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Anabolic-Androgenic Activities of Some Dimethylhydrazone Derivatives of Testosterone.* (26912)

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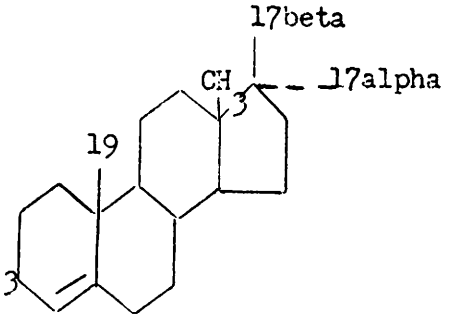
Undesirable masculinization has long been a liability to the use of testosterone and its esters as anabolic agents. The introduction of 17alpha-ethyl-19-nortestosterone(1) marked the initial step in separation of the androgenic from the anabolic component, while retaining the latter activity. In their recent review of the anabolic steroids Berczeller and Kupperman(2) included in their listing of the clinical problems in which these drugs are indicated such situations as the agonadal state, osteoporosis, postoperative catabolism, chronic administration of corticosteroids, retarded growth in pediatrics, and aging in geriatrics. The authors point out further that, in spite of the numerous clinical indications for anabolic agents, "a universally acceptable anabolic agent without untoward side effects has yet to be developed."

Clinton *et al.*(3) reported synthesis of several steroidal [3,2-c] pyrazoles with varied endocrinologic activities. Arnold *et al.*(4) and Potts *et al.*(5) have described the anabolic-androgenic activities of andro-stanazole (17beta-hydroxy-17alpha-methylandrostano-[3,2-c]pyrazole). In light of the high order of activity of this compound it was of interest to examine the testosterone-like activity of certain dimethyl-hydrazone derivatives of testosterone.

Methods. The compounds (Table I) were administered subcutaneously as solutions in sesame oil in a volume of 0.1 cc per day. Anabolic-androgenic activities were determined in castrated, 21-day-old male rats of the Mead Johnson colony (McCollum strain) by noting the increases in weight of the levator ani muscle, seminal vesicle, and ventral prostate gland, as described by Hershberger *et al.*(6). The relative anabolic-androgenic activities were calculated as follows: L.A./V.P. ratio = (levator ani response of test group—L.A. response of control)—(ventral prostate response of test group—V.P. response of control)(6).

* The compounds described herein were synthesized by Dr. R. H. Wiley, Dept. of Chemistry, Univ. of Louisville, Ky.

TABLE I. Structural Relations of Certain Dimethylhydrazone Derivatives of Testosterone.



Compound name	Compound No.	3	17	19
testosterone dimethylhydrazone	I	$(CH_3)_2N-N=$	-OH beta	CH_3
methyltestosterone dimethylhydrazone	II	$(CH_3)_2N-N=$	$-CH_3$ alpha; -OH beta	CH_3
19-nortestosterone dimethylhydrazone	III	$(CH_3)_2N-N=$	-OH beta	H
cis testosterone dimethylhydrazone	IV	$(CH_3)_2N-N=$	-OH alpha	CH_3
testosterone propionate	V	=O	$-O-C:O-C_2H_5$ beta	CH_3
norethandrolone (Nilevar®)	VI	=O	$-C_2H_5$ alpha; -OH beta	H

Estrogenic activity was assayed by the method of Lauson *et al.*(7), measuring the increase in uterine weight in 21-23-day-old, female rats. Progestational activity was studied by the deciduoma test in adult, ovariectomized rats as outlined by Zarrow *et al.* (8), using an intraluminal injection of 1.0 mg histamine dihydrochloride in the right horn as the pseudofertilization stimulus.

Results. Of these 4 dimethylhydrazone derivatives of testosterone only compound II exhibited significant androgenic activity on both the seminal vesicle and ventral prostate gland (Table II). Compound III showed relative, but insignificant, anabolic activity. On an absolute basis compound III was not even of the order of potency of testosterone propionate (V) and was considerably weaker than the highly active anabolic agent, norethandrolone (VI; Nilevar®). Undoubtedly as a reflection of the fact that it is a 19-nortestosterone derivative, the data obtained with compound III did reveal a relatively greater anabolic component than in compounds I, II, IV, or V. In this regard the rats receiving compound III actually showed a slight decrease in the response of the seminal vesicle. The only other compound, including norethandrolone (VI), with which this was evident was compound IV, whose overall activity was in fact negligible. As with compound VI, compound III was less active orally than parenterally.

Similar dimethylhydrazone derivatives of pregnenolone, progesterone, and ethisterone showed no progestational activity in the deciduoma test in estrone-primed, ovariectomized rats, and estrone dimethylhydrazone was only 1/32nd as active as estrone itself in increasing the uterine weight of immature rats.

Discussion. This relatively small series of dimethylhydrazone derivatives of the 3 principal types of sex hormones can not be construed as sufficiently large to draw extensive conclusions regarding structure-activity relationships. However, the relatively negative findings are sufficiently clear-cut to indicate that substitution of the dimethylhydrazone moiety tends to reduce markedly the

TABLE II. Anabolic-Androgenic Activities of Certain Dimethylhydrazone and Other Steroids.

Compound No.	Dose, μg/rat s.c.*	No. rats	Anabolic response (levator ani)	Androgenic response (ventral prostate)	Androgenic response (seminal vesicle)	L.A./V.P. ratio‡
			% final body wt × 1000†			
I	0	10	25.6 ± 1.5‖	6.8 ± .4‖	6.6 ± .6‖	—
	25	9	25.8 ± 1.1	9.1 ± .3	5.6 ± 1.4	.09
	200	9	30.2 ± 1.7	25.3 ± 2.3	10.2 ± .8	.25
II	0	9	30.1 ± 1.7	11.9 ± 1.3	8.3 ± .4	—
	25	10	32.3 ± 1.7	24.9 ± 3.2	13.4 ± 1.7	.17
	200	10	34.2 ± 2.4	64.7 ± 8.9	39.6 ± 4.6	.08
III	0	8	31.8 ± 2.0	10.6 ± .5	10.5 ± 1.0	—
	25	9	31.6 ± 1.4	11.3 ± .7	9.7 ± .8	−.3
	200	10	35.6 ± 2.9	15.6 ± 1.8	8.1 ± .8	.76
III	0	8	31.7 ± 1.0	11.0 ± .6	9.1 ± .5	—
p.o.§	100	10	34.4 ± 1.9	15.0 ± .9	9.8 ± .4	.68
	400	10	35.5 ± 1.6	17.6 ± 1.5	9.8 ± .8	.58
IV	0	8	26.7 ± 1.3	11.2 ± .4	8.6 ± 1.2	—
	25	10	27.0 ± 1.7	9.9 ± .5	8.8 ± .8	−.23
	200	10	26.8 ± 1.0	17.4 ± 1.4	7.5 ± .4	.02
V	0	52	27.0 ± .6	10.4 ± .5	8.5 ± .3	—
	12.5	17	32.5 ± 1.4	38.0 ± 1.9	24.3 ± 1.3	.17
	100	22	52.8 ± 1.8	93.9 ± 2.9	86.1 ± 2.6	.3
VI	0	9	27 ± 1.9	10.8 ± 1.3	8.1 ± .4	—
	12.5	10	52.2 ± 7.8	13.5 ± 1.1	8.5 ± 1.0	9.3
VI	0	10	28.5 ± 1.8	9.5 ± .6	7.9 ± .4	—
p.o.§	25	9	31.0 ± 1.2	10.6 ± .7	9.3 ± 1.0	2.3
	200	10	39.2 ± 1.7	12.5 ± .9	7.2 ± 1.0	3.6

* Animals dosed s.c. immediately post-castration and for 6 days thereafter, and sacrificed 24 hr following 7th dose; dosage vol 0.1 cc; controls received 0.1 cc vehicle (sesame oil) only.

† % final body wt × 1000 = (tissue wt in g ÷ final body wt in g) × 100000.

‡ L.A./V.P. ratio calculated as described by Hershberger *et al.* (text ref. No. 6) to obtain index of anabolic activity: (levator ani response of test − L.A. response of control) ÷ (ventral prostate response of test − V.P. response of control).

§ Note oral route; methocel vehicle; ingestion vol 0.25 cc.

‖ Mean ± S.E.

inherent activity of the parent compound. In the case of compound III, the hydrazone substituent did not block completely the qualitative separation of anabolic-androgenic activities that is characteristic of 19-nortestosterones.

That some arrangement of the –N–N= grouping on the steroid is not absolutely deleterious to the molecule is evident when one compares the data reported by Potts *et al.* (5) for andro-stanazole with that described herein for compound II, the compound in this series structurally most comparable to the cyclized andro-stanazole. The two sets of data indicate that andro-stanazole is infinitely more anabolic than compound II, but the latter is 1.5 to 2.5 times more androgenic. Exactly how the cyclic nature of the =CH–NH–N= system in andro-stanazole favors anabolic activity, especially upon oral administration, is uncertain, but the data reported herein indicate that the open-chained hydrazone system is more likely to cause a reduction in activity.

Summary. 1. Eight dimethylhydrazone derivatives of the sex hormones have been examined for anabolic, androgenic, estrogenic, and progestational activities in standard assay procedures in rats. 2. The dimethylhydrazone substituent induced a significant reduction in absolute activity relative to the parent compound, but it did not obliterate the qualitative separation of anabolic-androgenic activities in the 19-nortestosterone derivative.

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Identification of Heterozygous Carriers of Gargoylism. (26913)

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In recent years gargoylism (Hurler's syndrome, dysostosis multiplex) has been found to be a hereditary disorder of acid mucopolysaccharide (AMPS) metabolism(1-3). Thorough evaluation of pedigrees of affected families has indicated 2 forms of gargoylism: the autosomal recessive and the sex-linked recessive types(4). Many patients with overt gargoylism can be identified by an elevated urinary level of total AMPS(2-4) and by the presence of abnormal acid mucopolysaccharides such as chondroitin sulfate B(2-4) (ChS-B) and heparin monosulfate(3,5) (HMS) in their urine. Up to the present time, however, it has not been possible to identify chemically the heterozygous carriers of the gargoylism disease trait.

For the characterization of acid mucopolysaccharides, Hoffman and co-workers(6) have used the hexuronic acid carbazole/orcinol (C/O) color extinction ratio. In an analogous manner we have used the carbazole (7)/naphthoresorcinol(8) (C/N) color ratio. We obtained a C/N color ratio of 13.6 for chondroitin sulfate A* (ChS-A), 0.6 for chondroitin sulfate B*, 14.0 for chondroitin sulfate C* (ChS-C), and 12.2 for heparin monosulfate†. The C/O color ratios reported by Hoffman and co-workers(6) for ChS-A and ChS-C are only about 4 times the C/O ratio for ChS-B whereas the C/N color ratios we obtained for ChS-A and ChS-C are about 20 times the C/N ratio for ChS-B. A low C/N ratio appears to be characteristic of ChS-B.

The usefulness of the AMPS C/N ratio was discovered in our search for better methods of detecting the ChS-B and the elevated level of total AMPS in the urine of patients with clinical gargoylism. In some cases a simple quantitative index of the AMPS in urine was found to be inadequate for chemical verification of an abnormal AMPS excretion. Addition of the C/N ratio as an index of urine AMPS quality to the index of AMPS quantity has proved to be much more specific than the quantitative index alone. Our results indicate that patients with suspected gargoylism as well as their normal parents and other relatives with the heterozygous trait of gargoylism can now be identified.

Our method, performed on a single urine specimen, involves preliminary dialysis to remove most of the smaller uronic acid-containing molecules; concentration of the AMPS by precipitation with 5% cetyltrimethyl-ammonium bromide according to the DiFerrante and Rich(9) procedure; and determination of the AMPS hexuronic acid by the carbazole method of Dische(7) and the naphthoresorcinol method of Pelzer and Staib(8). The quantity and quality of the AMPS is then represented respectively as a

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