SYNTHESIS AND ESTROGENIC ACTIVITY SCREENING OF SOME 6,9-DISUBSTITUTED ESTRADIOL DERIVATIVES

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Received January 11, 2005
Accepted April 3, 2005

Oxidation of estradiol dipropionate (**1**) with chromium(VI) oxide-3,5-dimethylpyrazole complex yielded 9α-hydroxy-6-oxoestra-1,3,5(10)-triene-3,17β-diyl dipropionate (**2**) and 6-oxoestra-1,3,5(10)-triene-3,17β-diyl dipropionate (**3**). Dehydration of compound **2** with phosphorus(V) oxide or acetic anhydride gave 6-oxoestra-1,3,5(10),9(11)-tetraene-3,17β-diyl dipropionate (**5**). Reduction of compounds **2** and **5** with sodium borohydride afforded 3,6β,9α-trihydroxyestra-1,3,5(10)-triene-17β-yl propionate (**4**) and 3,6β-dihydroxyestra-1,3,5(10),9(11)-tetraene-17β-yl propionate (**6**), respectively. The action of thionyl chloride on compound **2** yielded 6-hydroxyestra-1,3,5(10),6,8-pentaene-3,17β-diyl dipropionate (**7**). Biological tests in vivo of these compounds showed a moderate antiestrogenic activity of compound **4**.

Keywords: Steroids; Estradiol derivatives; Equilenine derivatives; Estrogenic activity; Antiestrogenic activity; Oxidations.

Breast cancer represents one of the most frequent cancers in women. In view of the finding that some types of breast cancers are estrogen dependent their treatment is based on anti-estrogens, substances competing with endogenous estrogens for the receptor binding sites[1]. In the frame of a broader project directed towards obtaining potential anti-estrogens we have reported the preparation of a series of estradiole and estrone derivatives[2–8]. Some of these compounds showed a moderate antiestrogenic activity[3,5,6]. The aim of this paper was the synthesis of the B-modified estradiol derivatives and testing their biological activity on experimental animals.

EXPERIMENTAL

Melting points were determined using a Büchi SMP 20 apparatus and are uncorrected. Infrared 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 250E spectrometer operating at 250 MHz (proton) and 62.5 MHz (carbon) and are reported in ppm (δ-scale) downfield from a tetramethylsilane internal standard; coupling constants (*J*) are given in Hz. Mass spectra were recorded on a Finnigan MAT 8230 instrument, using chemical ionization (isobutane) or electron impact (70 eV) technique. All reagents used were of analytical grade. All solutions were dried over anhydrous sodium sulfate.

9α-Hydroxy-6-oxoestra-1,3,5(10)-triene-3,17β-diyl Dipropionate (**2**) and 6-Oxoestra-1,3,5(10)-triene-3,17β-diyl Dipropionate (**3**)

To dry methylene chloride (160 ml) chromium(VI) oxide (19.3 g, 0.19 mol) was added and the suspension was cooled to 0 °C. To the cooled suspension 3,5-dimethylpyrazole (18.3 g, 0.19 mol) was added at stirring, and estradiol dipropionate (**1**; 3.71 g, 9.7 mmol) 15 min later. The reaction mixture was stirred at 0 °C for 5 h, and then left at room temperature for the next two days. After that, to the reaction mixture previously cooled to 0 °C, 5 M NaOH (80 ml, 0.4 mol) was added and the content stirred at the same temperature for 1 h. The methylene chloride layer was washed sequentially with 2 M HCl, water and saturated solution of NaCl. After drying the solvent was evaporated. The brown oily residue (3.28 g) was chromatographed on a silica gel column (65 g) in toluene–EtOAc (9:1).

Compound **2**. Yield 1.80 g (45%), m.p. 149–150 °C. IR: 3526 (OH); 1760 and 1730 (C=O, ester); 1670 (C=O, C-6); 1605 (ArC–C). ^{1}H NMR ($CDCl_3$): 0.82 s, 3 H (3 × H-18); 1.15 t, 3 H, *J* = 7.5 (C**H**$_3$CH$_2$COO at C-3); 1.24 t, 3 H, *J* = 7.5 (C**H**$_3$CH$_2$COO at C-17); 1.60 s, 1 H (OH at C-9); 2.35 q, 2 H, *J* = 7.5 (CH$_3$C**H**$_2$COO at C-3); 2.60 q, 2 H, *J* = 7.5 (CH$_3$C**H**$_2$COO at C-17); 4.80 t, 1 H, *J* = 8.2 (H-17); 7.24 dd, 1 H, $J_{2,4}$ = 2.5, $J_{1,2}$ = 8.5 (H-2); 7.57 d, 1 H, $J_{1,2}$ = 8.5 (H-1); 7.67 d, 1 H, $J_{2,4}$ = 2.5 (H-4). ^{13}C NMR ($CDCl_3$): 8.90 and 9.14 (2 × **C**H$_3$CH$_2$COO); 11.08 (C-18); 22.65 (C-15); 27.40 and 27.55 (2 × CH$_3$**C**H$_2$COO); 31.97; 32.44; 37.15; 40.95; 42.69; 42.90; 69.02 (C-9); 81.76 (C-17); 120.40, 125.59, 126.99 (Ar C-H); 132.96 and 144.79 (Ar C); 150.51 (C-3); 172.71 and 174.47 (2 × CH$_3$CH$_2$**C**OO); 197.21 (CO, C-6). MS, *m/z*: 414 (M^+), 358 (M^+ – CH_3CHCO), 340 (M^+ – H_2O – CH_3CHCO). For $C_{24}H_{30}O_6$ (414.5) calculated: 69.56% C, 7.25% H; found: 69.32% C, 7.45% H.

Compound **3**. Yield 0.32 g (10%), m.p. 109–111 °C. IR: 3080 (ArC–H); 1766 and 1734 (C=O, ester); 1688 (C=O, C-6); 1616 (ArC–C); 1494, 1210, 1088, 900. ^{1}H NMR ($CDCl_3$): 0.84 s, 3 H (3 × H-18); 1.15 t, 3 H, *J* = 7.5 (C**H**$_3$CH$_2$COO at C-3); 1.27 t, 3 H, *J* = 7.5 (C**H**$_3$CH$_2$COO at C-17); 2.32 q, 2 H, *J* = 7.5 (CH$_3$C**H**$_2$COO at C-3); 2.59 q, 2 H, *J* = 7.5 (CH$_3$C**H**$_2$COO at C-17); 4.73 t, 1 H, *J* = 8.2 (H-17); 7.25 dd, 1 H, $J_{2,4}$ = 2.5, $J_{1,2}$ = 8.5 (H-2); 7.44 d, 1 H, $J_{1,2}$ = 8.5 (H-1); 7.73 d, 1 H, $J_{2,4}$ = 2.5 (H-4). ^{13}C NMR ($CDCl_3$): 8.96 and 9.17 (2 × **C**H$_3$CH$_2$COO); 11.79 (C-18); 22.89; 25.22; 27.32; 27.57; 27.68; 36.35; 39.42; 42.88; 43.73; 49.63; 81.81 (C-17); 119.84, 126.60 and 126.91 (Ar C-H); 133.50 and 144.08 (Ar C); 149.29 (C-3); 172.79 and 174.37 (2 × CH$_3$CH$_2$**C**OO); 196.76 (CO, C-6). MS, *m/z*: 398 (M^+), 342 (M^+ – CH_3CHCO), 286 (M^+ – 2 CH_3CHCO). For $C_{24}H_{30}O_5$ (398.5) calculated: 72.35% C, 7.53% H; found: 72.47% C, 7.30% H.

3,6β,9α-Trihydroxyestra-1,3,5(10)-triene-17β-yl Propionate (**4**)

To the solution of compound **2** (0.1 g, 0.24 mmol) in methanol (8 ml) sodium borohydride (0.1 g, 2.65 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and then poured into water (10 ml) and extracted with ether. After drying of the extract and removal of the solvent, the crude mixture was separated by flash chromatography (benzene–EtOAc, 1:1). The result was compound **4** (0.073 g, 83.4%) in the form of white crystals, m.p. 177 °C (hexane–acetone). IR: 3404 (OH); 1713 (C=O); 1606 (ArC–C); 1213 (C–O); 755. ^{1}H NMR (DMSO-d_6): 0.73 s, 3 H (3 × H-18); 1.02 t, 3 H, J = 7.5 (C**H**$_3$CH$_2$COO); 1.2–1.85 group of signals, 10 H (H-16, H-12, H-11β, H-8, H-7); 2.0 m, 1 H (H-14); 2.16–2.35 group of signals, 3 H (H-11α, CH$_3$C**H**$_2$COO); 4.24 s, 1 H (OH at C-9); 4.44 m, 1 H (H-6); 4.61 t, 1 H, $J_{17,16a}$ = 8.2, $J_{17,16b}$ = 8.5 (H-17); 5.20 d, 1 H, J = 7.8 (OH at C-6); 6.57 dd, 1 H, $J_{2,4}$ = 2.4, $J_{1,2}$ = 8.5 (H-2); 6.91 d, 1 H, $J_{2,4}$ = 2.4 (H-4); 7.20 d, 1 H, $J_{1,2}$ = 8.5 (H-1); 9.23 bs, 1 H (OH at C-3). ^{13}C NMR (DMSO-d_6): 9.38 (**C**H$_3$CH$_2$COO); 11.37 (C-18); 22.83 (C-15); 27.29 (CH$_3$**C**H$_2$COO); 27.43 (C-16); 30.98 (C-7); 32.33 (C-11); 32.73 (C-12); 40.34 (C-8); 41.81 (C-14); 42.68 (C-13); 68.34 (C-6); 68.84 (C-9); 81.95 (C-17); 113.42 (C-4); 114.00 (C-2); 126.34 (C-1); 133.44 (C-10); 142.34 (C-5); 156.34 (C-3); 173.89 (CH$_3$CH$_2$**C**OO). MS, *m/z*: 342 (M^+ – H_2O), 324 (M^+ – 2 H_2O), 235, 208. For $C_{21}H_{28}O_5$ (360.4) calculated: 69.97% C, 7.83% H; found: 69.72% C, 7.78% H.

6-Oxoestra-1,3,5(10),9(11)-tetraene-3,17β-diyl Dipropionate (**5**)

Method A. To the suspension of phosphorus(V) oxide (0.45 g, 3 mmol) in benzene (30 ml) compound **2** (0.11 g, 0.26 mmol) was added, and the reaction mixture stirred at room temperature for 2 h. After that the mixture was poured to water (30 ml), the layers were separated and the organic layer was washed with a saturated solution of $NaHCO_3$, dried, and the solvent removed. The resulting crude product (0.09 g, 87.4%) in the form of light-yellow crystals was recrystallized from hexane, affording pure compound **5**, m.p. 102–103 °C.

Method B. A solution of compound **2** (0.10 g, 0.24 mmol) in acetic anhydride (5 ml) was refluxed for 20 h. Then the reaction mixture was cooled to room temperature and poured into water (50 ml). The aqueous solution was neutralized by carefully adding solid $NaHCO_3$ and the mixture extracted with CH_2Cl_2 (3 × 20 ml). The combined organic extracts were washed with water, dried, and the solvent removed. The raw oily product was purified by flash chromatography (toluene–EtOAc, 12:1), affording pure compound **5** (0.06 g, 63%). IR: 1770 and 1725 (C=O, ester); 1685 (C=O); 1200, 1155. ^{1}H NMR ($CDCl_3$): 0.85 s, 3 H (3 × H-18); 1.15 t, 3 H, J = 7.5 (C**H**$_3$CH$_2$COO at C-3); 1.25 t, 3 H, J = 7.5 (C**H**$_3$CH$_2$COO at C-17); 2.35 q, 2 H, J = 7.5 (CH$_3$C**H**$_2$COO at C-3); 2.60 q, 2 H, J = 7.5 (CH$_3$C**H**$_2$COO at C-17); 4.85 t, 1 H, J = 7.5 (H-17); 6.45 t, 1 H, J = 5.1 (H-11); 7.25 dd, 1 H, $J_{1,2}$ = 8.5, $J_{2,4}$ = 2.5 (H-2); 7.70 m, 2 H (H-1, H-4). ^{13}C NMR ($CDCl_3$): 8.99 and 9.21 (2 × **C**H$_3$CH$_2$COO); 11.81 (C-18); 23.67; 27.46; 27.63; 27.70 (CH$_3$**C**H$_2$COO); 38.15; 39.30; 41.30; 43.25; 47.92; 81.87 (C-17); 119.27 (C-11); 125.31, 125.95 and 127.29 (C-1, C-2 and C-4); 131.54 (C-9); 132.71 and 138.24 (C-5 and C-10); 150.06 (C-3); 172.75 and 174.40 (2 × CH$_3$CH$_2$**C**OO); 196.50 (C-6). MS, *m/z*: 396 (M^+), 341, 340, 266. For $C_{24}H_{28}O_5$ (396.5) calculated: 72.70% C, 7.12% H; found: 72.55% C, 6.98% H.

3,6β-Dihydroxyestra-1,3,5(10),9(11)-tetraene-17β-yl Propionate (**6**)

To a solution of compound **5** (0.10 g, 0.25 mmol) in methanol (10 ml), sodium borohydride (0.10 g, 2.65 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and then poured into water (70 ml). The white precipitate was filtered, washed with water, and dried, to give 0.078 g (91%) of **6**, which after recrystallization from hexane–acetone system melted at 164–165 °C. IR: 3544 and 3438 (OH); 3024 (ArC–H); 1731 (C=O); 1606 (ArC–C); 1242 (C–O); 874 and 813 (C=CH). ^{1}H NMR (CD_3COCD_3): 0.82 s, 3 H (3 × H-18); 1.09 t, 3 H, *J* = 7.8 (C**H**$_3$CH$_2$COO); 2.34 q, 2 H, *J* = 7.8 (CH$_3$C**H**$_2$COO); 4.36 d, 1 H, *J* = 7.2 (OH at C-6); 4.62–4.80 m, 2 H (H-6, H-17); 6.07 bd, 1 H, *J* = 5.0 (H-11); 6.67 dd, 1 H, $J_{1,2}$ = 8.7, $J_{2,4}$ = 2.5 (H-2); 7.46 d, 1 H, $J_{2,4}$ = 2.5 (H-4); 7.46 d, 1 H, $J_{1,2}$ = 8.5 (H-1); 8.44 s, 1 H (OH at C-3). ^{13}C NMR ($CDCl_3$): 9.25 (**C**H$_3$CH$_2$COO); 12.00 (C-18); 23.95; 27.54; 27.80; 37.07; 38.19; 39.08; 41.13; 46.85; 68.99 (C-6); 82.49 (C-17); 112.54 and 115.36 (Ar CH); 118.22 (C-11); 125.21 (Ar CH); 126.58 (C-9); 133.86 and 140.10 (Ar C); 155.33 (C-3); 174.74 (CH$_3$CH$_2$**C**OO). MS, *m/z*: 342 (M^+), 324 ($M^+ - H_2O$), 235, 208. For $C_{21}H_{26}O_4$ (342.4) calculated: 73.66% C, 7.65% H; found: 73.31% C, 7.52% H.

6-Hydroxyestra-1,3,5(10),6,8-pentaene-3,17β-diyl Dipropionate (**7**)

Compound **2** (0.95 g, 2.3 mmol) was dissolved in dry pyridine (5 ml) and then a solution of thionyl chloride (0.7 ml, 9.2 mmol) in pyridine (3 ml) was added while stirring. After stirring at room temperature for 10 min, the reaction mixture was poured to water (50 ml) and extracted with benzene (4 × 20 ml). The extracts were dried, benzene evaporated, and the pyridine residues were removed by co-evaporation with benzene. The obtained yellow oil was purified by flash chromatography to yield compound **7** (0.3 g, 34%), m.p. 201 °C. IR: 3450 (OH); 1760 and 1715 (C=O, ester); 1610 (ArC–C); 1185. ^{1}H NMR ($CDCl_3$): 0.85 s, 3 H (3 × H-18); 1.15 t, 3 H, *J* = 7.5 (C**H**$_3$CH$_2$COO at C-3); 1.25 t, 3 H, *J* = 7.5 (C**H**$_3$CH$_2$COO at C-17); 2.40 q, 2 H, *J* = 7.5 (CH$_3$C**H**$_2$COO at C-3); 2.65 q, 2 H, *J* = 7.5 (CH$_3$C**H**$_2$COO at C-17); 4.90 t, 1 H, *J* = 7.5 (H-17); 5.60 s, 1 H (OH); 6.50 s, 1 H (H-7); 7.25 dd, 1 H, $J_{1,2}$ = 8.5, $J_{2,4}$ = 2.5 (H-2); 7.85 m, 2 H (H-1, H-4). ^{13}C NMR ($CDCl_3$): 9.21 and 9.37 (2 × **C**H$_3$CH$_2$COO); 11.33 (C-18); 23.61; 23.87; 27.91 and 28.34 (2 × CH$_3$**C**H$_2$COO); 34.19; 42.62; 46.37; 81.65 (C-17); 108.05, 113.60, 121.34, 122.50, 123.67, 124.82, 131.06 and 135.54 (Ar C and CH); 147.14 (C-6); 149.75 (C-3); 173.75 and 174.87 (2 × CH$_3$CH$_2$**C**OO). MS, *m/z*: 396 (M^+), 341, 340. For $C_{24}H_{28}O_5$ (396.5) calculated: 72.70% C, 7.12% H; found: 72.44% C, 7.15% H.

11-{[3,17β-Bis(propionyloxy)estra-1,3,5(10),6,8-pentaen-6-yl]oxy}-*N*-butyl-*N*-methylundecanamide (**8**)

11-Bromo-*N*-butyl-*N*-methylundecanamide[8] (0.93 g, 2.8 mmol) and compound **7** (0.57 g, 1.4 mmol) were dissolved in methylene chloride (13 ml), and a solution of NaOH (0.085 g, 2.1 mmol) and tetrabutylammonium bromide (0.45 g, 1.4 mmol) in water (13 ml) was added. The two-phase system was stirred vigorously at room temperature for 10 h. The organic layer was separated, washed with water until neutral, dried and evaporated to dryness. The dark brown oily residue was chromatographed on a silica gel column (50 g, petroleum ether–acetone, 9:1), yielding 0.215 g (23%) of pure compound **8** as a colorless oil. IR: 2940, 2867 (C–H); 1768 and 1742 (C=O, ester); 1650 (NC=O); 1608 (ArC–C); 1188 (C–O). ^{1}H NMR ($CDCl_3$): 0.77 s, 3 H (3 × H-18′); 0.95 m, 3 H (CH$_3$ from Bu); 1.19 t, 3 H, *J* = 7.6 (C**H**$_3$CH$_2$COO at C-3′); 2.39 q, 2 H, *J* = 7.6 (CH$_3$C**H**$_2$COO at C-3′); 2.67 q, 2 H, *J* = 7.5

($CH_3C\mathbf{H}_2COO$ at C-17′); 2.91 and 2.97 2 s, 3 H (N-CH_3); 3.25 and 3.36 2 t, 2 H, J = 7.3 (CH_2NCO); 4.07 t, 2 H, J = 6.5 (OCH_2); 4.90 t, 1 H, J = 7.2 (H-17′); 6.54 s, 1 H (H-7′); 7.24 dd, 1 H, $J_{1',2'}$ = 9.1, $J_{2',4'}$ = 2.5 (H-2′); 7.89 d, 1 H, $J_{1',2'}$ = 9.1 (H-1′); 7.92 d, 1 H, $J_{2',4'}$ = 2.5 (H-4′). ^{13}C NMR ($CDCl_3$): 9.19 and 9.36 (2 × $\mathbf{C}H_3CH_2COO$); 11.36 (C-18′); 13.94 (CH_3 from Bu); 20.02; 20.14; 23.76; 23.90; 25.19; 25.57; 26.26; 27.86; 27.90; 28.37; 29.35; 29.48; 29.54; 29.61; 30.71; 33.07; 33.40; 33.73; 34.22; 35.38; 42.76; 46.75; 47.47; 49.83; 68.19 (OCH_2); 81.63 (C-17′); 104.20, 113.68, 121.48, 121.99, 124.62, 125.06, 130.98 and 135.52 (Ar C and CH); 147.37 (C-6′); 153.22 (C-3′); 172.94 and 173.06 (NC=O from two conformers); 173.40 and 174.71 (2 × $CH_3CH_2\mathbf{C}OO$).

Uterotrophic and Antiuterotrophic Assays

The methods used were similar to those described in the literature[9,10]. Immature Wistar strain female rats (21–23 days old) were randomly divided into groups of six to eight animals each. The animals were treated by subcutaneous injection once a day for three consecutive days with 0.1 ml of a solution of the test compound in olive oil, either solely or in combination with estradiol dibenzoate (EB). The control group obtained the vehicle only. The total administered amounts of tested compounds were 0.5, 5 or 25 mg/kg body weight, whereas the EB dose was 0.03 mg/kg body weight. The animals were killed 24 h after the last injection. The adhering fat was removed from uteri 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 were calculated from the ratio of values recorded for treated and control animals thus:

$$\% \text{ agonism} = (C - A) \times 100/(B - A)$$

and

$$\% \text{ antagonism} = (B - D) \times 100/(B - A),$$

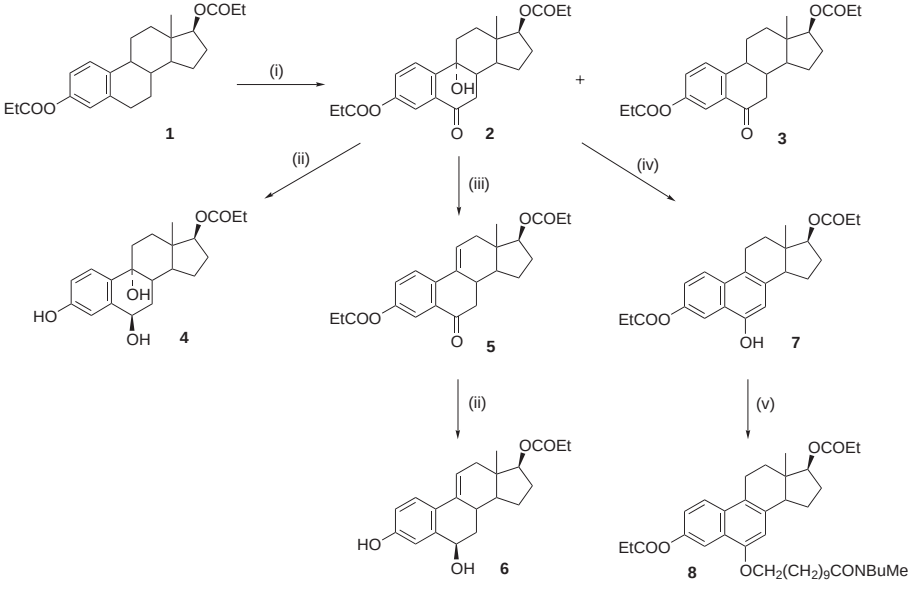
where A, B, C and D are uterine wet weights, corrected for differences in body weight, i.e. (mg/100 g body weight) for the vehicle alone, EB, test compound alone, or test compound plus EB groups, respectively.

RESULTS AND DISCUSION

The starting compound in the synthesis of B-modified estradiol derivatives was estradiol dipropionate (**1**; Scheme 1), which in the first step was oxidized with the CrO_3–3,5-dimethylpyrazole complex[11]. This gave 9α-hydroxy-6-oxoestra-1,3,5(10)-triene-3,17β-diyl dipropionate (**2**) as the main reaction product (45%) and 6-oxoestra-1,3,5(10)-triene-3,17β-diyl dipropionate (**3**) in a yield of 10%. With the aim of improving the yield of compound **2** it was attempted to use some other oxidizing agents such as pyridinium chlorochromate[12], CrO_3–pyridine complex[13] and *tert*-butyl chromate[14]. However, either a large portion of the starting compound remained or a complex reaction mixture was isolated. Compound **2** was reduced with $NaBH_4$

with a simultaneous hydrolysis of the C-3 ester function, yielding 3,6β,9α-trihydroxyestra-1,3,5(10)-triene-17β-yl propionate (**4**) in a yield of 83.4%.

On the other hand, dehydration of compound **2** with P_2O_5 in benzene at room temperature during 2 h or by heating with Ac_2O at reflux for 20 h, afforded 6-oxoestra-1,3,5(10),9(11)-tetraene-3,17β-diyl dipropionate (**5**) in a yield of 87.4 and 63.0%, respectively. Reduction of the carbonyl group of compound **5** with $NaBH_4$, which was also accompanied by the hydrolysis of the C-3 ester function, gave 3,6β-dihydroxyestra-1,3,5(10),9(11)-tetraene-17β-yl propionate (**6**) in a yield of 91%.



(i) CrO_3-3,5-dimethylpyrazole, CH_2Cl_2; (ii) $NaBH_4$, MeOH; (iii) P_2O_5, benzene or Ac_2O; (iv) $SOCl_2$, pyridine; (v) 11-bromo-*N*-butyl-*N*-methylundecanamide, tetrabutylammonium bromide, NaOH, CH_2Cl_2, H_2O

SCHEME 1

A stereochemical assignment of the hydroxy groups at C-6 in both compounds **4** and **6** was resolved by NOE experiments. The ball-stick model of both compounds indicates to a possible NOE between the OH at C-6 and H-8, since the distance between the β-oriented hydroxy group and the H-8 is less then 3 Å. Indeed, a 2.4% NOE enhancement was observed for the signal of H-8 (1.58 ppm) when the OH (5.20 ppm) signal of **4** was irradiated. Similarly, a strong NOE (23%) was observed for the H-8 (2.18 ppm), after an irradiation of the OH (4.36 ppm) signal in **6**. Since both alcohols **4** and **6** have the same configuration at C-6, it is clear that the hydride delivery during the reduction of both keto groups occurs from the α-side.

Dehydration of compound **2** with $SOCl_2$, followed by enolization of the C-6 keto group, yielded 6-hydroxy-17α-dihydroequilenine derivative **7**. Compound **8** was obtained by alkylation of the hydroxy group of compound **7** with 11-bromo-*N*-butyl-*N*-methylundecanamide[8] (obtained from 11-bromoundecanoic acid and butyl(methyl)amine via corresponding acyl chloride) carried out in the two-phase CH_2Cl_2–H_2O system under alkaline conditions, using tetrabutylammonium bromide as a phase-transfer catalyst.

Studies of estrogenic activity by uterotrophic method[9] encompassed compounds **2**–**4** and **6**–**8**, whereas compounds **2**, **4**, **6** and **8** were also tested for antiestrogenic activity using antiuterotrophic method[10]. Results are presented in Table I.

It is evident from the table that compound **4** (3,6β,9α-trihydroxy derivative) exhibited the highest percentage of antagonism (49.46%). Compound **2** (6-oxo-9α-hydroxy derivative) in the lower dose showed certain antago-

TABLE I
Agonistic and antagonistic effects of tested compounds in immature rat uterine weight test

Compound	Dose mg/kg	*n*	Agonistic effect %, mean ± SEM	*n*	Antagonistic effect %, mean ± SEM
2	0.5	8	37.68 ± 3.46[a]	6	21.79 ± 6.91
	5	8	52.99 ± 3.27[a]	6	11.89 ± 10.88
	25	8	63.19 ± 4.67[a]	6	9.64 ± 7.34
3	0.5	8	103.42 ± 4.13[a]		–
	5	8	106.46 ± 6.46[a]		–
	25	8	108.57 ± 8.17[a]		–
4	0.5	7	1.72 ± 2.78		–
	5	6	26.36 ± 2.43	8	48.97 ± 3.44[a]
	15	7	40.87 ± 4.83[a]	6	49.46 ± 5.47[a]
6	0.5	7	5.35 ± 4.51		–
	15	6	73.38 ± 5.66[a]	7	3.22 ± 5.07
7	0.5	8	56.08 ± 4.59[a]		–
	5	8	75.70 ± 5.52[a]		–
	25	7	106.75 ± 6.91[a]		–
8	0.5	8	3.02 ± 2.05		–
	5	6	13.93 ± 3.76		–
	25	8	37.25 ± 2.37[a]	8	–5.65 ± 7.02
Tamoxifen	5	7	39.78 ± 3.13		–
	25	8	39.36 ± 4.09	7	62.80 ± 2.13

Significance: [a] $p < 0.05$ vs corresponding control (Mann–Whitney non-parametric test).

nistic activity (21.79%). In view of the fact that compound **8** showed a negligible estrogenic activity it can be supposed that the side chain at C-6 assumes such a conformation that the binding of the phenol OH group at C-3 to the corresponding receptor sites is blocked. In our experimental condition, compound **4** tested in the same experiments with tamoxifen showed significant antagonistic effect. Although applied doses of compound **4** and tamoxifen were different (15 vs 25 mg/kg), both agonistic (40.87 ± 4.83 vs 39.36 ± 4.09) as well as antagonistic (49.46 ± 5.47 vs 62.80 ± 2.13) effects were comparable with tamoxifen. Also, on the basic of the results of the antiuterotrophic test it could be concluded that the α-oriented hydroxy group at the C-9 position (compounds **2** and **4**) increased antagonistic effect.

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

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