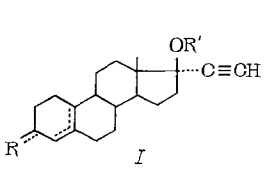
SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE 17-ACETATE AND DIACETATE OF 6-METHYLPREGNA-4,6-DIENE-3β,17α-DIOL-20-ONE

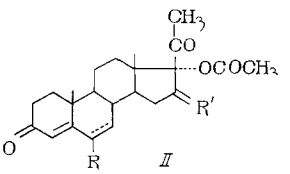
G. M. Kadatskii, G. S. Grinenko, A. I. Terekhina, L. I. Lisitsa, I. V. Ganina, and E. I. Gorenburgova

UDC 615.256.52

Gestagens, chiefly represented by the following two steroid groups: estrane, i.e., 19-norsteroids (I) and pregnane, i.e., progesterone derivatives (II), are used in the treatment of dysfunctional uterine hemorrhages, amenorrhea, endometriosis, and other gynecologic diseases [1].



I



II

R = R' = H, Δ^4: lynestrenol
R = $OCOCH_3$, R' = CH_3CO, Δ^4: ethynodiol diacetate
R = O, R' = H, $\Delta^{5(10)}$: norethynodrel
R = O, R' = $COCH_3$, Δ^4: norethisterone acetate
R = O, R' = H, Δ^4: (13-ethyl)-noregestrel
R = CH_3, R' = H_2: medroxyprogesterone acetate
R = CH_3, R' = H_2, Δ^6: megestrol acetate
R = Cl, R' = H_2 Δ^6: chlormadinone acetate
R = CH_3, R' = CH_2, Δ^6: melengestrol acetate
R = Cl, R' = CH_2, Δ^6: chlorsuperlutin acetate

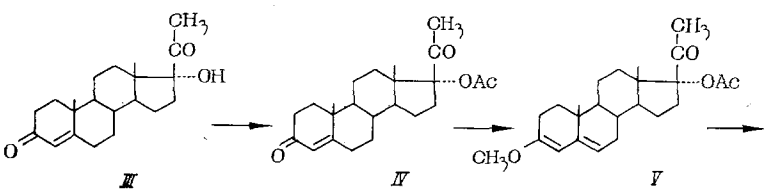
Profound changes in the progesterone molecule result in the creation of highly active oral preparations. It turned out that the most effective change was one which promoted the slowing of metabolic transformations at positions 6, 7, 17, and 16 of the steroid nucleus by introducing a Δ^6-double bond substituent at position 6 (alkyl, halogen, etc.) as well as introduction of a 17α-acylhydroxy group and a 16-alkyl or alkylidene substituent.

The present paper reports the effect of reduction of the 3-keto group in 6-methylpregna-4,6-dien-17α-ol-3,20-dione acetate (X) to a 3β-hydroxyl group on progestational activity.

Modifications in the direction of transforming the Δ^4-3-keto group into a 3-hydroxyl group were studied in a pregnane and estrane series [2]. This resulted in no appreciable increase in physiologic activity. However, discovery of the ability of these groups to interconvert in vivo 17α-hydroxyprogesterone, e.g., (3α,17α-dihydroxy-pregn-4-en-20-one is yielded after perfusion through bovine adrenals) [3] demonstrates the possibility of increasing the duration of the preparations' activity in the body.

Production of 3-hydroxy-$\Delta^{4,6}$-compounds also opens up the possibility of altering the duration and character of the preparations' activity as a result of acylating the hydroxyl group with different acids.

Using the following scheme we succeeded in synthesizing compounds XI and XII with the above-mentioned modification from 17α-hydroxyprogesterone:



III IV V

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow; Research Institute for Biological Testing of Chemical Compounds, Moscow Region. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 13, No. 6, pp. 63-68, June, 1979. Original article submitted November 30, 1978.

0091-150X/79/1306-0617$07.50

TABLE 1. Gestagenic Activity (scored according to the McPhail Scale) of Compounds XI and XII Compared to the Effect of Megastrol Acetate in Infant Rabbits

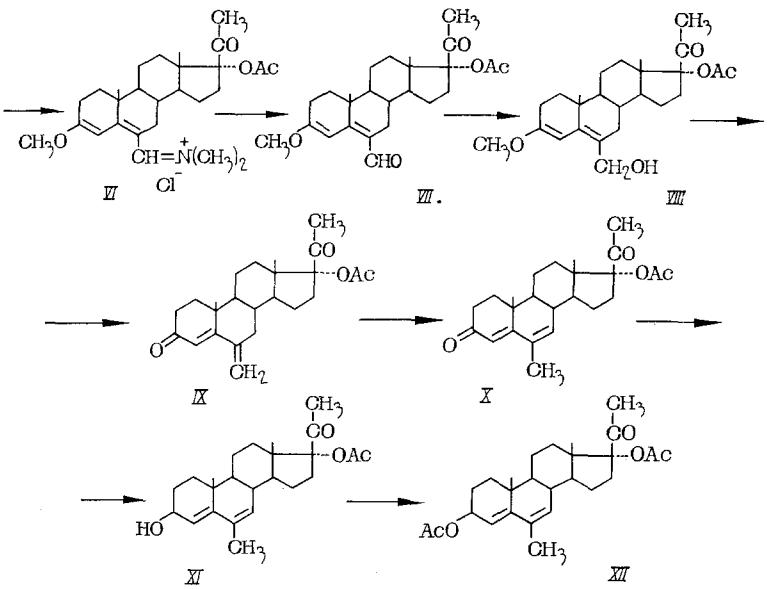
Preparation	Dose, mg per animal				
	0,4	0,04	0,008	0,004	0,0004
XI	4	4	4	2	0
XII	4	4	4	2	0
Megestrol acetate	4	4	4	2,5	0

Note. There were 3-4 rabbits in each group.

TABLE 2. Duration of the Gestagenic Effect (determined by the McPhail Scale Score) of Compounds XI and XII Compared to the Effect of Megestrol Acetate Following a Single Administration in a Dose of 5 mg per Animal

Preparation	Time elapsed after admin., days				
	3	7	10	14	17
XI	4	4	4	1,5	0
XII	4	4	4	1,7	0
Megestrol acetate	4	4	2,5	0,5	0

Note. There were 3-4 rabbits in each group.



In accordance with the method for synthesizing 6-methylenepregn-4-en-17α-ol-3,20-dione acetate (IX) [4], hydroxyprogesterone was acetylated by acetic anhydride in the presence of sulfosalicyclic acid in a boiling benzene solution. At the end of the acetylation reaction, the 3-enol acetate byproduct was hydrolyzed with hydrochloric acid. The hydroxyprogesterone acetate (IV) yield was 95%. The latter was converted into the methyl ester of the dienol V by treating IV with methylorthoformate in a benzene–methanol medium in the presence of sulfosalicyclic acid. The reaction was carried out in such a way that the methylformate formed during the process was driven off. The yield for the formation of the 3-methyl ester V was as high as 94%. Formylation was achieved by using Wilsmeier's reagent, prepared from phosphorus oxychloride and dimethylformamide, in excess as a solvent. The reaction product was hydrolyzed with a potassium carbonate solution. The 3-methyl ester of the 6-formyl substituted VII which was formed was reduced with sodium borohydride to the corresponding carbinal VIII, which without extraction was dehydrated by acid hydrolysis of the 3-methyl ester

of dienol. This resulted in the production of the 6-methyl derivative IX with a yield of 78%. The latter was isomerized under hydrogen transfer conditions, i.e., boiling with cyclohexene in an alcohol solution of sodium acetate in the presence of a catalyst, palladium on carbon [5]. Obtained at a yield of up to 93%, 3-keto-4,6-diene X was reduced with sodium borohydride in a methanol solution as opposed to the lithium tri(tert-butoxy)-aluminum hydride suggested for this purpose [6, 7]. Under these conditions the reduction reaction occurs with strict selectivity yielding up to 95% 3β-hydroxy-4,6-diene (XI) as a result of shielding of the 20-keto group by the 17α-acetoxyl residue. According to the results of thin-layer chromatography, contamination by the corresponding 3,20-diol did not exceed 1%. Preservation of the $\Delta^{4,6}$-diene system of the double bond during the course of this reaction is supported by UV-spectrum findings (λ_{max} 242 nm, ε = 24,500). Acetylation of XI with acetic anhydride in pyridine at room temperature results in the production of the diacetate XII with 90% yield after crystallization from isopropyl alcohol.

EXPERIMENTAL PHARMACOLOGY

Compounds XI and XII were investigated for their gestagenic, antiandrogenic, anabolic, estrogenic, thymolytic, anti-inflammatory, and mineralocorticoid activity. Their gestagenic effect was evaluated by stimulation of secretory changes in the endometrium of sexually immature rabbits [8, 9], by their influence on deciduoma development in ovariectomized mice [10], and by the pregnancy maintenance test in ovariectomized rats calculating the ratio between the number of implantation sites and the number of live fetuses [11]. Duration of the gestagenic effect was determined by their effect on the endometria of infant rabbits after a single injection of the compound in a dose of 5 mg/kg subsequent to previous adminstration of folliculin (0.5 μg per animal per day for 7 days). The effects were recorded on days 3, 7, 10, 14, and 17 after the test substance was administered [12].

The methods of investigating the other types of activity have been described previously [13]. The compounds' hormonal action was compared to the effects of megestrol acetate. In all of the experiments the substances were administered subcutaneously in an oil solution in a volume of 0.1 ml per mouse, 0.2 ml per rat, and 0.5 ml per rabbit.

The experiments performed showed that both test substances possess high gestagenic activity. As is apparent from Table 1, the Clauberg-McPhail test showed the same activity for compounds XI and XII as well as megestrol acetate: The dose causing the maximum effect was 0.008 mg for all of the substances studied. Comparative evaluation of the duration of the compounds' gestagenic action (Table 2) showed that substances XI and XII were more effective than megestrol acetate on days 10-14 after a single administration.

The stimulating effect of the substances on deciduoma development in ovariectomized mice was investigated after preliminary administration of folliculin (estrone) in a dose of 0.5 g per animal per day for 4 days. The compounds were administered from day 5 to 14 in a dose of 1 mg. On the 10th day after the start of folliculin administration, the left horn of the uterus was traumatized by suturing with 10 mm of silk thread. The animals were sacrificed 24 h after the last injection of the test substance, and the effect was measured by the degree to which the traumatized uterine horn had increased in weight. As is evident from Table 3, compound XII and megestrol acetate do not differ significantly in effectiveness, while substance XI is less active.

Comparison of the effects of compound XII and megestrol acetate in the pregnancy maintenance test in ovariectomized rats showed them to be equally effective. When the preparations were administered to ovariectomized rats in a dose of 5 mg per animal in combination with estradiol dipropionate (0.1 mg/kg) from day 12 through day 19 of pregnancy, approximately 70% of the fetuses survived. The fetal and placental weights of the rats which had received the substances were approximately the same as the controls.

When the antiandrogenic activity of the compounds was investigated, it was found (Table 4) that compound XI has a pronounced effect when compared to the effect of megestrol acetate. Substance XII does not cause a definite decrease in the androgenic action of testosterone propionate.

Compounds XI, XII, and megestrol acetate have roughly the same uterotrophic effect. When the substances are administered for 3 days in a dose of 10 μg/kg, the uterine weight of the infant mice increases two to three times, and the organ's moisture content increases by 5-7%.

Compound XI revealed antiestrogenic activity. When administered for 3 days in a dose of 1 mg/kg, it reduced the increase in folliculin-stimulated (10 μg/kg) uterine weight by approximately 50%. When substance XI was administered in a dose of 10 mg/kg for 4 days to adrenalectomized rats, it brought about an approximately 40% reduction in thymus weight.

TABLE 3. Effect of Compounds XI, XII, and Megestrol Acetate on Deciduoma Development in Ovariectomized Mice

Preparation	Weight of the intact uterine horn, mg		Weight of the traumatized uterine horn, mg		Increase in weight of the traumatized uterine horn, %
	$M \pm m$	P	$M \pm m$	P	$(M \pm m)$
Control	19,4±1,4	—	28,6±1,4	—	33,3±2,4
XI	30,8±4,4	<0,05	66,0±6,2	<0,001	120,9±17,6
XII	37,1±3,6	<0,001	162,4±14,4	<0,001	363,7±15,6
Megestrol acetate	33,8±4,8	<0,02	115,2±12,1	<0,001	259,9±47,2

Note. There were 8-9 mice in each group.

TABLE 4. Antiandrogenic Activity of Compounds XI and XII Compared with the Effect of Megestrol Acetate and Cyproterone Acetate

Preparation	Weight of the animals, g		Organ weight, mg per 100 g body weight $(M \pm m)$		
	initial	final	seminal vesicles	ventral prostate	muscle
—	60	64	10,5±0,5	12,3±1,7	14,0±0,9
Testosterone propionate (TP)	61	73	79,5±6,8	77,5±8,7	43,1±2,6
Cyproterone acetate + TP	61	66	19,1±1,3*	23,5±4,6*	16,4±2,1*
Megestrol acetate + TP	60	72	38,8±3,6*	54,8±1,8*	25,7±1,3*
XI + TP	61	71	53,3±6,5*	43,5±3,7*	21,0±1,8*
XII + TP	62	80	66,2±4,2	58,7±2,9	41,0±4,3

*Reliability of the difference has been calculated with reference to groups which received TP.

Note. 1. There were five to six animals in each group. 2. TP was administered in a dose of 2 mg/kg, the preparations in a dose of 20 mg/kg.

Neither compounds XI or XII, nor megestrol acetate, have any androgenic (10 mg/kg), anabolic (10 mg/kg), anti-inflammatory (10 mg/kg), or mineralocorticoid (2 mg/kg) activity. Compound XII and megestrol acetate have no antiestrogen (1 mg/kg) or thymolytic (10 mg/kg) activity.

Thus, the results of our research indicate that compound XI has high gestagenic activity and shows a marked antiandrogenic and weak uterotrophic, antiestrogenic, and thymolytic effect.

Compound XII displayed high gestagenic activity compared to that of megestrol acetate, but was surpassed by the latter in duration of action. Considering the higher selectivity of its hormonal action compared to megestrol acetate (antiandrogenic activity practically absent), compound XII may be of practical value as a gestagenic drug.

Correlation of the chemical structure and biological activity of the series of compounds investigated enables us to draw the following conclusions. Reduction of the 3-keto group to a 3-hydroxyl group in 6-methylpregna-4,6-dien-17α-ol-3,20-dione acetate results in an increase in the duration of the gestagenic effect, and display of a thymolytic and antiestrogenic effect (comparison of megestrol acetate with XI). Acetylation of the 3-hydroxyl group in compound XI is accompanied by a considerable decrease in antiandrogenic activity and a disappearance of the thymolytic and antiestrogenic effects (comparison of compound XI with XII).

EXPERIMENTAL CHEMISTRY

6-Methylpregna-4,6-dien-17α-ol-3,20-dione 17-Acetate (X). A suspension of 5.5 g of 6-methylpregn-4-en-17α-ol-3,20-dione 17-acetate [4], 0.27 g of 5.5% palladium on carbon, 1.1 g of sodium acetate, 0.07 g of cyclohexene, and 100 ml of ethyl alcohol are heated at the boiling point while stirring for 1.5 h. When isomerization is complete (checked by UV-spectrum) the catalyst is filtered out, and the alcoholic filtrate is concentrated in a vacuum. Methylene chloride is added to the residue, and the undissolved sodium acetate is

filtered out. After driving off the methylene chloride and crystallizing the residue in alcohol, 5 g of X are obtained with a melting point of 216-217°C, $[\alpha]_D^{20}$ + 12°, $E_{1\,cm}^{1\%}$ 630 at 288 nm. Reports in the literature [5] give a melting point of 214-216°C, λ_{max} 287.5 nm, ε = 25,120.

6-Methylpregna-4,6-3β,17α-diol-20-one 17-Acetate (XI). To a stirred suspension of 5 g of 6-methylpregna-4,6-diene-17α-ol-3,20-dione acetate in 100 ml of methanol 0.5 g of sodium borohydride are added over a 5-min period. After 15 min of additional stirring, the reaction mixture is diluted with water. The suspension is stirred for 30 min to complete crystallization, the precipitate is filtered out, rinsed with water on the filter, and dried out. The result is 4.72 g of impure XI, melting point 192-203°C, $[\alpha]_D^{20}$ −53.6° (2% solution in chloroform). After crystallization from aqueous isopropyl alcohol, XI is obtained with a melting point of 203-205°C, $[\alpha]_D^{20}$ −56.3° (2% solution in chloroform). Reports in the literature [12, 13] give a melting point of 190-205°C and 198-204°C, $[\alpha]_D^{20}$ −56.5°.

6-Methylpregna-4,6-diene-3β,17α-diol-20-one Diacetate (XII). While stirring at room temperature for 16 h, 5 g of unpurified XI are acetylated with a mixture of 10 ml of acetic anhydride and 10 ml of pyridine. The solution obtained is decanted into a mixture of hydrochloric acid, water, and ice and stirred for 1 h. The precipitate which forms is filtered out and rinsed with a solution of hydrochloric acid, then with water till neutral, dried out, and crystallized from aqueous isopropyl alcohol. The result is 4.36 g of XII with a melting point of 196-197°C, $[\alpha]_D^{20}$ −78° (2% solution in chloroform). Reports in the literature [12] give a mp of 197-199°C, and $[\alpha]_D^{20}$ −81°.

LITERATURE CITED

1. V. Petrow, Chem. Technol., 7, 563 (1977).
2. D. J. Marshall, P. F. Morand, et al., J. Med. Chem., 7, 355 (1964).
3. H. Levy, T. Saito, and S. Takeyama, Biochem. Biophys. Acta, 69, 198 (1963).
4. Author's Abstract No. 433,138 (USSR), Otkrytiya, No. 23, 61 (1974).
5. D. Burn, D. N. Kirk, and V. Petrov, Tetrahedron, 21, 1619 (1965).
6. P. V. Sollman, Pat. 3,126,399 (USA).
7. Pat. 1,055,534 (England).
8. C. Clauberg, Klin. Wschr., No. 9, 2,004 (1930).
9. M. C. McPhail, J. Physiol. (Lond.), 83, 10 (1934).
10. J. Mijake, Steroids, 2, 739 (1963).
11. J. Jochim and M. Zarrow, Fertil. and Steril., 12, 263 (1961).
12. K. Junkmann, Arch. Exp. Path. Pharmakol., No. 223, 244 (1954).
13. A. I. Terekhina, Z. I. Istomina, L. I. Lisitsa, et al., Khim. Farm. Zh., No. 10, 14 (1975).

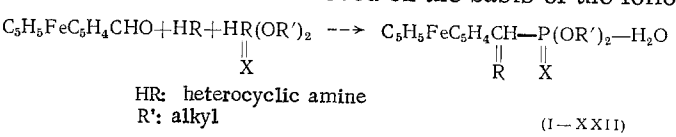
SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF FERROCENYLAMINOMETHYLPHOSPHONATES AND FERROCENYLAMINOMETHYLTHIOPHOSPHONATES CONTAINING HETEROCYCLIC RADICALS

Yu. L. Volyanskii and V. I. Boev

UDC 615.281:547.558.1

As research into the antimicrobial activity of organophosphorus compounds containing a ferrocenyl nucleus grew [1], it seemed worthwhile to produce a series of new ferrocenylaminomethylphosphonates and ferrocenylaminomethylthiophosphonates containing heterocyclic radicals.

Synthesis of 22 substances in the series was achieved on the basis of the following reaction:



Novokuznetsk Institute for the Advanced Training of Physicians. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 13, No. 6, pp. 68-73, June, 1979. Original article submitted October 31, 1978.