Some derivatives of pregna-D_6'-pentaranes as possible antagonists of progesterone

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Some derivatives of progestins of the pregna-D_6'-pentarane series have been obtained and examined for their possible hormonal and antihormonal activity in the Clauberg—McPhail assay as well as in the pregnancy maintenance test in ovariectomized rabbits. 16α,17α-Cyclohexanoprogesterones of the 19-methyl and 19-nor series (**1—4**) saturated in ring A, which are inactive as progestins, exhibited a remarkable antiprogesterone effect. They decreased the McPhail index under combined administration with progesterone in a dose-dependable manner and completely inhibited the action of the latter in the pregnancy maintenance test.

Key words: pentarane, progestin, Clauberg—McPhail assay, antihormonal action, progesterone.

Pregna-D′-pentaranes are modified progestins with an additional D′ carbocycle in the 16α,17α-positions. These compounds have high progestational activity and contraceptive effects; pregna-D_6'-pentaranes with cyclohexano ring D′ are the most active.[1] They exhibit high affinity for the rabbit and calf uterine progesterone receptor.[2,3] A study of the structure-activity relationships in the pentarane series showed that different micromodifications of the D_6'-pentarane molecule can lead to an increase in progestational activity or its decrease and complete disappearance.[4]

This study demonstrates that D_6'-pentaranes can also display antihormonal properties (see also the previous communication[5]). In this work the results of the synthesis of pregna-D_6'-pentaranes with a saturated A ring and a study of their hormonal and antihormonal activities are presented.

Results and Discussion

16α,17α-Cyclohexano-5α-pregnanes of the 19-methyl and 19-nor series (**1** and **2**, respectively) were prepared by stereospecific reduction of the Δ^4-bond in molecule **5** by lithium in ammonia followed by Jones oxidation of the 20ξ-hydroxy group in resulting **6**.[6] Compound **1** was also prepared from 16-dehydropregnanolone acetate (**7**). The Lewis acid-catalyzed condensation of the latter with butadiene according to the procedure developed by us[1] led to cycloadduct (**8**) in a high yield, catalytic hydrogenation of which gave saturated ketoacetate (**9**). Hydrolysis of 3β-acetate and subsequent oxidation of the 3β-hydroxy group afforded *trans*-A/B-3,20-dione **1**.[7]

16α,17α-Cyclohexano-5β-pregnanes **3** and **4** were obtained by catalytic hydrogenation of the Δ^4-bond in (**5a,b**) in the presence of acid[8] (Scheme 1).

Pentarane **10** with a 16β-methyl group was synthesized starting from the corresponding cycloadduct (**11**)[9] by hydrogenation of the double bond in the D′ ring and the standard introduction of the Δ^4-3-keto group in the A ring of the saturated product (**12**) (Scheme 2).

We examined the activity of pentaranes **1—4**, **10**. The procedures of the tests were analogous to those described in Ref. 10 (see Experimental). Compounds **1—4** and **10** proved to be inactive both in the Clauberg—McPhail assay (dosage range 0.0008—4.0 mg/kg day; subcutaneous and oral administration) and in the pregnancy maintenance test in ovariectomized rabbits (subcutaneous injection of 0.2 mg/kg day of the tested compounds did not preserve blastocysts in comparison to the same dose of progesterone, which preserved 60 % of blastocysts).

The antiprogestational activity of pentaranes **1—4** was studied mainly after oral administration. In each case the compound was tested for its ability to inhibit the endometrial response due to subcutaneous administration of 0.4 mg/kg day of progesterone to immature female rabbits (the anti-Clauberg—McPhail assay). The results of these experiments are given in Table 1. As can be seen from the Table, progesterone itself at this dose has a progestational effect evaluated by the McPhail index equal to 3.5. Under combined administration of progesterone and the tested compounds within the dosage range represented in Table 1 there was a remarkable decrease in the McPhail index as the dose of the pentarane introduced increased. Thus, pentaranes **1—4** exhibited a dose-dependent antiprogestational effect.

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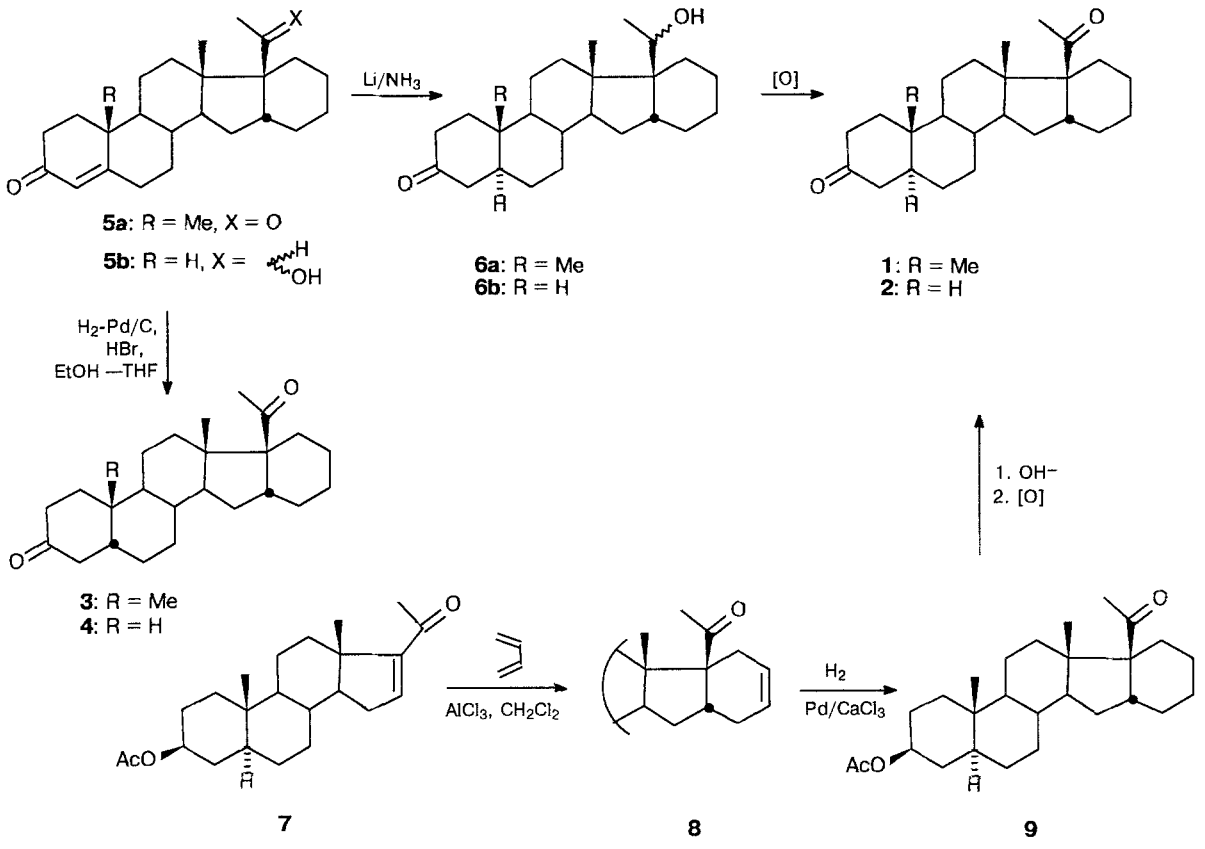
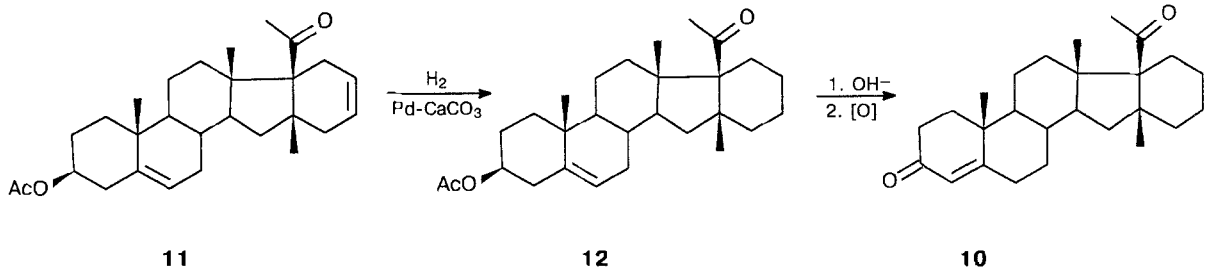
Scheme 1



Scheme 2



5α-Pentaranes **1** and **2** were the most potent: as the pentarane dose increased the McPhail index decreased more than 50 %; 5β-pentaranes **3** and **4** displayed lower antiprogestational activity. It should be noted that pentaranes **1—4** begin exhibiting their antiprogestational action at very low doses (*e.g.*, 0.0008 mg/kg day for **1** and **2**; the decrease of the McPhail index is from 3.51 to 2.3 and 2.5, respectively).

5α-Pentarane **1** was also tested for its ability to inhibit the progestational action of a very active synthetic progestin — megestrole acetate (6-methyl-6-dehydro-17α-hydroxyprogesterone acetate) (Table 2). Under combined oral administration of 0.1 mg/kg day of the latter and increasing doses of compound **1** a decrease in the McPhail index took place. This fact demonstrates the antiprogestagenic effect of **1** in this case as well.

The ability to maintain pregnancy in animals lacking endogenic progesterone is known to be characteristic of "real" progestins and, in particular, of pregna-D_6'-pentaranes with high progestational activity in the Clauberg—McPhail assay.[4] We examined the ability of 5α-pentarane **1** to inhibit the positive effect of progesterone in the pregnancy maintenance test in ovariectomized rabbits. In the control group of animals after ovariectomy pregnancy was not preserved at all.

Table 1. Antiprogestational activity of pregna-D_6'-pentaranes **1—4** (with subcutaneous injection of 0.4 mg/kg day of progesterone)

Compound	Dose per day /mg kg^{-1}	McPhail index
Progesterone	0.4	3.51±0.05
1[a]	0.0008	2.30±0.00
	0.004	2.30±0.00
	0.01	2.20±0.20
	0.02	2.00±0.19
	0.1	1.70±0.20
1[b]	0.0008	2.50±0.00
	0.004	2.30±0.00
	0.02	2.30±0.20
	0.1	1.80±0.05
	0.4	1.70±0.20
2[b]	0.0008	3.00±0.00
	0.004	2.90±0.10
	0.01	2.80±0.10
	0.02	2.15±0.20
	0.1	2.10±0.16
	0.4	1.50±0.06
3[b]	0.0008	3.00±0.12
	0.004	2.80±0.05
	0.01	2.60±0.10
	0.02	2.50±0.12
	0.1	2.20±0.15
	0.4	2.20±0.15
	1.0	2.00±0.19
	5.0	1.30±0.22
4[b]	0.0008	2.75±0.12
	0.004	2.69±0.07
	0.01	2.50±0.12
	0.02	2.40±0.08
	0.4	2.15±0.11
	1.0	2.00±0.18

[a] Subcutaneous administration of tested compound.
[b] Oral administration of tested compound.

Table 2. Antiprogestational activity of 16α,17α-cyclohexano-5α-pregnane-3,20-dione **1**

Compound	Dose per day /mg kg^{-1}	McPhail index
Megestrole acetate[a]	0.1	3.25±0.03
1[b]	0.1	2.70±0.20
	0.4	2.50±0.10
	4.0	2.20±0.10

[a] Oral administration.
[b] Subcutaneous administration.

Thus, combined subcutaneous administration of 0.2 mg/kg day of progesterone and 1 mg/kg day of pentarane **1** resulted in complete inhibition of the progesterone effect, evaluated as 60 % after a single administration.

At the same time, 16β-methyl-16α,17α-cyclohexanoprogesterone **10**, which is inactive as a progestin, did not block the positive action of progesterone in the pregnancy maintenance test.

Thus, we found that for 5*H*-compounds **1—4**, after elimination of the Δ^4-bond from progestationally active 16α,17α-cyclohexanoprogesterone a reversal of the activity profile takes place and a distinct antiprogestational effect is demonstrated.

On the whole, D_6'-pentaranes with a saturated A ring, which are inactive as progestins, exhibit an antiprogestational effect, whereas pentarane **10**, having no progestational activity due to other structural alterations, does not manifest such an effect. The antiprogestational action of pentaranes with saturated A ring seems to be explained by changes in their binding to progesterone receptors. The pentaranes studied could be considered to be more like partial antagonists than classic antihormones.

Experimental

All melting points were measured on a Boetius hot-stage apparatus. Mass-spectra were obtained using a Varian CH-6 MAT instrument (the energy of ionizing electrons was 70 eV, direct introduction into the ionizing camera). The ^1H NMR spectra were recorded on a Bruker WM-250 (250 MHz) spectrometer in $CDCl_3$ solutions. TLC was performed on microplates (Silica gel LS 5/40μ), visualized with iodine vapors. All column chromatography separations were carried out on "Woelm" Silica gel (200 mesh).

16α,17α-Cyclohexano-5α-pregnane-3,20-dione (1). A solution of 0.15 g of 16α,17α-cyclohexanopregn-4-en-3,20-dione (**5a**) in 15 mL of THF was added over 2 min at −50 °C to a stirred solution of 0.032 g of Li in 50 mL of NH_3. After 6 min the reaction mixture was quenched with 0.10 g of NH_4Cl, and after evaporation of NH_3, it was diluted with water and extracted with CH_2Cl_2. The residue obtained after the removal of the solvent and drying *in vacuo*, was dissolved in 20 mL of acetone and treated with stirring at 10 °C with 0.5 mL of Jones reagent. Then the stirring was continued for an additional 10 min, and the excess of Jones reagent was decomposed with Pr^iOH and ice water was added. The precipitate was separated by filtration, dried, and crystallized from acetone to give 0.123 g of **1**; m.p. 198—200 °C (from acetone), which was identical to the sample of **1** prepared from **10**.[7]

16α,17α-Cyclohexano-5α-19-norpregnane-3,20-dione (2). Using the above procedure, 0.28 g of 16α,17α-cyclohexano-19-norpregn-4-en-20ξ-ol-3-one **5b** gave 0.23 g of **2**; m.p. 198—205 °C (from acetone).

16α,17α-Cyclohexano-5β-pregnane-3,20-dione (3). A solution of 0.5 g of **5a** in 20 mL of THF—EtOH (1 : 1) and 0.06 mL of conc. HBr was hydrogenated in the presence of 0.1 g 5 % Pd/C at atmospheric pressure. The catalyst was filtered off, 1 mL of water was added to the filtrate and the mixture was kept at room temperature for 0.5 h. The residue obtained after evaporation of the solvents was recrystallized several times from a CH_2Cl_2—hexane mixture to give 0.35 g of **3**; m.p. 195—203 °C. ^1H NMR, δ: 0.69 (s, 3 H, 18-Me); 1.02 (s, 3 H, 19-Me); 2.13 (s, 3 H, 21-Me); 2.96 (m, 1 H, H—C(16)).

16α,17α-Cyclohexano-5β-19-norpregnane-3,20-dione (4). Using the above procedure, 0.455 g of **4** was prepared from 0.6 g of **5b**, m.p. 175—180 °C (from CH_2Cl_2—hexane). ^1H NMR, δ: 0.71 (s, 3 H, 18-Me); 2.13 (s, 3 H, 21-Me); 2.97 (m, 1 H, H—C(16).

16β-Methyl-16α,17α-cyclohexanopregn-4-en-3,20-dione (10). A solution of 0.346 g of **11** in 60 mL of EtOH was hydrogenated in the presence of 0.1 g 5% $Pd/CaCO_3$. The yield of **12** was quantitative; m.p. 175—177 °C (from ether—hexane). ^1H NMR, δ: 0.93 (s, 3 H, 18-Me); 1.02 (s, 3 H, 19-Me); 1.34 (s, 3 H, 16-Me); 2.04 (s, 3 H, 3-OAc); 2.13 (s, 3 H, 21-Me); 4.61 (m, 1 H, 3-H); 5.38 (m, 1 H, H—C(6)). MS, *m/z*: 386 $M-AcOH^+$. Calculated for $C_{28}H_{42}O_3$, 426.63.

A mixture of 0.32 g of **12** in 50 mL of MeOH and 0.105 g of KOH in 2 mL of water was refluxed for 0.5 h and neutralized with dil. HCl after cooling. Most of the MeOH was evaporated *in vacuo*. The residue was diluted with water, and the resulting precipitate was separated and dried. The raw 3-hydroxyderivative (0.22 g) thus formed was oxidized by the Oppenauer procedure (0.3 g of $Al(OPr^i)_3$, 3 mL of cyclohexanone, 20 mL of toluene, reflux, 3 h). The crude product **10** was purified by column chromatography (elution with a benzene—ether mixture 5→25 %) m.p. 180—183 °C (from ether—hexane). ^1H NMR, δ: 0.95 (s, 3 H, 18-Me); 1.18 (s, 3 H, 19-Me); 1.33 (s, 3 H, 16-Me); 2.12 (s, 3 H, 21-Me); 5.75 (br.s, 1 H, H—C(4)).

Clauberg—McPhail assay*.[10] Immature female rabbits weighing 800—1000 g were primed with 5.0 μg of subcutaneous 17β-estradiole benzoate in sunflower oil per day for 5 days. After 6 days, various daily doses of compounds **1—4**, **10** were administered subcutaneously (or *per os*) in sunflower oil once a day for 5 consecutive days (dosage range in mg/kg, see general part). The animals were sacrificed by intravenous air embolism 24 h after the last steroid administration and the uteri were dissected and underwent standard histological work-up. The endometrial response was evaluated visually according to McPhail by degrees from 0 to 4. The experimantal data were treated by regression analysis.

In each assay a control group of animals, treated only by sunflower oil, was used. The number of animals for each test compound per experiment was 25 rabbits.

Anti-Clauberg—McPhail assay. ***a.*** The procedure used was analogous to that of the above mentioned test but beginning on the 7th day of an experiment, simultaneously with the tested compounds **1—4** 0.4 mg/kg day of progesterone was subcutaneously administered for 5 days. Subcutaneous administration of this dose of progesterone itself gave a progestational effect, evaluated by the McPhail index as equal to 3.51 (Table 1).

b. The procedure used was analogous to that of ***a*** but beginning on the 7th day of an experiment 0.1 mg/kg day of megestrole acetate was orally administered for 5 days simultaneously with the subcutaneously administered pentarane **1**. The oral administration of 0.1 mg/kg day of megestrole acetate alone gave a progestational effect, evaluated by the McPhail index as equal to 3.25 (Table 2).

Pregnancy maintenance assay. Female rabbits weighing 3000—3500 g were mated during their estrus; bilateral ovariectomy was performed 18 h later. The number of ruptured follicles was determined during the operation. Progesterone in a dose of 0.2 mg/kg day was subcutaneously injected on the day of the ovariectomy and for 5 consecutive days to one group of rabbits; the same procedure was performed on the other group of rabbits but simultaneously with progesterone, 0.1 mg/kg day of pentarane **1** (or **10**) was administered. On 7th day the rabbits were sacrificed. The uteri were removed, washed with saline solution, and the number of blastocysts was counted. The degree of pregnancy survival was evaluated from the rate of ovulated follicles.

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