SYNTHESIS OF 17β-ACYLOXY-17α-ETHYNYL-5α-ANDROST-2-ENES AND THEIR EFFECT ON OVULATION IN EXPERIMENTS

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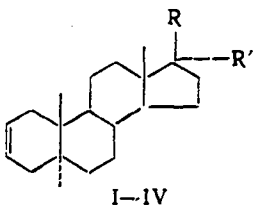
The search for ovulation stimulators used for the treatment of disfunctions of the ovaries, particularly endocrine forms of infertility, a number of hormone-dependent tumors, and various hormonal disorders, had led to obtaining highly effective preparations in the triphenylethylene series (clomiphene citrate, tamoxifen) [1, 2]. Compounds with similar activity have been found in the steroid series, viz., 6-chloropregna-1,4,6-trien-20-one and 17-epiestradiol 3-methyl ester [9], the activity of which, however, is inferior to that of clomiphene citrate. Valuable biological and therapeutic properties have been detected for the synthesized 17α-ethynyl-5α-androst-2-en-17β-ol (I) and its 17β-acetate (II) [7]: the use of low doses stimulates the activity of the hypophysis and gonads, as in the case of clomiphene citrate, and, when used in high doses, I and II give a gonad-inhibiting effect due to depression of the gonadotropic activity of the hypophysis. These properties are used in medical practice in the treatment of a number of endocrine tumors. According to the data in a patent [7], high activity of I was discovered in experiments involving the treatment of adenocarcinoma of the mammary glands of mice and ascitic tumors of the ovaries of female rats. Compound II displays antiparathyroid properties in the treatment of experimentally induced hyperplasia of not only the parathyroid gland but also of bone; the activity of II is comparable to the activity of ethynylestradiol.

We have accomplished the synthesis of 17α-ethynyl-5α-androst-2-en-17β-ol (I) and its esters from an intermediate in the transformation of tigogenin, viz., 5α-androst-2-en-17-one (IV). In addition to the free 17-β-alcohol (I) and its acetate (II), we obtained the propionate (III) in order to study the effect of the acyl residue on the specificity and duration of the activity of the indicated compounds.

The reaction of IV with acetylene in the presence of potassium isobutoxide in toluene gave I in 80% yield. The esterification of I with acetic anhydride in acetic acid in the presence of anhydrous zinc chloride gave the 17β-acetate (II) of I, and the propionylation of I with propionyl chloride in chloroform-dimethylaniline (3:1) gave the 17β-propionate (III). The yields were 78% and 80%, respectively.

We studied the biological activity of II and III as compared with clomiphene citrate, which has been authorized for medical use as an ovulation stimulator [1].



I—IV

R = OH (I); $OCOCH_3$ (II); $OCOC_2H_5$ (III);
R′ = —C≡CH (I—III);
R + R′ = O (IV).

EXPERIMENTAL CHEMICAL PART

The PMR spectra of solutions of the compounds in $CDCl_3$ were recorded with a μ-4H-100 spectrometer; the chemical shifts are presented on the δ scale, and the position of the signal of tetramethylsilane (TMS) was taken as 0.00 ppm. The mass spectra were obtained with a MAT-112 mass spectrometer at an ionizing voltage of 70 eV with direct introduction of the samples

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TABLE 1. Effect of 3-Day Administration of the Indicated Compounds on the Ovulatory Function of Rats

Compound	No. of egg cells washed out, M ± m	State of the ovaries as compared with the control
Control	10,6±0,5	Normal
Clomiphene	15,8±0,7	»
I	13,9±0,6	Many growing follicles
II	13,2±0,5	Normal
III	13,6±0,5	Many growing follicles.

Note. $P < 0.05$ in all cases.

into the mass spectrometer. The course of the reactions and the purity of the compounds were monitored by thin-layer chromatography on Silufol plates in hexane—ether (5:3) with development with a 1% solution of vanillin in 10% aqueous perchloric acid solution with heating.

17α-Ethynyl-5α-androst-2-en-17β-ol (I). A stream of dry acetylene was passed for 2 h through a solution of potassium isobutoxide (from 3 g of potassium hydroxide and isobutyl alcohol) in 15 ml of toluene at 15-18°C, after which a solution of 1 g of 5α-androst-2-en-17-one in 7 ml of toluene was added to the resulting solution of potassium acetylide, and acetylene was passed through the mixture for 2 h. Water (5 ml) was added to the resulting solution of potassium acetylide, and acetylene was passed through the mixture for 2 h. Water (5 ml) was added to the resulting cooled (to 3-5°C) reaction mass, and the aqueous mixture was acidified to pH 1.0 with 10% sulfuric acid. It was then stirred for 10 min, after which the organic layer was separated, and the aqueous layer was extracted with toluene. The combined toluene layers were washed with water until the wash water was neutral and then evaporated in vacuo. The residue was crystallized from hexane—acetone to give 0.8 g (80.36%) of I with mp 174-176°C (according to [8], mp 175-178°C).

17α-Ethynyl-5α-androst-2-en-17β-ol Acetate (II). A 0.2-g sample of anhydrous zinc chloride and 0.2 ml of a 4% solution of hydrochloric acid in acetic acid were added at 10-12°C to a suspension of 1 g of I in 10 ml of acetic acid and 2 ml of acetic anhydride, after which the reaction mixture was stirred at room temperature for 1.5 h. Water (40 ml) was then added, and the mixture was stirred for 1 h The precipitate was removed by filtration, washed with water until the wash water was neutral, dried, and recrystallized from ethanol to give 0.89 g (78.07%) of II with mp 128-129°C. PMR spectrum, δ, ppm: 0.77 (18-CH_3), 0.86 (19-CH_3), 2.04 ($OCOCH_3$), 2.59 (—C≡CH), 5.6 (2-H). According to the data in [6], this compound had mp 128-129°C.

17α-Ethynyl-5α-androst-2-en-17β-ol Propionate (III). A 15-ml sample of propionyl chloride was added to a solution of 1 g of I in 45 ml of dry chloroform and 15 ml of dry dimethylaniline, and the mixture was allowed to stand at room temperature for 24 h. The chloroform was then removed in vacuo, and the residue was poured into 200 ml of water containing 20 ml of concentrated hydrochloric acid. The resulting precipitate was removed by filtration, washed with water, dried, and recrystallized from ethanol to give 0.95 g (80.03%) of III with mp 103-105°C. PMR spectrum, δ, ppm: 0.77 (18-CH_3); 0.86 (19-CH_3); 1.31; 2.32 ($OCOC_2H_5$); 2.58 (—C≡CH); 5.6 (2-H). Mass spectrum, m/z: 354 (M^+;, 339 $(M - CH_3)^+$, 325 $(M - C_2H_5)^+$, 298 $(M - C_2H_5CO)^+$, 283 $(M - C_2H_5COCH_3)^+$.

EXPERIMENTAL PHARMACOLOGICAL PART

A study of the ability of I-III to stimulate ovulation was made on mature female rats with masses of 160-180 g (90 experiments on 74 animals) and mature female rabbits with masses of 3.0-3.5 kg (92 experiments on 66 animals). The results were treated statistically by the Student method in the Montsevichute-Eringene modification [4]. The reliability of the data obtained was estimated with $P < 0.05$.

In experiments of the I series an aqueous suspension of the investigated compound was administered in a dose of 4 mg/kg with a probe into the stomachs of mature female rats with

TABLE 2. Effect of Single-Shot Administration of the Investigated Compounds on the Ovulatory Function and Masses of the Target Organs of Rabbits (M ± m)

Compound	No. of ovulated follicles	Masses of the organs, mg/kg		
		uteri	ovaries	adrenal glands
Control	10,1±0,7	2612,9±109,4	79,8±9,7	186,5±10,4
Clomiphene	12,0±1,4	1875,6±256,7*	174,3±16,4*	176,2±9,4
I	11,7±0,8	2243,1±18,3	95,9±8,7	195,1±0,8
II	14,8±3,4*	2145,8±188,7	118,4±25,4	175,4±3,4
III	10,6±1,3	1533,5±299,6*	84,1±10,0	160,5±10,8

*Note that $P < 0.05$ as compared with the control.

an established 4-5-day estral cycle in the proestrus stage. Clomiphene citrate in the same dose was used as the standard. A 0.2-ml sample of a physiological solution was administered to control-group animals. After 24 h from the onset of the estrus stage in the rats, the animals were decapitated under ether narcosis. The ovulated egg cells were washed out from the oviducts. The state and number of these egg cells were evaluated visually under a binocular magnifier.

In analyzing the data obtained one may note that one one of the investigated compounds, including clomiphene, displayed an effect that stimulates ovulation in the case of the single-shot administration of a dose of 4 mg/kg.

In experiments of the II series the investigated preparations and clomiphene in a dose of 4 mg/kg were administered in the course of 3 days via the method described above, timing the first administration to the diestrus-1 stage. The character of ovulation was evaluated in the estrus stage. The data obtained are presented in Table 1.

It is apparent from Table 1 that both clomiphene and all three of the androstane derivatives reliably ($P < 0.05$) intensify the ovulatory function of the experimental animals; this is expressed in an increase in the number of ovulated egg cells as compared with the control. Since the propionate and the free alcohol significantly stimulated the growth of the younger generations of follicles, one may assume for them the existence of a more prolonged stimulating effect as compared with clomiphene.

In experiments of the III series on female rabbits a 1.2% solution of copper acetate in distilled water was administered intravenously to the rabbits in heat to induce ovulation by the laboratory method adopted in [3]. The investigated substances in a dose of 4 mg/kg in the form of finely dispersed suspension were administered simultaneously through a probe into the stomachs of the animals. Animals that had received an intravenous injection of copper acetate served as the control. After 24 h, the numbers of ovulated follicles in the animals were determined, and the masses of the uteri, ovaries, and adrenal glands were also measured. The data obtained are presented in Table 2.

It is apparent from Table 2 that, except for II, the investigated compounds (including the standard) did not stimulate the ovulatory function of the experimental animals in the case of single-shot administration. Clomiphene and propionate III gave rise to a reliable (as compared with the control) decrease in the mass of the uterus in the experimental animals. An increase in the mass of the ovaries was observed for animals that had received clomiphene.

To ascertain the peculiarities of the effect of the investigated substances on ovulation induced by copper acetate we formulated a series of experiments with 2-day administration of I-III in the same dose.

The data obtained are presented in Table 3. It is apparent that all of the investigated compounds increased the number of ovulated follicles, particularly the propionate and acetate. Consequently, 2-day administration of the substances activated the ovulatory function of the experimental animals to a greater degree than 1-day administration. In addition, the investigated androstane derivatives caused a significant decrease in the mass of the uterus and an increase in the mass of the adrenal gland in the experimental animals.

TABLE 3. Effect of the Investigated Compounds on the Ovulatory Function and Masses of the Target Organs of Rabbits in the Case of 2-Day Administration (M ± m)

Compound	No. of ovulated follicles	Masses of the organs, mg/kg		
		uteri	ovaries	adrenal glands
Control	10,0±0,7	2612,9±109,4	79,8±9,7	186,5±10,3
Clomiphene	12,1±1,2	2513,5±97,4	188,4±15,4*	207,7±20,8
I	12,8±1,6*	1594,7±28,3	179,9±26,8*	207,1±16,3
II	14,6±1,0*	1500,0±200,2*	166,7±15,6*	217,2±9,6
III	14,8±0,6*	1377,0±61,1*	169,5±11,0*	158,1±4,9*

*Note that $P < 0.05$ as compared with the control.

In experimental series IV compounds I-III in a dose of 4 mg/kg were administered to rabbits for 20 h prior to ovulation induced by copulation. An effect of stimulation of the ovulatory function under the influence of the investigated compounds was also demonstrated in these experiments. Whereas an average of 9.2 ± 0.5 egg cells ovulated in the control-group animals, 12.0 ± 0.5 egg cells ovulated under the influence of the "free alcohol," 12.1 ± 0.3 egg cells ovulated under the influence of the acetate, and 13.8 ± 0.4 egg cells ovulated under the influence of the propionate.

To pin down the mechanism of the effect of the investigated preparations we carried out a series of experiments involving the determination of the level of gonadotropins in the blood plasma of the experimental animals by a radioimmunochemical method [5]. In the experiments we used rabbits that had received the test preparations in the course of 2 days.

It was demonstrated that all of the investigated compounds increased the level of lutenizing hormone (LH); the most pronounced effect was observed in the group of animals that had received the propionate. Whereas the LH level in the control-group animals was 167.0 ± 1.0 ng/ml, its concentration increased to 273.4 ± 47.2 ng/ml under the influence of the "free alcohol" (I), as compared with 310.1 ± 54.2 ng/ml under the influence of the acetate (II) and 353.4 ± 58.0 ng/ml under the influence of the propionate (III). Clomiphene caused a weaker increase in the LH level in the blood plasma as compared with the androstane derivatives; it was only 251.2 ± 35.6 ng/ml. In all cases the increase in the LH level was reliable with $P < 0.05$.

The changes in the level of follicle-stimulating hormone (FSH) in the blood plasma of the experimental animals were as follows: 2.9 ± 0.7 μg/ml in the control group; 0.75 ± 0.03 μg/ml ($P < 0.05$) in the animals that had received clomiphene; under the influence of III the FSH level increased to 4.3 ± 0.5 μg/ml ($P < 0.05$), while I and II did not affect the level of this hormone; the FSH level was 2.1 ± 0.6 μg/kg under the influence of the "free alcohol" and 2.7 ± 0.4 μg/kg under the influence of the acetate.

Thus, on the basis of this investigation, it was established that all of the investigated compounds are capable of increasing the LH level in the blood plasma of animals; one may note the tendency for a change in the percentage of gonadotropins under the influence of the preparations as a function of the size of the radical in the 17β position. The maximum LH level was noted in animals that had received the propionate, a less significant LH level was observed for those that had received the acetate, and an even slighter LH level was noted for those that had received the "free alcohol".

The preparations acted similarly to clomiphene with respect to the dose and the duration of administration. Single-shot administration of both the test substances and the standard was ineffective. More prolonged administration (2- and 3-day) stimulated the ovulatory function of the experimental animals, although the activation effect obtained should be regarded as a threshold value. It is possible that the stimulating activity is associated with the effect of the preparations on the level of gonadotropins.

Thus the introduction of a propyl radical in the 17β position of 17α-ethynyl-5α-androst-2-en-17β-ol intensifies the ability of the compound to activate the gonadotropic function of the hypophysis.

LITERATURE CITED

1. E. M. Vikhlyaeva, F. S. Badaeva, and K. S. Mitin, Aksuh. Gin., No. 7, 6-39 (1981).
2. M. P. Dement'eva, N. Ya. Ass, and É. K. Voznyi, Klin. Med., No. 5, 50-52 (1979).
3. V. V. Korkhov, Estrogen–Progestins and Regulation of the Reproductive Function [in Russian], Leningrad (1979).
4. E. V. Montsevichute-Éringene, Pat. Fiziol., No. 4, 71-78 (1964).
5. G. P. Nikiforova, A. S. Antonov, O. G. Krivosheev, and V. A. Isachenkov, Probl. Éndokrinol., 23, No. 1, 74-79 (1977).
6. US Patent No. 3226405 (1965); Chem. Abstr., 64, 6722.
7. Belgian Patent No. 823594 (1975); Chem. Abstr., 84, 685.
8. A. Bowers, A. D. Cross, J. A. Edwards, et al., J. Med. Chem., 6, 156-161 (1963).
9. P. Y. Keller, M. Ruppen, W. E. Schreiner, and H. J. Wyss, Gynaecologia (Basel), 168, 425-427 (1969).

GASTROPROTECTIVE AND ADRENOPROLONGING PROPERTIES OF PHENOLCARBOXYLIC ACIDS

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The presence of distinct and strong gastroprotective properties has been established for the flavonoids for which one of the essential structural peculiarities is the presence of two hydroxyl groups in the ortho-position to one another in the A or B ring (quercetin, myristin, gerbacetin, 4'-methoxyscutellarein, etc.) [1-6, 9]. However, in gnaphaloside A, isolated from *Gnaphalium uliginasum* L. and exhibiting gastroprotective and adrenoprolonging properties, two hydroxyl groups are present only in the caffeic acid radical [4]. In view of this, the purpose of the present work was a comparative evaluation of the ability of a number of natural phenolcarboxylic acids to limit the formation of experimental destruction of the stomach in mice and rats. From the standpoint of detecting structure-action patterns, it is justified to use compounds close to phenolcarboxylic acids (benzoic acid and its derivatives, propyl gallate, quercetin).

Methods of production and evaluation of stomach lesions in mice after intraperitoneal injection of reserpine (2.5 mg/kg) or immobilization stress were described in our earlier works [1, 8]. In these series of experiments, sometimes significant differences were noted in the number of mice that died, which was considered in the summary evaluation of the effectiveness of the compounds. In Wistar rats (200 males weighing 130-150 g), erosions of the stomach were produced by intramuscular injection of butadione (200 mg/kg) after two-day deprivation of food. The erosions were classified as large (more than 2.5 mm in diameter) and band-like (5 mm long), medium (1.5-2.5 mm in diameter), and small (less than 1.5 mm). The same 11 indices were subjected to statistical treatment as in the experiments on mice [1-6, 9]. The experiments on rats and mice (620 individuals of both sexes of the SHR strain) were conducted in the autumn to winter period of 1984-1985 by a blind method. Two series of experiments were conducted on reserpinized or immobilized mice, and their data were compared and combined.

The activities of the following compounds were compared: benzoic (I), p-hydroxybenzoic (II), p-aminobenzoic (III), vanillic (IV), veratric (V), o-coumaric (VI), salicylic (VII), p-aminosalicylic (VIII), caffeic (IX), and gallic (X) acids, propyl gallate (XI), and quercetin (XII). Compound IX was used in a dose of 60 mg/kg, while the rest were administered internally in equimolar doses in a 5% solution of dimethyl sulfoxide for seven days; the mice received the doses twice a day for three days. In an individual series of experiments, in the study of IX, the dose was reduced to 30 mg/kg, which is equimolar to 50 mg/kg quercetin (XII). Considering the possibility of inhibition of catechol ortho-methyltransferase (COMT) by certain phenol-carboxylic acids, we studied their adrenoprolonging effects according to our modification [2-6] of the method of F. de Eds [10]. The compound IX was introduced into the bath with an isolated segment of rabbit large intestine in a concentration of $1 \cdot 10^{-5}$ g/ml, while the other compounds (8-12 experiments with each) were introduced in equimolar

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