

SIGNIFICANCE OF THE HYDROXYL GROUPS OF STEROIDS IN
PROMOTING GROWTH*

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PLATE 30

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In the hypophysectomized rat which is guarded from contact with steroids in the ration and environment, the uterus and vagina are atrophic but they remain highly responsive to the difunctional steroids (1) which promote their growth and hence compose an assay system. Utilizing it we have found that functional groups at positions 3 and 17 in the steroids mentioned participate in the induction of growth, but that these physiologically active centers are equivalent neither in quantitative effects nor in the type of growth which they evoke. The state of oxidation at both sites and the degree of saturation of the A and B rings have much to do with determining the quantity and the pattern of the growth response.

In order to evaluate the importance of the individual structural elements of steroid hormones in accelerating cell growth, an investigation was made of steroids possessing an oxygen function at either position 3 or position 17 but not at both sites. The results to be set forth in this present paper demonstrate that in such monofunctional steroids of the androstane series the possession of a β -hydroxyl at a specific site, position 17, is essential for the stimulation of growth. In the phenolic estrane series, however, a compound, 17-desoxy-estradiol, with a single hydroxyl group at position 3 is active in promoting growth, a confirmation of earlier work (2).

A series of monofunctional steroids was investigated by Kochakian (3) with respect to their effects on the growth of the kidney, the seminal vesicle, and the prostate of castrate mice. The compounds tested were androstan-3 α -ol; androstan-3 β -ol; 5-androsten-3 β -ol; androstan-17 β -ol; 17-methylandrostan-17 β -ol; androstan-17-one; and 4-androsten-3-one. Kochakian (3) found that none of these steroids, administered by implantation as pellets, had biological activity, but the absorption of the compounds from the subcutaneous tissues was low. To overcome the difficulty of

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poor solubility, Kochakian (4) subsequently studied the effects of the oral administration of 17-methylandrostan-17 β -ol (0.1 to 0.2 mg. per gm. of food) for a 30 day period and observed that this compound was highly active in promoting growth of the kidney and, to a lesser extent, the prostate and seminal vesicles.

Prelog, Ruzicka, and Wieland (2) reported that 17-desoxyestradiol was moderately active in causing estrus in ovariectomized rats. It has been stated that 3-desoxyequilenin (5) has weak estrogenic properties, but this effect was not observed by Bachman and Wilds (6).

Methods

Biological.—The methods employed were those described in an accompanying paper (1). The hypophysectomized rats were injected with compounds, in sesame oil, at a dose of 1 mg. daily for 7 days beginning at age 38 days; necropsy was performed at age 45 days. The uterine weight ratio is the average of the weight of the uterus of 4 injected rats divided by a similar value of the uterus of 4 rats receiving the solvent alone. Most of the compounds were administered at 2 levels of dosage, 1 mg. and 3 mg. *per diem*.

When the ventral prostate was visible in the gross, its lobes were excised at necropsy and promptly weighed on a torsion balance.

Chemical.—Some of the steroids¹ employed in this study were obtained through the generosity of other investigators as follows: androstan-3-one, 2-androsten-3-one and androstan-3 β -ol, from Dr. T. F. Gallagher, Sloan-Kettering Institute for Cancer Research; androstan-17 β -ol, Dr. F. A. Travers, Ciba Pharmaceutical Products, Inc.; androstan-17-one, Dr. Abraham White, Chemical Specialties Co., Inc.

Certain steroids were synthesized in this laboratory by methods to be described (7) in detail elsewhere: androstan-3 α -ol, androstan-3 β -ol, 4-androsten-3 β -ol, 5-androsten-3 β -ol, androstan-17 β -ol, 4-androsten-17 β -ol, 5-androsten-17 β -ol, 3,5-cycloandrostan-17 β -ol, etiocholan-17 β -ol, 4-androsten-3-one, and 17-desoxyestradiol.

RESULTS

Weight of the Vagina, Prostate, and Uterus.—An increase of the ratio of uterine weight above unity was always accompanied by an increase in the weight of the vagina as well. Increased weights of the uterus and vagina occurred after the administration of the following compounds in the androstane series (Table I): Androstan-17 β -ol (I); 4-androsten-17 β -ol (II); and 5-androsten-17 β -ol (III). All other C₁₉-steroids in this series were inactive in so far as they failed to cause an increase of weight of these tissues.

Two of the compounds, androstan-17 β -ol (I) and 4-androsten-17 β -ol (II) caused growth of the prostatic glands (range of weight 7.2 to 10.4 mg.) to such extent that these vestigial structures became visible in the gross; the epithelium was cylindrical when examined with a microscope (Fig. 6).

An increased weight of the vagina and uterus occurred after the administration of 17-desoxyestradiol (XIV), but the quantities (Table II) required to produce growth were larger than the quantity that was needed with estradiol-17 β (XV).

¹ The chemical formulae of the steroids used in this paper are provided in Text-fig. 1.

TABLE I
The Uterine Weight Ratio and the Weight of the Vagina Following the Administration of 19-Carbon Steroids

No.	Compound	Uterine weight ratio*	Average weight of vagina
			mg.
I	Androstan-17 β -ol	3.0	55.7
II	4-Androsten-17 β -ol	1.4	26.2
III	5-Androsten-17 β -ol	1.4	25.1
IV	3,5-Cyclandrostan-17 β -ol	1.0	17.8
V	Etiocholan-17 β -ol	0.9	22.0
VI	Androstan-17-one	1.0	19.3
VII	2-Androsten-17-one	1.0	22.0
VIII	Androstan-3 α -ol	1.0	19.8
IX	Androstan-3 β -ol	1.0	22.4
X	4-Androsten-3 β -ol	1.0	21.8
XI	5-Androsten-3 β -ol	1.0	19.2
XII	4-Androsten-3-one	1.0	18.9
XIII	Androstan-3-one	1.0	20.9
	Sesame oil (control)	1.0	21.6

* Average weight of uteri of 4 rats injected with the compound divided by the value obtained from a similar group injected with the solvent alone.

TABLE II
The Weight of the Uterus and Vagina Following the Administration of 17-Desoxyestradiol and Estradiol-17 β

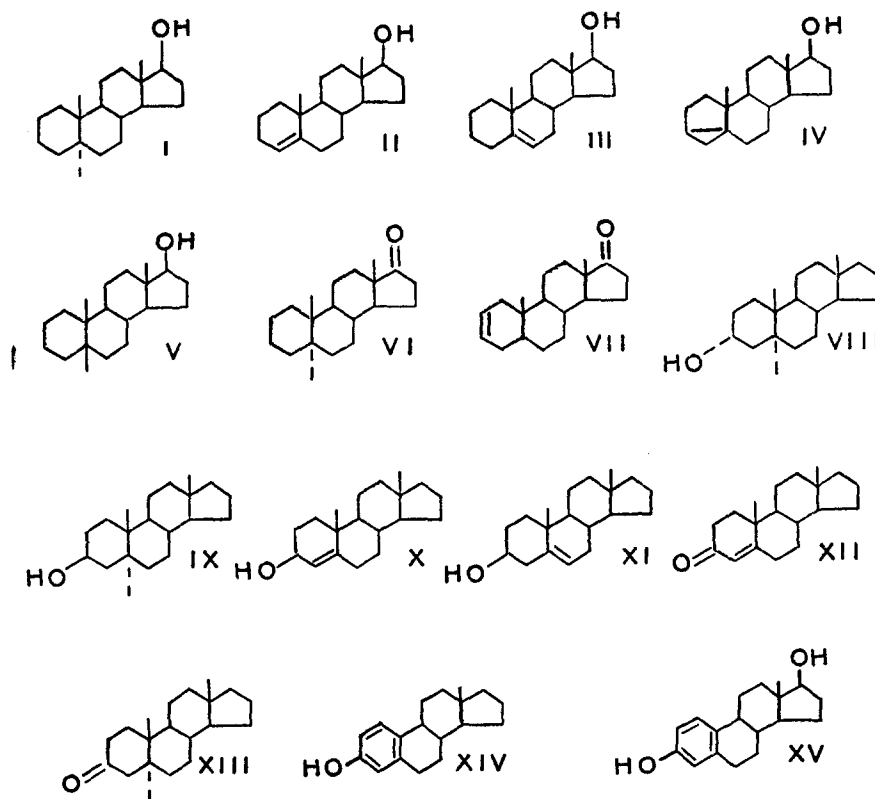
No.	Compound	Daily amount	Weight of uterus*	Weight of vagina*
		μ g.	mg.	mg.
XIV	17-Desoxyestradiol	1	21.9	23.9
	17-Desoxyestradiol	2.5	50.7	31.2
XV	Estradiol-17 β	0.005	24.5	24.4
	Estradiol-17 β	0.01	61.4	34.0
	Sesame oil		19.3	21.6

* Each value the average of 4 rats.

Action of Monofunctional Steroids on the Vaginal Epithelium.—Keratinization or stratification of the vaginal epithelium did not follow the administration of any monofunctional steroid in the C₁₉-series. In this series all the compounds which promoted growth of the vaginal epithelium produced an identical cellular pattern, with growth both of the basal cells and of the

mucous cells. Growth of this sort occurred after the administration of androstan-17 β -ol (Fig. 1); 4-androsten-17 β -ol (Fig. 2); 5-androsten-17 β -ol (Fig. 3). No other compound listed in Table I induced growth in the vaginal epithelium.

The administration of 17-desoxyestradiol at a daily level of 1 μ g. caused growth both of the basal cells and the mucous cells. An increase in dosage



TEXT-FIG. 1

of this compound to 2.5 μ g. *per diem* induced keratinization of the epithelium (Fig. 5).

DISCUSSION

The site of the functional group proved to be critical for the induction of growth by monofunctional steroids of the androstane series. Growth occurred only when the active group was present at position 17; steroids with an identical group at position 3 were devoid of the capacity to promote growth.

Further the state of oxidation of the chemical group at position 17 was critical in determining activity, in the growth process, of monofunctional steroids of the androstane series. Whereas the presence of a β -oriented hydroxyl group at position 17 conferred considerable activity in promoting growth, compounds with a ketone group at C₁₇ or C₃ were devoid of this capacity (Fig. 4).

In addition to possessing the 17 β -hydroxyl group the steroid nucleus must have certain geometric requirements in order to accelerate growth processes. Highest activity was observed in the saturated androstane compound with the 5 α -configuration (I). In contrast to observations with difunctional steroids (1) the presence of a Δ^4 or Δ^5 double bond (II, III) lowered the activity somewhat whereas 17 β -hydroxysteroids with the 5 β -configuration (V) or the *i*-steroid structure (IV) are completely inactive.

The influence of the 17 β -hydroxyl group on physiologic processes was further illustrated by the growth of the ventral prostate which followed the administration of androstan-17 β -ol (I), or 4-androsten-17 β -ol (II). Testosterone is known to have the property of inducing growth of the prostatic glands of female rats (8,9).

In sum, the capacity of the monofunctional steroids in the C₁₉-series to induce growth of the female genital tract would seem to require (a) the presence of a hydroxyl group at (b) a critical site, position 17, in a molecule which has (c) closely restricted geometric specifications.

In contrast to the C₁₉-steroids the promotion of growth by phenolic steroids of the estrane series does not require the presence of the 17 β -hydroxyl group although its presence increased their activity. In confirmation of previous findings (2) 17-desoxyestradiol markedly stimulated uterine growth and induced keratinization in the vagina, although larger quantities were required than with estradiol-17 β .

CONCLUSIONS

The presence of a 17 β -hydroxyl group endows the simple androstane molecule with the ability to produce growth of the uterus, vagina, and prostate of the female hypophysectomized albino rat. It appears that hydrogen atoms at position 17 are of critical importance since related compounds with a ketone group at this site are inactive. Monofunctional steroids with a hydroxyl or a ketone group at position 3 likewise are devoid of activity.

If a phenolic A-ring is present in monofunctional steroids the 17 β -hydroxyl group is not obligatory for growth. Proliferation of the uterus and vagina were found to follow the administration of 17-desoxyestradiol.

BIBLIOGRAPHY

1. Huggins, C., Jensen, E. V., and Cleveland, A. S., *J. Exp. Med.*, 1954, **100**, 225.
2. Prelog, V., Ruzicka, L., and Wieland, P., *Helvet. chim. acta*, 1945, **28**, 250.
3. Kochakian, C. D., *Am. J. Physiol.*, 1949, **158**, 51.

4. Kochakian, C. D., *Proc. Soc. Exp. Biol. and Med.*, 1952, **80**, 386.
5. Prelog, V., and Fuhrer, J., *Helvet. chim. acta*, 1945, **28**, 583.
6. Bachman, W. E., and Wilds, A. L., *J. Am. Chem. Soc.*, 1940, **62**, 2084.
7. Jacobson, H. I., Pratt, R., Myers, T. C. and Jensen, E. V., data to be published.
8. Korenchevsky, V., *J. Physiol.*, 1937, **90**, 371.
9. Deanesley, R., and Parkes, A. S., *Lancet*, 1938, **2**, 606.

EXPLANATION OF PLATE 30

The photomicrographs are of paraffin sections stained with hematoxylin and eosin. The compounds had been injected into hypophysectomized rats from age 38 to 44 days and necropsy was performed at age 45 days. The tissue, the compound injected with the daily dosage, and the magnification are stated.

FIG. 1. Vaginal epithelium of a rat injected with androstan-17 β -ol, 1 mg. Proliferation of the superficial and deep layers of the mucosa is shown. \times 500.

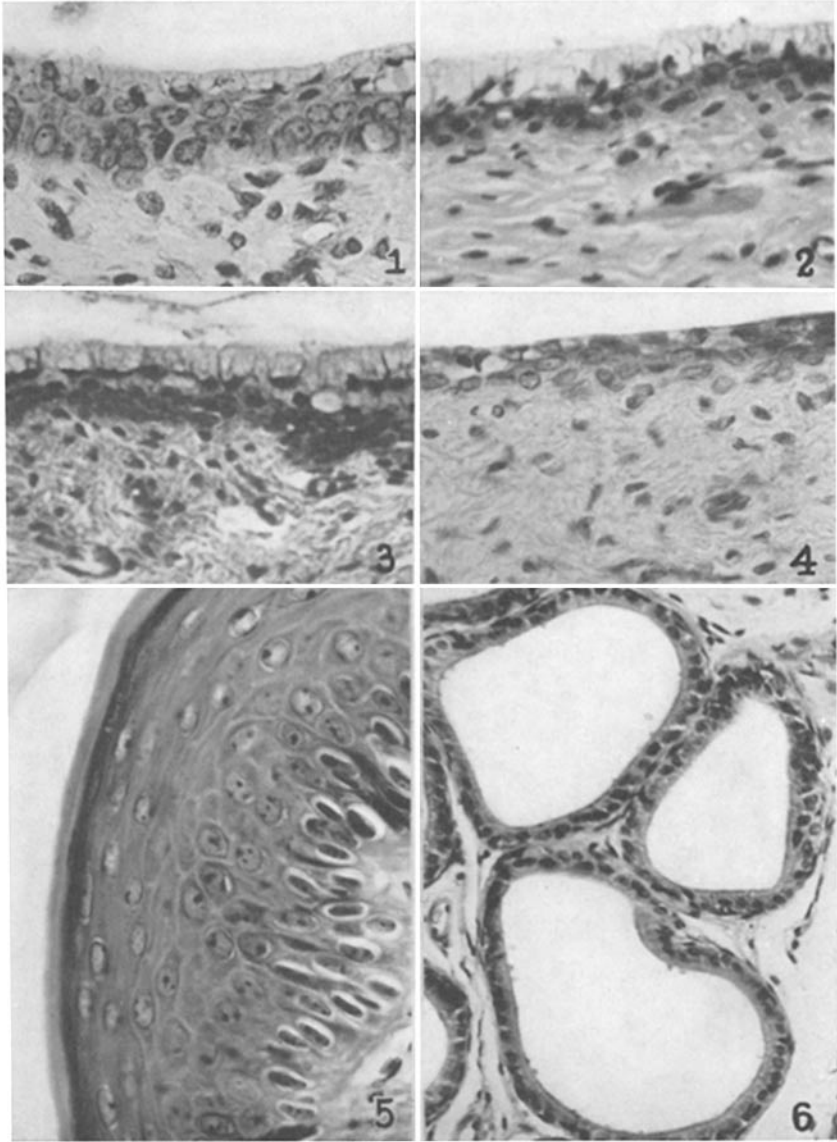
FIG. 2. Vaginal mucosa of a rat injected with 4-androsten-17 β -ol, 1 mg. While growth of the epithelium has occurred it is less than in rats injected with androstan-17 β -ol; see Fig. 1. \times 500.

FIG. 3. Vaginal mucosa of a rat injected with 5-androsten-17 β -ol, 3 mg. Growth of the epithelium induced by this compound is less than that which followed administration of androstan-17 β -ol; see Fig. 1. \times 500.

FIG. 4. Vaginal mucosa of a rat injected with androstan-17-one, 3 mg. This compound did not promote growth of the vaginal epithelium. \times 500.

FIG. 5. Extensive growth of the deep layer of the vaginal mucosa with keratin formation on the surface occurring in a rat injected with 17-desoxyestradiol, 2.5 μ g. \times 500.

FIG. 6. Proliferation of the female prostate of the rat induced by the administration of 4-androsten-17 β -ol, 1 mg. \times 275.



(Huggins and Jensen: Hydroxyl groups of steroids in promoting growth)