J.Serb.Chem.Soc. 68(10)707–714(2003)
JSCS – 3089

UDC 547.92+542.913+548.735:66.099.73
Original scientific paper

Synthesis, crystal structure and antiaromatase activity of 17-halo-16,17-*seco*-5-androstene derivatives

KATARINA PENOV-GAŠI[1*#], SRDJAN STOJANOVIĆ[1#], MARIJA SAKAČ[1#], EVGENIJA DJURENDIĆ[1#], SUZANA JOVANOVIĆ-ŠANTA[1#], SLOBODANKA STANKOVIĆ[2], NEBOJŠA ANDRIĆ[3] and MIRJANA POPSAVIN[1#]

[1]Department of Chemistry, Faculty of Science, University of Novi Sad, 21000 Novi Sad, Trg Dositeja Obradovića 3, (E-mail: marijas@ih.ns.ac.yu), [2]Department of Physics, Faculty of Science, University of Novi Sad, 21000 Novi Sad, Trg Dositeja Obradovića 4 and [3]Department of Biology, Faculty of Science, University of Novi Sad, 21000 Novi Sad, Trg Dositeja Obradovića 2, Serbia and Montenegro

(Received 14 April, revised 2 June 2003)

Abstract: Starting from 3β-acetoxy-15-cyano-17-oxo-16,17-*seco*-5-androstene (**2**) and 3β-acetoxy-15-cyano-17-hydroxy-17-methyl-16,17-*seco*-5-androstene (**11**), new 17-halo-derivatives (**5**–**10** and **13**) were obtained. The fluoro derivative **5** was obtained from 17-tosylate **4** in reaction with tetrabutylammonium fluoride. The structure of the 17-iodo-devitive **10** was unambiguously proved by the appropriate X-ray structural analysis. Compounds **5**–**10**, as well as **12** and **13**, were tested for possible anti-aromatase activity, whereby only compound **9**, with bromine as the C-17 substituent, induced 19.4 % inhibition of aromatase activity compared to the control.

Keywords: 17-halo derivatives of 5-androstene, D-*seco*-steroids, aromatase inhibitors.

INTRODUCTION

Aromatase is the enzyme responsible for catalyzing the conversion of androgens to estrogens in the last step of estrogen biosynthesis. The inhibition of aromatase is a specific route for the control of estrogen levels and estrogen-dependent disturbances.[1,2] Compounds that inhibit aromatase have potential applications in the treatment of advanced estrogen-dependent tumors, such as breast cancer, endometrial cancer, prostatic hyperplasia, and prostate cancer. A number of steroids may inactivate aromatase by diverse interactions with the enzyme.[3,4] In a previous paper[5] we described the synthesis of some 17-oxo-16,17-*seco* and 17-hydroxy-16,17-*seco* derivatives of 5-androstene as potential aromatase inhibitors.

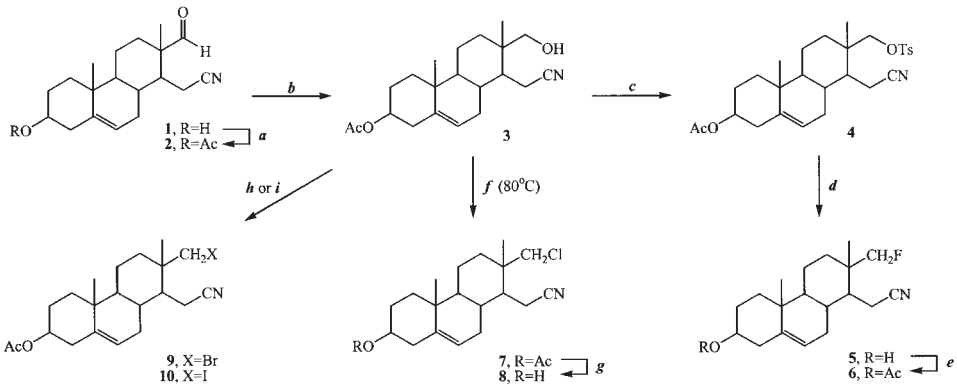
With the aim of studying the anti-aromatase activity of some other 17-substituted 5-androstenes, several new 17-halo-16,17-*seco* derivatives were synthesized using similar reagents as in the estrane series.[6]

* Author for correspondence

\# Serbian Chemical Society active member.

RESULTS AND DISCUSSION

Several new 17-halo-derivatives (**5**–**10** and **13**) were prepared using two independent reaction schemes. One scheme utilizes 3β-hydroxy-15-cyano-17-oxo-16,17-*seco*-5-androstene (**1**[7]) as the starting material, which is converted to the 3β-acetoxy derivative **2** (Scheme 1). Its treatment with sodium borohydride in ethanol under reflux for 45 min gave the *seco*-cyano alcohol **3** in 61 % yield. The reaction of **3** with *p*-toluenesulphonyl chloride in pyridine at room temperature for 70 h afforded the derivative **4** in 51 % yield. Substitution of the *p*-toluenesulphonyl group in **4** with fluorine (using tetrabutylammonium fluoride) in ethyl methyl ketone under reflux for 48 h gave the corresponding 17-fluoro-16,17-*seco* derivative 5, isolated in a yield of 52 %. The latter was transformed into the acetate **6** with acetic anhydride and pyridine.



Scheme 1. **a**, Ac_2O, Py; **b**, $NaBH_4$, EtOH; **c**, TsCl, Py; **d**, $Bu_4NF{\times}3H_2O$, EtCOMe; **e**, Ac_2O, Py; **f**, CCl_4, Ph_3P, benzene; **g**, KOH, MeOH; **h**, CBr_4, Ph_3P, benzene; **i**, I_2, Ph_3P, imidazole, Py.

Compound **3** was also used as the starting material to prepare 17-chloro-16,17-*seco*-derivative **7**. The reaction of compound **3** with tetrachloromethane and triphenylphosphine in benzene at 80 ºC for 60 min, gave, after column chromatography, compound **7** in 70 % yield. Compound **8** was obtained after deacetylation of **7** with potassium hydroxide in methanol. Similarly, 17-bromo-16,17-*seco*-5-androstene derivative **9** was obtained from **3** and tetrabromomethane and triphenylphosphine in 71.5 % yield.

Treatment of **3** with iodine, triphenylphosphine and imidazole in pyridine at 60 ºC for 2 h gave derivative **10** in 76 % yield.

The structures of the new compounds were determined by NMR and IR-spectroscopy. In addition, an X-ray analysis of **10** was performed (Fig. 1).

The X-ray crystal structure analysis of **10** showed an *anti* orientation of the C_{14}–C_{15} (β) and C_{13}–C_{17} (α) bonds. It can be assumed that the cyano function and iodine atom have the maximum distance which decreases steric hindrance.

The NMR spectra of the newly synthesized compounds **4**–**9** showed a diastereotopic relationship for the C-17 protons. Namely, in the NMR spectra of compounds **4** (tosylate), **7**, **8** (chlorides), and **9** (bromide), these protons appear as AB quartets, having different coupling constants. In the case of the fluoro derivatives **5** and **6**, two signals (2×*dd*) for the

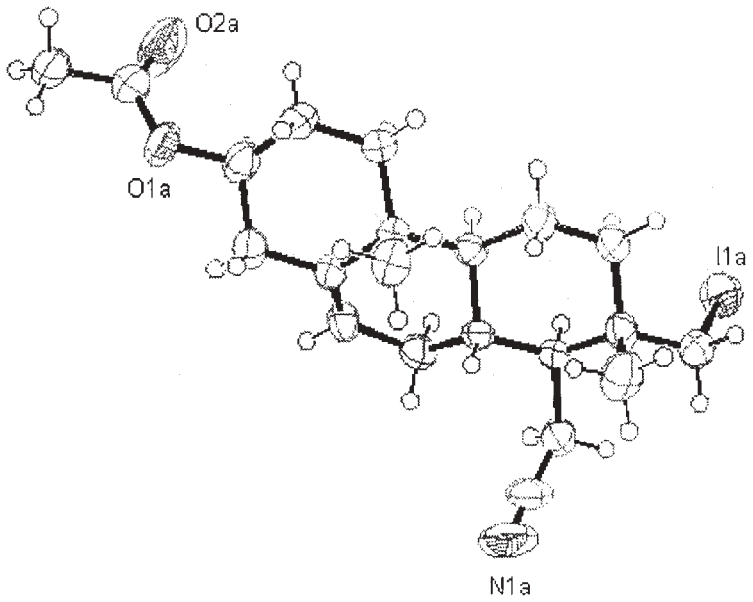


Fig. 1. Perspective view of the molecular structure of compound **10**.

C-17 protons are present, whereas in the case of iodo derivative **10**, the signal for the C-17 protons appears as a singlet.

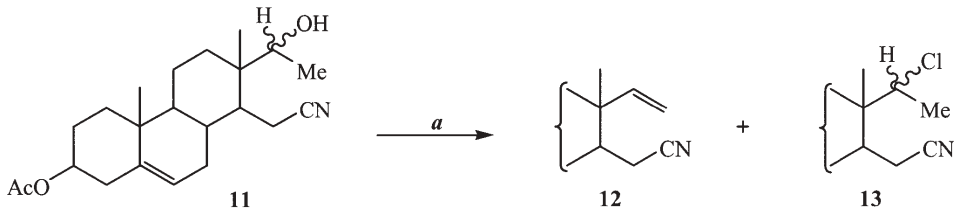
Probably, the reason for such characteristics of D-seco derivatives **4**–**9** is the existence of a quasi ring D which can be formed *via* dipole to dipole interaction between $C_{16}^{\delta+} \equiv N$ and $C_{17}–X^{\delta-}$, thus partially preventing free rotation around the C_{13} to C_{17} bond.[8] In the case of iodo derivative **10**, possessing the least polarized and the longest C-halogen bond, this relationship practically disappears, so that the C-17 protons behave as equivalent.

The ^{13}C chemical shifts of the C-17 fluoro- (90.8 ppm), chloro- (**7**: 54.5 and **8**: 54.6 ppm), bromo- (45.6 ppm) and iodo- (23.9 ppm) derivatives are in accordance with the "heavy atom" effect phenomena, which is additional confirmation of the structure of these halogen derivatives.

The starting material for the second pathway, to synthesize 17-halo-17-methyl-16,17-*seco*-5-androstene derivatives, was 3β-acetoxy-15-cyano-17-hydroxy-17-methyl-16,17-*seco*-5-androstene (**11**), obtained by a multistage synthesis described in a previous paper[5] (Scheme 2).

Compound **11** reacted with tetrachloromethane and triphenylphosphine in benzene at 60 ºC for 4 h to give a mixture of two products, olefin **12** in moderate yield (43 %) and 17-chloro-17-methyl-16,17-*seco*-15-cyano derivative **13** in low yield (27 %). The mixture was separated on a silica gel column. Attempts to prepare the 17-bromo-17-methyl– or 17-iodo-17-methyl-16,17-*seco*-5-androstene derivatives were unsuccessful: these derivatives were always obtained as a mixture with olefin **12**, which could not be separated by column chromatography.

The novel compounds synthesized in this study (**5**–**10** and **12**, **13**) were tested for possible anti-aromatase activity using the denucleated ovarian fraction from PMSG-pretreated



11 **12** **13**

Scheme 2. **a**, CCl_4, Ph_3P, benzene.

female rats (PMSG = pregnant mare serum gonadotrophin). Details of the assay procedure have been published elsewhere.[5] For screening purpose, compounds were tested at a single concentration (5 μM). The results showed that only compound **9**, with bromine as the C-17 substituent, induced 19.4 % inhibition of aromatase activity compared to the control (Table I). In a previous study,[5] it was demonstrated that 15-cyano-17-methyl-16,17-*seco*-4-androstene-3,17-dione showed a relatively high anti-aromatase activity in the same assay system as in this study. The IC_{50} value was 0.42 μM, which is about 3 times higher than the IC_{50} for aminoglutethimide (AG). This compound at a dose of 5 μM induced 75 % inhibition of aromatase activity.[5]

TABLE I. Effects of 17-halo-16,17-*seco*-derivatives on the aromatase activity in the denucleated fraction of ovaries from PMSC pretreated rats

Compond (5 μM)	Aromatase activity/% *vs.* control
5	114.1 ± 4.8
6	109.3 ± 8.2
7	124.6 ± 15.0
8	100.8 ± 9.1
9	80.6 ± 14.1
10	103.7 ± 13.5
12	121.2 ± 15.2
13	98.4 ± 10.2

Purified denucleated fractions of ovaries from PMSG pretreated female rats (0.366 mg proteins) were incubated in the presence of saturation (500 nM) concentrations of the substrate testosterone and NADPH (1 mM) and in the absence (control) or presence of the different androstene derivatives for measuring the aromatase activity. The estradiol level was determined by RIA. The results are presented as the percentage of inhibition *vs* control. The numbers represent the mean ± SEM of 6 replicates.

On the other hand, 3β-hydroxy-15-cyano-17-keto-17-methyl-16,17-*seco*-5-androstene at a dose of 5 μM expressed 49 % inhibition of aromatase activity compared to the control, with an IC_{50} of 5.45 μM.[5] These results suggest that the introduction of halogens at position 17 did not increase the activity toward aromatase.

EXPERIMENTAL

Melting points were determined using a Büchi SMP 20 apparatus and were not corrected. Infrared spectra (ν in cm^{-1}) were recorded on a Perkin-Elmer 457 spectrometer. NMR spectra were taken on a Bruker AC 250E spectrometer operating at 250 MHz (proton) and 62.9 MHz (carbon), and are reported in parts per million downfield from the tetramethylsilane internal standard. All reagents used were commercially available analytical grade substances.

*3β-Acetoxy-15-cyano-17-oxo-16,17-*seco*-5-androstene (**2**)*

3β-Hydroxy-15-cyano-17-oxo-16,17-*seco*-5-androstene (**1**; 2.70 g; 8.96 mmol) was dissolved in pyridine (60 mL) and acetic anhydride (90 mL; 54.1 mmol) was added. The reaction mixture was heated to reflux for 90 min, then poured into ice-cold water (about 200 mL), and the crystalline precipitate of **2** (2.24 g) was filtered off. Pure compound **2** was obtained after column chromatography (silica gel, 225 g; benzene/ethyl acetate 4:1) and recrystallization from methanol in a yield of 1.69 g (63 %; m.p. 131 ºC; white crystals).

IR: 3000–2840, 2250, 1730, 1700, 1480, 1380, 1240, 1020. ^{1}H-NMR ($CDCl_3$): 1.06 (*s*, 3H, H-19); 1.23 (*s*, 3H, H-18); 2.04 (*s*, 3H, Ac); 4.61 (*m*, 1H, H-3); 5.40 (*m*, 1H, H-6); 9.38 (*s*, 1H, CHO). ^{13}C-NMR ($CDCl_3$): 13.3 (C-18); 17.5; 18.8; 19.2 (C-19); 21.4 (Ac); 27.5; 31.4; 31.6; 32.8; 36.6; 36.8; 37.7; 41.0; 48.7; 49.8; 73.4 (C-3) ; 118.5 (C-16); 121.1 (C-6); 139.4 (C-5); 170.5 (Ac); 204.9 (C-17). Elemental analysis: Calcd. (%) for $C_{21}H_{29}NO_3 \times 1/2H_2O$: C, 71.56; H, 8.58; N, 3.97. Found: C, 71.37; H, 9.09; N, 3.83.

*3β-Acetoxy-15-cyano-17-hydroxy-16,17-*seco*-5-androstene (**3**)*

3β-Acetoxy-15-cyano-17-oxo-16,17-*seco*-5-androstene (**2**; 1.94 g; 5.67 mmol) was dissolved in ethanol (120 mL) and sodium borohydride (0.388 g; 10.10 mmol) was added. The reaction mixture was stirred and heated to reflux for 45 min, then poured into ice-cold water (about 150 mL). The crude product (1.34 g) was precipitated, filtered off, and purified by column chromatography (silica gel, 125 g; toluene/ethyl acetate 4:1). Recrystallization from diethyl ether gave 1.19 g (61 %) of pure compound **3** (m.p. 164 ºC; white crystals).

IR: 3650–3200, 2960, 2250, 1730, 1260, 1050. ^{1}H-NMR ($CDCl_3$): 0.94 (*s*, 3H, H-18); 1.04 (*s*, 3H, H-19); 2.04 (*s*, 3H, Ac); 3.43 (AB_q, 2H, J_{gem} = 10.7 Hz, H-17); 4.61 (*m*, 1H, H-3); 5.38 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 15.4 (C-15); 16.1 (C-18); 19.1 (C-19); 20.0; 21.4 (Ac); 27.6; 31.9; 31.9; 35.4 (C-12); 36.6; 36.9 (C-10); 37.7; 38.2; 43.4; 49.1; 71.2 (C-17); 73.7 (C-3); 119.7 (C-16); 121.4 (C-6); 139.4 (C-5); 170.6 (Ac). Elemental analysis: Calcd. (%) for $C_{21}H_{31}NO_3$: C, 73.01; H, 9.04; N, 4.05. Found: C, 72.84; H, 9.24; N, 4.36.

*3β-Acetoxy-15-cyano-17-p-toluenesulphonyloxy-16,17-*seco*-5-androstene (**4**)*

3β-Acetoxy-15-cyano-17-hydroxy-16,17-*seco*-5-androstene (**3**, 0.29 g; 0.74 mmol) was dissolved in anhydrous pyridine (4 mL) and *p*-toluenesulphonyl chloride (0.70 g; 3.66 mmol) was added in several portions at 0 ºC. The obtained reaction mixture was stirred for 70 h at room temperature, then poured into ice and water (about 20 mL). The crude product **4** (0.26 g) was precipitated and filtered off. Pure compound **4** was isolated by column chromatography (silica gel, 26 g; benzene/ethyl acetate 8:1 and 5:1) followed by crystallization from a mixture of hexane/acetone (3:1) in a yield of 0.214 g (51 %; m.p. 164 ºC; white crystals).

IR: 2960, 2250, 1730, 1360, 1260, 1190, 1050, 980, 840, 670, 550. ^{1}H-NMR ($CDCl_3$): 0.95 (*s*, 3H, H-18); 1.02 (*s*, 3H, H-19); 2.04 (*s*, 3H, Ac); 2.47 (*s*, 3H, Ts); 3.73 (AB_q, 2H, J_{gem} = 10.0 Hz, H-17); 4.67 (*m*, 1H, H-3); 5.36 (*m*, 1H, H-6); 7.38 (*d*, 2H, Ts); 7.79 (*d*, 2H, Ts). ^{13}C-NMR ($CDCl_3$): 15.2 (C-15); 16.1 (C-18); 19.1 (C-19); 19.7; 21.4 (Ac); 21.7 (Ts); 27.5; 31.5; 31.6; 35.3 (C-12); 36.5; 36.8 (C-10); 37.7; 38.2; 42.2; 48.7; 73.5 (C-13); 76.3 (C-17); 118.7 (C-16); 121.2 (C-6); 127.9 (Ts); 130.0 (Ts); 133.2 (Ts); 139.3 (C-5); 145.2 (Ts); 170.5 (Ac). Elemental analysis: Calcd. (%) for $C_{28}H_{37}NO_5S$: C, 67.30; H, 7.46; N, 2.80; S, 6.42. Found: C, 67.22; H, 7.63; N, 3.03; S, 6.29.

*3β-Hydroxy-15-cyano-17-fluoro-16,17-*seco*-5-androstene (**5**)*

3β-Acetoxy-15-cyano-17-*p*-toluenesulphonyloxy-16,17-seco-5-androstene (**4**; 0.20 g; 0.38 mmol) was dissolved in ethyl methyl ketone (6 mL) and tetrabutylammonium fluoride trihydrate (0.65 g; 0.69 mmol) was

added. The reaction mixture was stirred and heated to reflux for 48 h, then poured into ice and water (about 20 mL). The crude yellow product (0.21 g) was precipitated and filtered off. The mother liquor was extracted with dichloromethane (5×30 mL) and the combined organic phases were dried over Na_2SO_4 and evaporated to yield a further 0.091 g of product. Purification of the crude product (0.30 g) by column chromatography (silica gel, 30 g; benzene/ethyl acetate 4:1) yielded 0.071 g (55.5 %) of **5** as white crystals. Recrystallization from methanol yielded pure compound **5** (52 %; m.p. 112 ºC).

IR: 3700–3100, 2950, 2250, 1420, 1120–1000, 700. ^{1}H-NMR ($CDCl_3$): 1.00 (*d*, 3H, J = 1.8 Hz, H-18); 1.04 (*s*, 3H, H-19); 3.53 (*m*, 1H, H-3); 4.07 (*dd*, 1H, J_{gem} = 9.6 Hz, J_{HaF} = 47.5 Hz, H_a-17); 4.24 (*dd*, 1H, J_{gem} = 9.6 Hz, J_{HbF} = 48.6 Hz, H_b-17); 5.37 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 14.8 (*d*, 1C, J_{CF} = 6.4 Hz, C-18); 15.8 (*d*, 1C, J_{CF} = 3.1 Hz, C-15); 19.2 (C-19); 19.7; 31.4; 31.9; 34.8 (*d*, 1C, J_{CF} = 4.0 Hz, C-12); 36.8 (C-10); 36.9; 38.2 (*d*, 1C, J_{CF} = 15.8 Hz, C-13); 41.9; 43.7 (*d*, 1C, J_{CF} = 1.6 Hz, C-14); 49.0; 71.5 (C-3); 90.8 (*d*, 1C, J_{CF} = 176.2 Hz, C-17); 119.1 (C-16); 120.4; 140.4 (C-5). Elemental analysis: Calcd. (%) for $C_{19}H_{28}NFO$: C, 70.55; H, 9.35; N, 4.33. Found: C, 70.85; H, 9.06; N, 4.56.

3β-Acetoxy-15-cyano-17-fluoro-16,17-seco-*5-androstene (**6**)*

3β-Hydroxy-15-cyano-17-fluoro-16,17-*seco*-5-androstene (**5**; 0.050 g, 0.16 mmol) was dissolved in pyridine (1 mL) and acetic anhydride (2 mL; 0.98 mmol) and the mixture was stirred at room temperature for 4 h. It was then poured into water (10 mL) and extracted with dichloromethane (3×20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification of the obtained yellow oil (0.090 g) by column chromatography on silica gel (9 g, toluene/ethyl acetate 8:1) yielded 0.048 g (84 %) of **6** as white crystals. Recrystallization from methanol gave the pure **6** in a yield of 0.042 g (73 %; m.p. 170 ºC).

IR: 2960, 2250, 1720, 1430, 1360, 1240, 1030. ^{1}H-NMR ($CDCl_3$): 1.01 (*d*, 3H, J = 2.4 Hz, H-18); 1.05 (*s*, 3H, H-19); 2.04 (*s*, 3H, Ac); 4.09 (*dd*, 1H, J_{gem} = 9.8 Hz, J_{HaF} = 47.6 Hz, H_a-17); 4.24 (*dd*, 1H, J_{gem} = 9.8 Hz, J_{HbF} = 48.8 Hz, H_b-17); 4.61 (*m*, 1H, H-3); 5.39 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 14.8 (*d*, 1C, J_{CF} = 6.4 Hz, C-18); 15.7 (*d*, 1C, J_{CF} = 2.7 Hz, C-15); 19.1; 19.6 (C-19); 21.4 (Ac); 27.5; 29.7; 31.7; 34.7 (*d*, 1C, J_{CF} = 3.7 Hz, C-12); 36.6; 36.8; 37.7; 38.2 (*d*, 1C, J_{CF} = 15.5 Hz, C-13); 43.6 (C-14); 48.9; 73.6 (C-3); 90.8 (*d*, 1C, J_{CF} = 176.7 Hz, C-17); 119.1 (C-16); 121,3 (C–6); 139.4 (C-5); 170.6 (Ac). Elemental analysis: Calcd. (%) for $C_{21}H_{30}NFO_2 \times CH_3OH$: C, 69.62; H, 9.03. Found: C, 69.60; H, 9.03.

3β-Acetoxy-15-cyano-17-chloro-16,17-seco-*5-androstene (**7**)*

3β-Acetoxy-15-cyano-17-hydroxy-16,17-*seco*-5-androstene (**3**; 0.10 g, 0.33 mmol) was dissolved in anhydrous benzene (3 mL), cooled to 0 ºC and triphenylphosphine (0.26 g, 0.99 mmol) and tetrachloromethane (0.4 mL; 1.41 mmol) were added. The reaction mixture was heated to 80 ºC for 60 min, then cooled and methanol (10 mL) was added to destroy the excess of reactants. It was then poured into water (20 mL), acidified to pH 5 with hydrochloric acid (6 M) and extracted with dichloromethane (3×20 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and concentrated *in vacuo* to give crude **7** (0.110 g) as a yellow oil. Pure compound **7** was obtained by column chromatography (silica gel, 11 g, benzene/ethyl acetate 20:1) and crystallization from methanol in a yield of 0.073 g (70 %; m.p. 140 ºC; white crystals).

IR: 2950, 2250, 1720, 1460, 1390, 1070, 730. ^{1}H-NMR ($CDCl_3$): 1.05 (*s*, 3H, H-19); 1.08 (*s*, 3H, H-18); 2.04 (*s*, 3H, Ac); 3.44 (AB_q, 2H, J_{gem} = 11.6 Hz, H-17); 4.61 (*m*, 1H, H-3); 5.38 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 15.0 (C-15); 18.2 (C-18); 19.2 (C-19); 20.0; 21.4 (Ac); 27.5; 31.6; 31.7; 35.5; 36.5 (C-12); 36.8 (C-10); 37.7; 38.3 (C-13); 42.6 (C-14); 48.8; 54.5 (C-17); 73.6 (C-3); 118.8 (C-16); 121.2 (C-6); 139.4 (C-5); 170.6 (Ac). Elemental analysis: Calcd. (%) for $C_{21}H_{30}NClO_2$: C, 69.31; H, 8.31; N, 3.85. Found: C, 69.07; H, 8.22; N, 3.99.

3β-Hydroxy-15-cyano-17-chloro-16,17-seco-*5-androstene (**8**)*

3β–Acetoxy-15-cyano-17-chloro-16,17-*seco*-5-androstene (**7**; 0.030 g, 0.083 mmol) was dissolved in methanol (2 mL) and potassium hydroxide (0.021 g, 0.38 mmol) was added. The reaction mixture was stirred and heated to reflux for 90 min, then poured into water (10 mL) and extracted with dichloromethane (5×20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4. After evaporation of the solvents and purification of the residue by column chromatography (silica gel, 4 g; benzene/ethyl acetate 6:1), com-

pound **8** was obtained in a yield of 0.025 g (87 %, m.p. 164 ºC; white crystals) after recrystallization from methanol.

IR: 3700–3100, 2950, 2250, 1420, 1120–1000, 950, 700. ^{1}H-NMR ($CDCl_3$):1.04 (*s*, 3H, H-19); 1.07 (*s*, 3H, H-18); 3.44 (AB_q, 2H, J_{gem} = 11.5 Hz, H-17); 3.54 (*m*, 1H, H-3); 5.37 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 15.1 (C-15); 18.2 (C-18); 19.3 (C-19); 20.1; 31.5; 31.8; 35.6 (C-12); 36.8 (C-10); 36.9; 38.4 (C-13); 41.9; 42.9 (C-14); 49.0; 54.6 (C-17); 71.6 (C-3); 118.9 (C-16); 120.4 (C-6); 140.5 (C-5). Elemental analysis: Calcd. (%) for $C_{19}H_{28}NClO \times 1/2CH_3OH$: C, 69.31; H, 8.94. Found: C, 69.54; H, 8.74.

3β-Acetoxy-17-bromo-15-cyano-16,17-seco-*5-androstene (**9**)*

3β-Acetoxy-17-hydroxy-16,17-*seco*-5-androstene (**3**; 0.030 g, 0.10 mmol) was dissolved in anhydrous benzene (2 mL), cooled to 0 ºC and triphenylphosphine (0.30 g, 1.15 mmol) was added, then tetrabromomethane (0.20 g, 0.60 mmol) was added portion wise with stirring and the mixture was heated to reflux for 2 h. The reaction mixture was cooled, methanol (5 ml) was added (to destroy the excess reactants), then poured into water (10 mL) and acidified to pH 5 with hydrochloric acid (6 M). The crude product was extracted with dichloromethane (4×20 mL) and the combined organic phases were dried over Na_2SO_4. After evaporation *in vacuo*, the crude product (0.039 g) was purified by column chromatography (silica gel, 4 g, benzene/ethyl acetate 6:1) to give 0.025 g (71.5 %) of **9** as a colourless oil.

IR: 2960, 2260, 1730, 1460, 1380, 1260, 1060, 920, 830, 620. ^{1}H-NMR ($CDCl_3$): 1.05 (*s*, 3H, H-19); 1.13 (*s*, 3H, H-18); 2.04 (*s*, 3H, Ac); 3.36 (AB_q, 2H, J_{gem} = 10.8 Hz, H-17); 4.61 (*m*, 1H, H-3); 5.38 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 15.0 (C-15); 18.4 (C-18); 19.2 (C-19); 20.1; 21.4 (Ac); 27.5; 31.7; 31.8, 36.4; 36.5 (C-12); 36.8 (C-10); 37.5, 37.7 (C-13); 43.7 (C-14); 45.6 (C-17); 48.9; 73.6 (C-3); 118.7 (C-16); 121.2 (C–6); 139.4 (C-5); 170.5 (Ac).

3β-Acetoxy-15-cyano-17-iodo-16,17-seco-*5-androstene (**10**)*

3β-Acetoxy-15-cyano-17-hydroxy-16,17-*seco*-5-androstene (**3**; 0.050 g, 0.15 mmol) was dissolved in anhydrous pyridine (2 mL), cooled to 0 ºC, then triphenylphosphine (0.368 g, 1.41 mmol), imidazole (several crystals) and iodine (0.178 g, 0.70 mmol) in portions were added. The reaction mixture was stirred and heated to 60 ºC for 2h, then cooled and methanol (5 mL) was added to destroy the excess reactants. It was then poured into water (20 mL), neutralized with hydrochloric acid (6 M) and extracted with dichloromethane (5×20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the residue (0.081 g) by column chromatography (silica gel, 8 g, benzene/ethyl acetate 30:1 and 20:1) gave 0.058 g (87.5 %) of **10** in the form of a clear oil. Recrystallization from ethyl acetate gave **10** as white crystals in a yield of 0.050 g (76 %, m.p. 143 ºC).

IR: 2960, 2250, 1730, 1450, 1400, 1380, 1270, 1220, 1050, 610. ^{1}H-NMR ($CDCl_3$): 1.05 (*s*, 3H, H-19); 1.16 (*s*, 3H, H-18); 2.04 (*s*, 3H, Ac); 3.23 (*s*, 2H, H-17); 4.60 (*m*, 1H, H-3); 5.38 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 15.0 (C-15); 18.2 (C-18); 19.2 (C-19); 20.4; 21.4 (Ac); 23.9 (C-17); 27.5; 31.7; 32.2; 36.1; 36.5 (C-12); 36.8 (C-10); 37.7; 38.3 (C-13); 45.6 (C-14); 48.9; 73.6 (C-3); 118.7 (C-16); 121.2 (C-6); 139.4 (C-5); 170.5 (Ac). Elemental analysis: Calcd. (%) for $C_{21}H_{30}NIO_2$: C, 55.39; H, 6.64; N, 3.08. Found: C, 55.20; H, 6.73; N, 3.01.

3β-Acetoxy-15-cyano-16,17-seco-*5,17(20)-androstadiene (**12**) and 3β-acetoxy-15-cyano-17-chloro-17-methyl-16,17*-seco-*5-androstene (**13**)*

To a solution of 3β-acetoxy-15-cyano-17-hydroxy-17-methyl-16,17-*seco*-5-androstene (**11**, 0.20 g, 0.56 mmol) in anhydrous benzene (15 mL) at 0 ºC triphenylphosphine (0.61 g, 2.33 mmol) and tetrachloromethane (0.4 mL, 1.12 mmol) were added dropwise. The reaction mixture was stirred and heated to 60 ºC for 4 h, then cooled and methanol (10 mL) was added to destroy the excess reactants. It was then poured into ice and water (about 20 mL) and acidified with hydrochloric acid (6 M) to pH 5, then extracted with dichloromethane (5×20 mL). After drying over Na_2SO_4 and evaporation of the solvents, the residue (1.04 g) was chromatographed on a silica gel column (100 g, petroleum ether/acetone/ethyl acetate, 4.7:0.4:0.15). Compound **12** was obtained in a yield of 0.081 g (43 %; m.p. 129 ºC, white crystals) after recrystallization from a mixture of dichloromethane/*n*-hexane, 2:7 and compound **13** was obtained in a yield of 0.065 g (34 %) in the form of a colourless oil.

Compound **12**: IR 3000–2830, 2240, 1740, 1640, 1250, 920. ^{1}H-NMR ($CDCl_3$): 1.05 (*s*, 3H, H-19); 1.08 (*s*, 3H, H-18); 2.04 (*s*, 3H, Ac); 4.62 (*m*, 1H, H-3); 5.07 (*dd*, 1H, J_{gem} = 1.0 Hz, J_{trans} = 17.1 Hz, CH=C**H**$_2$); 5.09 (*dd*, 1H, J_{gem} = 1.0 Hz, J_{cis} = 11.1 Hz, CH=C**H**$_2$); 5.38 (*m*, 1H, H-6); 5.60 (*dd*, 1H, J_{cis} = 11.1 Hz, J_{trans} = 17.1 Hz, C**H**=CH$_2$). ^{13}C-NMR ($CDCl_3$): 15.7 (C-18); 16.7; 19.2 (C-19); 20.1; 21.4 (Ac); 27.6 (C-17); 32.0; 36.6; 36.8; 37.8; 39.3; 40.7; 47.1; 49.2; 73.6 (C-3); 113.2 (CH=**C**H$_2$); 119.6 (C-16); 121.4 (C-6); 139.4 (C-5); 148.3 (**C**H=CH$_2$); 170.5 (Ac). Elemental analysis: Calcd. (%) for $C_{22}H_{31}NO_2$; C, 77.38; H, 9.15; N, 4.10. Found: C, 77.15; H, 9.77; N, 4.83.

Compound **13**: ^{1}H-NMR ($CDCl_3$): 1.04 (*s*, 3H, H-18); 1.14 (*s*, 3H, H-19); 1.48 (*d*, 3H, *J* = 6.8 Hz, CH_3); 2.04 (*s*, 3H, Ac); 4.20 (*q*, 1H, *J* = 6.8 Hz, H-17); 4.61 (*m*, 1H, H-3); 5.38 (*m*, 1H, H-6). ^{13}C-NMR ($CDCl_3$): 15.0; 18.9 (C-18); 19.0 (C-19); 19.1 (C-20); 19.6; 21.4 (Ac); 27.5; 30.0; 32.0; 33.3; 36.6; 36.9; 37.6; 41.1; 44.7; 49.1; 67.3 (C-17); 73.6 (C-3); 118.9 (C-16); 121.1 (C-6); 139.6 (C-5); 170.6 (Ac).

Acknowledgment: This research was supported by the Ministry of Science, Technologies, and Development of the Republic of Serbia (Grant No. 1896).

ИЗВОД

СИНТЕЗА, КРИСТАЛНА СТРУКТУРА И АНТИАРОМАТАЗНА АКТИВНОСТ 17-ХАЛО-16,17-*seco*-5-АНДРОСТЕНСКИХ ДЕРИВАТА

КАТАРИНА ПЕНОВ-ГАШИ[1], СРЂАН СТОЈАНОВИЋ[1], МАРИЈА САКАЧ[1], ЕВГЕНИЈА ЂУРЕНДИЋ[1], СУЗАНА ЈОВАНОВИЋ-ШАНТА[1], СЛОБОДАНКА СТАНКОВИЋ[2], НЕБОЈША АНДРИЋ[3] и МИРЈАНА ПОПСАВИН[1]

[1]Департман за хемију, Природно-математички факултет, Универзитет у Новом Саду, Трг Доситеја Обрадовића 3, 21000 Нови Сад (E-mail: marijas@ih.ns.ac.yu), [2]Департман за физику, Природно-математички факултет, Универзитет у Новом Саду, Трг Доситеја Обрадовића 4, 21000 Нови Сад и [3]Департман за биологију, Природно-математички факултет, Универзитет у Новом Саду, Трг Доситеја Обрадовића 2, 21000 Нови Сад

Полазећи од 3β-ацетокси-15-цијано-17-оксо-16,17-*seco*-5-андростена (**2**) синтетизовани су нови 17-хало деривати: 3β-хидрокси-15-цијано-17-флуоро-16,17-*seco*-5-андростен (**5**) и његов 3β-ацетокси аналог (**6**), 3β-ацетокси-15-цијано-17-хлоро-16,17-*seco*-5-андростен (**7**) и његов 3β-хидрокси аналог (**8**), 3β-ацетокси-17-бромо-15-цијано-16,17-*seco*-5-андростен (**9**) и 3β-ацетокси-15-цијано-17-јодо-16,17-*seco*-5-андростен (**10**). С друге стране, полазећи од 3β-ацетокси-15-цијано-17-хидрокси-17-метил-16,17-*seco*-5-андростена (**11**) синтетизован је 3β-ацетокси-15-цијано-17-хлоро-17-метил-16,17-*seco*-5-андростен (**13**) у ниском приносу. Главни производ у овој реакцији био је 3β-ацетокси-15-цијано-16,17-*seco*-5,17(20)-андростадиен (**12**). Структура 17-јодо деривата (**10**) потврђена је рендгенско-структурном анализом. Једињења **5**–**10**, као и **12** и **13** тестирана су на антиароматазну активност, при чему је само 17-бромо дериват (**9**) инхибирао у извесном проценту активност ензима ароматазе у односу на контролу.

(Примљено 14. априла, ревидирано 2. јуна 2003)

REFERENCES

1. A. M. H. Brodie, *J. Steroid Biochem. Molec. Biol.* **49** (1994) 281
2. W. C. Schwarzel, W. G. Kruggel, H. Brodie, *J. Endocrinol.* **92** (1973) 866
3. S. Li, E. J. Parish, *JAOCS* **73** (1996) 1435
4. A. M. H. Brodie, V. C. O. Njar, *J. Steroid Biochem. Molec. Biol.* **66** (1998) 1
5. K. Penov Gaši, S. Stanković, J. Csanadi, E. Đurendić, M. Sakač, Lj. Medić-Mijačević, O. Arcson, S. Stojanović, S. Andrić, D. Molnar Gabor, R. Kovačević, *Steroids* **66** (2001) 645
6. S. Jovanović-Šanta, V. Pejanović, J. Petrović, *J. Serb. Chem. Soc.* **64** (1999) 393
7. D. Miljković, J. Petrović, M. Stajić, M. Miljković, *J. Org. Chem.* **38** (1973) 3585
8. J. A. Petrović, V. M. Pejanović, D. A. Miljković, J. T. Hranisavljević, *Steroids* **55** (1990) 276.