Antihormonal properties of some new A-homo-B, 19-dinor steroids of the androstane series

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On solvolysis of Westphalen-type steroids with a leaving group in the position 6β (e.g., 2), products of elimination (followed by rearrangement and fragmentation of the steroid skeleton) were prepared (e.g., 4 and 5). These products were subsequently converted to suitable analogs of the compound, which has been reported to promote hair growth (1). Compounds 11 to 13 exhibited strong antiandrogenic activity in vivo; however, this activity could not be interpreted either in terms of inhibition of 5α-reductase or by strong binding to an androgen receptor. (*Steroids* **57**:460–463, 1992)

Keywords: steroids with modified skeleton; androgen receptor; antiandrogens; 5α-reductase inhibitor; testosterone; dihydrotestosterone

Introduction

Both antiandrogens and 5α-reductase inhibitors have found their way among therapeuticals; nevertheless, some practical goals once expected of them have not been fully reached. We have been preparing and testing various analogs or hormones with modified steroid skeleton to find a relationship between a particular structural modification of an androgen hormone and its antihormonal activity.[1–5] When Shiseido Co.[6] filed its patent application claiming that azulenone derivative **1** stimulated hair growth, we interpreted this finding as the result of possible inhibitory activity on 5α-reductase or of antiandrogenic activity. As we had available a method of transforming type **2** steroids into type **4** dienes,[7] we decided to prepare and test analogs of steroid hormones that resembled the structure of compound **1** in A and B rings and that also had C and D rings identical to those of testosterone or methyltestosterone.

Earlier[8–10] we carried out hydrogenation of the diene **4** to a mixture of more stable tetrahydro derivatives that were only then either oxidized (e.g., to hydroxy ketone **11**) or treated with methyl lithium and then oxidized (to hydroxy ketones **12** and **13**). One of the products of the hydrogenation was originally formulated[9,10] with reversed configuration at carbons 4a and 5; the currently given structures are now in full agreement with x-ray diffraction (the correction will be published later).

As the conversion of Westphalen-type steroids (e.g., **3**) to diene **4** could be carried out by, for example, lithium aluminum hydride, we anticipated that it could be achieved by treatment with methyl lithium as well; the two-fold role of the reagent (initiation of the rearrangement, conversion of the 17-ketone into a tertiary carbinol) would reduce the number of steps leading to the biologically more acceptable 17α-methyl-17β-alcohols **6**, **12**, and **13**.

As the active azulenone **1** is highly unsaturated, it seemed desirable to prepare and test unsaturated intermediates as well; their preparation could be envisaged only in a strictly oxygen-free medium.

Experimental

Chemistry

Melting points were determined with a Kofler block and are uncorrected. Optical measurements were carried out in chloroform. Infrared spectra were recorded on a Zeiss UR20 instrument, and ^{1}H nuclear magnetic resonance (NMR) spectra were measured on a Tesla B 467 (60 MHz) instrument in deuteriochloroform and are reported in parts per million on the δ scale, from tetramethylsilane. Solvents for the given reactions were freshly distilled from lithium aluminum hydride prior to use. All reactions were conducted under a rigorously dried nitrogen atmosphere. "Methyltrienolone" (17α-methyl-17β-hydroxyestra-4,9,11-trien-3-one) and [17α-^{3}H]methyltrienolone (specific radio-

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activity, 87.6 Ci/mmol) were obtained from Roussel Uclaf (Romainville, France), [1,2-^{3}H]testosterone (specific radioactivity, 54 Ci/mmol) was from Amersham International (UK), and testosterone propionate and other nonradioactive steroids were from Koch and Light (Amersham, Buck., UK) or Sigma (USA).

The following compounds were prepared according to the literature: 17β-hydroxy-4aβ-methyl-A-homo-B, 19-dinor-5α, 10α-androstan-3-one (**11**),[9] 17β-hydroxy-4aβ, 17-dimethyl-A-homo-B, 19-dinor-5α, 10α-androstan-3-one (**12**),[10] 17β-hydroxy-4aα, 17-dimethyl-A-homo-B, 19-dinor-5α, 9β-androstan-3-one (**13**),[7] and 4a-methyleno-3β, 10-oxido-A-homo-B, 19-dinor-5β-androstane-9,17β-diol (**14**).[11]

3β-Acetoxy-4a-methyl-A-homo-B, 19-dinor-3, 4-seco-5β-androst-9-en-17-one (5). A solution of methanesulfonate **3**[8] (2.10 g, 3.96 mmol) in ether (100 ml) was slowly added under nitrogen at room temperature to a stirred solution of lithium aluminum hydride (600 mg, 16 mmol) in tetrahydrofuran (25 ml). After refluxing for an additional 3 hours, the reagent was decomposed with moist ether and then with several drops of saturated aqueous solution of sodium sulfate. The inorganic material was removed by filtration through a column of anhydrous sodium sulfate and the filtrate was concentrated in vacuo. The dry residue was acetylated with acetic anhydride (4 ml) in pyridine (6 ml) at 20 C for 18 hours under nitrogen. The product was dissolved in acetone (25 ml) containing p-toluenesulfonic acid (400 mg). After 18 hours standing under nitrogen the solution was diluted with the same volume of toluene, concentrated to the original volume, washed with a solution of potassium hydrogen carbonate, then with water, dried over anhydrous sodium sulfate, and separated by flash chromatography (silica gel, toluene/ethyl acetate, 30 : 1). The first major component (mp, 93 to 95 C) was identical to an authentic sample of **4**[10] (740 mg, 57% yield). The more polar component was compound **5** (262 mg, 20% yield), $[\alpha]_D$ = +169° (c = 0.9), IR (CCl_4): 3,075, 1,651, 1,642, 995 (C=CH_2), 1,745, 1,408 (—$COCH_2$—), 1,745, 1,244 (—OCO—) cm^{-1}. ^{1}H NMR: δ 0.93 (s, 3H, 18—CH_3), 1.59 (bs, 3H, C=C-CH_3), 2.01 (s, 3H, CH_3CO), 3.20 (bd, 1H, $W_{1/2}$ = 15 Hz, 5β-H), 3.95 (t, J = 6.5 Hz, 2H, 3-H), 4.65 (bs, $W_{1/2}$ = 3.5 Hz, 2H, C=CH_2). Analysis calculated to $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.09, H, 9.41.

4a,17-Dimethyl-A-homo-B, 19-dinor-3, 4-seco-5β-androst-9-ene-3,17β-diol (7). A solution of compound **5** (1.1 g, 3.33 mmol) in tetrahydrofuran (6 ml) was added dropwise to a solution of methyllithium (1 M, 15 ml, 15 mmol) stirred under reflux condenser in nitrogen atmosphere. After 20 hours the reagent was decomposed by pouring it on ice (approximately 100 g) acidified with hydrochloric acid (1.5 ml, 15 mmol). An oily product was extracted with chloroform and the extract was washed successively with a potassium hydrogen carbonate solution (6%), then water, and dried over sodium sulfate. Flash chromatography of the product (silica gel, toluene/ethyl acetate, 10 : 1) afforded compound **7** (433 mg, 44% yield), $[\alpha]_D$ = +110° (c = 1.0); IR ($CHCl_3$): 3,075, 1,652, 1,644 (CH_2=C), 3,620, 1,062, 1,047 (OH) cm^{-1}; ^{1}H NMR: δ 0.93 (s, 3H, 18-CH_3), 1.18 (s, 3H, 17α-CH_3), 1.58 (s, 3H, C=C—CH_3), 3.21 (bd, $W_{1/2}$ = 14 Hz, 1H, 5-H), 3.60 (t, J = 6 Hz, 1H, 3α-H), 4.62 (bs, $W_{1/2}$ = 4 Hz, 2 H, C=CH_2). Analysis calculated for $C_{20}H_{32}O_2$: C, 78.89; H, 10.60. Found: C, 78.73; H, 9.54.

4a-Methylene-17α-methyl-A-homo-B,19-dinor-5β-androst-9-ene-3β,-17β-diol (6)

From 3β-acetoxy-4a-methylene-A-homo-B,19-dinor-5β-androst-9-en-17-one (4): In the same manner the ketone **4**[10] (420 mg, 1.28 mmol) was treated with methyllithium (1 M, 7 ml, 7 mmol). Flash chromatography (silica gel, toluene/ethyl acetate, 10 : 1) afforded compound **6** (245 mg, 63% yield), mp 150 to 153 C (toluene); [α] = 43° (c = 0.9); IR ($CHCl_3$): 3,075, 1,650, 1,638 (C=CH_2), 3,610, 1,031 (OH) cm^{-1}; ^{1}H NMR: δ 0.92 (s, 3H, 18-CH_3), 1.16 (s, 3H, 17α-CH_3), 3.32 (bd, $W_{1/2}$ = 16 Hz, 1H, 5-H), 3.73 (m, $W_{1/2}$ K 19 Hz, 1 H, 3α-H). Analysis calculated for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.16; H, 10.26.

From 3β-acetoxy-6β-methanesulfonyloxy-5-methyl-19-nor-5β-androst-9-en-17-one (9): A solution of compound **9**[8] (1.1 g, 2.59 mmol) in tetrahydrofuran (6 ml) was added dropwise to a stirred solution of methyllithium in ether (1 M, 15 ml, 15 mmol) and was refluxed under nitrogen for 4 hours. The reagent was decomposed and worked up as above. Flash chromatography yielded only 78 mg (10%) of compound **9** (IR and ^{1}H NMR spectra were identical with those of the above sample).

Biologic methods

Bioassay for antiandrogenic activity. A standard method based on simultaneous administration of testosterone propionate (5 μmol) and of the tested compound (50 μmol) to castrated male mice (seven animals in each group) for 3 weeks, followed by weighing of seminal vesicles and kidneys, was used for testing the antiandrogenic and antirenotropic activity in vivo.[1-4] The effects of tested compounds were related to those of cyproterone acetate. For full details of the method used refer to ref. 3.

Binding to the androgen receptor. The technique previously used for determination of epitestosterone interaction with androgen receptors was applied here.[1] Briefly, increasing concentrations of either methyltrienolone or tested steroids (from 0.2 to 20 nmol/l) were incubated with rat prostate cytosol (as a supernatant 105,000 × g from the homogenate of prostates from castrated rats) labeled with [17α-^{3}H]methyltrienolone at 0 C for 16 hours. Bound and free steroids were separated by adsorption on dextran-coated charcoal. The apparent intrinsic association constant for methyltrienolone-receptor interaction was assessed from the Scatchard plot in the modification of Chamness and McGuire.[12] It amounted on average to 2.8 × 10^9 L/mol. Relative-binding affinities were determined in terms of competitor concentrations required to displace 50% of the labeled ligand using log-logit plot (IC_{50} values).

5α-Reductase assay. The assay system described by Brooks et al.[13] was applied for screening the compounds as potential 5α-reductase inhibitors. Briefly, rat prostates were homogenized in Tris-HCl buffer (20 mmol/l, pH 7.0, containing 1 mmol/L each of magnesium chloride, dithiothreitol, and EDTA and 0.25 mol/L sucrose) and the homogenate was centrifuged at 800 × g to obtain the crude pellet. Tested steroids or progesterone as a reference compound, 0.5 nmol each, were incubated in the mixture containing pellet suspension equivalent to 45 mg of tissue, testosterone substrate (0.17 nmol/tube) admixed with [^{3}H]testosterone (10,000 dpm/tube), dihydrotestosterone (49 μmol/l), and NADPH-generating system in a final volume of 2.1 ml at 37 C under shaking for 15 minutes. The reaction was stopped by extraction with ethyl acetate. Following evaporation the samples were admixed with nonradioactive testosterone and androstenedione, and were divided into two equal portions for separation of metabolites. This was achieved by chromatography either on a thin layer of silica gel (Alufor F_{254} plates, Merck, Darmstadt, Germany) in cyclohexane/ethyl acetate (2 : 1) or by high-performance liquid chromatography using isocratic elution with acetonitrile-water (47 : 53) on 5μ reverse C18 phase and Czechoslovak HPLC systems (model 3001, Laboratory Devices, Prague, Czechoslovakia). The average yields of 5α-dihydrotestosterone formed were calculated from the radioactivities corresponding

Table 1 Organ weights[a] related to the total body weight of castrated mice[b] treated with testosterone propionate either alone or in combination with tested steroids

Compound	Seminal vesicles (mg/100 g)	Kidney (g/100 g)
None	41 ± 8	1.10 ± 0.41
Testosterone propionate	310 ± 95	1.50 ± 0.20
6	183 ± 88*	1.34 ± 0.77
7	179 ± 114*	1.30 ± 0.70
11	147 ± 59**	1.25 ± 0.98
12	150 ± 37**	1.35 ± 1.4
13	128 ± 60**	1.16 ± 1.01
14	223 ± 101	1.36 ± 1.0
Cyproterone acetate	160 ± 78**	1.61 ± 0.17

[a] Significance against the effect of testosterone propionate: ** $P < 0.01$, * $P < 0.05$.
[b] Seven animals were used in each group.

Table 2 In vitro competition of tested steroids with methyltrienolone for rat prostate cytosol receptor and their effect on prostatic 5α-reductase activity

Compound	Relative binding affinity to receptor[a] (%)	Average yields of dihydrotestosterone[b] (%)
None	—	14
Progesterone	—	8.4
Methyltrienolone	100	—
11	0.7	17
12	3.0	17
13	1.2	14

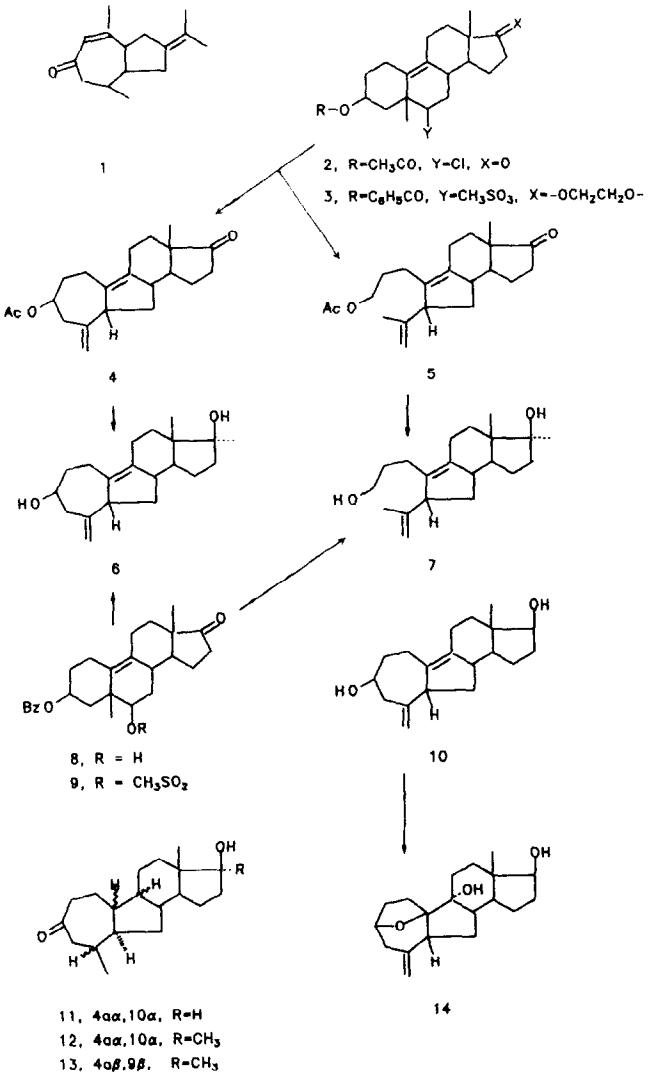
[a] Methyltrienolone or tested compounds in three concentrations (0.2, 2, and 20 nmol/l), each in duplicate, were incubated with rat prostate cytosol labeled with [^{3}H]methyltrienolone. Using log-logit plot, the concentrations required to displace 50% of specifically bound radioligand were calculated and related to that of methyltrienolone. An average intrinsic association constant for the methyltrienolone-receptor interaction was 2.8×10^9 L/mol.
[b] The average yields of dihydrotestosterone following incubation of testosterone (0.17 nmol/tube) with tested compounds (0.5 nmol/tube each) and with tissue preparation of rat prostates containing 5α-reductase, under reductive conditions at 37 C for 15 minutes in a final volume of 2.1 ml. The data represent mean values from three incubation experiments followed by two independent techniques of separation of metabolites (thin-layer chromatography and high-performance liquid chromatography). The average coefficient of variation for calculation of the yields of 5α-reduced metabolites was 12.4%.

to testosterone and dihydrotestosterone, measured by liquid scintillation spectrometry.

Results and Discussion

17α-Methyl-17β-alcohols **6** and **7** were prepared by action of methyllithium on ketones **4** and **5**, respectively, when the reaction was carried out in ether under nitrogen with strict exclusion of oxygen.

17α-Methyl-4a-methylene-A-homo-B, 19-dinor-5β-androst-9-ene-3β,17β-diol (**6**) was prepared by an alternative route in which methyllithium reacted with 3β-acetoxy-6β-methanesulfonyloxy-5-methyl-19-nor-5β-androst-9-en-17-one (**9**). Because of the low yield (10%), however, the shortcut in the synthesis is not useful.



Scheme 1

The results of biologic testing are given in Table 1. Out of six steroids tested, three were as active as or even more active than cyproterone acetate (**11** to **13**), as demonstrated by a significant ($P < 0.01$) decrease of relative weights of seminal vesicles from castrated mice treated with testosterone propionate. None of these compounds exerted antirenotrophic properties.

As shown in Table 2, none of these steroids inhibited 5α-reductase, as measured by dihydrotestosterone yields following incubation of testosterone with an enzyme contained in a subcellular fraction from the homogenate of rat prostates using two independent methods. Steroids **11**, **12**, and **13** exhibited the slight but distinct ability to displace methyltrienolone from its binding to an androgen receptor in a dose-related way (Table 2).

It should be kept in mind, however, that in in vivo experiments the tested compounds were administered at 10 times the dose of the agonist, and it was thus possible that even such low affinities could account for their biologic activity.

Since the hypothesis that the biologic activity of the above steroids may be due to their effect on 5α-reductase was not confirmed, a further plausible explanation of their activity is their in vivo metabolism to antiandrogenically active products. A survey of their structures (Scheme 1) indicates that such active compounds could be the products of 1,2-dehydrogenation of 3-oxosteroids in an expanded A ring, structurally more similar to the azulenone **1**, by which this study had been inspired.

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