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A full conformational characterization of antiandrogen cortexolone-17α-propionate and related compounds through theoretical calculations and nuclear magnetic resonance spectroscopy

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Cortexolone-17α-propionate is a topical antiandrogen under investigation for the treatment of androgen related skin disorders. A full conformational characterization was realized, in comparison with others steroidal androgens and antiandrogens, by means of theoretical calculations at the B3LYP/6-31G(d) level supported by high field NMR analyses. All the studied molecules showed a good overlay nevertheless the different functional groups present in the skeleton of the molecules drive the individual biological profile.

Introduction

The skin capability of synthesizing and converting androgens is well documented.[1] The 5α-reductase-catalyzed transformation of testosterone **1** affords dihydrotestosterone (DHT, **2**), the most active metabolite involved in many androgen-related skin disorders such as hirsutism, androgen alopecia and acne.[1-5] The treatment of these disorders can be realized either inhibiting the 5 α -reductase or antagonizing the binding of testosterone and DHT at the androgen binding sites.[6] Known androgen antagonists, such as finasteride **6** or cyproterone acetate **7**, when systemically administered, show beneficial effects in the management of these skin disorders together with an interference with the hormonal environment in male and female patients.[7] Several years ago we investigated[8] the antiandrogenic activity of a family of 17α-esters of cortexolone (17α,21-dihydroxy-4-pregnene-3,20-dione **3**), an intermediate of the glucocorticoids biosynthesis, devoid of endocrine function, with the exception of a weak glucocorticoid activity.[9] Among the studied esters 17α-propionate **4** showed a strong local antiandrogenic activity in the hamster flank organ test[10] being, on the contrary, ineffective when subcutaneously injected by repeated administrations in animals even at very high doses.[8] The systemic anti-androgenic activity of 17α-propionate of cortexolone was assessed by its ability to decrease the weight of the androgen-dependent organs (ventral prostate, seminal vesicles) stimulated by the injection of testosterone propionate (TP).[8]

The absence of systemic antiandrogenic effects could be explained considering that the propionate, after percutaneous application, is quickly hydrolyzed by the skin and plasma esterases into the inactive parent cortexolone (**3**) getting through the 21-propionate **5**.

The topical activity of compound **4** is higher than that of finasteride **6** and about equivalent to that of cyproterone acetate **7**.

Taking into account the topical activity of propionate **4** joined to the lack of systemic activity, we planned to compare this 17α-ester with the well known antiandrogen cyproterone acetate **7** and with the natural androgens, testosterone **1** and dihydrotestosterone **2**, from a conformational point of view.

In fact the conformation of a biologically active compound plays a central role when it interacts with the target, for example a receptor or an enzyme; among the possible conformations only one could be able to stimulate the biological response as we also reported in a previous work.[11] The choice of the compounds to be compared with propionate **4** was driven by its antiandrogenic activity. In fact testosterone (**1**) and dihydrotestosterone (**2**) are antagonized both by compounds **4** and **7** with the same action mechanism that not implicates the interference with the 5α-reductase. The conformational characterization was realized by means of theoretical calculations validated by the ^{1}H and ^{13}C NMR complete signals assignment, as in the case of our previous studies of steroidal compounds.[11]

Results and discussion

Biological activity of cortexolone-17α-propionate (4)

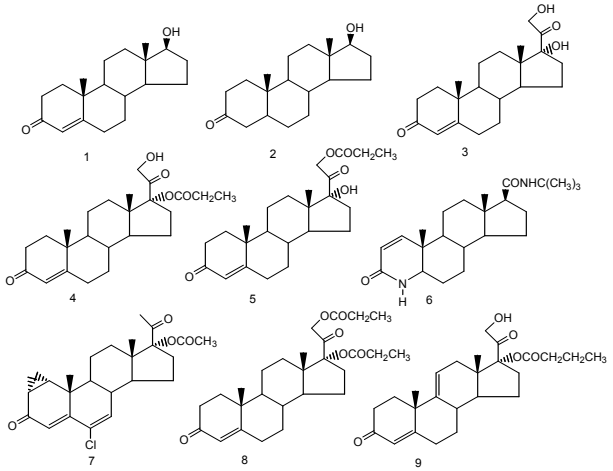

Fig. 1. Structures of the studied compounds.

Local activity and mechanism of action of cortexolone-17α-propionate (4)

The local activity of **4** was established by means of the hamster's flank organ test.[8] The results are summarized in Table 1 (for a more exhaustive discussion see reference 8). The antiandrogenic activity of **4** is dose-related; the parent cortexolone (**3**) is devoid

of effect; when compared to other androgen antagonists, administered at the fixed dose (400 μg), compound **4** resulted two times more active than finasteride (**6**) and about as active as cyproterone acetate (**7**).

Table 1. Local antiandrogenic activity in hamster's flank organ.

Topical treatment (acetone 0.05 mL)	Daily dose [a]	Flank organ inhibition (%)[b]
Cortexolone (**3**) + TP	400 + 4	0
Finasteride (**6**) + TP	400 + 4	71*
Cyproterone acetate (**7**) + TP	400 + 4	93**
Cortexolone-17α-propionate (**4**) + TP	100 + 4	40*
Cortexolone-17α-propionate (**4**) + TP	200 + 4	78*
Cortexolone-17α-propionate (**4**) + TP	400 + 4	84**

*$P<0.05$, **$P<0.01$. [a](μg/animal, antiandrogen + androgen). [b]The local antiandrogenic activity of **4** and other tested compounds **3**, **6**, **7** was expressed as the percent inhibition of the flank organ enlargement induced by the topical application of testosterone propionate (TP) alone.

As concerns the mechanism of action, compound **4,** compared to finasteride (**6**) the well-known inhibitor of 5α-reductase, did not inhibit the conversion of testosterone (**1**) to DHT (**2**) in reconstructed human epidermis (Cosmo R & D personal communication), thus resulting devoid of inhibitory activity on the 5α-reductase, as shown by the studied [^{14}C]-testosterone metabolism after 24h transepidermal diffusion (Figure 2).

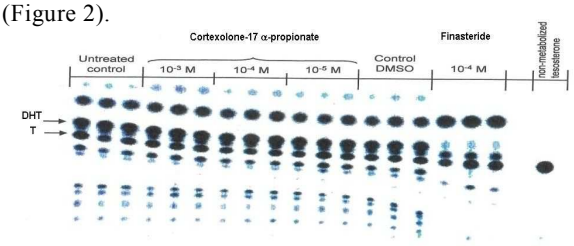

Fig. 2. [^{14}C]-testosterone (T, **1**) methabolism and DHT (**2**) production in presence of cortexolone-17α-propionate (**4**) or finasteride (**6**).

Additional experiments (Cosmo R & D personal communication) showed that, in binding affinity test to androgen-receptor of human prostate cancer cells, compound **4** inhibited the specific binding of [^{3}H] methyltrienolone (R1881) to the androgen receptor with K_i value of 4.0E-08, and IC_{50} value of 5.0E-08 M. As a consequence, compound **4** should be considered as an antiandrogen acting at the androgen-receptor level.

Systemic activity of cortexolone-17α-propionate (4)

Systemic antiandrogenic activity of **4** was evaluated in male castrated rats primed with TP. After repeated subcutaneous injections of 0.2-1 and 5 mg/animal, compound **4** was completely unable to antagonize the stimulating effect of TP on the target organs ventral prostate, seminal vescicles, preputial glands.[8] Also parent cortexolone (**3**) resulted devoid of activity in this test.[8] Compound **4** resulted also endowed with glucocorticoid activity (adrenals and thymus weight inhibition), detectable only at the highest tested dose equivalent to about 100 mg/kg b. wt. Considering the very high dose required to obtain the glucocorticoid activity, this effect should be minimal and of little importance when the product is administered by topical route.[8] In addition, in the antigonadotropic activity test 17α-propionate **4**, even injected at the highest dose, is completely devoid of activity on gonadotropins hypersecretion, as compared to progesterone. These data demonstrate that compound **4** is a peripherally selective antiandrogen not affecting CNS.[8]

Metabolism of cortexolone-17α-propionate (4)

The absence of systemic activity can be explained by a rapid hydrolysis of 17α-ester affording the inactive cortexolone (**3**). Cortexolone-17α-propionate (**4**), when incubated in rat plasma, was rapidly converted (50%) to free cortexolone (**3**) within 2 hours of incubation and completely metabolized within 8 hours (Table 2). The acyl group initially undergoes a non enzymatic migration to the 21-position and subsequently an esterase-catalyzed hydrolysis affording inactive cortexolone (**3**). The formation of intermediate **5** was elucidated incubating **4** at 37.8 °C up to 8 hours in absence or in presence of 50 μg/mL of enzymatic inhibitor dichlorvos. When the incubation was performed in the presence of the inhibitor, a large amount of cortexolone-21-propionate (**5**) was detected, thus confirming that the biotransformation of 17α-propionate **4** to cortexolone (**3**) takes place by the production of 21-propionate **5**, as effect of non enzymatic migration of the propionyl group from the 17α position to the 21 position, and subsequent hydrolysis of 21-propionate (**5**) to cortexolone (**3**).

Table 2. Metabolism of **4** in rat plasma.

Incubation time	Cortexolone-17α-propionate (**4**) (%)	Cortexolone (**3**) (%)
0'	100	0
5'	95-90	5-10
15'	95-90	5-10
30'	90-80	10-20
1h	80-60	20-40
2h	50	50
4h	40-20	60-80
8h	10-0	90-100

An analogous metabolic profile of **4** was observed in human plasma incubation (Table 3).

Table 3. Metabolism of **4** in human plasma.

Incubation time	Cortexolone-17α-propionate (**4**) (%)	Cortexolone-21-propionate (**5**) (%)	Cortexolone (**3**) (%)
0'	99.6	0.5	0.0
30'	93.2	6.5	0.4
1h	85.2	13.2	1.7
2h	67.2	25.3	7.5
4h	30.3	33.8	35.9
6h	11.3	23.8	64.9

In rat skin homogenate the metabolic transformation of **4** to cortexolone (**3**) reached the peak (40-44.7 %) within 8-16 hours, and was maintained stable during the remnant incubation period up the 24 hours (Table 4).

Table 4. Metabolism of **4** in rat skin homogenate.

Incubation time	Cortexolone-17α-propionate (**4**) (%)	Cortexolone-21-propionate (**5**) (%)	Cortexolone (**3**) (%)
0'	99	0.5	0
5'	99	0.5	0
15'	99	0.5	0
30'	98.5	0.5	0.5
1h	89.5	5	5
2h	69.5	15	15
4h	59.5	10	30
8h	49.5	10	40
16h	44.75	10	44.75
24h	44.75	10	44.75

*Biological activity of cortexolone-21-propionate (**5**) and cortexolone-17α,21-dipropionate (**8**)*

In Table 5 is reported the local antiandrogenic activity of compounds **5** and **8** resulting in the ability of the tested steroids (400 μg) to inhibit the enlargement of the hamster's organ test in turn produced by administration of TP (4 μg). The 21-monoesterification of cortexolone (**3**) leads to compound **5** nearly devoid of activity like parent cortexolone (**3**). The 17α,21-diesterification (compound **8**) reduces the antiandrogenic activity granted by the 17α-esterification of compound **4** (84% of inhibition, Table 1).

Table 5. Local antiandrogenic activity of **4** related compounds **5**, **8** and **9** in hamster's flank organ.

Compound	Flank organ inhibition (%)[a]
Cortexolone-21-propionate (**5**)	29
Cortexolone-17α,21-dipropionate (**8**)	57
9,11-Dehydrocortexolone-17α-butyrate (**9**)[11]	85

[a]Ability of the tested steroids (400 μg) to inhibit the enlargement of the hamster's organ flank produced by the administration of 4 μg of testosterone propionate.

*Biological activity of 9,11-dehydrocortexolone-17α-butyrate (**9**)*

The 9,11-dehydrocortexolone-17α-butyrate (**9**), based on the results of the screening phase, was identified as the most potent topical anti-androgen among the 17α-monoesters; in fact it showed a 85% ability to inhibit the enlargement of the hamster's flank organ produced by topical administration of TP (Table 5), while the corresponding saturated 17α-butyrate showed a 76% ability.

Differently from the 17α-monoesters of cortexolone, 9,11-dehydrocortexolone-17α-butyrate (**9**), was found endowed with systemic activity in the rat after subcutaneous injection.[12]

Compound **9** was also discovered to be a potent inhibitor of gonadotropins hypersecretion, thus mimicking the profile of activity of cyproterone acetate (**7**), which blocks the androgen-receptor interaction and simultaneously reduces serum testosterone through its antigonadotropic action.[13,14] The presence of a double bond at position 9,11 of cortexolone, modifying the spatial conformation of steroids rings, could be responsible of the systemic and increased topical activity of **9**.[12]

Conformational Properties

The above observations prompted us to study the conformational properties of compound **4** and of the related compounds to establish and compare their preferred conformations. To this aim other known androgenic or antiandrogenic steroids were simultaneously analyzed including testosterone (**1**) and its most potent metabolite DHT (**2**) active in the skin, cortexolone (**3**) and its derivatives **8** and **9** and cyproterone acetate (**7**).

Conformational properties of compounds **1** and **2**

An extensive exploration of the conformational space of compounds **1** and **2** was carried out through DFT calculations at the B3LYP/6-31G(d) level. Attention was focused on the tetracyclic system and on the possibility of inversion of the hexacyclic rings. Compound **1** prefers conformation **1A** in which ring A assumed a half-chair geometry due to the presence of the double bond between C4 and C5. Conformation **1Ab** differs from **1A** (Figure 3) only for the orientation of the hydroxyl group at C17 and it is less stable by 0.27 kcal/mol. These two geometries account for more than 97% of the overall population (Table 6). The inversion of A ring (**1B**) determined the obtainment of a geometry less stable by about 2 kcal/mol, while the B and C rings inversion gave two geometries (**1C**, **1D**) less stable by 6.05 and 11.13 kcal/mol, respectively (Table 6). Concerning compound **2**, the preferred conformation **A** showed the expected chair geometry of the hexacyclic rings and a twisted geometry of ring D. The inversion of ring A is easiest than the others, but the corresponding conformation (**2B**) is less stable by more than 3 kcal/mol, leaving **2A** and the analogous **2Ab** conformation the only ones significantly populated; in fact, they account for more than 99% of the overall population. The two geometries differ only for the orientation of the hydroxyl group at C17 that has two accessible orientations with a small difference in energy (0.31 kcal/mol). The preferred one is that with $\tau_1 \approx 170°$ (**2A**) (Figure 3 and Table 6).

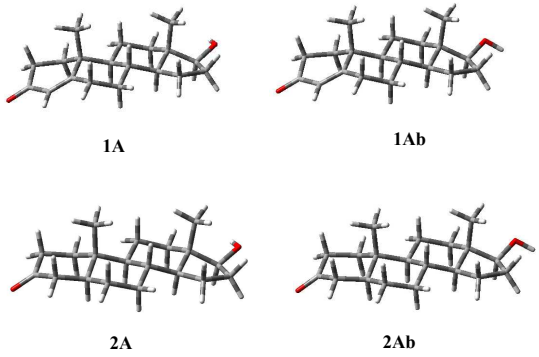

Fig. 3. Three-dimensional plots of the most populated conformations of compounds **1** and **2**.

Some short contacts characterize the conformational preferences of the rings of **2**: H-2ax/CH_3-10 (2.81 Å), and H-4ax/CH_3-10 (2.76 Å) for A ring; H-6ax/CH_3-10 (2.72 Å), H-8ax/ CH_3-10 (2.86 Å) for B ring conformation; H-11ax/CH_3-10 (2.73 Å), H-11ax/CH_3-13 (2.76 Å), and H-8ax/ CH_3-13 (2.78 Å) for C ring; H-15ax/CH_3-13 (2.90 Å) for ring D.

Conformational properties of compounds **3**, **4** *and* **7**-**9**

The conformational space of compounds **3**, **4**, **7**, **8** and **9** was analyzed at the same level as above. Analogously to **1**, ring A presents, in all the cases, an unsaturation between C4 and C5, that forces it to assume a half-chair geometry. In Table 6 the most representative conformations are reported. The preferred conformation of compounds **3**, **4**, **7**, **8** and **9** (Figures 4 and 5), located in the present study, corresponds, in the tetracyclic skeleton, to the geometry already determined at the same level of calculations for progesterone and a group of 13-ethylsteroids and related estrogens modeled in previous papers.[11] In fact, as it can be seen from Table 6, they show very close values of torsional angles (τ_{A-C}) and puckering coordinates.

The energy profiles for rotation around the C17-C20, and C17-O single bonds, defined by τ_1, and τ_2, were obtained and the preferred orientations determined. The C17-C20 bond showed a quite balanced distribution of its possible orientations, with the presence, for all compounds, of two significantly populated geometries, that present $\tau_1 \approx 160$, and $\tau_1 \approx -10$, respectively. For **3** and **8** conformation **Ab**, with $\tau_1 \approx -10$, is favoured by 0.63 and 1.29 kcal/mol, respectively, while **4** and **9** prefer the other orientation by 0.46 and 0.25 kcal/mol, respectively. Concerning the C17-O bond, in the case of **4**, **8**, and **9**, a significant preference was observed for the orientation characterized by $\tau_2 \approx -60°$, with the other higher in energy by 4-6 kcal/mol, while in **3** the hydroxyl group bonded at C17 shows two orientations related to the value of τ_1, being preferred τ_2 = -45for τ_1 = 146, τ_2 = -159 for τ_1 = -24. In compound **8**, the hydroxyl at C21, present in **3**, **4**, and **9**, is esterified, with the obtainment of a second propionate group. Considering the oxoethylpropionate bonded at C17 the torsional angle τ_3 describes the relative orientation of the two carbonyl groups of the chain. The second one is perpendicular to the first one ($\tau_3 \approx 90°$), whatever the orientation of this latter (see τ_1).

A careful analysis of the conformational freedom of the tetracyclic skeleton allowed to determine the facility of inversion of the three hexacyclic rings. The A ring inversion from the 1α,2β-half-chair to the 1β,2α-half-chair conformation (**3**, **4**, **8**, **9A** → **3**, **4**, **8**, **9B)** is the easiest among all the possible ring inversions. In the case of compounds **3**, **4**, **8**, the A ring inversion determines the obtainment of a conformation less stable by about 2 kcal/mol. For compound **9** the same inversion is easier giving a conformation **9B** with an energy value of 1.34 kcal/mol. So the percentage contribution of the 1β,2α-half-chair conformation to the overall population is double in the case of **9** respect to the other ones, although the 1α,2β-half-chair conformation remains widely preferred.

As regards B ring inversion, the obtained conformations **C**, presenting a relative energy of about 4-6 kcal/mol, respectively, do not give any contribution to the overall population. The ring C inversion of **9** is not possible because of the presence of the double bond, while for **3**, **4** and **8** is obtained a conformation (**D**) higher in energy than the global minimum by more than 10 kcal/mol.

In **3**, **4**, **8** and **9** the preference of ring A for the 1α,2β-half-chair conformation is characterized by the short contact H-2ax/CH_3-19 (2.90 Å). The contacts H-6ax/CH_3-19 (2.91 Å), H-8ax/CH_3-19 (2.87 Å) confirm the B ring conformation; contacts H-8ax/CH_3-18 (2.74 Å), H-11ax/CH_3-19 (2.70 Å), H-11ax/CH_3-18 (2.73 Å) assure the C ring geometry, while contact H-15ax/CH_3-18 (2.90 Å) the D ring conformation.

Finally, the different orientations of τ_1 could be verified through contacts H-21b/H-12eq (2.48, 2.29, 2.36 Å, respectively, for **3**, **4**, and **8**) for conformation **A**; H-21a,b/CH_3-18 (2.63, 2.82, 2.90 Å), H-21a/H-16 (2.42, 2.25, 2.27 Å), and H-21b/H-16 (2.37, 2.43, 2.46 Å) for conformation **Ab**.

Analogously, conformation **A** of compound **9** presents the contacts: H-2ax/CH_3-19 (2.85 Å) for ring A; H-6ax/CH_3-19 (2.94 Å) for ring B; H-21a/H-12eq (2.39 Å) for τ_1 = 156. The second orientation of τ_1 could be verified through contacts: H-21b/CH_3-18 (2.88 Å), H-21b/H-16 (2.24 Å), H-21a/H-16 (2.48 Å).

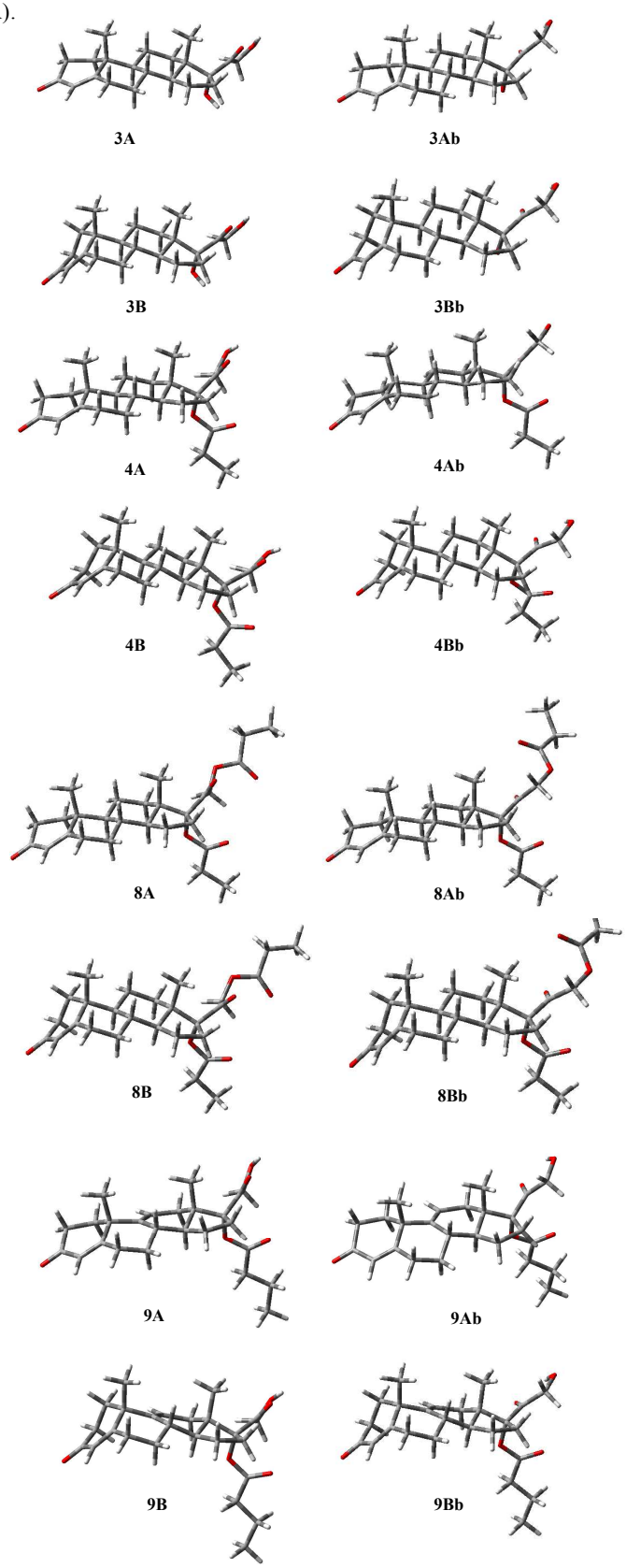

Fig. 4. Three-dimensional plots of the most significant conformations of compounds **3**, **4**, **8** and **9**.

Compound **7** shows a rigid structure and the only degree of conformational freedom is the inversion of C ring. Rings A and B could not be inverted because of the presence of the cyclopropanic ring and the double bond, respectively. Nevertheless, the C ring inversion determined the obtainment of conformations with relative energy of about 13 kcal/mol, giving not contribution to the overall population. So, only two geometries, **7A**, and **7Ab** are populated (Figure 5). Conformation **A** of compound **7** presents the following contacts: H-11β/CH_3-18 (2.24 Å), H-11β/CH_3-19 (2.29 Å), H-8/CH_3-19 (2.76 Å), H-9/CHa-cPr (2.66 Å).

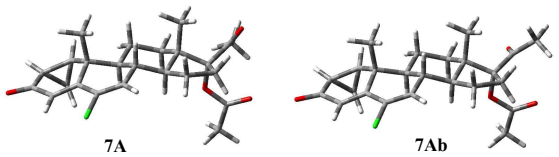

Fig. 5 Three-dimensional plots of the most significant conformations of compound **7**.

On the basis of crystallographic studies[15] performed on dihydrotestosterone (**2**) complexed with the ligand-binding domain of the wild-type androgen receptor, both the carbonyl oxygen atom bonded at C3 and the hydroxyl group at C17 of this molecule actively contribute to stabilize the obtained complex. The A ring conformation influences the orientation of the carbonyl group that deeply affects the binding of the entire molecule.

A docking study performed on **7**,[16] into the homology model for the glucorticoid receptor ligand binding domain, revealed that in the active site it assumes conformation **A**.

The superimposition of the heavy atoms of the tetracyclic system of the preferred conformations of compounds **3**,**4** and **7-9** (Figure 6) put in evidence that the presence of the hydroxyl group or the ester chain, bonded to C21 in compounds **3**, **4**, **8** and **9** does not affect the orientation of the substituent at C17.

Compounds **3**, **4**, **8** and **9** show a very good overlay, also concerning the O3 atoms that are perfectly coincident, in spite of the presence in **9** of a double bond on ring C. Conversely, the overlay shows that in **7** the O3 atom is differently oriented and diverges with a distance d(O-O) ≈ 1.0 Å.

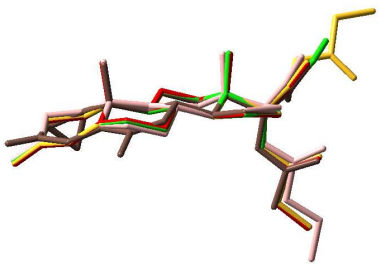

Fig. 6. Overlap of preferred conformations of compounds **3** (green shading), **4** (red shading), **8** (yellow shading), **9** (pink shading), and **7** (brown shading) obtained through rms fitting of the heavy atoms of the tetracyclic system.

NMR data

Complete ^{1}H and ^{13}C NMR signal assignments (Tables 7-9) of the spectra of compounds **3, 4** and **7-9** were achieved using a combination of 1D and 2D (COSY, HSQC and NOESY) experiments recorded in $CDCl_3$ at 298K. In general, starting from characteristic H-4, H-7 or H-11 olefinic protons it was possible to assign the resonances of all the other protons of the studied steroids on the basis of their 2D spectra. First of all H-8 was assigned through COSY correlations from H-4 of compounds **3**, **4**, **8** and **9** or from H-7 of compound **7**. Then, it was possible to discriminate between H-9 and H-14 (showing two very clear and distinctive HSQC cross peaks accounting for C-H protons) on the basis of H-9/H-11 coupling, being H-11β assigned on the basis of its NOESY cross peak with 19-CH_3 (previously distinguished from 18-CH_3 that showed NOESY correlation with the characteristic 21-protons). Also H-1, and consequently H-2, resonances were assigned through NOESY cross peak of H-1β and 19-CH_3. Finally, the analysis of 18- and 19-CH_3 NOESY cross peak network was especially useful for the assignment of the α or β configuration of geminal protons (see Table 7) of all the studied compounds. Even if some protons in the ^{1}H NMR spectra resonated as complex multiplets (see Table 7), many signals resulted well resolved and their coupling could be measured. The obtained values are reported in Table 9 in comparison with the calculated constants of compounds **3, 4** and **7-9**. For each populated conformer the ^{1}H vicinal coupling constants were calculated with the electronegativity-modified Karplus relationship[17] and were weighted averaged on the basis of the population percentages. The experimental and the calculated values resulted in close agreement. The following noe contacts were observed in the NOESY spectra of the studied compounds. Compound **3**: CH_3-19/ H-1β, H-2β, H-6β, H-11β and H-8; CH_3-18/ H-11β, H-12β, H-15β, H-16β, H-21b and H-8; H-21a/ H-12β and H-16β; H-21b/ H-16 β; H-7α/ H-9 and H-14. Compounds **4** and **8**: CH_3-19/ H-1β, H-2β, H-6β, H-11β and H-8; CH_3-18/ H-11β, H-12β, H-15β, H-16β, H-21a and H-8; H-21b/ H-12β and H-16β; H-21a/ H-16β; H-8/ H-15β; H-7α/ H-9 and H-14. Compound **9**: CH_3-19/ H-1β, H-2β, H-6β, H-11 (3,80 Å calcd) and H-8; CH_3-18/ H-11 (4,00 Å calcd), H-12β, H-15β, H-16β, H-21a and H-8; H-21b/ H-12β and H-16β; H-21a/ H-16β; H-8/ H-15β; H-7α/ H-9 and H-14. Compound **7**: CH_3-19/ H-1, H-11β and H-8; CH_3-18/ H-11β, H-12β, H-15β, H-16β, CH_3-21 and H-8; CH_3-21/ H-12β and H-16β; H-8/ H-15β; H-9/CHa-cPr.

These noe data, such as the experimental values of the ^{1}H vicinal coupling constants, supported the calculated preferred conformations. In particular, almost all these contacts correspond to distances of <3 Å as measured on the computed (Figures 4 and 5) most populated conformations of compounds **3, 4** and **7-9**

Tab. 6 Geometrical features, relative energies, and equilibrium percentages of the selected conformations of compounds **1-4** and **7-9**.

	E_{rel} (kcal/mol)	%	τ_A (°)[a]	τ_B (°)[b]	τ_C (°)[c]	τ_1 (°)[d]	$\tau_{1'}$ (°)[e]	τ_2 (°)[f]	τ_3 (°)[g]	Ring puckering coordinates										
										A ring			B ring			C ring			D ring	
										Q	ϕ_2	θ	Q	ϕ_2	θ	Q	ϕ_2	θ	q_2	ϕ_2
1A	0.00	59.8	-54	54	-54	172				0.44	16	54	0.54	167	7	0.54	272	6	0.46	187
1Ab	0.27	37.7	-54	54	-54	64				0.44	16	54	0.54	167	7	0.57	271	6	0.46	187
1B	1.88	2.5	55	55	-54	172				**0.44**	**203**	**125**	0.58	347	5	0.58	294	5	0.46	187
1C	6.05	0.0	-48	-51	-54	172				0.45	347	54	**0.72**	**261**	**84**	0.59	335	5	0.47	188
1D	11.13	0.0	-54	53	45	174				0.45	12	55	0.56	12	55	**0.73**	**324**	**79**	0.49	193
2A	0.00	62.8	-51	54	-54	173	-	-	-	0.54	285	10	0.57	320	4	0.57	275	5	0.47	188
2Ab	0.31	36.9	-51	54	-54	64	-	-	-	0.54	285	10	0.58	319	4	0.57	275	5	0.46	187
2B	3.15	0.3	28	55	-54	173	-	-	-	**0.77**	**265**	**85**	0.56	210	4	0.57	276	5	0.46	188
2C	11.83	0.0	-45	-42	-54	173	-	-	-	0.56	270	19	**0.71**	**274**	**77**	0.60	344	5	0.47	188
2D	11.50	0.0	-49	55	45	174	-	-	-	0.54	278	12	0.59	216	5	**0.73**	**215**	**78**	0.50	193
3A	0.63	24.8	-54	54	-54	146	175	-45	-	0.44	17	54	0.54	165	6	0.57	270	4	0.48	187
3Ab	0.00	71.7	-54	54	-54	-24	-169	-159	-	0.44	17	54	0.54	169	6	0.57	271	5	0.48	188
3B	2.58	0.9	55	55	-55	147	175	-44	-	**0.44**	**203**	**125**	0.58	350	5	0.58	302	3	0.48	188
3Bb	1.96	2.6	55	55	-54	-24	-169	-158	-	**0.44**	**203**	**125**	0.58	349	5	0.58	299	4	0.48	188
3Cb	6.14	0.0	-48	-51	-54	-24	-169	-158	-	0.45	347	54	**0.72**	**261**	**84**	0.59	339	5	0.48	189
3Db	10.55	0.0	-54	53	45	-25	-169	-156	-	0.44	11	54	0.56	182	13	**0.74**	**324**	**78**	0.51	193
4A	0.00	66.0	-54	54	-55	156	163	-65	-	0.44	17	54	0.54	163	6	0.57	271	3	0.47	190
4Ab	0.46	30.3	-54	54	-54	-13	-172	-75	-	0.44	17	54	0.54	166	7	0.57	271	5	0.46	188
4B	1.93	2.6	**55**	54	-55	156	163	-65	-	**0.44**	**202**	**125**	0.58	345	5	0.58	298	3	0.47	190
4Bb	2.44	1.1	**55**	55	-54	-13	-172	-75	-	**0.44**	**203**	**125**	0.58	352	5	0.58	301	4	0.46	188
4C	6.20	0.0	-49	**-51**	-55	156	164	-65	-	0.45	348	53	**0.72**	**261**	**84**	0.59	353	4	0.48	192
4D	10.59	0.0	-54	53	**45**	157	168	-64	-	0.44	13	55	0.56	183	12	**0.73**	**325**	**78**	0.49	199
8A	1.29	9.8	-54	54	-54	155	150	-65	95	0.44	17	54	0.54	166	6	0.57	268	4	0.46	190
8Ab	0.00	85.8	-54	54	-53	-11	-179	-75	77	0.44	16	54	0.54	165	6	0.57	278	5	0.46	185
8B	3.17	0.4	55	55	-55	156	149	-66	96	**0.44**	**203**	**125**	0.58	353	5	0.58	312	3	0.47	191
8Bb	1.81	4.0	55	54	-53	-11	-179	-75	77	**0.44**	**202**	**125**	0.58	346	5	0.58	301	4	0.47	185
8Cb	6.10	0.00	-48	-51	-54	-11	-179	-75	77	0.45	347	54	**0.72**	**261**	**84**	0.59	344	5	0.47	187
8Db	10.25	0.00	-54	53	43	-12	-179	-74	77	0.46	22	49	0.56	183	13	**0.73**	**323**	**78**	0.49	192
9A	0.00	55.0	-55	56	-16	154	162	-66	-	0.44	15	54	0.50	117	12	0.51	266	52	0.45	187
9Ab	0.25	36.0	-55	56	-15	-13	-172	-76	-	0.44	14	54	0.50	116	12	0.51	267	52	0.45	183
9B	1.34	5.7	**56**	54	-16	156	163	-65	-	**0.45**	**203**	**125**	0.55	17	10	0.51	267	51	0.45	184
9Bb	1.68	3.2	**56**	54	-16	-13	-172	-76	-	**0.45**	**203**	**125**	0.55	20	10	0.51	267	51	0.44	183
9C	3.77	0.1	-50	**-57**	-14	157	162	-76	-	0.46	348	54	**0.70**	**264**	**89**	0.50	272	49	0.45	186
7A	0.00	82.0	-6	**-1**	-54	156	-	-67	-	0.30	301	81	0.49	259	51	0.58	316	3	0.47	191
7Ab	0.90	18.0	-6	**-1**	-54	-10	-	-76	-	0.30	301	81	0.49	259	51	0.59	314	4	0.46	188

a) τ_A: C10-C1-C2-C3; b) τ_B: C5-C6-C7-C8; c) τ_C: C9-C11-C12-C13; d) τ_1: C16-C17-O-H for **1** and **2**, C16-C17-C20-C21 for **3**, **4**,**7-9**; e) $\tau_{1'}$: C17-C20-C21-O; f) τ_2: C16-C17-O-H for **3**, C16-C17-O-C17' for **4**, **7-9**; g) τ_3: C20-C21-O-C22.

Tab. 7. ^{1}H NMR chemical shifts (ppm) of compounds **3, 4,** and **7-9**

^{1}H	**3**	**4**	**7**	**8**	**9**
1			1.70		
1α	1.66	1.71	-	1.70	2.06-2.17
1β	2.00	2.02	-	2.02	2.06-2.17
2			2.00		
2α	2.32	2.32	-	2.34	2.43-2.50
2β	2.39	2.41	-	2.40	2.43-2.50
CHa (cPr)	-	-	0.86	-	-
CHb (cPr)	-	-	1.26	-	-
4	5.70	5.72	6.16	5.72	5.74
6α	2.26	2.27	-	2.27	2.35
6β	2.38	2.38	-	2.38	2.56
7	-	-	6.20	-	-
7α	1.08	1.10	-	1.08	1.16
7β	1.85	1.85	-	1.84	2.01
8	1.59	1.60	2.31	1.62	2.22
9	0.95	1.00	1.45	0.99	-
11	-	-	-	-	5.52
11α	1.62	1.65	1.94	1.65	-
11β	1.38	1.40	1.55	1.45	-
12α	1.72	1.89	2.03	1.88	2.75
12β	1.40	1.54	1.61	1.74	1.76
14	1.70	1.67	1.96	1.67	1.85
15α	1.80	1.76	1.89	1.72	1.94
15β	1.37	1.35	1.44	1.34	1.45
16α	1.57	1.85	1.82	1.84	1.97
16β	2.66	2.81	2.98	2.82	2.80
17 ($OCOCH_2$)	-	2.34	-	2.35	2.28
17 ($OCOCH_2C\mathit{H}_3$)	-	1.12	-	1.13	-
17 ($OCOCH_2C\mathit{H}_2$)	-	-	-	-	1.62
17 ($OCOCH_2CH_2C\mathit{H}_3$)	-	-	-	-	0.93
17 ($OCOCH_3$)	-	-	2.09	-	-
17 OH	2.45	-	-	-	-
18 (CH_3)	0.68	0.66	0.71	0.74	0.61
19 (CH_3)	1.16	1.17	1.21	1.16	1.32
21 (CH_3)	-	-	2.04	-	-
21a	4.28	4.21	-	4.59	4.24
21b	4.64	4.26	-	4.87	4.28
21 ($OCOCH_2$)	-	-	-	2.45	-
21 ($OCOCH_2C\mathit{H}_3$)	-	-	-	1.15	-
21 OH	3.09	3.03	-	-	3.04

Tab. 8. ^{13}C NMR chemical shifts (ppm) of compounds **3, 4,** and **7-9**

^{13}C	**3**	**4**	**7**	**8**	**9**
1	35.68	35.70	26.07	35.67	33.83
2	33.87	33.91	25.22	33.92	34.22
CH_2 (cPr)	-	-	12.29	-	-
3	199.58	199.26	197.94	199.40 (199.04)	199.05
4	123.94	124.07	120.51	123.98	124.17
5	170.93	170.33	155.22	170.61	169.01
6	32.73	32.65	130.23	32.69	32.70
7	31.98	31.91	136.51	31.91	32.13
8	35.59	35.57	38.33	35.57	37.48
9	53.28	53.16	47.70	53.14	144.20
10	38.54	38.53	38.70	38.54	40.98
11	20.52	20.50	20.78	20.57	118.25
12	30.08	30.46	31.02	30.24	32.35
13	48.57	47.72	47.24	47.79	46.28
14	50.28	50.93	48.77	50.94	48.16
15	23.70	23.89	23.23	23.77	24.65
16	34.52	30.77	30.30	30.78	30.52
17	89.00	93.77	96.18	94.86	93.33
17 (OCO)	-	174.05	170.55	174.20 (174.02)	173.24
17 ($OCOC\mathit{H}_3$)	-	-	21.17	-	-
17 ($OCOC\mathit{H}_2$)	-	27.77	-	27.10 (27.90)	36.24
17 ($OCOCH_2C\mathit{H}_3$)	-	8.85	-	8.97 (8.90)	-
17 ($OCOCH_2C\mathit{H}_2$)	-	-	-	-	18.31
17 ($OCOCH_2CH_2C\mathit{H}_3$)	-	-	-	-	13.57
18	14.99	14.29	14.19	13.75	14.31
19	17.36	17.39	22.83	17.35	26.20
20	212.33	206.21	203.61	199.40 (199.04)	206.22
21	67.42	66.93	26.43	66.88	66.91
21 (OCO)	-	-	-	174.20 (174.02)	-
21 ($OCOC\mathit{H}_2$)	-	-	-	27.10 (27.90)	-
21 ($OCOCH_2C\mathit{H}_3$)	-	-	-	8.97 (8.90)	-

Discussion

Cortexolone-17α-propionate (**4**) is a steroid endowed with a strong local antiandrogenic activity but it is devoid of systemic antiandrogenic activity and it does not affect gonadotopins hypersecretion. Cortexolone-17α-propionate (**4**) does not inhibit the conversion of testosterone (**1**) to DHT (**2**) in reconstructed human epidermis, thus resulting devoid of activity on the 5α-reductase. Nevertheless, compound **4** competes with the androgens at androgen receptor level, thus its strong antiandrogenic activity is attributable to this mechanism of action. As a consequence it is under investigation in the management of acne and alopecia.

In the present work a conformational comparison of **4** with testosterone (**1**), active testosterone metabolite DHT (**2**) and antiandrogen cyproterone acetate (**7**) (similar to **4** for the action mechanism direct on androgen receptor) was done by means of theoretical calculations, supported by their complete high field NMR characterization. In addition the comparison was extended to the related compounds cortexolone (**3**), devoid of activity, cortexolone-17α,21-dipropionate (**8**), less active, and Δ^9-butyrate **9**, active both topically and systemically.

The conformational characterization showed that all compounds are similar (see Figure 6), minor differences being observed: cyproterone acetate (**7**) has the 3-carbonyl group differently oriented and the presence of the 9,11-double bond hampers the C ring inversion in compound **9**. However the 3-carbonyl group orientation (**7** *vs* **4**, Table 1) and the conformation of C ring (**9** *vs* **4**, Tables 1 and 5) do not influence the extent of the local antiandrogenic activity of the tested compounds. On the contrary, the presence of 17α-ester group, which resulted always oriented in the same way from the conformational study, seems to be mandatory for a good inhibitory activity (**3** *vs* **4**, Table 1). In conclusion the cortexolone series compounds **3-5** and **8**, cyproterone acetate (**7**), and Δ^9-17α-butyrate **9** share the same skeleton conformation but show different antiandrogenic activities due to the presence of an acyl chain linked to the 17α-hydroxyl. The same skeleton conformation of the examined compounds is consistent with the capability of each of them to interact, even with different outcomes, with the androgens receptors. Furthermore the absence of systemic activity of **4** could be explained by its metabolic fate, *i.e.* the esterases-catalyzed hydrolysis of the acyl chain, after the rapid migration from 17 to 21 position.

Tab. 9. ^{1}H NMR coupling constants (Hz) of compounds **3, 4,** and **7-9**.

J	**3**	**3**	**4**	**4**	**7**	**7**	**8**	**8**	**9**	**9**
	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc
1α,1β	13.4		13.4		-		13.4		n.d.	
1α,2α	4.5	4.0	5.0	3.9	-		n.d.	3.9	n.d.	3.9
1α,2β	n.d	13.5	n.d	13.5	-		n.d.	13.6	n.d.	13.6
1β,2α	3.2	2.9	3.3	2.9	-		2.7	2.9	n.d.	2.9
1β,2β	5.0	3.4	5.0	3.4	-		4.8	3.4	n.d.	3.5
2β,2α	16.7		16.7		-		n.d.		n.d.	
1, 2	-		-		7.9	10.5	-		-	
1, CHa (cPr)	-		-		6.4	9.5	-		-	
1, CHb (cPr)	-		-		7.9	10.3	-		-	
2, CHa (cPr)	-		-		4.5	8.2	-		-	
2, CHb (cPr)	-		-		8.9	10.4	-		-	
CHa,CHb(cPr)	-		-		4.8		-		-	
6α,6β	14.6		14.6		-		14.5		14.4	
6α,7α	4.3	3.7	4.2	3.7	-		4.0	3.7	3.9	3.3
6α,7β	2.4	2.9	2.4	2.9	-		2.4	2.9	2.6	3.1
6β,4	n.d.		n.d		-		n.d.		1.9	
6β,7α	12.0	13.2	11.5	13.3	-		12.0	13.3	14.4	13.4
6β,7β	n.d.	3.8	5.0	3.8	-		n.d.	3.8	4.8	3.5
7α,7β	13.0		13.7		-		14.0		12.4	
7α,8	12.0	12.3	12.5	12.3	-		12.0	12.4	12.5	12.4
7β,8	n.d.	3.4	3.3	3.2	-		3.3	3.2	4.7	3.2
7,8	-		-		2.0	1.0	-		-	
8,9	10.8	12.1	11.0	12.3	9.8	12.9	10.8	12.9	-	
8,14	n.d.	12.1	11.0	12.3	10.0		10.8		10.8	
8,12α	-		-		-		-		2.9	
8,12β	-		-		-		-		2.0	
11,8	-		-		-		-		2.0	
11,12α	-		-		-		-		2.9	2.0
11,12β	-		-		-		-		5.9	6.4
9,11α	4.2	3.4	4.0		3.0		4.1		-	
9,11β	11.8	12.3	12.5		12.8		13.0		-	
11α,12α	n.d.	3.8	4.3	3.8	n.d.		4.3	4.0	-	
11α,12β	n.d.	2.8	2.9	2.9	2.7		n.d.	2.6	-	
11β,12α	n.d.	13.2	13.0	13.2	12.8		13.2	13.1	-	
11β,12β	n.d.	4.1	4.2	4.0	4.0		4.2	4.4	-	
11α,11β	n.d.		13.4		12.8		13.2		-	
12α,12β	n.d.		13.0		12.6		13.0		17.0	
14,15α	n.d.	5.8	7.0	5.4	n.d.		n.d.		7.7	6.1
14,15β	n.d.	11.6	11.8	11.8	11.0		11.3		11.0	11.3
15α,16α	9.4	11.0	9.4	11.8	9.2		n.d.	12.0	9.4	11.0
15α,16β	3.0	2.3	2.6	2.1	2.4		2.5	2.6	3.0	2.7
15α,15β	n.d.		11.8		11.6		11.3		17.0	
15β,16α	6.2	5.4	6.5	5.7	6.0		6.5	4.7	5.0	4.9
15β,16β	11.5	12.0	11.8	11.8	10.2		11.3	12.0	12.5	12.0
16α,16β	14.8		16.0		15.7		15.7		15.2	
21a,21b	19.8		18.2		-		16.5		18.3	
21a, OH	4.5		4.9		-		-		4.9	
21b, OH	4.5		4.9		-		-		4.8	

Experimental

Materials and methods

All the cortexolone derivatives were from internal source. Others steroids, Sephadex LH-20 and all reagents and solvents were purchased from Sigma Aldrich, Milan (Italy); Extrelut® NT columns were obtained from Merck.

Biological tests

Animals. Female Syrian golden hamsters and Wistar Han rats of both sexes (Charles River, Italy) were used in the experiments. The experiments were approved by the local Institutional Ethical Committee, and were conducted in agreement with the EEC Directive 86/609 and with the Italian Legislative Decree 116/27.01.1992 concerning the protection of animals used for experimental purpose or other scientific scope.

Hamster's flank organ test. Female Syrian golden hamsters, 60-90 g b.wt., were treated for 21 consecutive days with cortexolone-17α-propionate (**4**) and related compounds **5**, **8**, **9** directly applied onto the right flank organ at the daily dose ranging from 100 to 400 μg. The products were dissolved in 0.05 mL acetone solution containing 4 μg of TP. Parent cortexolone (**3**), progesterone, cyproterone acetate (**7**) and finasteride (**6**) were also tested. Control groups of animals treated with TP alone have been also included. The antiandrogenic activity of the compounds was assessed by their ability to reduce the enlargement of the flank organs induced by TP alone.

Systemic antiandrogenic and glucocorticoid activity. Male castrated Wistar rats, 45-50 g b.wt., were subcutaneously injected, for seven consecutive days, with cortexolone-17α-propionate (**4**) or with 9,11-dehydrocortexolone-17α-butyrate (**9**) (0.2-1 and 5 mg), and with TP 40 μg. Parent cortexolone (**3**) was also tested at daily dose of 5 mg. Control groups of untreated or treated animals with TP alone have been also included. Antiandrogenic activity of **4** and **9** was evaluated by their ability to antagonize the androgenic effect induced by TP on the target androgenic organs: ventral prostate, seminal vesicles, preputial glands. The weight reduction of adrenals and thymus was assumed as glucocorticoid activity index.

Antigonadotropic activity. Wistar rats of both sexes, 50-60 g b.wt., were employed. The males were castrated and, on the following day, they were surgically joined with intact females in latero-lateral parabiosis. In this model the castration induces a prompt increase of males gonadotropins which, in turn, reach the intact partner females and stimulate the ovarian growth. Starting on the day after parabiosis, each male was subcutaneously injected with cortexolone-17α-propionate (**4**) or with 9,11-dehydrocortexolone-17α-butyrate (**9**) at doses of 1 and 5 mg. Progesterone was assumed as standard control and was administered, with the same procedure, at doses of 0.5 and 2 mg. The treatment lasted for eight consecutive days,

after which the couples were autopsied. The ovaries were isolated and weighed. The inhibitory activity against gonadotropins hypersecretion, induced by castration in the males, was evaluated by the ability of **4** and **9** to antagonise the ovarian weight increase.

Metabolism

Metabolism in rat and human plasma. Samples of rat plasma were incubated at 37.8°C with cortexolone-17α-propionate (**4**) (0.4 mg/mL) for 8 hours in presence or absence of 50 µg of enzymatic inhibitor dichlorvos. Determination of **4**, free cortexolone (**3**), and cortexolone-21-propionate (**5**) were done at 5', 15', 30' and at 1-2-4-8 hours of incubation period by HPLC. Samples of human plasma were incubated with cortexolone-17α-propionate (**4**) (0.4 mg/mL) at 37.8°C for 6 hours. Determination of **4**, free cortexolone (**3**), and cortexolone-21-propionate (**5**) were done at 30' and at 1-2-4-6 hours of incubation period by HPLC.

Metabolism in rat skin homogenate. Dorsal skin samples of male rats (Harlan Laboratories, Italy) were omogenated and incubated at 36.5 °C with cortexolone-17α-propionate (**4**) (0.4 mg/mL). Determination of **4**, free cortexolone (**3**) and cortexolone-21-propionate (**5**) were done at 30' and at 1-4-8-16-24 hours of incubation period by HPLC.

Determination of 3, 4 and 5 by LC-MS and HPLC analyses

Instruments - HPLC analysis were performed on a Merck-Hitachi L-6200. A LiChrospher 100 RP-18 (Merck) column (244 mm x 4 mm i.d, 5µm) was employed; acetonitrile-water (65/35 v/v) was used at flow rate of 1 mL/min, at room temperature. Detector wavelength was set at 240 nm. Mass spectrometry analysis was carried out using LCQ^{DECA} ion trap mass analyser (TermoQuest, San Jose, USA) with an electrospray ionization ESI in negative ion mode interface. The HPLC apparatus comprised Thermo Finningan Mat P 4000 series pump and vacuum degasser. The method was adapted from the method developed for HPLC-UV assay (same column, flow rate and mobile phase). Data were processed with Xcalibur software 1.1. The optimized parameters were as follows: source voltage 5.00 kV; sheath gas flow rate 50; capillary voltage -15 V; capillary temp 250 °C.

Preparation of samples. The samples obtained from incubations were loaded onto an Extrelut® NT column; the steroids were eluted with ethyl acetate (3 x 5 mL). The combined organic extracts were evaporated under a stream of nitrogen at 50 °C. The dried extracts were dissolved in cyclohexane/ethyl acetate (7:3, 0.5 mL) and purified by gel chromatography on Sephadex LH-20 column using cyclohexane/ethyl acetate (7:3) as eluant; first eluted 15 mL were collected and taken to dryness under a stream of nitrogen.

LC-MS analysis The samples were dissolved in acetonitrile (1 mL) and an amount (15 µl) was analyzed by LC-MS. The observed peaks were identified, by comparison with standard samples, as cortexolone (**3**), cortexolone-17α-propionate (**4**) and cortexolone-21-propionate (**5**). The three standard solutions (1 mg/mL) were previously injected (5µl); the retention time and the observed m/z are reported below:

Std	M	RT (min)	m/z
3	346.21	3.48-3.89	345.0 [M-1], 691.3 [2M-1]
4	402.24	5.38-5.67	803.1 [2M-1]
5	402.24	7.05-7.59	803.2 [2M-1]

In order to quantify the percentage of each compound the samples were analyzed by HPLC

HPLC analysis. The quantitative analysis was performed by HPLC, using the same samples analyzed by LC-MS, the results being expressed as area %: the three standard solutions and the samples of plasma and of skin homogenates were analyzed in double.

Statistical analyses The data collected in the animal studies have been statistically analyzed for significant differences according to Student's test. A P value <0.05 was considered statistically significant.

Computational methods

All calculations were carried out using the Gaussian03[18] program package. The conformational space of compounds **1-4**, **7-9** was explored through optimization of all the possible starting geometries which were optimized within the DFT approach at the B3LYP level with the 6-31G(d) basis set. All the degrees of conformational freedom were considered, in particular the possible existence of different conformations at the A, B, and C hexacyclic rings as well as the orientation of the groups bonded at C17. Several conformations were located for each compound and the corresponding percentage contributions to the overall population were determined through the Boltzmann equation. The geometry of the A-C rings is described through two kind of descriptors, i.e., a significant torsion angle for each ring, τA, τB, τC, and the ring puckering coordinates determined according to Cremer and Pople.[19] Vibrational frequencies were computed at the same level as above in order to verify that the optimized structures were minima. The ^{1}H vicinal coupling constants were calculated with the electronegativity-modified Karplus relationship[17] and were weighted averaged on the basis of the population percentages.

NMR spectroscopy

All NMR spectra were recorded at 298 K with a Bruker AVANCE-500 spectrometer operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C, respectively, using a 5 mm single pulsed field gradient (z-PFG) broadband reverse probe. Chemical

shifts are reported on the δ (ppm) scale and are relative to chloroform signals (7.24 for ^{1}H and 77.0 ppm, central line, for ^{13}C spectra respectively). Compounds **3**, **4** and **7-9** (about 10 mg) were dissolved in $CDCl_3$ (0.5 mL) under N_2, and their assignments were given by a combination of 1D and 2D COSY, HSQC and NOESY experiments, using standard Bruker pulse programs. Z-PFGs were used to obtain ^{1}H-^{1}H COSY and HSQC spectra. The pulse widths were 7.50 μs (90°) and 14.5 μs (90°) for ^{1}H and ^{13}C respectively. Typically 32768 data points were collected for one-dimensional spectra. Spectral widths were 11.45 ppm (5733 Hz) for ^{1}H NMR (digital resolution: 0.17 Hz per point) and 259.84 ppm (32680 Hz) for ^{13}C NMR (digital resolution: 1.0 Hz per point). 2D experiments parameters were as follows. For ^{1}H-^{1}H correlations: relaxation delay 2.0 s, 1024 × 1024 data point matrices (512 experiments to 1024 zero filling in F1, 1024 in F2), 2 or 16 transients in each experiment for COSY and NOESY respectively, spectral width 6.0 ppm (3004.8 Hz). The NOESY spectra were generated with a mixing time of 1.0 s and acquired in the TPPI mode. There were not significant differences in the results obtained at different mixing times (0.5 – 1.5 s). For ^{13}C-^{1}H correlations (HSQC): relaxation delay 2.5 s, 1024 × 1024 data point matrices (512 experiments to 1024 zero filling in F1, 1024 in F2), 2 transients in each experiment, spectral width 6.0 ppm (3004.8 Hz) in the proton domain and 180.0 ppm (22638.6 Hz) in the carbon domain. All 2D spectra were processed with the Bruker software package.

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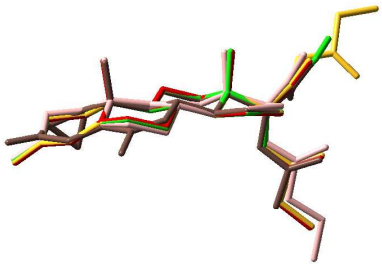

Theoretical calculations and high field NMR analyses were used in order to characterize the conformational properties of the topical androgen Cortexolone-17α-propionate **(red shading)** in comparison with other steroidal androgens and antiandrogens.