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BIOLOGICAL ACTIVITY OF TRANSFORMED STEROIDS. XIX.* SYNTHESIS AND HORMONAL ACTION SPECTRUM OF 6α-METHYL-16α,17α-CYCLOBUTANOPROGESTERONE

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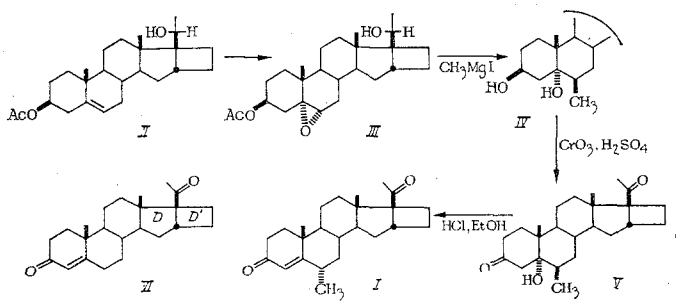
It is assumed that the introduction of substituents at position 6 of the molecule of true progestagens leads to a strengthening of their hormonal activity [1]. In fact we have shown particularly that the introduction of a halogen atom (F, Cl) and a methyl group into position 6 of the molecule of D'_6-pentarane, which is a representative of the class of highly active progestomimetics, leads to a strengthening of the oral progestagenic activity and a clear strengthening of the contraceptive action [2-4]. On the other hand there is information in the literature that in the retroprogesterone series (substituted 17α-hydroxy-9α,10α-progesterones) substitution at C_6 reduces progestogenic activity in comparison with the unsubstituted analog [5].

With the aim of studying the influence of substitution at C_6 on the hormonal activity in the D'_4-pentarane series, 6α-methyl-16α,17α-cyclobutanoprogesterone (I) has been obtained by us and its biological activity has been studied. As was shown by us previously in [6] 16α, 17α-cyclobutanoprogesterone (VI) possesses marked hormonal activity but less than the corresponding cyclohexano derivative.



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The 3-acetate of 16α,17α-cyclobutanopregn-5-en-3β,20β-diol (II) [7] was selected as starting material. Epoxidation of the 5,6 double bond in the latter with monoperphthalic acid led to the 5,6-epoxide of (II) in 80% yield. On boiling (III) with an excess of CH_3MgI in toluene the triol (IV) was obtained in high yield. Oxidation of (IV) with chromic anhydride in sulfuric acid led to 6β-methyl-16α,17α-cyclobutanopregnan-5α-ol-3,20-dione (V) in 90% yield. Removal of the 5α-hydroxy group and isomerization of the 6-methyl group in (V) was carried out in one stage by boiling in ethanol with concentrated hydrochloric acid. The structures of the obtained compounds were demonstrated by the combination of the physicochemical analysis data.

EXPERIMENTAL CHEMISTRY

Melting points were determined on a Kofler block. IR spectra were measured on a UR-10 instrument (East Germany) in KBr. PMR spectra were obtained on a Tesla BS-497 spectrometer (Czechoslovakia) in $CDCl_3$ solution relative to TMS. Mass spectra were obtained on a Varian MAT CH-6 instrument (Switzerland). TLC was carried out on microplates of silica gel type L (5-40 μm). Silica gel type KSK (200-250 mesh) free from iron was used for columns.

16α,17α-Cyclobutano-5α,6α-epoxypregnan-3β,20β-diol 3-Acetate (III). An 8% solution (80 ml) of monoperphthalic acid in ether was added to a solution of (II) (0.8 g: 2.0 mmole) [7] in CH_2Cl_2 (15 ml). The mixture was kept at 5-8°C for 20 h, then diluted with ether (150 ml), treated with saturated Na_2CO_3 solution, the organic layer washed with water, and dried over anhydrous $CaCl_2$. After removal of the solvent the residue was crystallized from a mixture of ether and acetone. Compound (III) (0.73 g: 87%) was obtained having mp 156-160°C. IR spectrum, ν_{max} cm^{-1}: 1250, 1730, 3450. PMR spectrum, δ, ppm: 0.64 s (18-CH_3), 1.04 s (19-CH_3), 1.32 d (21-CH_3, J = 6 Hz), 1.94 s (3-OAc), 2.86 d(H C_6, J = 3.5 Hz). Found, %: C 73.04; H 9.53. $C_{27}H_{40}O_5$. Calculated, %: C 72.94; H 9.08.

6β-Methyl-16α,17α-cyclobutanopregnan-3β,5α,20β-triol (IV). An ether solution of MeMgI (1 g Mg and 20 ml MeI in 100 ml ether) was added in a stream of argon to a solution of (III) (0.8 g: 2 mmole) in toluene (80 ml). The solution was concentrated to one half volume and the residue maintained at 75°C for 1.5 h, then cooled, and neutralized with saturated NH_4Cl solution. The organic layer was separated, washed with water, and dried over $MgSO_4$. After removal of the solvent the residue was recrystallized from a mixture of hexane and ether. Compound (IV) (0.57 g: 76%) was obtained having mp 163-168°C. IR spectrum, ν_{max} cm^{-1}: 1050, 3420. PMR spectrum, δ, ppm: 0.73 s (18-CH_3), 1.04 (19-CH_3), 1.20 d (6-CH_3, J = 6 Hz), 1.32 d (21-CH_3, J = 6 Hz). Found, %: C 76.89; H 10.88. $C_{24}H_{40}O_3$. Calculated, %: C 76.55; H 10.71.

6β-Methyl-16α,17α-cyclobutanopregnan-5α-ol-3,20-dione (V). An oxidizing mixture (12 ml) (13.36 g CrO_3, 11.5 ml H_2SO_4, and water to 50 ml total volume) was added at 0-5°C to a stirred solution of (IV) (0.5 g: 1.3 mmole). After 30 min the reaction mixture was poured into water, extracted with chloroform, the organic layer washed with water, dried, and the solvent removed in vacuum. Compound (V) (0.48 g: 94%) was obtained having mp 224-228°C (hexane—acetone). IR spectrum, ν_{max} cm^{-1}: 1680, 1710, 3415. PMR spectrum, δ, ppm: 0.62 s (18-CH_3), 1.18 d (6-CH_3, J = 5 Hz), 1.23 s (19-CH_3), 2.03 s (21-CH_3). Found, %: C 77.83; H 9.31. $C_{24}H_{36}O_3$. Calculated, %: C 77.37; H 9.71.

6α-Methyl-16α,17α-cyclobutanopregn-4-en-3,20-dione (I). A mixture of (V) (0.3 g: 0.8 mmole), concentrated hydrochloric acid (0.3 ml), and ethanol (15 ml) was maintained at 50°C for 40 min. After cooling, the mixture was poured into cold water (100 ml), and extracted with $CHCl_3$. The organic layer was washed with water, dried, and the solvent removed in vacuum. The residue was chromatographed on a column of SiO_2. After elution with a mixture of petroleum ether—acetone (92:8), (I) (0.09 g: 33%) was obtained having mp 164-167°C (from methanol). IR spectrum, ν_{max}, cm^{-1}: 1610, 1675, 1690. PMR spectrum, δ, ppm: 0.68 s (18-CH_3), 1.12 s (19-CH_3), 1.00 d (6-CH_3, J = 6 Hz), 2.07 s (21-CH_3), 5.72 m (H-C_4). Found, %: C 81.22; H 9.48. $C_{24}H_{34}O_2$. Calculated, %: C 81.31; H 9.67.

EXPERIMENTAL PHARMACOLOGY

Progestagenic Activity. A modified Clauberg method was used to determine progestagenic activity [8]. Immature female rabbits of weight 600-800 g prepared by administration of folliculin (at 2.5 μg in sunflower oil for 5 days) were treated with the test compounds intramuscularly (i.m.) or intragastrically (i.g.). Daily doses of progestagens (in 0.4 ml sunflower oil) were also administered for 5 days from the 7th day after the beginning of the

TABLE 1. Progestagenic Activity of D^{1}_{4}-Pentaranes

Compound	Clauberg–McPhail test			Relative activity	% blastocyte survival (daily dose 0.2 mg/kg i.m.)
	route of administration	studied dose range, mg/kg	ED_{50}, mg/kg		
Progesterone	i.m.	4—0,025	0,26	1	60
VI	i.m.	4—0,008	0,057	4,5	
	i.g.	4—0,04	1,41	0,2	27,6
I	i.m.	0,4—0,04	0,18	1,4	
	i.g.	4—0,4	4,14	0,06	19,2

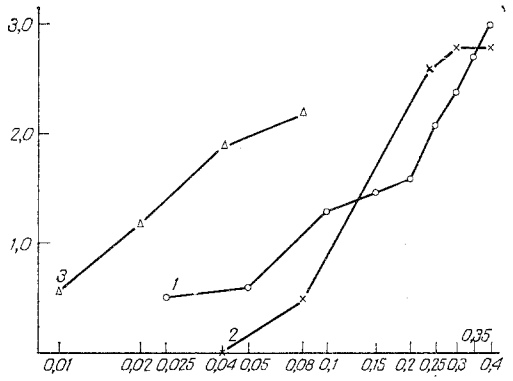

Fig. 1. Progestagenic activity of compounds (I, VI) and progesterone in the Clauberg–McPhail test after intramuscular administration. Dose (mg/kg in logarithmic scale) is given on the abscissa and McPhail index on the ordinate. 1) Progesterone; 2) compound (I); 3) compound (VI).

experiment. On the day after the last administration of a preparation animals were killed and after the standard histological treatment the extent of the secretory changes of the endometrium was investigated. Assessment was made on the 4 point McPhail system [9]. Progesterone (intramuscular) was used as standard. The biological activity was estimated as the ED_{50} corresponding to a McPhail index of 2.

Pregnancy Maintenance Test. Mature rabbits of weight 3.15 ± 0.075 kg were mated during estrus and bilateral ovariectomy was carried out 18 h later. The number of bursting follicles was determined at operation. On the day of ovariectomy and for five succeeding days test substances were administered intramuscularly at a dose of 0.2 mg/kg in an oil solution. Animals were killed on the 7th day, uteri removed, washed with physiological solution, and the number of blastocytes counted. The degree of pregnancy maintenance was assessed as the percentage of washed blastocytes in relation to the number of ovulated follicles.

Contraceptive Effect. The contraceptive effect was studied in combination with mestranol (estrogen at a dose of 0.06 mg/kg, progestagen 3 mg/kg, ratio 1:50) as aqueous suspensions on administration i.g. for 14 days. Experiments were carried out on female rats with a 4-5 day estral cycle. Contraceptive activity was judged as the absence of pregnancy on the 20th day after detecting sperm in vaginal smears.

Antiovulatory Activity. The antiovulatory activity was determined in mature female rabbits of mass 3.15 ± 0.84 kg in which ovulation had been induced by the intravenous administration of copper acetate at a dose of 4 mg/kg [10].

RESULTS AND DISCUSSION

The results of experiments on the study of progestagenic activity are shown in Table 1. The experimental data of the Clauberg–McPhail test were treated using the regression equation:

$$y = a + b \lg x,$$

where y is the McPhail index, x progestagen dose in mg/kg, a and b are regression coefficients.

The points located on the steepest portions of the dose–response curve were taken into consideration for calculations.

Results are shown in Fig. 1 of the investigation of progestagenic activity as the relationship of McPhail index and log dose. The ED_{50} is given in Table 1 as is the relative activity of the studied compounds (the activity of progesterone was taken as 1) and results of the study of pregnancy maintenance in ovariectomized rabbits.

As is evident from Table 1 and Fig. 1 the introduction of a 6α methyl group into the molecule of (VI) leads not only to an increase of its progestagenic action but also causes a definite fall in it. On studying the contraceptive action of (I) in combination with mestranol negative results were obtained. It should be noted that (VI) also eliminated the contraceptive effect [6, 11]. At the same time (I) at a dose of 0.5 mg/kg completely suppressed ovulation, the same action was also shown by the combination of 0.5 mg/kg (I) with 0.01 mg/kg mestranol. Ovulation was completely suppressed in 50% rabbits, the mean number of ovulated follicles in rabbits with maintained ovulation was 1.75 ± 0.6 in comparison with 9 ± 0.1 in controls. Unsubstituted (VI) gave a weaker antiovulatory effect. At a dose of 0.5 mg/kg ovulation was suppressed completely in 33% rabbits, in rabbits maintaining ovulation the number of ovulated follicles was 5 ± 0.5. The same results were obtained on using a combination of (VI) with mestranol.

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