ANTI-ANDROGENIC ACTIVITY OF 17,17-DIMETHYL-18-NORANDROST-13-ENES

Albert Segaloff, M.D. and R. Bruce Gabbard, Ph.D.

Division of Endocrine Research, Alton Ochsner Medical Foundation,
New Orleans, Louisiana

Received May 19, 1964

ABSTRACT Retropinacolic elimination of hydroxyls of 17-methyl-17β-hydroxy steroids with attendant migration of C-18 methyl group to C-17 has been utilized as a means of preparation of 17,17-dimethyl-18-nor-androst-13-enes.

Biologic data are presented to show that these compounds are active androgen antagonists which compare favorably to other known anti-androgens.

The availability of agents to counteract the peripheral effects of natural hormones would advance many areas of medicine, particularly if truly selective agents could be found.

The discovery of an effective antagonist for the renal effects of aldosterone has been a great boon in the handling of many edematous states (1). Unfortunately, we do not as yet have antagonists for other steroidal hormones which can be given to man and produce the effects of withdrawal of the hormonal steroid.

Agents which inhibit the complete synthesis of potent hormonal steroids are known but as yet appear to be useful only as diagnostic tools.

Therefore, we thought it important and interesting to attempt to find potent androgen antagonists (anti-androgens).

The problem of finding effective anti-androgens is a complex one. Indeed, effective ones may already exist, but we are not aware of them either because they have not been tested or because we do not have adequate systems for finding them. For example, there are many people who are certain that estrogens and androgens are mutually antagonistic,

but this is not the case. Indeed, although there may be some evidence of occasional peripheral antagonism, in general they are more complementary. The ideal antagonists for androgens would be compounds which produce the effects of castration. To the best of our knowledge such agents are not known. Therefore, attempts have been made to find compounds which antagonize one or another of the biologic properties of androgens.

It can be difficult to analyze antihormone activity for a series of compounds. Analysis depends on the end points employed and correlations usually cannot be found between two end points. For example, if one assesses the ability of a series of materials to inhibit the uterotrophic effect of estrogen in mice, one readily finds compounds which are effective and can be arranged in order of relative activity. On the other hand, if one employs a method which inhibits the effects of estrogen on the vagina, one also finds a series of effective compounds but they are generally different from the anti-uterotrophic ones (2).

There are a few compounds shown to be more or less effective in antagonizing properties of androgens. Generally, these tests have employed the ability to inhibit the effect of androgen on the comb growth of the single comb White Leghorn chick, although an increasing number of studies is being done in mammals, particularly in the rat and more recently in the mouse (3,4). These studies have delineated two compounds. The first is a purely synthetic compound, Ro 2-7239, studied extensively by Dorfman. It is an anti-androgen in both the chick and the rat, is related to the steroids but missing ring D. The other reported anti-androgen is A-norprogesterone (5).

Our own recently reported study (6) indicated very strongly that the oxygen function at C-17 of steroids is essential for a high

order of androgenic activity. We believe it represents a major site for attachment to the "activity enzyme" in target tissues. Since simple removal of this oxygen function does not lead to potent anti-androgens, we felt it important to substitute a methyl group for the oxygen function. It was believed that the methyl group would not be a binding site for an enzyme substrate but would block the active site of the enzyme in the region of C-17. In order to assist the fit it was thought advantageous to remove the angular methyl group, C-18. Accordingly, a series of 18-nor-17,17-dimethyl-androst-13-enes were synthesized (Table I).

Materials and Methods

We have employed two major bioassays in our search for anti-androgens, the comb of the testosterone-treated chick and the ventral prostate of the testosterone-treated rat. Our firm belief is that it is better to employ small amounts of androgen which produce significant stimulation, since we are interested in finding compounds able to inhibit completely the effect of the androgen.

We use single comb White Leghorn cockerels brought to the laboratory on the day of hatching and started on study the next day. We have either used the Dorfman method (3) of giving testosterone enanthate, 0.5 mg. as a single injection at the start of therapy, or have applied testosterone directly to the comb. The steroids are dissolved in mineral oil which is then diluted with 99 volumes of ethyl ether. This solution of a suitable concentration is applied to the comb, using a 0.25 tuberculin syringe and a 27 gauge needle, in a volume of 0.05 ml. each day for 7 days. The chicks are sacrificed with ether 48 hours after the last application. They are weighed and the combs excised with standard 6 inch, sharp and blunt operating scissors applied as closely as possible to the skull.

The rat assay employs immature male rats of the homozygous Fischer strain bred in our laboratories and weighing 44-55 grams when they are castrated under ether anesthesia. On the day of castration they are started on the first of 7 daily injections and are autopsied 24 hours after the last injection. The steroids are given subcutaneously in 0.1 ml. of sesame oil. At sacrifice the ventral prostate, the right seminal vesicle and the levator ani muscle are dissected out and weighed. In this assay we generally employed 1 microgram of testosterone per day.(6) We have had no compounds to date sufficiently anti-androgenic so that we felt warranted in the routine use of greater doses of testosterone. The testosterone is always given subcutaneously and the antagonist either injected at a separate site or given orally.

Table I (a)

		m.p.	$[\alpha]_D^{18°}$
RBG 10	17,17-Dimethyl-18-norandrosta-4,13-dien-3-one (b)	69-72°	+ 41°
RBG 33	17,17-Dimethyl-18-norandrosta-4,13-dien-3β-ol	137-140°	- 19°
RBG 44	17,17-Dimethyl-4-hydroxy-18-norandrosta-4,13-dien-3-one	119-122°	+ 3°
RBG 45	2α,17,17-Trimethyl-18-nor-5α-androst-13-en-3-one (c)	125-129°	- 5°
RBG 46	17,17-Dimethylgona-4-13-dien-3-one (d)	93-96°	- 14°
RBG 50	2α,17,17-Trimethyl-18-norandrosta-4,13-dien-3-one	82-86°	+ 53°
RBG 58	17,17-Dimethyl-18-nor-5α-androst-13-en-3-one (e)	134-135°	- 8°
RBG 59	17,17-Dimethyl-18-nor-5β-androst-13-en-3-one	oil (f)	- 7° (f)
RBG 64	17,17-Dimethyl-18-nor-5β-androst-13-en-3α-ol	116-117°	- 4°
RBG 83	17,17-Dimethyl-18-norandrosta-5,13-dien-3β-ol (g)	132-135°	-188°
RBG 107	17,17-Dimethyl-A,18-dinorandrosta-4,13-dien-3-one	oil (f)	- 61° (f)

(a) All compounds except the oils were recrystallized from dilute ethanol. All melting points were determined on a Köfler microstage. All optical rotations were determined with an O. C. Rudolph Model 131 polariscope, 1 dm. tubes, ethanol as solvent except when noted to the contrary.

(b) Reported (11): m.p. 66-70°

(c) Reported (11): m.p. 118-121°

(d) Reported (10): m.p. 91-93° $[\alpha]_D$ -12°

(e) Reported (12): m.p. 141-142° $[\alpha]_D$ -2°

(f) Could not be crystallized after repeated attempts. Optical rotations in chloroform. Thin layer chromatography (silica gel, petroleum ether 100%) disclosed a single spot.

(g) Reported (9): m.p. 129-131° $[\alpha]_D$ -190.2°

Results

Before presenting the results with our own compounds we would like to discuss results in our assay systems with the previously reported androgen antagonists mentioned above.

We have assayed A-norprogesterone both by our methods as outlined above and by using the androgen amounts, routes and rats as reported by the previous workers (5). We were able to confirm their findings that A-norprogesterone is non-androgenic, even in amounts up to 25 mg. per day per rat. On the other hand, calculated on the basis of ventral prostate weights, they were able to achieve from 47 to 96% inhibition of the testosterone effect with doses of A-norprogesterone of 1, 5 and 25 mg. In our hands, using our Fischer rats, such dosage of A-norprogesterone yielded inhibitions of 22 to 39 and 46% (Table II).

Employing day-old, single comb White Leghorn chicks given a single injection of 0.5 mg. testosterone enanthate, we were able to achieve 75% inhibition of the comb growth increment with 0.5 mg. of A-norprogesterone as opposed to 100% reported by the Squibb group.

Tables III and IV present representative assays of the many we have done with RBG 10.

Table V is a summary of the assays which we have performed on the eleven 17,17-dimethyl-18-nor steroids which we have prepared. We have in every case used the same stimulating dose of testosterone. For the local chick comb inhibiting assays we have always used 1 mg. of the inhibitor applied to the chick comb, whether the stimulator is given as 1 μg. also applied to the chick comb but at a different time, or as the single injection of 0.5 mg. of testosterone enanthate. In the rat parenteral assay we have employed either 1 or 10 mg. of the inhibiting steroid per day. In general, where 1 mg. is shown in the table it is because the 10 mg. dose was androgenic. The same is true of the rat

Table II

Rat Anti-Androgen Assay

Fischer Males, 45-55 gms.

Compound	No. Rats	Daily Dose	Levator Ani (mg)±S.D.	Seminal Vesicle (mg)±S.D.	Ventral Prostate (mg)±S.D.	Inhibition*
Controls	5	--	11.1±0.8	3.1±0.2	10.6±0.5	
Testosterone	5	1γ	17.8±2.6	4.8±0.2	17.2±3.4	
	5	10γ	20.0±3.0	6.8±0.2	27.4±2.5	
	5	100γ	34.6±7.8	19.8±6.2	49.4±4.5	
A-Nor-progesterone	5	1 mg	21.6±3.1	4.6±0.8	11.7±0.5	
	5	5 mg	13.7±3.5	4.2±0.7	11.9±2.1	
	5	25 mg	18.2±5.8	6.4±0.5	13.0±2.1	
A-Nor-progesterone + testosterone	5	1 mg) 100γ)	33.0±3.9	20.2±3.9	40.8±8.8	22%
A-Nor-progesterone + testosterone	5	5 mg) 100γ)	27.1±2.9	17.6±2.3	34.4±5.2	39%
A-Nor-progesterone + testosterone	5	25 mg) 100γ)	24.0±2.6	15.8±2.3	31.4±2.7	46%

* As per cent of increment in ventral prostate weight

oral assay, where the stimulating testosterone is given subcutaneously and the antagonist given orally.

Each of these assays has been repeated at least 3 times and with other dosage levels, but it was not felt that presentation of all the data would improve the points being made. The data presented are considered as typical. The rat data are calculated on the basis of inhibition of ventral prostate stimulation.

It is immediately apparent from perusal of the table that even employing these small amounts of testosterone we rarely achieved complete inhibition with certain anti-androgens, and these instances were when the anti-androgens were applied locally to the chick comb.

Also apparent from Table V is the fact that there is no consistency among the 4 assays employed. The highest scoring compound is RBG 10. Nor is there any real consistency among the compounds in the

Table III

Chick Anti-Androgen Assay

Compound	No. Chicks	Dose/Day	Comb wt. (mg) ± S.D.	Inhibition
Controls	15	---	36.3±12.2	
Testosterone	15	1γ	60.8±14.8	
	15	10γ	95.2±24.3	
Testosterone enanthate	15	0.5 mg i.m. x 1	82.4±21.3	
RBG 10	15	316γ	25.8±6.4	
	15	1 mg	29.7±7.8	
RBG 10 + testosterone	15	316γ) 1γ)	31.3±14.5	100%
RBG 10 + testosterone	15	1 mg) 1γ)	36.9±13.2	100%
RBG 10 + testosterone enanthate	15	1 mg) 0.5 mg i.m.) x 1)	55.3±16.4	59%

various assays. For example, RBG 58 (5α-dihydro derivative) is the only compound that did not appear to inhibit either of the chick comb assays, produced only 10% inhibition when given parenterally in the rat, yet has consistently achieved our highest inhibition when administered orally to the rat.

We are unable to correlate the distribution of inhibitions observed with the detailed structure of the compound. On the other hand, removal of C-19 or changing ring A to the five-carbon A-nor ring does not greatly change the overall distribution of anti-androgenic activity, provided that the 4-ene-3-ketone group is retained. On the other hand, the introduction of a 2α-methyl group sharply decreases overall anti-androgen activity.

Chemistry

Retropinacolic elimination of a 17-hydroxy group with attendant migration of the C-18 methyl group to C-17 has been utilized as a means of preparation of 18-nor steroids (7). This type of rearrangement was first observed by Cohen and coworkers (8) for 17α-methylestradiol-3-methyl ether. For the androstane series, Tortorella and coworkers (9) reported the retropinacol rearrangement of 17-methyl-5α-androstane-3β,17β-diol and 17-methylandrost-5-ene-3β,17β-diol to 17,17-dimethyl-18-nor-5α-androst-13-en-3β-ol and 17,17-dimethyl-18-norandrosta-5,13-dien-3β-ol respectively.

Table IV

Rat Anti-Androgen Assay

Compound	No. Rats	Daily Dose	Levator Ani (mg) ±S.D.	Seminal Vesicle (mg) ±S.D.	Ventral Prostate (mg)±S.D.	Inhibition *
Controls	5	--	8.8±1.8	2.5±0.4	8.4±0.6	
Testosterone	5	1γ s.c.	13.6±1.5	3.6±0.7	15.8±3.1	
	5	10γ s.c.	15.0±2.6	5.6±1.2	21.8±1.4	
RBG 10	5	10 mg s.c.	13.4±2.6	2.5±0.7	9.4±1.0	
	5	10 mg p.o.	12.8±0.6	2.5±0.5	10.3±0.7	
RBG 10 + testosterone	5	10 mg s.c.) 1γ s.c.)	11.6±1.9	2.7±0.4	10.6±1.0	70%
RBG 10 + testosterone	5	10 mg p.o.) 1γ s.c.)	13.0±1.8	2.6±0.8	10.4±1.4	73%

* As per cent of increment in ventral prostate weight

Table V

Anti-Androgen Assays

	Chick Local			Rat Parenteral		Rat Oral	
		INHIBITION		INHIBITION		INHIBITION	
Compound	Dose mg/day	Testosterone 1 μg/day-local	Test. enanthate 0.5 mg i.m. x 1	Dose mg/day	Testosterone 1 μg/day s.c.	Dose mg/day	Testosterone 1 μg/day s.c.
RBG 10	1 mg	100%	59%	10 mg	70%	10 mg	73%
RBG 33	1 mg	55%	---	10 mg	0	10 mg	67%
RBG 44	1 mg	65%	---	1 mg	0	1 mg	49%
RBG 45	1 mg	11%	---	10 mg	0	10 mg	74%
RBG 46	1 mg	82%	89%	10 mg	65%	10 mg	43%
RBG 50	1 mg	27%	---	10 mg	17%	10 mg	29%
RBG 58	1 mg	0	0	10 mg	10%	10 mg	86%
RBG 59	1 mg	100%	24%	10 mg	10%	10 mg	24%
RBG 64	1 mg	22%	28%	1 mg	36%	1 mg	6%
RBG 83	1 mg	92%	5%	10 mg	25%	10 mg	75%
RBG 107	1 mg	90%	67%	10 mg	21%	10 mg	61%

The location of the double bond between C-13 and C-14 for the retropinacol rearranged products of 17β-hydroxy-17-methyl steroids has been confirmed by nuclear magnetic resonance data (NMR) for 17,17-dimethylgona-4,13-dien-3-one (10), 17,17-dimethyl-18-norandrosta-5,13-dien-3β-ol (9), 17,17-dimethyl-18-norandrosta-4,13-dien-3-one and 2α,17,17-trimethyl-18-nor-5α-androst-13-en-3-one (11). Because of the above mentioned reports backed up by NMR data, that Wagner-Meerwein rearrangements of representative 17β-hydroxy-17-methyl steroids consistently lead to 17,17-dimethyl-18-nor-13-eno steroids, all of the compounds described in this report were assigned the 17,17-dimethyl-18-nor-13-ene structure.

While infrared absorption data (Table VI) would not be conclusive for rearranged compounds containing the 4-ene or 5-ene in addition, because of the possibility of interference by the C-4-H band of 4-enes around 11.5μ or the C-6-H band of 5-enes around 12.5μ, the spectra of the 5α or 5β saturated compounds did not show any bands between 11 and 15μ that would have indicated a double bond with hydrogen atoms, thus ruling out the possibility that a double bond with other than 13-ene (no hydrogens on the double bond, therefore no bands between 11-15μ) could have anomalously arisen from the Wagner-Meerwein rearrangements.

Chemistry - Experimental

Nomenclature, melting point and specific optical rotation data of the compounds are presented in Table I. Infrared absorption data of the rearranged compounds and their starting products are presented in Table VI.

Preparation of 17,17-dimethyl-18-norandrost-13-enes

Hydrochloric Acid Method: Concentrated hydrochloric acid, 50 ml. for each gram 17β-hydroxy-17-methyl steroid used, was stirred with the appropriate steroid. Within 5 minutes after solution was effected, the rearranged product precipitated out. A sufficient amount of distilled water was added to complete precipitation, the solid was collected by filtration under reduced pressure, washed free of acid by a sufficient amount of distilled water and recrystallized from dilute ethanol. Prepared by this method was RBG 10 - 80% yield.

Acetic Acid-Hydrochloric Acid Method: The appropriate 17β-hydroxy-17-methyl steroid was dissolved in glacial acetic acid, 2 ml. for every gram steroid used, and concentrated hydrochloric acid of an equal volume was then added. After 30 minutes at room temperature a sufficient amount of distilled water was added to complete precipitation of the rearranged product. The workup of the precipitate was the same as previously described. Prepared by this method were: RBG 10 (55% yield), RBG 44 (80% yield), RBG 45 (68% yield), RBG 50 (70% yield), RBG 58 (70% yield), RBG 59 (74% yield), RBG 64 (18% yield) and RBG 107 (52% yield).

Hydrochloric Acid-Ethanol Method: The appropriate 17β-hydroxy-17-methyl steroid was vigorously refluxed in an ethanolic solution of hydrochloric acid, 4 ml. ethanol and 1 ml. concentrated hydrochloric acid for each gram steroid used, for 30 minutes. Afterwards, the solution was diluted with a sufficient amount of distilled water to complete precipitation and the precipitate was worked up as usual. Prepared by this method was RBG 83 (94% yield).

17,17-Dimethyl-18-norandrosta-4,13-dien-3β-ol (RBG 33)

Eight grams of 17,17-dimethyl-18-norandrosta-4,13-dien-3-one (RBG 10) was dissolved in 80 ml. ethanol and treated with 2 g. sodium borohydride previously dissolved in 10 ml. distilled water. After 30 minutes the solution was poured into 300 ml. distilled water containing 30 ml. glacial acetic acid. The precipitated solid was collected by

Table VI. Infrared (a) Data of 17-Methyl-17β-hydroxy Steroids and Their Rearranged Products

	OH Str.	CH Str.	C=O Str.	C=C Str. (b)	OH Bend	CH Rock (C=C) (b)
17-Methyl-17β-hydroxyandrost-4-en-3-one	2.80	3.40	6.03	6.22	8.68	11.45
RBG 10	--	3.42	6.01	6.23	--	11.43,11.60
17-Methyl-4,17β-dihydroxyandrost-4-en-3-one	2.80,2.97	3.40	6.05	6.18	8.74(d)	11.40
RBG 44	2.95(c)	3.40	6.05	6.18	-- (d)	11.30
2α,17-Dimethyl-17β-hydroxy-5α-androstan-3-one	2.83	3.40	5.90	--	8.60	--
RBG 45	--	3.40	5.86	--	--	--
17-Methyl-17β-hydroxyestr-4-en-3-one	2.92	3.40	6.05	6.24	8.71	11.35
RBG 46	--	3.40	6.03	6.22	--	11.15
2α,17-Dimethyl-17β-hydroxyandrost-4-en-3-one	2.87	3.40	6.03	6.20	8.60	11.35
RBG 50	--	3.43	6.05	6.22	--	11.35
17-Methyl-17β-hydroxy-5α-androstan-3-one	2.82	3.32	5.87	--	8.69	--
RBG 58	--	3.36	5.82	--	--	--
17-Methyl-17β-hydroxy-5β-androstan-3-one	2.88	3.37	5.83	--	8.71	--
RBG 59	--	3.37	5.82	--	--	--
17-Methyl-5β-androstane-3α,17β-diol	2.95	3.37	--	--	8.62(f)	--
RBG 64	2.88(e)	3.35	--	--	-- (f)	--
17-Methyl-androst-5-ene-3β,17β-diol	2.90,3.00	3.35	--	5.95(g)	8.62(h)	12.48(g)
RBG 83	2.90(e)	3.33	--	5.95(g)	-- (h)	12.27(g)
17-Methyl-17β-hydroxy-A-norandrost-4-en-3-one	2.86	3.38	5.99	6.20	8.72	11.83
RBG 107	--	3.38	5.90	6.19	--	11.82

(a) Perkin-Elmer Infracord (R) model 137, KBr pellets, except for the oils (RBG 59, RBG 107) which were analyzed as film on NaCl cells. The starting product is given first, then its rearranged product (RBG no.) follows. Assignment of a given band to a particular structural feature was made after consulting the Colthup chart (13). (b) 4-ene except when noted to the contrary. (c) 4-Hydroxyl. (d) In addition, there are bands at 8.62-8.65 and 9.25-9.30μ either or both of which may be assigned to the 4-hydroxyl. (e) 3-Hydroxyl. (f) In addition, there is a 9.60μ band present for both compounds which may be assigned to the 3α-hydroxyl (equatorial). (g) 5-ene. (h) In addition, there is a band between 9.45-9.50μ for both compounds which may be assigned to the 3β-hydroxyl (equatorial).

filtration under reduced pressure and recrystallized from dilute ethanol to afford 5.8 g. (72% yield) of the title compound.

Discussion

It would appear that the premise of the transposition of the 18-methyl group to the 17 position with removal of the 17-oxygen function of a series of androgens indeed affords the production of compounds which are capable of inhibiting the androgenicity of administered testosterone in both the chick and the rat. Unfortunately, however, the antagonist/testosterone ratios of even the most effective compounds are so high that it would require unreasonably large doses to adequately inhibit androgenicity in man.

Summary

A series of 11 steroidal androgen antagonists (all 18-nor-17,17-dimethyl steroids) have been prepared. The most active anti-androgen of this series was RBG 10 (17,17-dimethyl-18-norandrosta-4,13-dien-3-one).

ACKNOWLEDGEMENTS

This study was supported by grant CA 03603-07 from the National Institutes of Health, Public Health Service.

For the steroid starting materials supplied to us for this study we wish to thank the Cancer Chemotherapy National Service Center of the National Institutes of Health, as well as the Squibb Institute for Medical Research (E. R. Squibb and Sons), Syntex Corporation and the Ciba Pharmaceutical Company.

A preliminary report on some of this material was given at the International Congress on Hormonal Steroids, Milan, Italy, 1962 (14).

REFERENCES

1. Kagawa, C.M., Cella, J.A. and Van Arman, C.G.: SCIENCE 126:1015 (1957)
2. Emmens, C.W., Cox, R.I. and Martin, L.: RECENT PROGRESS IN HORMONE RESEARCH 18, ed. G. Pincus, Acad. Press, N.Y. 1962, p. 415
3. Dorfman, R.I.: METHODS IN HORMONE RESEARCH II, ed. R.I.Dorfman, Acad. Press, N.Y. 1962, p. 315

4. Dorfman, R.I.: ACTA ENDOCRIN. 45:40 (1964)
5. Lerner, L.J., Bianchi, A. and Borman,A.: PROC. SOC. EXP. BIOL. and MED. 103:172 (1960)
6. Segaloff, A. and Gabbard, R.B.: ENDOCRINOLOGY 71:949 (1962)
7. Johns, W.F.: J. ORG. CHEM. 26:4583 (1961)
8. Cohen, A., Cook, J.W. and Hewett, C.L.: J. CHEM. SOC. 445 (1935)
9. Tortorella, V., Lucente, G. and Romeo, A.: ANNAL. CHIM. (Rome) 50:1198 (1960)
10. Kirdani, R., Dorfman, R.I. and Nes, W.R.: STEROIDS 1:219 (1963)
11. Caspi, E. and Pitiak, D.M.: CAN. J. CHEM. 41:2294 (1963)
12. Pelc, B.: COLLECTION CZECH. CHEM. COMMUN. 29:1029 (1964)
13. Colthup, N.B.: J. OPT. SOC. AM. 40:397 (1950)
14. Segaloff, A. and Gabbard, R.B.: EXCERPTA MEDICA 51 (Abstract) Internat'l Congress on Hormonal Steroids, Milan 1962, p. 216