SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW 16,17-SECOESTRONE DERIVATIVES

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Starting from estrone 3-benzyloxy-17β-hydroxyestra-1,3,5(10)-trien-16-one oxime (**3b**) was synthesized, which underwent Beckmann fragmentation giving the 3-benzyloxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4b**). Sodium borohydride reduction of this compound afforded 3-benzyloxy-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**5b**). The deprotection of the 3-hydroxy group was achieved by action of hydrogen upon derivatives **4b** and **5b** in presence of Pd/C as a catalyst, yielding 3-hydroxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4a**) and 3,17-dihydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**5a**). In biological tests on experimental animals, compounds **4a**, **4b**, **5a** and **5b** showed virtually a complete loss of estrogenic activity, whereas compounds **4a**, **5a** and **5b** exhibited moderate antiestrogenic effect.

Key words: Steroids; Secosteroids; Hormones; Estrogenes; Estrogenic activity; Beckmann fragmentation reaction; Hydrogenolysis.

In the frame of a broader project directed towards obtaining potential antiestrogens, the preparation of a series of new 16,17-secoestrone derivatives with methoxy function at C-3 has been reported previously[1]. The aim of this paper was the synthesis of two new D-secoestrone derivatives bearing a free C-3 hydroxy function and testing of their biological activity on experimental animals.

EXPERIMENTAL

Melting points were determined in open capillary tubes on a Büchi SMP apparatus and are uncorrected. Infrared spectra (wave numbers in cm^{-1}) were recorded in KBr pellets or as film on a Perkin–Elmer M457 spectrophotometer. NMR spectra were taken on a Bruker AC 250E spectrometer (^1H at 250.13 MHz, ^{13}C at 62.5 MHz) and are reported in ppm downfield from

a tetramethylsilane internal standard; symbols s, bs, d, dd, q and m denote singlet, broad singlet, doublet, double doublet, quartet and multiplet, respectively. Mass spectra were recorded on a Finnigan-MAT 8230 instrument, using electron impact (70 eV) or chemical ionization (isobutane) techniques; the first number denotes *m/z* value, and the ion abundances are given in parentheses.

3-Benzyloxyestra-1,3,5(10)-trien-17-one (**1b**)

To a solution of estrone (**1a**; 5.0 g, 18.5 mmol) in a mixture of freshly distilled methanol and acetone (1 : 1, 250 ml), potassium carbonate (5.0 g, 36.2 mmol) and benzyl chloride (25.0 ml, 217 mmol) were added and the mixture was refluxed for 8 h. After evaporation of the solvent, an excess of benzyl chloride was removed by steam distillation. The precipitate was colected by filtration, washed with water, and air-dried, giving crude **1b** (6.63 g, 99%). After crystallization from methanol, 5.40 g (81%) of pure compound **1b**, m.p. 132–133 °C (ref.[2] m.p. 135–136 °C) was obtained. IR: 3 020, 2 920, 1 605, 1 580, 1 500, 1 460, 1 290, 1 260, 1 240, 740, 700. ^{1}H NMR ($CDCl_3$): 0.98 (s, 3 H, 3 × H-18); 5.15 (s, 2 H, O-C**H**$_2$-C_6H_5); 6.70–7.40 (group of signals, 8 H, aromatic protons). ^{13}C NMR ($CDCl_3$): 13.83 (C-18); 76.49 (O-CH_2-C_6H_5); 156.85 (C-3); 220.86 (C=O). For $C_{25}H_{28}O_2$ (360.5) calculated: 83.29% C, 7.83% H; found: 83.50% C, 7.71% H.

3-Benzyloxyestra-1,3,5(10)-triene-16,17-dione 16-Oxime (**2b**)

Metallic potassium (0.96 g, 24.6 mmol) was dissolved in 2-methylpropan-2-ol (40 ml), **1b** (3.0 g, 8.3 mmol) was added and the mixture was stirred for 15 min at room temperature. Isopentyl nitrite (4.5 ml, 33 mmol) was then introduced and the stirring continued for 1 h. The mixture was left overnight and then poured into water (150 ml). The solid was filtered off and the filtrate neutralized with dilute HCl (1 : 4) and the white precipitate of crude **2** was collected by filtration, washed with water and air-dried (3.10 g). The crude **2b** was dissolved in a solution of KOH (1.30 g) in 50% aqueous ethanol (60 ml) and the solution neutralized with diluted HCl (1 : 4). The separated crystals were filtered off, washed with water until neutral and dried, yielding the pure **2b** (2.90 g, 89.5%), m.p. 196–198 °C (ref.[3] m.p. 193.5–195.5 °C). IR: 3 400, 2 910, 1 740, 1 610, 1 580, 1 500, 1 460, 1 390, 1 290, 1 240, 945. ^{1}H NMR (DMSO-d_6): 0.95 (s, 3 H, 3 × H-18); 4.85 (s, 2 H, O-C**H**$_2$-C_6H_5); 6.58–7.25 (group of signals, 8 H, aromatic protons); 12.82 (s, 1 H, C=NOH). ^{13}C NMR (DMSO-d_6): 13.82 (C-18); 69.37 (O-CH_2-C_6H_5); 155.16 (C=NOH); 156.32 (C-3); 205.15 (C=O). MS: 389 (4; M^+); 149 (93); 91 (76); 57 (91); 43 (100). For $C_{25}H_{27}NO_3$ (389.5) calculated: 77.09% C, 6.99% H, 3.60% N; found: 77.17% C, 7.16% H, 4.24% N.

3-Benzyloxy-17-hydroxyestra-1,3,5(10)-trien-16-one Oxime (**3b**)

Compound **2b** (1 g, 2.6 mmol) was dissolved under heating in a mixture of methanol (20 ml), methylene chloride (8 ml) and 1% aqueous solution of KOH (30 ml). To the cooled solution, $NaBH_4$ (0.95 g, 25.1 mmol) was added portionwise. The reaction mixture was stirred for 20 min at room temperature and then refluxed for 40 min. After cooling, acetic acid was added to pH 5 and the white precipitate collected and washed thoroughly with water (0.98 g, 98%, m.p. 193–195 °C). Recrystallization from methanol afforded analytically pure **3b**: 0.83 g (83%), m.p. 195–196 °C. IR: 3 600–3 100, 2 920, 1 610, 1 580, 1 500, 1 260, 1 080, 950. ^{1}H NMR ($CDCl_3$): 0.80 (s, 3 H, 3 × H-18); 3.68 (bs, 1 H, OH); 4.20 (s, 1 H, H-17);

5.13 (s, 2 H, O-C**H**$_2$-C$_6$H$_5$); 6.72–7.34 (group of signals, 8 H, aromatic protons); 8.87 (bs, 1 H, C=N-OH). ^{13}C NMR (CDCl$_3$): 11.22 (C-18); 69.93 (O-CH$_2$-C$_6$H$_5$); 81.70 (C-17), 156.81 (C-3); 165.59 (C=NOH). MS: 391 (19; M$^+$); 373 (5); 91 (100). For $C_{25}H_{29}NO_3$ (391.5) calculated: 76.70% C, 7.47% H, 3.58% N; found: 76.84% C, 7.26% H, 3.73% N.

3-Benzyloxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4b**)

Oxime **3b** (1.0 g, 2.56 mmol, finely ground and dried for 3 h at 90 °C) and 4-methylbenzenesulfonyl chloride (1.53 g, 8.0 mmol) were dissolved in pyridine (15 ml). The reaction mixture was kept at room temperature for 3 h and than poured into an excess of cold dilute HCl. The separeted precipitate of the crude **4b** was collected, washed with water and dried (0.95 g). Column chromatography on silica gel (70 g, toluene–ethyl acetate 95 : 5) afforded 0.72 g (72%) of pure compound **4b**, m.p. 137–138 °C. IR: 2 910, 2 220, 1 725, 1 620, 1 580, 1 290, 1 265, 1 020, 760. ^{1}H NMR (CDCl$_3$): 1.18 (s, 3 H, 3 × H-18); 2.95 (d, 2 H, 2 × H-15); 5.08 (s, 2 H, O-C**H**$_2$-C$_6$H$_5$); 6.78–7.35 (group of signals, 8 H, aromatic protons); 9.40 (s, 1 H, CHO). ^{13}C NMR (CDCl$_3$): 13.11 (C-18); 69.97 (O-CH$_2$-C$_6$H$_5$); 118.63 (C≡N); 156.94 (C-3); 204.76 (C=O). MS: 373 (19; M$^+$); 91 (100). For $C_{25}H_{27}NO_2$ (373.5) calculated: 80.39% C, 7.29% H, 3.75% N; found: 80.38% C, 7.15% H, 3.89% N.

3-Benzyloxy-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**5b**)

Nitrile **4b** (1.0 g, 2.7 mmol) was dissolved under heating in methanol (35 ml). To the cooled solution $NaBH_4$ (0.81 g, 22.2 mmol) was added portionwise. After stirring for 30 min at room temperature and refluxing for 20 min, the reaction mixture was diluted with water (100 ml). The white precipitate was filtered off, washed with water and dried, yielding 0.98 g of the crude **5b**. The product was purified by chromatography on a silica gel column (100 g, toluene–ethyl acetate 2 : 1), whereby 0.96 g (96%) of pure **5b**, m.p. 135–136 °C, was obtained. IR: 3 600–3 200, 2 920, 2 240, 1 610, 1 500, 1 280, 1 240, 1 020. ^{1}H NMR (CDCl$_3$): 0.95 (s, 3 H, 3 × H-18); 2.16 (d, 1 H, OH, J = 13.3); 2.54 (dd, 1 H, H-15$_a$, J_{gem} = 16.1, $J_{15a,14}$ = 6.8); 2.68 (dd, 1 H, H-15$_b$, $J_{15b,14}$ = 7.2); 3.44 (dd, 2 H, H-17, J_{gem} = 10.0, $J_{H,OH}$ = 12.5); 5.03 (s, 2 H, O-C**H**$_2$-C$_6$H$_5$); 6.78–7.35 (group of signals, 8 H, aromatic protons). ^{13}C NMR (CDCl$_3$): 15.47 (C-18); 69.87 (O-CH$_2$-C$_6$H$_5$); 70.90 (C-17); 119.94 (C≡N); 156.81 (C-3). MS: 375 (22; M$^+$); 91 (100). For $C_{25}H_{29}NO_2$ (375.5) calculated: 79.96% C, 7.79% H, 3.73% N; found: 79.81% C, 7.92% H, 3.92% N.

3-Hydroxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4a**) and
3,17-Dihydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**5a**)

To the solution of the corresponding benzyl ether **4b** and **5b** (1.0 mmol) in dichloromethane–methanol mixture (1 : 1, 10 ml), 10% Pd/C (0.24 g) was added. The suspension was stirred at room temperature for 12 h, *i.e.* 48 h under an atmosphere of hydrogen. After removing of the catalyst, the solvent was evaporated to dryness and the product isolated by column chromatography (25 g silica gel, toluene–ethyl acetate 4 : 1).

Compound **4a**: yield 41%, colourless oil. IR: 3 400, 3 300, 2 920, 2 250, 1 720, 1 650, 1 600, 1 505, 1 320, 1 120. ^{1}H NMR (acetone-d_6): 1.18 (s, 3 H, 3 × H-18); 6.62–7.15 (group of signals, 3 H, aromatic protons); 8.05 (s, 1 H, OH); 9.45 (s, 1 H, CHO). ^{13}C NMR (acetone-d_6): 13.45 (C-18); 17.87 (C-15); 120.08 (C≡N); 156.23 (C-3); 206.1 (C=O). For $C_{18}H_{21}NO_2$ (283.4) calculated: 76.28% C, 7.47% H, 4.96% N; found: 76.38% C, 7.46% H, 3.93% N.

Compound **5a**: yield 41%, m.p. 198–199 °C. IR: 3 600–3 100, 2 920, 2 250, 1 620, 1 505, 1 230, 1 020. ^{1}H NMR (acetone-d_6): 0.92 (s, 3 H, 3 × H-18); 3.28 (dd, 1 H, H-17$_a$, J_{gem} = 11.1, $J_{17a,OH}$ = 5.1); 3.55 (dd, 1 H, H-17$_b$, $J_{17b,OH}$ = 5.1); 6.62–7.12 (group of signals, 3 H, aromatic protons); 8.04 (s, 1 H, OH).^{13}C NMR (acetone-d_6): 15.79 (C-15); 16.44 (C-18); 71.08 (C-17); 120.87 (C≡N); 156.06 (C-3). MS: 343 (68; (M + *i*-Bu)$^+$); 342 (100; (M + *i*-Bu–1)$^+$); 303 (17); 286 (87; (M + 1)$^+$); 285 (38; M$^+$); 268 (17; (M + 1 – H_2O)$^+$). For $C_{18}H_{23}NO_2$ (285.4) calculated: 75.75% C, 8.12% H, 4.91% N; found: 75.62% C, 8.01% H, 5.08% N.

BIOLOGICAL TESTS

Immature Wistar strain female rats (21–23 days old) were randomly divided into groups of seven to ten animals. The animals were treated by subcutaneous injection once a day for 3 days with 0.1 ml of a solution of the test compound in olive oil, either solely or in combination with estradiol benzoate (EB). The control group obtained the vehicle only. The total administered amounts of compounds **4a**, **4b**, **5a** or **5b** were 240 μg, whereas the EB dose was 1.5 μg. The animals were killed on the fourth day. The uteri were removed, dissected free of adhering fat and blotted dry after expulsion of uterine fluid and the wet weight was recorded.

Percentage of agonist and antagonist activity in immature rat uterine weight assays was calculated from the ratio of values recorded in treated and control animals thus:

$$\% \text{ AGONISM} = (C - A) \cdot 100 / (B - A)$$

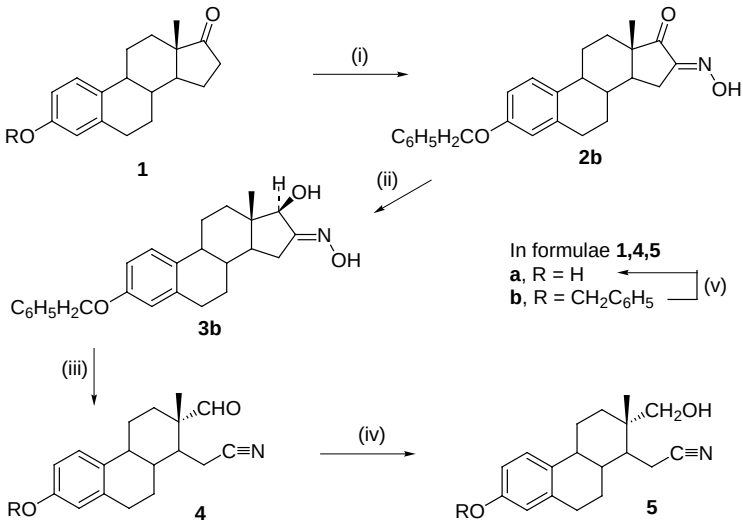
$$\% \text{ ANTAGONISM} = (B - D) \cdot 100 / (B - A)$$

where *A*, *B*, *C* and *D* are uterine wet weights, corrected for differences in body weight, *i.e.* (mg/100 g body weight) for vehicle alone, EB, test compound alone, and test compound plus EB, respectively.

RESULTS AND DISCUSSION

The estrone 3-benzyl ether **1b** (Scheme 1) was obtained in 99% yield (81% after crystallization) by treating estrone (**1a**) with benzyl chloride in the presence of potassium carbonate and then converted into the corresponding 16-oximino derivative **2b** using the known procedure[4]. In addition to **2b**, the crude reaction mixture contained a less polar compound, presumably the *Z*-isomer of **2b**. However, all attempts to isolate this minor product

failed, probably to its isomerization on silica. The reduction of ketone **2b** with sodium borohydride in methanol afforded 3-benzyloxy-17β-hydroxyestra-1,3,5(10)-trien-16-one oxime (**3b**) in a high yield. The β-configuration of OH function at C-17 was proved by NOE experiment.



(i) $(CH_3)_2CHCH_2CH_2ONO/(CH_3)_3COK/(CH_3)_3COH$, 89.5%; (ii) $NaBH_4$-1% aq. KOH/MeOH-CH_2Cl_2, 98%; (iii) TsCl/pyridine, 72%; (iv) $NaBH_4$/MeOH, 96%; (v) H_2-Pd/C/CH_2Cl_2-MeOH, 41% **4a**, 41% **5a**

SCHEME 1

The Beckmann cleavage of oximino alcohol **3b** was carried out under similar reaction conditions as earlier[5], yielding cyanoaldehyde **4b** in 95% yield, which was converted, by sodium borohydride reduction, into the corresponding secocyanoalcohol **5b**, in a high yield.

Deprotection of the 3-hydroxy function was performed by hydrogenolysis at room temperature and low hydrogen pressure, using Pd/C as catalyst, whereby high yields of 3-hydroxy-16,17-secoestrone derivatives **4a** and **5a** were obtained.

The estrogenic and antiestrogenic effects of compounds **4a**, **4b**, **5a** and **5b** were tested on experimental animals, using the uterotropic and antiuterotropic methods[6]. The differences in weights of uteri of treated and control animals served for the calculation of the agonistic and antagonistic effects[7] presented in Table I.

As can be seen from Table I, all the compounds exhibited an almost total loss of estrogenic activity and compounds **4b** and **5a** even prevented the ac-

tion of endogenous estrogens. On the other hand, compounds **4a**, **5a** and **5b** partially hindered the action of estradiol benzoate, behaving as moderate antagonists.

TABLE I
Agonistic and antagonistic effects of compounds **4a**, **4b**, **5a** and **5b**

Compound	Dose, mg/kg	Agonistic effect, %	Antagonistic effect, %
4a	5	4.13	15.96
4b	5	–10.35	0.42
5a	5	–2.06	21.07
5b	5	0.71	31.47

Further derivatizations of compounds **5b** with an aim of obtaining compounds with higher antagonistic action are under progress.

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REFERENCES

1. a) Petrović J. A., Pejanović V. M., Miljković D. A., Hranisavljević J. T.: *Steroids* **1990**, *55*, 276; b) Pejanović V.: *Ph.D. Thesis*. University of Novi Sad, Novi Sad 1991; c) Petrović J., Stepić T., Pejanović V., Medić-Mijacević Lj., Kovacević R., Andrić S., Stanković S.: *J. Serb. Chem. Soc.* **1998**, *63*, 113.
2. Schulster D., Whitehead J. K., Kellie A. F.: *Biochem. J.* **1964**, *93*, 512.
3. Huffman M. N., Lott M. H.: *J. Biol. Chem.* **1948**, 325.
4. Litvan F., Robinson R.: *J. Chem. Soc.* **1938**, 1997.
5. Miljković D., Petrović J.: *J. Org. Chem.* **1977**, *42*, 2101.
6. Emmens C. W. in: *Hormone Assay* (C. W. Emmens, Ed.), p. 408. Academic Press, New York 1950.
7. Wakeling A. E., Valcaccia B., Newboult E., Green L. R.: *J. Steroid Biochem.* **1984**, *20*, 111.