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Progesterone-Like Activity of a Series of 19-Nortestosterones. (23063)

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Hertz *et al.*(1) reported that 17α-ethynyl-19-nortestosterone is an orally effective progestational agent in rabbits, having an activity about 5 times that of 17α-ethynyltestosterone. Greenblatt(2) found the norcompound was also effective in women, when administrated orally. Ferin(3) has recently reported that 17-methyl-19-nortestosterone has progestational properties in women, and Overbeek(4) found this compound to be active in rabbits. Pincus *et al.*(5) extended the series, observing that 17-ethyl-19-nortestosterone also has progestational effects in rabbits.

In this laboratory, extensive studies on the biological properties of 19-nortestosterones carried out over the past several years, indicated a marked progestational activity of these compounds (Drill and Saunders).† The present paper deals with the progestational effects of a series of 19-nortestosterones in which the 17α side chain ranges from H to C_8H_{17}, with both saturated and unsaturated linkages.

Methods. White rabbits weighing about 2 kg were ovariectomized. After a 2 week recovery period, they were injected subcutaneously, daily for 6 days, with 5 μg estrone. On the day following the last injection the abdomen was opened and a 1-inch segment of each uterine horn was isolated after the method of McGinty *et al.*(6). The test substance, in 0.1 ml corn oil, was injected into one uterine horn while a like amount of corn oil was injected into the opposite horn as a control. Three days later, the animals were sacrificed, cross-sections of the uteri were fixed in Zenker's and prepared for histology. The degree of glandular proliferation was graded from +1 to +4. Additional studies were made, using intact, immature rabbits which had been primed with 6 daily injections of 5 μg estradiol each. They were then treated orally or subcutaneously with the test compound in oil, daily for 5 days. On the day after the last injection the animals were sacrificed and the uteri were studied histologically.

Results. Intrauterine assay. The average responses to the various doses are shown in Figs. 1-3. The ordinates are arbitrary scales on which 1 denotes that the endometrial glands are only small straight indentations, similar to those usually found in the estrogen-primed controls. Further development with slight branching of a few glands is designated 2, while 3 indicates that there are many branched glands. Grade 4 is reserved for those uteri which show so much branching that, in cross-section, many portions appear detached. The number of animals at each dose is also indicated. The average rating for 545 control, estrogen-primed uteri was 1.04. Only 2 of these control uteri had ratings of 3 and 4 respectively. In both of these cases the contralateral horn had been injected with a high dose of an active compound. A systemic effect, or leakage, may account for these false positives.

The activity of progesterone is shown in Fig. 1. It will be seen that a dose of 0.5 μg is adequate to produce a definite response, with an average value of 2.3. The data for

† Drill, V. A., and Saunders, F. J., *Proc. of a Conference on Anabolic Agents.* G. D. Searle & Co., Chicago, Apr. 9, 1956.

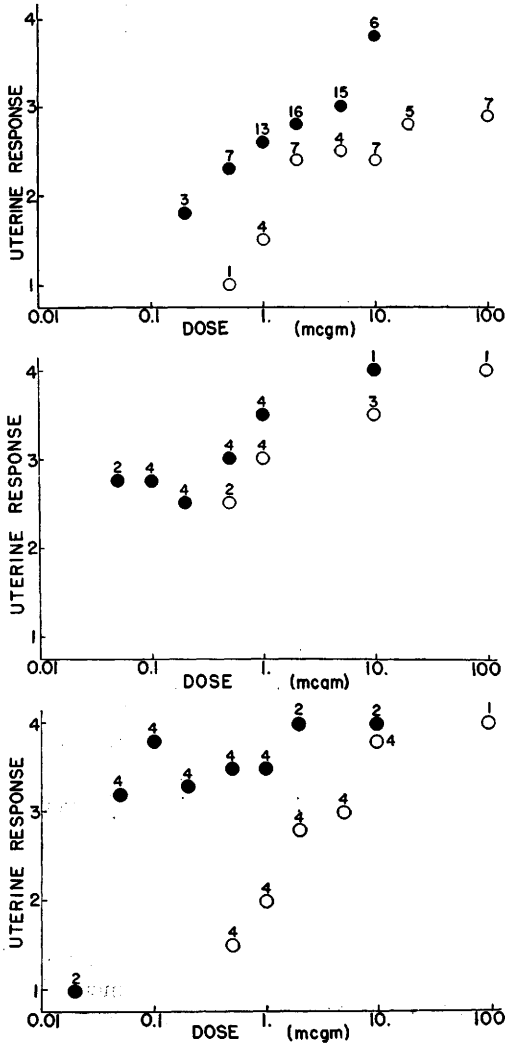

FIG. 1 (top). Responses of endometrial glands to local applications of progesterone (●) and 17-ethyl-19-nortestosterone (○). No. above points indicate No. of rabbits used.

FIG. 2 (middle). Responses of endometrial glands to local applications of 17-propyl-19-nortestosterone (●) and 17-octyl-19-nortestosterone (○). No. above points indicate No. of rabbits used.

FIG. 3 (bottom). Responses of endometrial glands to local applications of 17-butenyl-19-nortestosterone (●) and 17-allyl-19-nortestosterone (○). No. above points indicate No. of rabbits used.

19-nortestosterone and 17-methyl-19-nortestosterone are not presented, but in both cases, doses up to 100 μg were inactive, with average responses less than 1.5. On the other hand, 17-ethyl-19-nortestosterone gave responses indicating that this compound is about ¼ as active as progesterone in this assay. As the side-chain was lengthened, the activity was increased further, the propyl compound being about 5 times as active as progesterone (Fig. 2). However the octyl compound appeared to be somewhat less active than the propyl.

At least with the lower members of the series, unsaturated linkages in the side-chain appeared to decrease activity. The 17-vinyl and 17-ethynyl compounds were both inactive in this assay at doses up to 100 μg. The allyl compound was about ⅕ as active as the propyl (Fig. 3). The 17-butenyl compound is the most active tested to date (Fig. 3) but in preliminary tests the butyl compound appears to be nearly as potent. In the case of the butyl compound, the side-chain is straight; in the butenyl it appears to be branched.

17-Propyl-4,5-dihydro-19-nortestosterone and 11β-hydroxy-17-ethyl-19-nortestosterone were also found to be inactive at doses of 100 μg.

Systemic assay. Administered subcutaneously in the estrogen-primed immature rabbit (Clauberg Assay) a different order of activity was found. The minimal effective daily dose of progesterone was found to be 0.05 mg. 19-Nortestosterone was ineffective at 10 × this does but 17-methyl-19-nortestosterone was more active than progesterone. The 17-ethyl compound appeared to be 5 × as potent as progesterone. This value agrees with findings of Pincus *et al.*(5). However the butenyl compound, though more active than progesterone itself, was less active than 17-ethyl-19-nortestosterone. Table I shows the average ratings obtained with these compounds at the several doses, as well as the number of animals used.

Discussion. The group of nortestosterones studied here represents a wide range of biological activity. The androgenic and anabolic activities of some of this series have been reported(7). These data have been included in Table II to show the lack of correlation between the progestational response and androgenic or anabolic activity. While ana-

TABLE I. Endometrial Response in Estrogen-Primed Immature Rabbit after Subcutaneous Administration of Various Doses of Several Steroids (Clauberg Assay).

Compound	Daily dose, mg, subcut. 1.	.5	.2	.1	.05	.02	.01	.005
Progesterone	3.9 (4)	3.8 (2)	3.4 (4)	3.1 (7)	2.9 (8)	1.1 (8)		
19-nortestosterone	1.5 (4)	1.0 (1)	1.0 (2)					
17-methyl-19-nortestosterone	3.5 (2)	3.3 (4)	2.7 (6)	2.8 (6)	2.7 (6)	2.4 (6)	2.0 (3)	
17-ethyl-19- "	4.0 (1)	3.5 (2)	3.0 (3)	3.5 (4)	3.0 (6)	3.4 (5)	3.1 (6)	1.8(4)
17-vinyl-19- "	3.5 (2)			3.5 (2)				
17-allyl-19- "				3.3 (4)			3.0 (3)	
17-butenyl-19- "	3.3 (3)		3.9 (4)		3.7 (4)	3.3 (6)	2.2 (6)	1.0(4)
17-octyl-19- "			1.0 (4)	1.0 (4)			1.0 (4)	

Endometrial responses were graded from +1 to +4 (see text). When the avg response for 4 or more rabbits is above 2, the compound is considered active. No. in parentheses indicates No. of rabbits at that dose.

bolic and androgenic potencies were greatest among the lower members of the series, progestational effects were most marked in the higher members. In the saturated series, the methyl and ethyl derivatives as well as nortestosterone itself all have equivalent androgenic and anabolic potencies. However progestational effects increased markedly as the 17-side-chain was lengthened. This was especially noticeable in the intrauterine assay, where propyl and butyl compounds were found to be 5 times as active as progesterone and even the octyl compound showed marked activity.

TABLE II. Relative Activities of a Series of 19-Nortestosterones Compared to Several Standards.

Compound	Progestational potency Systemic	Intrauterine	Androgenic potency	Anabolic potency
Progesterone	100%	100%	<2	<5
Testosterone propionate		<1	100%	100%
19-nortestosterone	<10	"	6	100
19-nortest'ter'ne derivatives				
17-methyl-	250	<1	6	100
17-ethyl-	500	25	6	"
11β-hydroxy-17-ethyl-		<1	<2	<10
17-vinyl-	>50*	"	2	20
17-ethynyl-		"	estrogenic	1
17-propyl-	200*	500	<5	10
17-propyl-4,5-dihydro-	<5	<1	<2	<5
17-allyl-	500*	100	2	10
17-butyl-		500*	<2	<5
17-butenyl-	250	1000	2	5
17-octyl-	<25	100		

* Preliminary data.

The introduction of a double bond in the 2 carbon side-chain markedly reduced anabolic and androgenic activity. With a triple bond, these activities were further reduced and in fact the ethynyl compound shows some estrogenic effects. In both cases, progestational effects, as determined by intrauterine assay, were abolished although both compounds show endometrial gland proliferation when administered systemically. In the propyl series, introduction of a double bond in the side-chain (allyl) had little effect on androgenic or anabolic properties. As was the case with the 2 carbon side-chain, however, intrauterine assay showed a marked reduction in progestational activity. Administered systemically, both propyl and allyl compounds were significantly more potent than progesterone. Saturation of the 4-5 double bond of the propyl compound not only abolished its androgenic, anabolic and progestational effects but imparted to this compound anti-hypertensive properties.*

Several of the compounds were further tested for their ability to maintain pregnancy in ovariectomized rabbits. Does were bred and 10 to 14 days later they were ovariectomized. On the day of operation and continuing for 6 or 7 days they received 1 mg per kg of the various compounds daily, subcutaneously. They were sacrificed on the day after the last injection. All 9 control rabbits showed complete resorption of the fetuses with no apparent development of the fetus

* Data to be published by F. M. Sturtevant of this Division, 1957.

after spaying. On the other hand 14 of 15 rabbits treated with progesterone had 1 or more normal living fetuses at time of sacrifice. None of 5 rabbits treated with 19-nortestosterone showed any protection. With 17-methyl-19-nortestosterone 1 rabbit had living fetuses and the other 4 had some further placental development although no fetuses remained. With 17-ethyl-19-nortestosterone, 9 had one or more apparently normal fetuses. Two more showed some further development after spaying but the young were resorbing at the time of sacrifice. Three additional rabbits showed no protection. With 17-propyl-19-nortestosterone, 4 of the 5 rabbits showed normal development while the fifth showed decided fetal growth, although the young died before autopsy. The higher analogs have not yet been tested.

It has been pointed out that, compared to progesterone, some of these compounds are much more effective when administered systemically rather than directly into the uterus. Preliminary data indicate that the oral activity of this series, in general, is very marked with a high oral/parenteral ratio. These findings suggest that these norsteroids may be metabolized into more active compounds. Further studies on this series are in progress.

Summary. 1. Progestational activity of a series of 17-substituted 19-nortestosterone derivatives was investigated using both intrauterine and systemic assays in rabbits. Several of the substances studied possessed a high order of progestational activity. This activity was not related to either androgenic or anabolic potency of these compounds. Of the compounds studied 17-butenyl appeared to be the most potent, possessing about 10 times and 2½ times the activity of progesterone in intrauterine and systemic assays, respectively. 2. Progesterone-like activity of these 19-nortestosterones was further demonstrated by the ability of some members of the series to maintain pregnancy in ovariectomized rabbits.

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Effects of Aldosterone and Desoxycorticosterone on Tissue Electrolytes.* (23064)

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Desoxycorticosterone (DOC) has been used in replacement therapy for adrenal insufficiency for more than 20 years. A major portion of research on the salt-retaining function of the adrenal cortex has consisted in a comparison of the effects of adrenalectomy and administration of desoxycorticosterone acetate (DCA). The recent research on aldosterone, the natural hormone whose actions DOC mimics, has revealed not only quantitative but also qualitative differences between the 2 steroids(1). Inasmuch as the major biological role of these compounds probably is to modify electrolyte transport, it seemed of interest to compare the effects of the 2 steroids on cellular ion transport systems. For this purpose, the effects of administra-

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