SYNTHESIS AND BIOLOGICAL ACTIVITY OF METHYL DERIVATIVES OF dl-19-NOR-D-HOMOTESTOSTERONE

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In preceding communications [1] we have described the total synthesis of dl-19-nor-D-homotestosterone (I) and have shown [2] that its anabolic activity is twice that of dl-19-nortestosterone. In view of this, it appeared of interest to obtain some transformed derivatives of dl-19-nor-D-homotestosterone and to study their biological activity. It is known that the introduction of methyl groups into positions 2 and 16 markedly increases anabolic activity [3] in a number of natural androgens. Consequently, we decided to synthesize the 17-methyl derivative (corresponding to the 16-methyl analog of the natural series), and the 2-methyl, the 4-methyl, and the 2,17-dimethyl derivatives of dl-19-nor-D-homotestosterone and to study the influence of the methyl groups on anabolic and androgenic activity in this series. dl-17α-Methyl-D-homotestosterone (II) was obtained by the Wilds-Nelson reduction [4] of the methyl ether of 17α-methyl-D-homoestrone (III). The ketone III was prepared from the methyl ether of dl-D-homoestrone (IV) under the conditions described by Bachmann [5], i.e., by condensation with diethyl oxalate in the presence of sodium hydride, subsequent methylation with methyl iodide, and saponification to III, with an over-all yield of 53%.

Reduction of III with lithium and ethanol in liquid ammonia formed 3-methoxy-17α-methyl-D-homoestra-2, 5(10)-dien-17aß-ol (V) with a yield of 87%. Hydrolysis of the latter in chloroform in the presence of concentrated hydrochloric acid gave 17α-methyl-19-nor-D-homotestosterone (II) with a yield of 82%.

Similarly, the methylation of the ketone I by Bachmann's method gave dl-2α-methyl-19-nor-D-homotestosterone (VI) with a yield of 25%.

The methylation of I in position 4 was carried out by Kirk and Petrov's method [6] without the isolation of the intermediate thiophenyl derivative. The yield of dl-4-methyl-19-nor-D-homotestosterone (VII) was 24%.

dl-2α,17α-Dimethyl-19-nor-D-homotestosterone (VIII) was prepared in low yield (8%) by methylating the ketone II by Bachmann's method.

The structures of compounds II, III, and VI-VIII were confirmed by their NMR spectra, which exhibited both singlet signals of the protons of an angular methyl group at C-18 and doublets (three proton units) with a spin-spin splitting constant J = 7 Hz, corresponding to the methyl groups in positions 2α and 17α. The introduction of a CH_3 group into the 17α position has practically no influence on the chemical shift of the C-18 methyl group. The signal from the C-18 methyl group in the ketone III was at 1.13 ppm, which agrees with data on the increment of a 17a-keto group [7]. The anisotropic magnetic field of the 17a-CO group also affects the signal of the protons of the 17α-CH_3 group. Thus, while in ketones II and VIII the doublet signal from the 17α-CH_3 group appears at 0.92 ppm, on passing to the ketone III it is shifted to a field weaker by 0.11 ppm (1.03 ppm). The spectrum of the ketol VI has a signal from the C-18 angular methyl group and also a doublet at 1.06 ppm relating to the protons of the 2-CH_3 group. The signal of this methyl group appears in a weaker field than the signal of the protons of the 17α-CH_3 group because of the influence of the magnetic anisotropy of the Δ^4-3-keto grouping. In the case of VIII, which contains two methyl groups, the spectrum exhibits two doublets at 1.05 and 0.92 ppm, corresponding to the 2α-CH_3 and 17α-CH_3 groups.

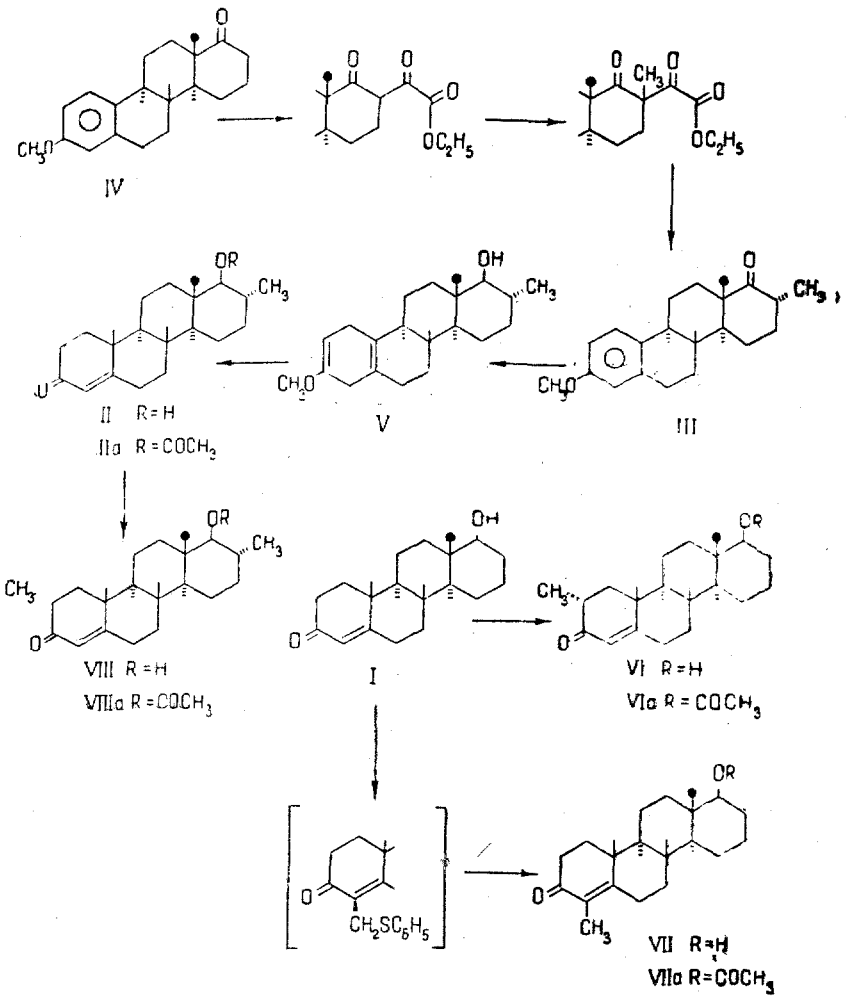
Biological studies

The androgenic activity of the compounds obtained was determined by the reaction of the ventral section of the prostate gland (ventr. prostata), and the anabolic activity from the reaction of one of the muscles of the perineal complex, musculus levator ani (m.l.a.). The experiments were carried out on sexually immature three-week old castrated white male rats by Herschberger's method [8]. The substances were administered (in physiological solution) subcutaneously for 7 days in a total dose of 0.35, 0.7, 3.5, 4.9, and in some cases 24.5 mg (per animal).

The anabolic index was calculated with respect to testosterone propionate (T.P.), the activity of which (both anabolic and androgenic) in the corresponding dose was taken as unity. In those cases where the absolute magnitudes of the anabolic and androgenic activities were small and the difference from the control was statistically insignificant, the corresponding indices were not calculated (Table 1).

As can be seen from Table 1, the anabolic activity of dl-19-nor-D-homotestosterone (I) is higher than or equal to that for testosterone propionate, while the androgenic effect of this compound is almost three times smaller. It is an interesting fact that the absolute and relative anabolic activities of dl-19-nor-D-homotestosterone (I) change less with an increase in the dose than those of testosterone propionate.

dl-17α-Methyl-19-nor-D-homotestosterone (II) had no anabolic and androgenic activity in doses of 0.35–4.9 mg and exhibited such activity only in a dose of 24.5 mg. In the case of dl-2α-methyl-19-nor-D-homotestosterone (VI) and dl-2α, 17α-dimethyl-19-nor-D-homotestosterone (VIII), both anabolic and androgenic activity were completely lacking even in a dose of 24.5 mg.



The greatest interest from our point of view was offered by dl-4-methyl-19-nor-D-homotestosterone (VII). In this compound, no anabolic and androgenic effects appeared in doses of 0.35 and 0.7 mg. However, when the dose was raised to 3.5 and 4.9 mg the anabolic effect rose considerably more rapidly than the androgenic effect. This is well shown from the figure, which compares the results in the change in the mean weights of the m. l. a. and the ventr. prost. as the dose of the compounds was increased. As can be seen, the absolute magnitude of the androgenic effect of dl-4-methyl-19-nor-D-homotestosterone (VII) rises with an increase in the dose, but it is several times less than those of testosterone propionate and dl-19-nor-D-homotestosterone (I). Thus, at a dose of 4.9 mg it is seven times less than that of testosterone propionate and half that of dl-19-nor-D-homotestosterone (I). At the same time, the absolute anabolic effect of this compound in a dose of 14.9 mg is similar to that of dl-19-nor-D-homotestosterone (I) and approximates that of testosterone propionate.

The same characteristics were also found for the acetates of the above-mentioned analogs.

Experimental

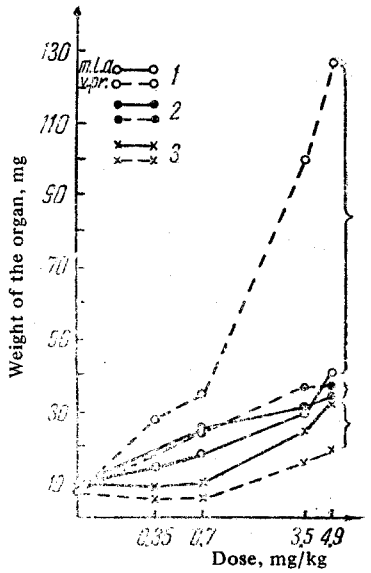
The course of the reaction and the completeness of the conversion were monitored by thin-layer chromatography (TLC) in nonfixed layers [9]. The adsorbent was alumina (Brockman activity grade III), layer thickness 1–1.5 mm, spots revealed in UV light or by the action of iodine vapor. The solutions were dried with anhydrous magnesium sulfate or sodium sulfate and were evaporated in vacuum (10–18 mm) at 30–40° C. Analytical samples of the substances were dried in vacuum (1–0.5 mm) at 60–70° C over phosphorus pentoxide. The melting points of the substances are not corrected. The UV spectra were taken on an SF-4 spectrophotometer in ethanol, the IR spectra on an IKS-21 or an H-800 instrument in paraffin oil, and the NMR spectra on a JNM C-60 spectrograph in chloroform. The values of the chemical shifts are given in δ units from tetramethylsilane as internal standard.

The methyl ether of dl-D-homoestrone (IV) and the dl-19-nor-D-homotestosterone (I) were obtained from methoxy-

tetralone by methods described previously [1, 10].

Methyl ether of dl-17α-methyl-D-homoestrone (III)

A mixture of 7 g of the methyl ether of dl-D-homoestrone (IV), 2.25 g of sodium hydride, 6.86 g of diethyl oxalate, and 140 ml of anhydrous benzene was stirred for 48 hr at 30–45° C in an atmosphere of nitrogen. The reaction mixture was decomposed by the successive addition of 7 ml of methanol and 200 ml of water (at a temperature not above 5° C) cooled to 0° C, neutralized with hydrochloric acid, and extracted with ether. The ethereal extracts were washed with water and evaporated in vacuum. The residue was treated with 7 g of anhydrous potassium carbonate and 21 ml of methyl iodide in 140 ml of dry acetone and the mixture was boiled for 48 hr. The hot solution was filtered, the precipitate was washed with hot acetone, and the filtrate was evaporated to dryness, after which the residue was dissolved in 200 ml of water and extracted with methylene chloride. The extract was evaporated and the residue was heated in vacuum (1–0.5 mm at 90° C) for 2 hr, mixed with a solution of sodium ethoxide (0.7 g of Na in 70 ml of ethanol), and left for 48 hr. The reaction mixture was diluted with 200 ml of water and extracted with methylene chloride. The extract was washed with water, dried, and evaporated, and the residue was crystallized from ethyl acetate. This gave 3.2 g of dl-17α-methyl-D-homoestrone (III) with mp 185–187° C. After crystallization the mother liquor gave another 0.6 g of III with mp 187–189° C. The total yield of III was 53%. An analytical sample had mp 189–191° C. IR spectrum: 1502, 1580 (aromatic ring), 1707 (17a-CO) cm^{-1}. NMR spectrum: 1.03 (17-CH_3), 1.13 (18-CH_3) ppm.



Comparative evaluation of the anabolic effect (with respect to the mean weight of the m. l. a.) and the androgenic effect (with respect to the mean weight of the v. pr.) of testosterone propionate (1), dl-19-nor-D-homotestosterone (2), and dl-4-methyl-19-nor-D-homotestosterone (3).

Found, %: C 80.63; H 8.78. Calculated for $C_{21}H_{28}O_2$, %: C 80.73; H 9.03.

17α-Methyl-3-methoxy-D-homoestra-2, 5(10)-dien-17aß-ol (V)

A solution of 1.7 g of the ketone III in 175 ml of absolute tetrahydrofuran and 125 ml of absolute ether was poured into 500 ml of liquid ammonia at −50 to −60° C, and after 10 min 3 g (20-fold excess) of lithium in the form of small pieces was added. The mixture was stirred at −70° C for 20 min and then 60 ml of absolute ethanol was added in drops over 30 min until the solution had been decolorized. The ammonia was evaporated off and the residue was decomposed with water at −5 to 0° C and extracted with ether. The extract was neutralized with solid carbon dioxide, washed with water, and dried, the solvent was distilled off, and the residue was filtered. Crystallization of the residue from methanol gave 1.5 g (87%) of 17α-methyl-3-methoxy-D-homoestra-2, 5(10)-dien-17aß-ol (V) with mp 145–147° C. An analytical sample had mp 149.5–150.5° C [from methanol–ethyl acetate (4:1)]. IR spectrum: 1672 (2C=C), 1700 (5C=C), 3440 (17aß-OH) cm^{-1}.

Found, %: C 80.12, H 10.00. Calculated for $C_{21}H_{32}O_2$, %: C 79.90; H 10.19.

Hydrolysis of the carbinol (V)

A solution of 0.6 g of the carbinol V in 6 ml of chloroform was stirred for 30 min with 0.6 ml of concentrated hydrochloric acid, neutralized with a saturated solution of sodium bicarbonate, and extracted with chloroform. The residue after the solvent had been distilled off was recrystallized from a mixture of acetone and petroleum ether (1:1), giving 500 mg (82%) of dl-17α-methyl-19-nor-D-homotestosterone (II) with mp 138–140° C. The melting point of an analytical sample was 139–140° C (from cyclohexane). IR spectrum: 1617 (C=C), 1668 (3-CO), 3420 (17aß-OH) cm^{-1}. NMR spectrum: 0.82 (18-CH_3), 0.92 (17-CH_3) ppm.

Found, %: C 79.46; H 10.18. Calculated for $C_{20}H_{30}O_2$, %: C 79.42; H 10.00.

The acetate of II (IIa) had mp 159–161° C (from methanol).

dl-2α-Methyl-19-nor-D-homotestosterone (VI)

A mixture of 7 g of dl-19-nor-D-homotestosterone (I), 2.25 g of sodium hydride, and 6.86 g of diethyl oxalate in 140 ml of anhydrous benzene was stirred at room temperature for 6 hr. The subsequent treatment was as described for the preparation of the ketone III. The reaction product was treated with 7 g of anhydrous potassium carbonate, 21 ml of methyl iodide, and 140 ml of dry acetone and the mixture was boiled for 48 hr. After similar working up, the methylation product was hydrolyzed with a solution of sodium ethoxide in ethanol (0.7 g of sodium in 70 ml of ethanol) at 20° C for 48 hr. This gave 1.6 g (25%) of dl-2α-methyl-19-nor-D-homotestosterone (VI) with mp 174–176° C. The melting

point of an analytical sample was 175–176° C (from acetone). IR spectrum: 1626 (C=C), 1670 (3-CO), 3430 (17a-OH) cm^{-1}. NMR spectrum: 0.83 (18-CH_3), 1.06 (2-CH_3) ppm.

Found, %: C 79.61; H 10.02. Calculated for $C_{20}H_{30}O_2$, %: C 79.42; H 10.00.

The acetate of (VI), (VIa), had mp 142–143° C.

Relative Anabolic Activities of dl-19-nor-D-Homotestosterone and its Methyl Derivatives

Compound	Total dose, mg	Weight of m.l.a. (mg)	Weight of v.pr. (mg)	Relative activity: anabolic	Relative activity: androgenic	Index referred to T.P.
Control	—	9.7±0.36	7.6±0.35	—	—	—
Testosterone propionate	0.35	14.2±1.1 *	27.3± 4.0 *	1	1	—
	0.7	18.2±1.9	34.5± 3.9	1	1	—
	3.5	29.6±1.3	100.0+ 9.5	1	1	—
	4.9	40.3±2.0	125.6±12.5	1	1	—
	24.5	38.2±2.0	142 ± 5.69	1	1	—
(I)	0.7	25.3±4.3 *	24.6± 4.8 *	1.4	0.7	2.0
	3.5	31.4±0.85	36.8± 3.3	1.06	0.36	2.9
	4.9	33.8±1.84	37.0± 2.32	0.83	0.29	2.8
	24.5	38.2±3.7	52.6± 3.1	1.0	0.37	2.7
(II)	0.35	8.0±0.23	7.7±1.87	—	—	—
	0.7	9.0±1.45	6.4±0.92	—	—	—
	3.5	13.8±1.03	8.6±1.2	—	—	—
	4.9	12.6±1.18	12.0±2.3	—	—	—
	24.5	25.3±0.66*	22.0±0.8*	0.66	0.15	4.4
(VI)	0.35	9.4±1.0	5.0±1.26	—	—	—
	0.7	8.0±0.63	7.6±0.61	—	—	—
	3.5	12.4±2.19	6.4±0.92	—	—	—
	4.9	8.0±1.14	6.6±0.74	—	—	—
	24.5	9.6±0.87	8.3±0.33	—	—	—
(VII)	0.35	9.2±1.48	5.6±0.92	—	—	—
	0.7	10.2±1.0	6.2±0.88	—	—	—
	3.5	24.4±3.2*	16.0±2.0*	0.8	0.16	5.0
	4.9	33.5±0.86*	15.5±0.86*	0.87	0.13	6.6
(VIII)	0.7	6.3±0.46	9.3±1.33	—	—	—
	3.5	7.7±0.87	7.7±0.3	—	—	—
	4.9	9.6±0.88	8.3±1.73	—	—	—
(IIa)	0.35	7.4±0.4	4.8±0.87	—	—	—
	0.7	7.2±1.22	3.6±0.26	—	—	—
	3.5	9.8±1.41	4.4±0.67	—	—	—
(VIa)	0.35	8.2±1.26	3.6±0.5	—	—	—
	0.7	8.0±0.4	3.6±0.74	—	—	—
	3.5	6.2±0.67	3.6±0.72	—	—	—
	4.9	7.3±0.86	5.3±0.46	—	—	—
	24.5	8.0±0.57	6.0±1.0	—	—	—
(VIIa)	0.35	8.6±1.94	3.0±0.26	—	—	—
	0.7	9.4±1.41	6.2±0.5	—	—	—
	3.5	18.8±0.37*	22 ±0.98*	0.6	0.22	2.7
	4.9	15.3±1.76*	17.3±4.65*	0.38	0.13	2.9
(VIIIa)	0.35	5.3±0.66	5.0±0.57	—	—	—
	0.7	5.6±0.33	5.0±0.57	—	—	—
	3.5	7.0±0.57	7.3±0.88	—	—	—
	4.9	6.5±0.28	7.5±0.86	—	—	—

*Difference from control significant ($P < 0.05$).

dl-4-Methyl-19-nor-D-homotestosterone (VII)

A mixture of 2 g of dl-19-nor-D-homotestosterone (I), 0.6 g of thiophenol, 1.6 g of 35% formaldehyde, and 1.6 g of triethylamine in 40 ml of ethanol was boiled for 50 hr. The mixture was poured into 30 ml of 10% sodium hydroxide solution and extracted with ether. After the solvent had been distilled off, the residue was extracted thrice with acetone, the extract was evaporated, and the product was subjected to desulfonation by boiling (5 hr) with 10 ml of a paste of Raney nickel in 50 ml of acetone.

The hot solution was filtered and the residue was washed with hot acetone (3 × 15 ml). The solvent was distilled off and the residue was crystallized from acetone–petroleum ether (1:1). This gave 500 mg (24%) of dl-4-methyl-19-nor-D-homotestosterone (VII) with mp 160–163° C. The melting point of an analytical sample was 163–164° C [from acetone–petroleum ether (1:1)]. IR spectrum: 1620 (C=C), 1680 (3-CO), 3380 (17a-OH) cm^{-1}. NRM spectrum: 0.84 (18-CH_3), 2.15 (4-CH_3) ppm.

Found, %: C 79.66; H 10.14. Calculated for $C_{20}H_{30}O_2$, %: C 79.42; H 10.00.

dl-2α, 17α-Dimethyl-19-nor-D-homotestosterone (VIII)

Three grams of dl-17α-methyl-19-nor-D-homotestosterone (III) was methylated under conditions analogous to those for the ketone VI. This gave 250 mg (8%) of dl-2α, 17α-dimethyl-19-nor-D-homotestosterone (VIII) with mp 160–167° C (from ethyl acetate). After four crystallizations from ethyl acetate, 170 mg of the ketol VIII with mp 180–181° C was obtained. UV spectrum: λ_{max} 240 mμ (log ε 4.30). IR spectrum: 1617 (C=C), 1660 (3-CO), 3340 (17-OH) cm^{-1}. NMR spectrum: 0.82 (18-CH_3), 0.92 (17-CH_3) 1.05 (2-CH_3) ppm.

Found, %: C 79.69; H 10.16. Calculated for $C_{21}H_{32}O_2$, %: C 79.70; H 10.19.

Summary

The synthesis of racemates of the 2α-methyl, 4-methyl, 17α-methyl, and 2α,17α-dimethyl derivatives of 19-nor-D-homotestosterone has been described. Biological tests have shown that the introduction of a CH_3 group into positions 2α and 17α totally suppresses both androgenic and anabolic activity. The introduction of a CH_3 group into position 4 leads to a divergence of the anabolic and androgenic effects.

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