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SYNTHESIS AND INVESTIGATION OF THE BIOLOGICAL PROPERTIES OF 6-OXA-8α-ANALOGS OF STEROID ESTROGENS CONTAINING A METHYL GROUP AT C-4

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4-Methyl-substituted 6-oxa-8α-analogs of steroid estrogens have been synthesized and the ^{1}H and ^{13}C NMR signals of these compounds have been completely assigned. It is shown that introduction of a methyl group in position 4 of steroids of the given stereochemical series leads to the loss of uterotropic and hypertriglyceridemic effects of the modified compounds. Steroids with these properties may have promise for the creation of vectors for transport into target organs of estrogens of other classes of compounds and inhibitors of enzymes responsible for the metabolism of hormones.

Keywords: 6-oxa-8α-analogs of steroid estrogens, their biological properties, NMR spectroscopy.

In recent decades there have been attempts to create inhibitors of enzymes responsible for the metabolism of steroid hormones. The most important condition for the choice of new potential preparations is the absence in them of hormonal effects [1-4]. To solve these problems it is necessary to know what modifications in the structures of steroid hormones or their analogs lead to a sharp decrease in their hormonal activity or its complete disappearance. It is also important that the chose compounds should not have side effects typical for estrogens. In particular, hypertriglyceridemic effects should be absent since these independent risk factors for cardiovascular diseases [5].

We have shown previously in experiments in ovariotomized rats that compound **1** possesses considerable hypertriglyceridemic activity, which is absent in compound **2** [6]. It is of interest to discover whether introduction of a methyl group in position 4 of other 6-oxa-8α-analogs of steroidal estrogens leads to a decrease in the negative influence of these substances on the triglyceride content.

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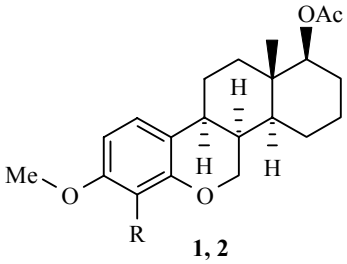
The 4-methyl-8α-analogs of estrogens **3** and **4** were chosen as model compounds for study since in the objective of obtaining optically active compounds is simpler in the case of compounds with a five-membered D ring. The biological activity of these compounds was compared with those of the "standard" substances **5** and **6** which have no substituents at position 4, and also with 17α-ethynylestradiol **7** which is frequently used in clinical practice. The synthesis of the required compounds is shown in the reaction scheme.



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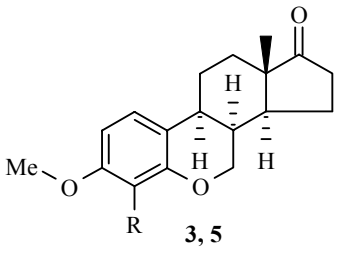

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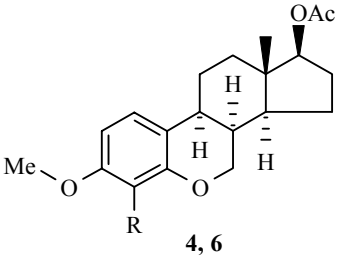

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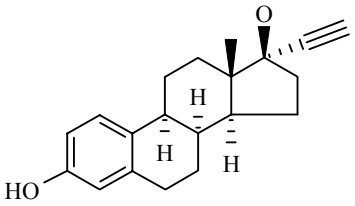

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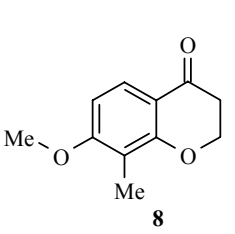
1, 5, 6 R = H; **2, 3, 4** R = Me



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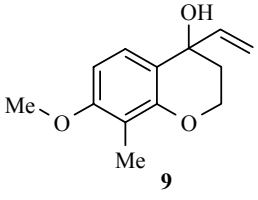

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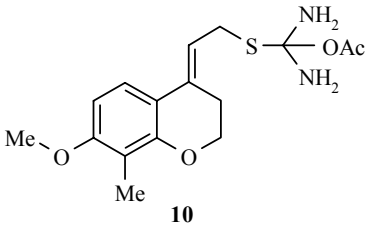

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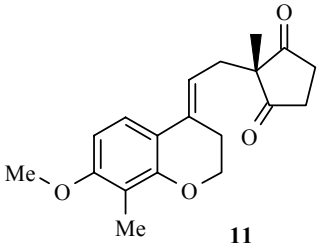

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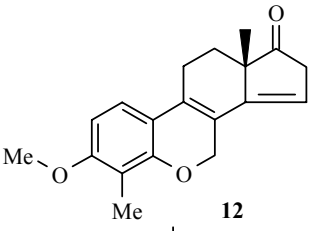

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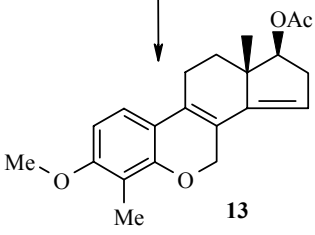

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TABLE 1. Investigation of Estrogenic, Osteoprotective, and Hypolipidemic Activity of Steroids **5** and **6** on Peroral Introduction to Ovariotomized Rats

Group of experimental rats	Change in mass of the body during the time of the experiment, g	Mass of the uterus, mg/100g mass of the body	Weight of ash from the femur/"Moist weight" of the femur	Cholesterol content in the blood serum, mg/dl	Content of triglycerides in the blood serum, mg/dl
Pseudo-operated	29.0 ± 3.2*	154 ± 4**	0.432 ± 0.007*	57.2 ±1.9*	57 ± 3**
Ovariotomized	62.0 ± 5.2	32 ± 1	0.403 ± 0.005	68.4 ± 2.4	38 ± 2
Ovariotomized, received					
steroid **7**	11.0 ± 2.9**	157 ± 8**	0.422 ± 0.005*	30.0 ± 1.7**	98 ± 9**
preparation **5**	45.5 ± 3.8*	72 ± 3**	0.425 ± 0.006*	51.9 ± 1.7*	77 ± 6**
preparation **6**	24.6 ± 3.8	115 ± 5**	0.423 ± 0.006*	37.2 ± 2.9**	76 ± 8**

* The signs "*" and "**" indicate the reliable differences between the groups of the ovariotomized rats ($p < 0.05$ and $p < 0.01$ respectively). The least squared errors and the differences of were calculated by the ANOVA program.

TABLE 2. Investigation of Estrogenic, Osteoprotective, and Hypolipidemic Activity of Steroids **3** and **4** on Peroral Introduction to Ovariotomized Rats

Group of experimental rats	Change in mass of the body during the time of the experiment, g	Mass of the uterus, mg/100 g mass of the body	Weight of ash from the femur/"Moist weight" of the femur	Cholesterol content in the blood serum, mg/dl	Content of triglycerides in the blood serum, mg/dl
Pseudo-operated	36±3*	156.7 ± 14.5**	0.419 ± 0.006*	47.4 ± 1.4*	59.9 ± 5.1*
Ovariotomized	65±4	18.9 ± 0.6	0.397 ± 0.006	64.3 ± 2.2	37.8 ± 1.5
Ovariotomized, received					
steroid **7**	9±5*	172.8 ± 7.2**	0.421 ± 0.008*	29.6 ± 2.6*	102.6 ± 8.8*
preparation **3**	64±5	19.3 ± 0.9	0.396 ± 0.005	65.8 ± 2.3	43.3 ± 2.9
preparation **4**	62±4	19.9 ± 1.0	0.390 ± 0.005	60.6 ± 2.7	42.7 ± 3.3

The signs "*" and "**" indicate the reliable differences between the groups of the ovariotomized rats ($p < 0.05$ and $p < 0.01$ respectively). The least squared errors and the reliable differences were calculated by the ANOVA program.

The isothiuronium salt **10** was prepared by a general method [7, 8] and its condensation with 2-methylcyclopentane-1,3-dione occurred readily to give the secocompound **11**, the cyclocondensation of which gave a high yield of 6-oxaestrapentaene **12**. Catalytic hydrogenation of this compound in benzene in the presence of Raney nickel with subsequent oxidation of the reaction products with Sarett's reagent gave steroid **3** in 39% yield based on the isothiuronium salt **10**.

Compound **4** was synthesized by reduction of the estrapentaene **12** with sodium borohydride, acetylation of the product with acetic anhydride in pyridine with subsequent hydrogenation if the acetate **13** in THF in the presence of Pd/C.

Analysis of the spectra of the analogs **3** and **4** by NMR spectroscopy with COSY, HSQC without decoupling from ^{13}C, COLOC, and NOESY according to [9] allowed the complete assignment of the signals in the ^{1}H and ^{13}C NMR spectra of these steroids and the assignment of their spatial structures. The "standards" **5** and **6** were synthesized by a known method [10].

Some biological properties of the analogs **3-6** were studied by experiments on ovariotomized Wistar rats in conditions of previous papers [6, 9] (Tables 1 and 2). It is easy to see that steroids **5** and **6** possess osteoprotective and hypocholesteremic effects with low uterotropic and hypertriglyceridemic effects which is preferable in comparison with 17α-ethynylestradiol (**7**) which is used clinically. Syntheses based on these compounds may be of interest in the search for new osteoprotective agents.

It follows from the data in Table 2 that the introduction of a methyl group in position 4 of 6-oxa-8α-analogs of steroid estrogens with a five-membered D ring leads to a decrease in the hypertriglyceridemic properties of the modified substances, hence in future one should track experimental attempts of the possibility of using them to transport into the body of estrogens of various classes and also to use them as inhibitors of various enzymes.

EXPERIMENTAL

Mass spectra were recorded on an MX-1321 instrument with a 200-210ºC temperature of the ionizing chamber. ^{1}H and ^{13}C NMR spectra were recorded at 295 K with a Bruker DPX-300 spectrometer (300 and 75 MHz respectively). For recording the ^{1}H NMR spectra of all the compounds, except the isothiuronium salts **10,** solutions of the substances (5-7 mg) in $CDCl_3$ (0.6 ml) were used and for the ^{13}C NMR spectra – 30-50 mg in the same volume. ^{1}H NMR spectra of compounds **10** were taken in $(CD_3)_2SO$ solution. Chemical shifts were measured with respect to TMS by allocating the signals of the solvent ($CDCl_3$–$CHCl_3$ 99.9:0.1) the standard values of 7.26 (^{1}H) and 76.90 ppm (^{13}C) with a precision no worse than ± 0.01 ppm). Homonuclear coupling constants were measured with a precision of ± 0.02 Hz from the ^{1}H NMR spectra after additional treatment of the lines by Lorentz-Gauss transformation.

7-Methoxy-8-methylchroman-4-one (8) was synthesized under conditions proposed in [11] for preparation of analogous compounds; mp 100-101ºC. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.06 (3H, s, 8-CH_3); 2.74 (2H, t, *J* = 6.4, H-3); 3.88 (3H, s, CH_3O); 4.52 (2H, t, *J* = 6.4, H-2); 6.58 (1H, d, *J* = 8.7, H-6); 7.79 (1H, d, *J* = 8.7, H-5). Found, %: C 68.77; H 6.35. $C_{11}H_{12}O_3$. Calculated, %: C 68.74; H 6.29.

4-(7'-Methoxy-8'-methylchromanylidene)ethylisothiuronium Acetate (10). A solution of compound **8** (12.7 g) in THF (80 ml) was added dropwise at 30ºC over 1 h to a solution of vinylmagnesium bromide prepared from 6.3 g magnesium in THF (145 ml). The reaction mixture was kept at room temperature for 12 h, then at 40ºC for 1 h. Powdered thiourea (3.6 g) and anhydrous acetic acid (55 ml) were added to the vinylcarbinol **9**, obtained by normal work up. The reaction mixture was stirred at room temperature for 4 h, the required product was precipitated by the addition of diethyl ether (400 ml). Yield of isothiouronium salt **10** 18.0 g (80%); mp 136-138ºC. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.77 (3H, s, CH_3COO); 1.94 (3H, s, 8'-CH_3);

2.64 (2H, t, *J* = 5.8, H-3); 3.75 (3H, s, CH_3O); 3.80 (2H, d, *J* = 7.8, CH_2); 4.14 (2H, t, *J* = 5.8, H-2); 5.97 (1H, t, *J* = 7.8, =CH); 6.57 (1H, d, *J* = 8.3, H-6); 7.40 (1H, d, *J* = 8.3, H-5). Found, %: C 56.50; H 6.65; N 8.45. $C_{16}H_{22}N_2O_4S$. Calculated, %: C 56.79; H 6.55; N 8.28%.

3-Methoxy-4-methyl-6-oxa-8,14-secoestra-1,3,5(10),9(11)-tetraene-14,17-dione (11). Isothiouronium salt **10** (4.7 g) and 2-methylcyclopentane-1,3-dione (4.7 g) were added to a 1:1 mixture of ethanol and water (100 ml) and the mixture was stirred for 36 h at room temperature. The precipitate was filtered off, washed with methanol, and dried in the air to give the secosteroid **11** (3.8 g, 87%); mp 75-77°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.18 (3H, s, 18-CH_3); 2.06 (3H, s, 4-CH_3); 2.54-2.80 (8H, m); 3.82 (3H, s, CH_3O); 4.78 (2H, t, *J* = 5.7, H-7); 5.67 (1H, t, *J* = 8.2, H-11); 6.47 (1H, d, *J* = 8.8, H-2); 7.26 (1H, d, *J* = 8.8, H-1). ^{13}C NMR spectrum, δ, ppm: 8.0, 19.3, 25.6, 34.2, 35.5, 55.6. 56.9, 66.4, 103.4, 111.9, 113.7, 115.1, 121.1, 132.8, 153.2, 158.1, 216.9. Found, %: C 72.47; H 7.28. $C_{19}H_{22}O_4$. Calculated, %: C 72.59; H 7.05.

3-Methoxy-4-methyl-6-oxaestra-1,3,5(10),8,14-pentaen-17-one (12). Conc. HCl (6 ml) was added to a solution of secosteroid **11** (3.3 g) in methanol (70 ml); the reaction mixture was boiled for 30 min, then kept for 1 day at 5°C. The precipitate was filtered off, washed with water and then methanol to give compound **12** (2.9 g, 93%); mp 165-167°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.17 (3H, s, H-18); 1.57-1.67 (1H, m); 2.03-2.11 (1H, m); 2.08 (3H, m, 4-CH3); 2.44-2.78 (1H, m); 2.95 (1H, dd, J_1 = 3.0 and J_2 = 23.4); 3.34 (1H, d, *J* = 23.4); 3.85 (3H, s, CH_3O); 4.83 (1H, d, *J* = 13.4); 5.02 (1H, d, *J* = 13.4); 5.74 (1H, t, *J* = 5.0, H-15); 6.50 (1H, d, *J* = 8.6, H-2); 7.07 (1H, d, *J* = 8.6, H-1). Found, %: C 77.11; H 7.03. $C_{19}H_{20}O_3$. Calculated, %: C 77.00; H 6.80.

3-Methoxy-4-methyl-6-oxa-8α-estra-1,3,5(10)-trien-17-one (3). Raney nickel (5g) was added to a solution of estrapentaene **12** (2.9 g) in benzene (270 ml); the mixture was hydrogenated for 40 min at a temperature of 80-120°C and a pressure of 80-180 atm. After normal work up, the reaction product was oxidized with Sarett's reagent made from chromium trioxide (2.5 g) in pyridine (25 ml). The required product was obtained by twice recrystallization from methanol, yield 1.4 g (48%); mp 152-154°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 6.90 (H-1); 6.47 (H-2); 4.06 (H-7β); 4.30 (H-7α); 2.51 (H-8α); 2.62 (H-9α); 1.98 (H-11α); 1.71 (H-11β); 1.43 (H-12α); 1.84 (H-12β); 2.19 (H-14α); 2.17 (H-15α); 1.98 (H-15β); 2.45 (H-16α); 1.92 (H-16β); 0.92 (H-18); 3.80 (CH_3O); 2.06 (4-CH_3). ^{13}C NMR spectrum, δ, ppm: 126.6 (C-1), 103.3 (C-2), 156.6 (C-3), 113.7 (C-4), 152.9 (C-5), 64.4 (C-7), 37.2 (C-8), 37.3 (C-9), 119.4 (C-10), 28.2 (C-11), 31.7 (C-12), 46.7 (C-13), 46.7 (C-14), 21.4 (C-15), 35.6 (C-16), 219.6 (C-17), 16.5 (C-18), 8.2 (4-CH_3), 55.7 (OCH_3). Mass spectrum, *m/z* (I_{rel}, %): 300 (100), 285 (3.5), 243 (3), 215 (44), 202 (10), 201 (13.5), 189 (7), 188 (5.5), 176 (67), 175 (63); 161 (10.5). Found, %: C 75.85; H 8.28. $C_{19}H_{24}O_3$. Calculated, %: C 75.97; H 8.05.

17β-Acetoxy-3-methoxy-4-methyl-6-oxa-8α-estra-1,3,5(10)-triene (4). Sodium borohydride (0.5g) was added to a solution of compound **12** (5g) in 1:1 dioxane–water (180 ml) and the reaction mixture was stirred for 3 h at room temperature. The excess reducing agent was decomposed with acetic acid, and the reaction product was acetylated with acetic anhydride under normal conditions [9]. Compound **13** was dried and then hydrogenated in the presence of 5% Pd/C in THF. The steroid **4** was crystallized from methanol; yield 3.2 g (55%); mp 164.5-167°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 6.90 (H-1); 6.46 (H-2); 4.07 (H-7β); 4.25 (H-7α); 2.37 (H-8α); 2.58 (H-9α); 1.88 (H-11α); 1.66 (H-11β); 1.34 (H-12α); 1.75 (H-12β); 1.80 (H-14α); 1.72 (H-15α); 1.64 (H-15β); 2.22 (H-16α); 1.51 (H-16β); 4.63 (H-17α); 0.84 (H-18); 3.79 (CH_3O); 2.07 (4-CH_3); 2.05 ($OCO\underline{C}H_3$). ^{13}C NMR spectrum, δ, ppm (*J*, Hz): 126.6 (C-1), 103.1 (C-2), 156.6 (C-3), 113.6 (C-4), 153.1 (C-5), 64.2 (C-7), 36.2 (C-8), 37.4 (C-9), 119.9 (C-10), 28.4 (C-11), 36.7(C-12), 41.5 (C-13), 45.5 (C-14), 22.2 (C-15), 26.9 (C-16), 82.2 (C-17), 13.6 (C-18), 8.2 (4-CH_3), 55.7 (OCH_3), 21.1 ($OCO\underline{C}H_3$), 170.9 ($O\underline{C}OCH_3$). Mass spectrum, *m/z* (I_{rel}, %): 344 (100), 301 (4), 285 (11), 269 (4), 243 (2), 227 (4), 215 (5), 202 (25), 189 (8), 176 (37), 175 (33), 161 (8), 151 (13). Found, %: C 73.17; H 8.37. $C_{24}H_{28}O_4$. Calculated, %: C 73.23; H 8.19.

Methyl Ether of 6-Oxa-8α-estrone (5) was made by a method descibed previously [10]; mp 148.5-150°C (mp 148-150°C [10]). The steroid showed no depression of the melting point in a mixture with a known sample. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.93 (3H, H-13); 1.43 (1H, H-12α); 1.68 (1H, H-11β); 1.84

(1H, H-12β); 1.90 (2H, H-15α, H-15β); 1.96 (1H, H-14α); 2.00 (1H, H-11α); 2.18 (1H, H-16α); 2.45 (1H, H-16β); 2.54 (1H, H-8α); 2.60 (1H, H-9α); 3.75 (3H, OCH_3); 4.07 (1H, H-7β); 4.22 (1H, H-7α); 6.38 (1H, H-4); 6.49 (1H, H-2); 6.99 (1H, H-1). ^{13}C NMR spectrum, δ, ppm: 129.45 (C-1), 107.37 (C-2), 158.85 (C-3), 100.84 (C-4), 154.79 (C-5), 71.92 (C-7), 43.27 (C-8), 39.04 (C-9), 119.13 (C-10), 29.05 (C-11), 32.42 (C-12), 46,73 (C-13), 48.37 (C-14), 24.26 (C-15), 36.33 (C-16), 220.40 (C-17), 16.20 (C-18), 55.12 (CH_3O). Found, %: C 75.46; H 7.79. $C_{18}H_{22}O_3$.Calculated, %: C 75.50; H 7.74.

17β-Acetoxy-3-methoxy-6-oxa-8α-estra-1,3,5(10)-triene (6) was obtained by a previously published method [10]: mp 115-116°C (mp 115-116°C [10]). ^{13}C NMR spectrum, δ, ppm: 130.04 (C-1), 107.24 (C-2), 158.76 (C-3), 101.28 (C-4), 155.14 (C-5), 64.04 (C-7), 36.05 (C-8), 36.89 (C-9), 119.45 (C-10), 28.05 (C-11), 36.56 (C-12), 41.34 (C-13), 45.29 (C-14), 22.08 (C-15), 26.68 (C-16), 81.94 (C-17), 13.50 (C-18), 55.10 (CH_3O), 20.99 ($OCO\underline{C}H_3$), 170.83 ($O\underline{C}OCH_3$), Found, %: C 72.75; H 8.11. $C_{20}H_{26}O_4$. Calculated, %: C 72.70; H 7.93.

Investigation of Uterotropic, Osteoprotective, and Hypocholesteremic Activities of the model compounds and their effects on the total weight of the experimental animals was carried out on ovariotomized Wistar line rats as carried out in [9]. Steroids **3-6** were introduced for 35 days *per os* in olive oil in a dose of 5 mg/kg of body mass per day, 17α-ethynylestradiol (**7**) in a dose of 0.1mg/kg of body mass per day.

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