ene-16-nitrile (**9**). White crystals (82%, m.p. 204 °C after crystallization from MeOH). IR: 3700–3120, 2240, 1730, 1240, 1040, 700. ^{1}H NMR (acetone-d_6): 0.93 s, 3 H (H-19); 1.02 s, 3 H (H-18); 2.04 s, 3 H (CH_3, Ac); 4.39 d, 1 H, J = 3.6 (H-17); 4.51 m, 1 H (H-3); 4.72 d, 1 H, J = 3.6 (OH-17); 5.42 m, 1 H (H-6); 7.23–7.40 m, 5 H (Ph). ^{13}C NMR (acetone-d_6): 16.8, 18.6, 19.5, 20.2 (CH_3, Ac); 27.4, 31.2, 31.9, 32.5, 36.4, 36.6, 37.6, 40.6, 42.2, 49.0, 73.2 and 78.2 (C-3, C-17); 119.8 (CN); 121.5, 126.8 (C-4′, Ph); 127.1 (C-3′, C-5′, Ph); 128.2 (C-2′, C-6′, Ph); 139.5 (C-1′, Ph); 142.1 (C-5); 169.4 (CO, Ac). For $C_{27}H_{35}NO_3 \cdot 0.25H_2O$ (426.1) calculated: 76.11% C, 8.40% H; found: 76.02% C, 8.37% H.

(17S)-17-Benzyl-3β,17-dihydroxy-16,17-secoandrost-5-ene-16-nitrile (**10**). Colorless oil (83%). IR: 3700–3150, 2250, 1070, 1040, 720. ^{1}H NMR ($CDCl_3$): 1.05 s, 3 H (H-19); 1.11 s, 3 H (H-18); 2.50 dd, 1 H, J_{gem} = 12.5, $J_{17,Ha}$ = 9.0 (Ha, C$\mathbf{H_2}$Ph); 2.91 d, 1 H, J_{gem} = 12.5 (Hb, C$\mathbf{H_2}$Ph); 3.54 m, 1 H (H-3); 3.59 d, 1 H, $J_{17,Ha}$ = 9.0 (H-17); 5.38 m, 1 H (H-6); 7.20–7.38 m, 5 H (ArH). ^{13}C NMR (DMSO-d_6): 14.8, 17.5 (C-18); 19.2 (C-19); 20.1, 31.5, 32.0, 32.1, 33.2, 36.7, 36.9, 37.9, 40.1, 41.9, 42.7, 49.0, 71.6 (C-3); 78.0 (C-17); 119.8 (CN); 120.6, 126.8 (C-4′, Bn); 128.9 (C-3′, C-5′, Bn); 129.3 (C-2′, C-6′, Bn); 139.1 (C-1′, Bn); 140.4 (C-5).

(17S)-3β-Acetoxy-17-benzyl-17-hydroxy-16,17-secoandrost-5-ene-16-nitrile (**11**). Colorless oil (90%). IR: 3600–3300, 2230, 1720, 1245, 1030, 730, 690. ^{1}H NMR ($CDCl_3$): 1.06 s, 3 H (H-19); 1.11 s, 3 H (H-18); 2.04 s, 3 H (CH_3, Ac); 2.50 dd, 1 H, J_{gem} = 12.5, $J_{17,Ha}$ = 9.0 (Ha, C$\mathbf{H_2}$Ph); 2.91 d, 1 H, J_{gem} = 12.5 (Hb, C$\mathbf{H_2}$Ph); 3.59 d, 1 H, $J_{17,Ha}$ = 9.0 (H-17); 4.61 m, 1 H (H-3); 5.39 m, 1 H (H-6); 7.20–7.39 m, 5 H (ArH). ^{13}C NMR (DMSO-d_6): 14.7, 17.5, 19.1, 20.0, 21.4, 27.6, 31.5, 31.8, 31.9, 36.6, 36.8, 37.9, 40.1, 42.6, 48.9, 73.7, 78.0, 119.8 (CN); 121.5, 126.8, 128.9, 129.3, 139.1, 170.5 (CO, Ac).

3β-Hydroxy-17aα-phenyl-17-oxa-17a-homoandrost-5-en-16-one (**12**)

A) Compound **8** (0.05 g, 0.13 mmol) was dissolved in ethylene glycol (3 ml) and KOH (0.295 g, 5.28 mmol) was added. The reaction mixture was refluxed for 4 h and then poured into water (6 ml), acidified with HCl (1:1) to pH 1 and extracted with CH_2Cl_2 (5 × 20 ml). The extract was dried and the solvent removed under reduced pressure, which resulted in crude product **12** (0.075 g) as yellow oil. The product was chromatographed on a silica gel column (8 g, CH_2Cl_2–MeOH 70:1) giving compound **12** (0.043 g, 77%; m.p. 216 °C after recrystallization from CH_2Cl_2–hexane 2:1) as white crystals.

B) By the same procedure as in *A*), compound **9** (0.05 g, 0.11 mmol) yielded the crude product **12** (0.062 g) in the form of yellow oil. Column chromatography on silica gel (7 g, benzene–EtOAc 5:1), afforded compound **12** (0.043 g, 91%) as white crystals. IR: 3700–3100, 1730, 1230, 1190, 1030, 760, 700. ^{1}H NMR ($CDCl_3$): 0.97 s, 3 H (H-19); 1.24 s, 3 H (H-18); 3.44 m, 1 H (H-3); 5.14 s, 1 H (H-17); 5.30 m, 1 H (H-6); 7.04–7.38 m, 5 H (ArH). ^{13}C NMR ($CDCl_3$): 18.2, 19.3, 19.5, 30.5, 31.3, 32.3, 33.0, 34.6, 35.4, 36.4, 36.6, 37.5, 41.9, 48.1, 71.5 (C-3); 91.5 (C-17); 120.4 (C-6); 127.4 and 128.0 (C-2′, C-3′, C-5′, C-6′, Ph); 128.1 (C-4′); 136.8 (C-1′, Ph); 140.5 (C-5); 171.0 (C-16). For $C_{25}H_{32}O_3$ (380.5) calculated: 78.91% C, 8.48% H; found: 79.23% C, 8.92% H.

17aα-Benzyl-3β-hydroxy-17-oxa-17a-homoandrost-5-en-16-one (**13**)

A) In the same way as described in *A*) for obtaining compound **12**, compound **10** (0.15 g, 0.38 mmol), KOH (0.86 g, 15.23 mmol) and ethylene glycol (8 ml) gave a crude product **13** (0.16 g) as yellow oil. The product was chromatographed on a silica gel column (16 g, CH_2Cl_2–MeOH 100:1), giving compound **13** (0.12 g, 78%; m.p. 190 °C after recrystallization from CH_2Cl_2–hexane 1:1) as white crystals.

B) In the same way as described in *A*) for obtaining compound **12**, compound **11** (0.10 g, 0.23 mmol), KOH (0.52 g, 9.20 mmol) and ethylene glycol (4 ml) gave a crude product **13** (0.14 g) as yellow oil. The product was chromatographed on a silica gel column (14 g, CH_2Cl_2–MeOH 100:1), giving compound **13** (0.08 g, 81%) as white crystals. IR: 3440, 1710, 1240, 1200, 1060, 1010, 750, 700. ^{1}H NMR (DMSO-d_6): 0.95 s, 3 H (H-18); 0.98 s, 3 H (H-19); 2.77 dd, 1 H, J_{gem} = 16.0, $J_{17,Ha}$ = 11.4 (Ha, C**H₂**Ph); 3.04 d, 1 H, J_{gem} = 16.0 (Hb, C**H₂**Ph); 3.26 m, 1 H (H-3); 4.20 d, 1 H, $J_{17,Ha}$ = 11.4 (H-17); 4.64 d, 1 H, J = 4.3 (OH); 5.28 m, 1 H (H-6); 7.20–7.32 m, 5 H (ArH). ^{13}C NMR (DMSO-d_6): 17.2, 19.1, 19.5, 29.9, 31.3, 31.5, 32.5, 32.9, 34.6, 36.2, 36.3, 36.6, 38.3, 42.0, 48.3, 70.0 (C-3); 89.0 (C-17a); 120.0 (C-6); 126.0 (C-4′, Ph); 128.1 (C-3′, C-5′, Ph); 129.4 (C-2′, C-6′, Ph); 138.7 (C-1′, Ph); 140.9 (C-5); 170.2 (C-16). For $C_{26}H_{34}O_3 \cdot 0.5H_2O$ (403.6) calculated: 77.38% C, 8.74% H; found: 77.68% C, 8.28% H.

General Procedure for Preparation of 17a-Homo Derivatives **12**, **13** and **16**–**19** from **2**, **3**, **14**, and **15**

Oximes **2**, **3**, **14**, or **15** (1 mmol) were dissolved in ethylene glycol (10 ml) and KOH (35–40 mmol) was added. The reaction mixture was vigorously stirred at reflux. When the reaction was completed (3 h for **15**, 4 h for **2** and **3**, 72 h for **14**) the mixture was diluted with water (200 ml), acidified with 2 M HCl to pH 1 and extracted with CH_2Cl_2. The organic phase was dried and the solvent was removed under reduced pressure. The obtained mixture was separated by silica gel column (for **12** benzene–AcOEt 5:1; for **13**, **16**, **18**, and **19** CH_2Cl_2–MeOH 99:1; for **17** toluene–AcOEt 2:1).

3β-Hydroxy-17aα-phenyl-17-oxa-17a-homoandrost-5-en-16-one (**12**). White crystals (68%, m.p. 216 °C after recrystallization from CH_2Cl_2–hexane 2:1).

17aα-Benzyl-3β-hydroxy-17-oxa-17a-homoandrost-5-en-16-one (**13**). White crystals (16.5%, m.p. 190 °C after recrystallization from CH_2Cl_2–hexane 1:1).

3β-Hydroxy-17aα-methyl-17-oxa-17a-homoandrost-5-en-16-one (**16**). White crystals (42%, m.p. 169 °C after recrystallization from CH_2Cl_2–hexane 2:1; lit.[5] m.p. 169 °C).

3β-Hydroxy-17-oxa-17a-homoandrost-5-en-16-one (**17**). White crystals (62%, m.p. 206–207 °C after recrystallization from CH_2Cl_2–hexane 1:1; lit.[6] m.p. 206–207 °C).

16-Amino-3β-hydroxy-17-phenyl-17a-homoandrosta-5,16-dien-17a-one (**18**). White crystals (80%, m.p. 317–318 °C after recrystallization from MeOH; lit.[4] m.p. 317–318 °C).

16-Amino-3β-hydroxy-17a-homoandrosta-5,16-dien-17a-one (**19**). White crystals (31%, m.p. 248–250 °C after recrystallization from MeOH; lit.[4] m.p. 248–250 °C).

X-ray Crystal Studies of **3** and **7**

Diffraction data (Table I) for compounds **3** and **7** were collected on an Enraf–Nonius CAD-4 and CCD Bruker Smart Apex diffractometer at 293 and 110 K, respectively, using MoKα radiation (λ = 0.71073 Å). Both structures were solved by direct methods (SHELXS97 [7]) and refined by full-matrix least-squares procedures (SHELXL97[7]). Non-hydrogen atoms were refined anisotropically. The H atoms were generated and refined as riding, with isotropic displacement fixed at $1.2U_{eq}$ or $1.3U_{eq}$ of parent atoms, or $1.5U_{eq}$ for the methyl-H atoms. Lorentz and polarization corrections were applied to the data. No absorption correction was made.

CCDC 263771 (for **3**) and 263772 (for **7**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

TABLE I
Crystallographic data, data collection and structure refinement for compounds **3** and **7**

Parameter	Compound **3**	Compound **7**
Formula	$C_{26}H_{35}NO_3$	$C_{28}H_{35}NO_3$
M_w	409.5	433.6
Crystal size, mm^3	0.08 × 0.14 × 0.20	0.07 × 0.18 × 0.22
Crystal description	colorless prism	colorless prism
Crystal system, space group	orthorhombic, $P2_12_12_1$	monoclinic, $P2_1$
a, Å; α, °	7.629(1); 90	11.477(4); 90
b, Å; β, °	12.854(1); 90	5.916(2); 105.14(3)
c, Å; γ, °	23.001(2); 90	17.783(9); 90
V, $Å^3$; Z	2255.6(4); 4	1165.5(8); 2
D_c, g cm^{-3}	1.206	1.235
$F(000)$; μ(MoKα), mm^{-1}	888; 0.078	468; 0.079
θ range, °; data completeness, %	1.81–25.23; 97.7	1.19–23.26; 99.9
Range of h,k,l	0/9, 0/15, 0/27	–12/12, –6/6, –19/19
No. of unique diffractions	2289	3350
No. of observed diffractions[a]	1665	2759
No. of parameters	277	292
R, wR for observed diffractions, %	3.83, 5.91	3.47, 7.32
R, wR for all data, %	6.25, 6.19	4.83, 9.34
GOF for all data	1.203	0.981
$(\Delta/\sigma)_{max}$	0.000	0.000
Residual electron density, e $Å^{-3}$	–0.127, 0.113	–0.154, 0.122

[a] Diffractions with $F_o > 2\sigma(F_o)$.

Biological Tests

Chemicals. Antiestradiol serum No. 244 was kindly supplied by Dr. G. D. Niswender (Colorado State University (CO), U.S.A.), pregnant mares serum gonadotrophin (PMSG) was obtained from the Veterinary Institute Subotica (Serbia and Montenegro), [1,2,6,7-^{3}H(*N*)]-estradiol from New England Nuclear (Belgium), NADPH from Sigma (St. Louis (MO), U.S.A.).

Animals, treatments, and assays. Preparation of denucleated ovarian fraction from PMSG pretreated rats and determination of aromatase activity in ovarian homogenate was carried out as described previously[3,4].

For preliminary assessment of potential antiaromatase activity, the synthesized compounds were added in two concentrations (1 and 20 μM) to the incubation mixture containing 500 nM testosterone as a substrate (saturated concentration; the estimated K_m for testosterone was 49 nM and V_{max} 5.76 pmol/min/mg protein).

Purified denucleated fraction of ovaries from PMSG pretreated female rats (≈0.2 mg protein/tube) was incubated at 37 °C for 15 min in the presence of saturated (500 nM) concentration of testosterone and NADPH (1 mM) in the absence (control) or presence of the two concentrations (1 and 20 μM) of compounds **12**, **13**, **16**, **17**. Each concentration was tested in 5 replicates. The estradiol level was determined by RIA.

RESULTS AND DISCUSSION

In our previous works[3,4] we synthesized 17α-phenyl[3] (**2**) and 17α-benzyl[4] (**3**) derivatives of androst-5-ene by adding phenyl- or benzyllithium with the 17-oxo group of 3β-hydroxy-16-(hydroxyimino)androst-5-en-17-one (**1**) (Scheme 1).

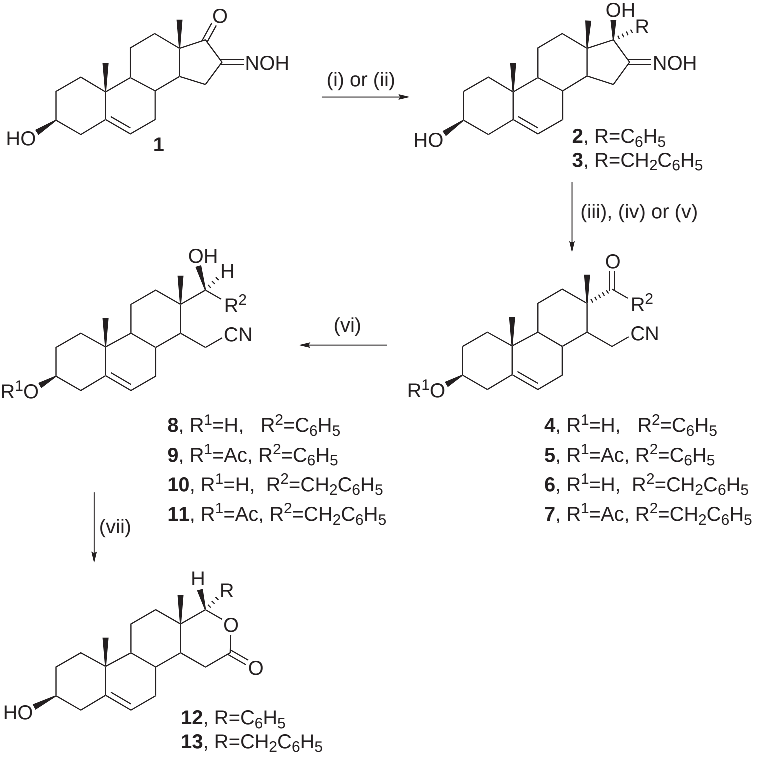
The Beckmann fragmentation of compound **2** with titanium(III) chloride, tosyl chloride or acetic anhydride yielded the seco-cyano ketones **4** and **5** [3,8], while the fragmentation of the hydroxyimino alcohol **3** with acetic anhydride in pyridine gave product **7**, whose hydrolysis produced compound **6** in an overall yield of 62% [4]. In the present work we achieved a better yield of compound **6** (72%) by modifying the fragmentation procedure using $TiCl_3$ in ethanol. The reaction was performed at the boiling temperature of the reaction mixture for 10 min, and not at room temperature, when compound **6** was obtained in a yield of 23% [9].

X-ray structure analysis confirmed the structure of compound **3** and α-orientation of the 17-benzyl group (Fig. 1). Also, the structure of the seco-cyano ketone **7** was unambiguously proved by X-ray structure analysis, a perspective view of the molecule being presented. The equatorial orientation of the 13-phenylacetyl and 14-cyanomethyl groups, as well as the axial orientation of the 13-methyl group are obvious (Fig. 2).

Reduction of the seco-cyano ketones **4**–**7** with sodium borohydride in MeOH yielded the corresponding 17-phenyl and 17-benzyl seco-cyano alcohols **8**–**11**.

Synthesis of the lactones **12** and **13** is presented in Scheme 1. Compound **8** or its 3β-acetyl derivative **9** reacted with KOH in ethylene glycol and acidification of the reaction mixture yielded lactone **12**. In the case of compound **9**, a simultaneous hydrolysis of the 3β-acetoxy group took place. Similarly, lactone **13** was obtained from the cyano alcohols **10** and **11**.

Configuration at the C-17 atom of lactone **12** was established by NOE experiment, namely, by irradiating the signal of the angular C-18 methyl group at 1.24 ppm. The NOE effect appeared on the singlet at 5.14 ppm, belonging to the H-17 atom, which confirmed that they are on the same side (β-orientation) of the molecule. Similarly, in the case of lactone **13**, the irradiation of the proton of the angular C-18 methyl group at 0.95 ppm produced the NOE effect of the doublet at 4.20 ppm belonging to the H-17



(i) PhLi, ether, THF, –10 °C; (ii) BnLi, THF, –10 °C; (iii) TsCl, Py, r.t.; (iv) Ac_2O, Py, reflux; (v) $TiCl_3$, HCl, H_2O, EtOH, reflux; (vi) $NaBH_4$, MeOH, reflux; (vii) KOH, $HOCH_2CH_2OH$, reflux

SCHEME 1

atom, which confirmed their β-orientation. From these results it was possible to establish the configuration at C-17 in seco-cyano alcohols **8**–**11**, as *R* for compounds **8** and **9**, and *S* for compounds **10** and **11** (Scheme 1). Here described stereochemical route of the sodium borohydride reduction is in agreement with our recently published results related to reduction of a similar 17-oxo compound in the estrone series[10].

In our previous work[6] a procedure was developed for preparing lactone **17** directly from hydroxyimino alcohol **15** by heating with KOH solution in ethylene glycol (Scheme 2). Applying the same reaction conditions, in this work we obtained lactones **12**, **13** and **16** directly from compounds **2**, **3** and **14**, whereby the previously described preparation procedure was shortened

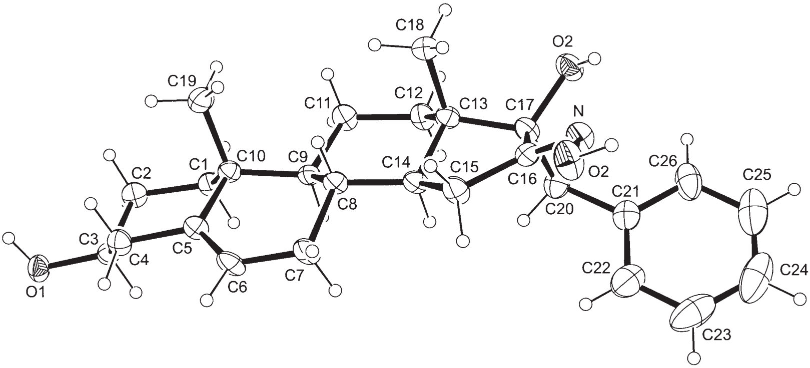


FIG. 1
Perspective view of the molecular structure of compound **3**

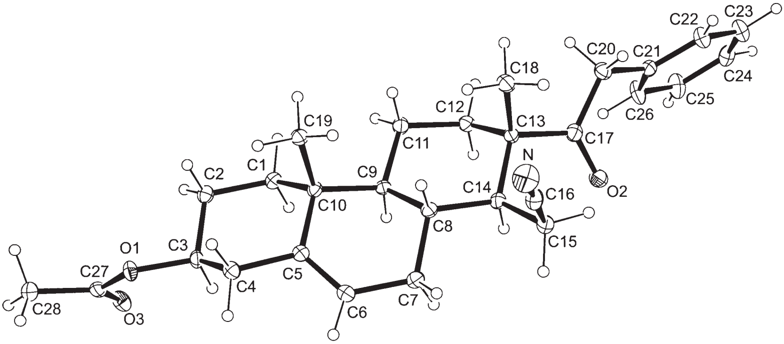
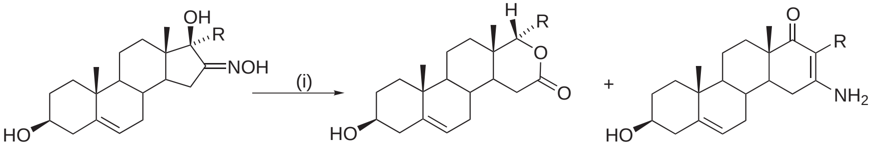


FIG. 2
Perspective view of the molecular structure of compound **7**

by two steps. Lactone **16** was obtained previously[3,5] from compound **14** in three reaction steps, in overall yield of 39%.

By analyzing the reaction of the hydroxyimino alcohols **2**, **3**, **14** and **15** with KOH in ethylene glycol it can be seen that in the case of 17-benzyl and 17-methyl derivatives of **3** and **14**, in addition to the lactones **13** and **16**, 16-amino-17a-homo derivatives **18** [4] and **19** [4] are also obtained (Scheme 2). The same reaction carried out with 17α-phenyl hydroxyimino alcohol **2** or 17α-unsubstituted alcohol **15** showed no formation of the corresponding 16-amino-17a-homo derivatives; instead, D-lactones **12** and **17** were formed.



2, 3, 14, **15**

12, **13**, **16**, **17**

18, **19**

In formulae

2, **12**, R = C_6H_5; **3, 13, 18**, R = $CH_2C_6H_5$; **14, 16, 19**, R = CH_3; **15, 17**, R = H

(i) KOH, $HOCH_2CH_2OH$, reflux

SCHEME 2

The obtained results indicate that the nature of the C-17 substituent in compounds **2**, **3**, **14** and **15** governs the formation of 16-amino-17a-homo derivatives and/or D-lactones. If the α-hydrogen atoms are present in the C-17 substituent ($CH_2C_6H_5$, CH_3), 16-amino-17a-homo derivatives are formed in a mixture with D-lactones, which are solely formed if the mentioned hydrogen atoms are not present. On the other hand, by choosing the base, solvent and reaction conditions it is possible to favor the formation of lactones or 16-amino-17a-homo derivatives. These results are in agreement with the mechanism of formation of 16-amino-17a-homo derivatives described in the previous paper[5] as well as with the mechanism of lactonization of hydroxyimino alcohols of the estrane series[11].

Biological Properties

In this study we tested lactones **12** and **13** for the potential anti-aromatase activity in the denucleated ovarion fraction from PMSG-pretreated female rats. In order to examine the effect of the 17aα substituents in this type of 17a-homolactones we compared the inhibitory effects of the lactones **12**, **13**, **16**, and **17**. Compounds were tested at different concentration as shown in Table II.

It can be concluded that phenyl (in **12**) and benzyl (in **13**) groups at the 17aα position increased only a little the inhibitory activity (63 and 59%, respectively) against aromatase compared with the 17aα-unsubstituted derivative **17** (49.2% of inhibition), observed at the substrate concentration of 1 μM. However, the methyl group (compound **16**) increased significantly the inhibitory activity (89%).

TABLE II
Inhibitory effects of androst-5-ene derivatives **12**, **13**, **16**, and **17** on the aromatase activity in the denucleated fraction of ovaries from PMSG pretreated rats

Inhibitor, conc. μM	Inhibition, %			
	12	**13**	**16**	**17**
20	95.6 ± 4.4	93.6 ± 4.5	95.9 ± 3.2	99.0 ± 1.0
1	62.8 ± 8.6	58.7 ± 10.0	88.7 ± 3.9	49.2 ± 4.2

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