Effect of 7α-Methylation on Biological Activities of Norethindrone. (29377)

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Methylation of hormonally active steroids has produced compounds useful in studying structure-activity relationships. Introduction of a 7α-methyl group in certain anabolic-androgenic agents has afforded a number of new compounds of considerable interest(1,2, 3). Recently, 7α-methylation of an orally active progestational agent has been accomplished(4). Biologic activities of this compound, 7α-methyl-17α-ethynyl-19-nortestosterone (U-13851) are described.

Methods and results. Uterotropic, androgenic, progestational, gonadotropin inhibiting and pregnancy inhibiting properties and effects on estrous cycles were assessed using methods previously described(3,5). Sprague-Dawley rats and New Zealand white rabbits were used. Purina Chow was fed *ad libitum*, environmental temperature was maintained at 78 ± 2°F and 14 hours of artificial light were provided daily.

Uterotropic activity was assessed in bilaterally ovariectomized immature rats following 10 days of treatment. U-13851 administered orally at doses of 0.5, 1.0, 5.0 and 10.0 μg/day induced substantial uterotropic responses qualitatively comparable to that observed with estradiol-17β, subcutaneously (Table I). Potency, in relation to estradiol-17β, was 0.018 (95% confidence limits = 0.014 to 0.22). In relation to orally administered 17-ethynyl-19-nortestosterone (norethindrone), U-13851 was 24 times as potent (95% confidence limits = 19 to 31). Administered concomitantly with 0.04 μg of estradiol (SQ), 250 μg of U-13851 (orally) augmented uterine weight response from 115 mg up to 172 mg.

TABLE I. Uterotropic Assay. Immature ovariectomized rat (5 rats/point).

Compound (route)	Daily dose (μg)	Avg uterine wt and range (mg)
U-13851 (oral)	.0	28* (26- 37)
	.5	39 (26- 51)
	1.0	56 (48- 63)
	5	121 (87-142)
	10	149 (140-162)
Norethindrone (oral)	25	73 (61- 91)
	50	88 (86- 92)
	100	105 (94-112)
Estradiol (SQ)	.02	56 (47- 64)
	.03	70 (62- 80)
	.04	92 (74-110)
	.07	133 (104-155)

* Individual standard deviations among the 12 groups were not significantly different at 5% level. Avg = ± 4.8.

A portion of this same sample of U-13851 was subsequently subjected to preparative paper chromatography in a Bush-3 system (6) which had been demonstrated capable of separating probable estrogenic impurities. No change in estrogenic potency of this latter preparation was observed when it was reassayed along with the original sample.

Progestational activity was assessed in estradiol benzoate primed immature (1 kg) rabbits. Test compounds were administered orally for 5 days. A total dose of 5 mg of U-13851 induced a 2.25 response (McPhail index)(7), or 2% of the endometropic potency of medroxyprogesterone acetate standard(8). Norethindrone, at total oral doses of 1.25, 2.5, 5 and 10 mg, gave response indices of 1.2, 2.7, 3.7 and 3.9 respectively, and was 5% as potent as medroxyprogesterone acetate.

Androgenic activity was assessed in immature bilaterally castrated rats following 10 days of oral treatment. Seminal vesicle and ventral prostate gland weights were obtained 1 day after last treatment. Only minimal seminal vesicle weight increases (maximal average gland weight of 27 ± 0.8 mg) were observed over a 16-fold range in doses of U-13851 (Table II). A modest increase in ventral prostate gland weights was noted, plateauing at about 90 mg. However, in neither case did the accessory gland weight responses parallel those induced with a standard oral androgen (methyltestosterone).

Gonadotropin inhibition activity was as-

TABLE II. Androgen Assay. Immature castrate rats treated for 10 days.

Compound	Daily oral dose (mg)	No. of rats	Avg gland wt and range (mg) Seminal vesicle	Ventral prostate	Body wt gain (g)
U-13851	.0	5	6 (6- 7)	37 (33- 40)	51
	.1	10	16 (12- 19)	66 (52- 97)	36
	.2	10	24 (17- 32)	82 (65-100)	33
	.4	15	23 (21- 31)	75 (72- 83)	30
	.8	5	23 (22- 27)	90 (78-113)	27
	1.6	5	27 (25- 29)	93 (83-104)	25
Methyltestosterone	1.0	10	24 (15- 33)	112 (88-135)	47
	2.0	10	40 (32- 50)	152 (135-182)	51
	4.0	10	86 (67-114)	256 (203-322)	49

TABLE III. Gonadotropin Inhibition Assay. Immature male rats treated for 10 days. Five rats/point.

Compound	Daily oral dose (μg)	Organ wt (mg ± S.D.) Testes	Seminal vesicle	Ventral prostate
U-13851	.0	1299 ± 49	19 ± 1.4	109 ± 3.6
	6 2	1246 ± 95	16 ± 2.2	105 ± 7.9
	12.5	883 ± 102	12 ± .4	77 ± 7.3
	25	797 ± 72	13 ± .7	78 ± 3.1
	50	392 ± 25	14 ± .5	69 ± 6.9
	100	479 ± 84	14 ± .4	64 ± 1.8
	200	542 ± 102	17 ± 1.0	69 ± 7.5
	400	431 ± 32	22 ± 1.8	79 ± 4.8
Norethindrone	125	1237 ± 36	17 ± 1.2	101 ± 6
	250	1152 ± 100	14 ± 1.8	102 ±10
	500	839 ± 125	10 ± 1.1	76 ± 5.9
	2000	501 ± 38	12 ± .8	75 ± 9.3

sessed in intact, immature male rats after 10 days of oral treatment. Testicular, seminal vesicle and ventral prostate weights (obtained at sacrifice 24 hours after last treatment) were depressed at doses above 12.5 μg of U-13851 and 500 μg of norethindrone (Table III). Based on testicular weight depression, U-13851 was 34 times as potent as norethindrone (95% confidence limits = 23 to 50).

Pregnancy inhibition activity was assessed in mature female rats. Compound administration was initiated during proestrus and was continued for a total of 7 doses. All females mated with males of known fertility within 24 hours of the first treatment. Rats were sacrificed 48 hours after last dose, then examined for implantation sites. Minimal doses causing complete inhibition of pregnancy for U-13851 were 50 μg subcutaneously and 100 μg orally (Table IV). For norethindrone these doses were 200 and 1000 μg, respectively.

TABLE IV. Inhibition of Pregnancy by U-13851 and Norethindrone when Administered for 7 Days Beginning in Proestrus.

U-13851 Daily dose (μg) and route	No. rats preg/No. treated	Norethindrone Daily dose (μg) and route	No. rats preg/No. treated
25 oral	7/7	500 oral	6/6
50 "	3/6*	1000 "	0/2
100 "	0/6	2000 "	0/3
25 subq	5/5	100 subq	4/5
50 "	0/3	200 "	0/3
100 "	0/3	5000 "	0/2
200 "	0/3		

* Three pregnant rats had a total of 9 implants. Avg No. of fetuses in untreated rats for this colony is 10.3.

Discussion. U-13851 possesses gonadotropin inhibiting, pregnancy inhibiting and uterotropic activities. In these aspects it is qualitatively comparable to norethindrone, but is 20 to 30 times as potent by oral administration. Since each of these parameters may reflect estrogenic properties of a com-

pound, this single activity could account for all effects observed with U-13851 in the above assays. For this reason, rigorous purification of U-13851 to remove any estrogenic impurities was effected. Comparative biologic studies of analytically pure and rigorously purified material showed that the uterotropic property of U-13851 did not result from estrogenic contamination but was an inherent property.

Norethindrone is considered to produce physiological and morphological responses similar to those of other progestogens. However, in contrast to enhancement of estrogenic potency observed following 7α-methylation of norethindrone, there was no indication of an increase in progestational potency with U-13851 in the McPhail assay.

Marked enhancement of androgenic, anabolic and myotropic potencies was observed after 7α-methylation of a number of C_{19} and 19-norsteroids(2,3,9). For example, 7α,17α-dimethyl-19-nortestosterone was 18 and 41 times as potent as methyltestosterone as an androgenic and myotropic agent, respectively. Estrogen and progestogen-estrogen preparations induce moderate seminal vesicle and ventral prostate gland weight increases reflecting increase in muscle and submucosal tissue which is in contrast to androgen-dependent hypertrophy of secretory epithelium (10,11,12). Although norethindrone has been reported to increase the weight of prostate and seminal vesicle glands beyond the range produced by estrogens and to have an androgenic potency equivalent to 2% of testosterone propionate(13,14), U-13851 (at daily doses up to 1.6 mg/rat) did not demonstrate typical androgenic activity. Thus, through 7α-methylation, U-13851 has provided a unique combination of substantially enhanced estrogenic potency in an orally effective progestogen without a concomitant increase in endometropic or androgenic properties.

Summary. A very substantial increase of oral gonadotropin inhibiting (34 ×), pregnancy inhibiting (10 ×) and uterotropic (24 ×) potencies was observed for 7α-methyl-17α-ethynyl-19-nortestosterone (U-13851) compared to 17-ethynyl-19-nortestosterone (norethindrone) even though progestational potency was not enhanced. Although anabolic and androgenic properties are greatly increased in some 7α-methyl derivatives of testosterone and 19-nortestosterone, U-13851 did not appear to produce a typical androgenic response in laboratory tests.

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