The Biological Profile of Three 19-Nortestosterone-3-Cyclopentyl Enol Ethers (34207)

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The synthesis of 3-enol ethers of Δ^4-3-ketosteroids was first reported by Ercoli and Gardi (1). Though many studies on enol ether analogues of progesterone (2–7) and certain androgens (2, 7, 8–11) have been reported, little is known about similar derivatives of 19-nortestosterone. Experiments with the 3-cyclopentyl enol ether of 17α-ethynyl-19-nortestosterone-17β-acetate [quingestanol acetate (QA)] showed that enol etherification generally enhances most biological activities as compared with its parent ketone (2, 4, 5, 7, 12). Substitution of a methyl or ethyl group for the ethynyl group and replacement of the acetate by a hydroxyl group has provided two additional enol ethers, 17α-methyl-19-nortestosterone-3-cyclopentyl enol ether (MCPE) and 17α-ethyl-19-nortestosterone-3-cyclopentyl enol ether (EC PE) for this comparative study.

Materials and Methods. All compounds were dissolved in sesame oil and administered orally except as noted. Sprague-Dawley derivative rats (K. G. Farms, Parsippany, N. J.) were maintained at 22–24°C in a "reversed" day–night room (dark 6 a.m.–6 p.m.; light 6 p.m.–6 a.m.). After at least 2 weeks for acclimatization, mature female rats were caged with fertile males between noon and 3 p.m. Copulation was determined by the presence of spermatozoa in the vaginal smear (day O of pregnancy).

The following biological parameters were studied:

(1) *Progestational activity* was determined according to the method of Clauberg (13) as modified by McPhail (14). New Zealand immature rabbits (800–1000 g) were primed subcutaneously once daily with 1.25 μg of estradiol-17β for 6 days. From days 7 to 11 the test compounds were administered orally along with a maintenance dose of 0.125 μg of estradiol-17β subcutaneously. Midsections of uteri removed on day 12 were fixed, stained, and scored.

(2) *Pregnancy maintenance* was determined in rats ovariectomized on day 8 of pregnancy. Animals were treated from days 8 to 19 and laparotomized on day 20. The number of normal and resorbed fetuses were counted and calculated as percentage of fetal survival. Progesterone (P) was injected subcutaneously in both of the above tests as a reference standard.

(3) *Uterotropic activity* was determined in intact, immature female rats. The animals were treated for 3 days. Ethynylestradiol (EE) was used as a reference standard. The uteri were removed on day 4 and weighed to the nearest 0.5 mg after fluid was pressed out.

(4) *Androgenic activity* was determined after both multiple and single dosing, employing castrated immature male rats. For multiple dosing, the rats were treated with each test compound or 17α-methyltestosterone (MT) for 7 days. Ventral prostate glands were removed on day 8 and weighed to the nearest 0.5 mg. The method of Meli (10, 11) was followed for single dosing. The steroids were administered orally at a dose of 2.5 mg or subcutaneously at a dose of 5.0 mg on the third day after castration. The ventral prostate weights were recorded for each group of 5 rats at 1, 2, 3, 6, and 12 days after oral dosing and at 1, 3, 5, 7, 14, 21, and

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28 days after subcutaneous injection.

(5) *Masculinization of female fetuses* was determined according to a modification of the method described by Revesz *et al.* (15). Pregnant rats, individually caged on wood chips, were treated from days 15 to 21 of pregnancy. At birth ano-genital distances were measured and sex was determined at autopsy. If parturition was delayed, the pups were removed by cesarean section. Pups with ano-genital distances of $\leq$2.0 mm were considered females; $\geq$3.0 mm, males; and between 2.1–2.9 mm, pseudohermaphrodites.

(6) *Inhibition of pituitary gonadotropin hypersecretion*[3] was determined according to Shipley (16) using intact female–castrated male parabiotic rats. The male partners were treated for 10 days and degree of inhibition was calculated from ovarian weights on day 11.

(7) *Antiovulatory activity* was assessed in rats by the method of Bennett *et al.* (17). The test compounds were administered for 15 days and on days 5–8, 3 ml of a 1% filtered solution of Dianil blue "2R" was injected intraperitoneally. All corpora lutea formed from ovulations on day 9 or later appear pink against a blue background in animals posted on day 16.

Results. Complete transformation of the rabbit endometrium from a proliferative to a secretory pattern could be induced by subcutaneous P (250 μg/animal/day) or a similar oral dose of ECPE. Doses 2–4 times as high of either QA or MCPE were required to induce similar changes.

When administered orally at the dose of 10 mg/animal/day, both QA and MCPE failed to maintain pregnancy in ovariectomized rats, while a fetal survival of more than 60% could be obtained following a similar dose of ECPE. Subcutaneous P (5 mg/animal/day) resulted in 95% fetal survival.

As compared to the uterine stimulation induced by EE (0.03 – 0.09–0.27 μg/animal/day), doses approximately 400 and 4500 times as high for QA or MCPE and ECPE, respectively, were required to produce a similar stimulation.

[3] These studies were conducted by The Endocrine Laboratories, Madison, Wisconsin.

As compared to oral MT (200–400–800 μg/animal/day), MCPE is about 3.5 times more effective than the former in stimulating ventral prostate growth while both QA and ECPE are about one half as effective.

After single oral administration, MCPE was significantly more androgenic than QA and ECPE on days 1, 3, 5, and 8 posttreatment ($p \leq 0.05$). There was no significant difference between QA and ECPE. Only for MCPE there was indication of prolonged androgenic activity (Fig. 1a). Following single subcutaneous injection, only MCPE showed prolonged androgenic activity and was significantly more active ($p \leq 0.05$) than any other treatment throughout the observation period. As for oral administration, there was no significant difference between QA and ECPE (see Fig. 1b).

Administered orally at doses ranging from 0.1 to 2.7 mg/animal/day, QA was approximately ⅙ and 1/15 as potent as ECPE and MCPE, respectively, in inducing masculinization of the rat female fetuses.

MCPE appears to have approximately 5 and 10 times more antigonadotropic activity than ECPE and QA, respectively (Table I).

As far as antiovulatory activity is concerned, MCPE is again the most active, followed by ECPE and QA (Table II).

Results of all the biologic parameters under consideration employing QA as a reference standard are summarized in Table III.

Discussion. The introduction of different groups at carbon 17 led to significant changes in both biological activity and profile of 19 nortestosterone-3-cyclopentyl enol ether.

The introduction of an ethyl group (ECPE) led to a decrease in estrogenicity and an increase in both progestational and pregnancy maintenance properties as compared with the methyl (MCPE) or the ethynyl (QA) substituents. However, these properties are not too different from those exhibited by the corresponding parent ketones (18).

The greatest effect of the substitution at carbon 17 was the considerable increase in androgenic activity exhibited by MCPE which following multiple oral dosing was 8

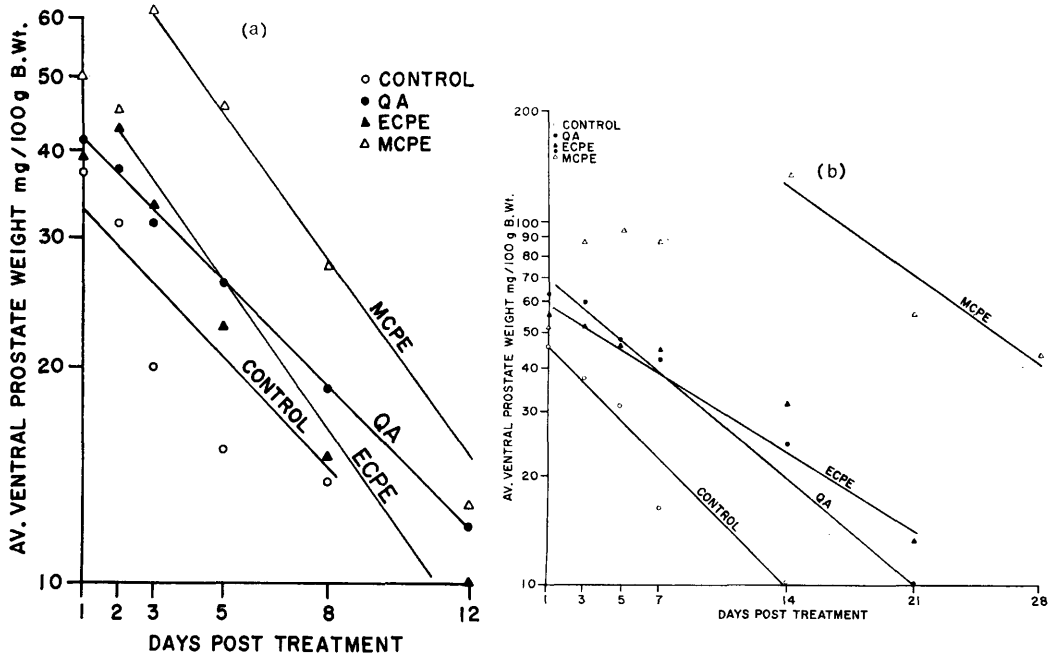

FIG. 1. Androgenic activity after a single administration of test compounds; each point represents value from five rats: (a) 2.5 mg po; (b) 5.0 mg sc.

times more effective than either ECPE or QA.

The methyl and ethyl substitutions were especially active in promoting female rat fetal masculinization, in terms of both total number of masculinized female fetuses and in number of mothers with such fetuses. ECPE in particular has more activity than would be expected from its androgenic property. These findings are in agreement with other data in the literature (15, 19) which similarly indicate that there is no direct relationship between these two properties.

The increased antigonadotropic and antiovulatory activities exhibited by MCPE, as compared to ECPE and QA, could be attributed to its greater androgenic activity.

It has been shown that storage in and release from body fat depots is the mechanism responsible for the increased activity of orally or subcutaneously administered 17α-methyltestosterone-3-cyclopentyl enol ether

TABLE I. Inhibition of Gonadotropin Hypersecretion in Parabiotic Rats.

Treatment	Dosage (mg/animal/day)	No. of pairs	Ovarian wt (mg; av ± SE)	Relative activities (C.L.P. = 0.95)
Control	—	4	256.0 ± 5.9	
QA	0.32	5	52.1 ± 4.5	100
	0.08	5	116.1 ± 6.5	
MCPE	0.08	5	28.8 ± 1.4	1020[a] (647–1612)
	0.02	5	52.5 ± 8.9	
	0.005	4	139.2 ±15.6	
ECPE	0.32	4	34.1 ± 4.7	170[a] (122–239)
	0.08	5	101.0 ± 5.0	
	0.02	5	165.4 ± 8.7	

[a] Significantly different from QA ($p \leq 0.05$).

TABLE II. Inhibition of Ovulation in the Rat.

Treatment	Dose (mg/animal/day)	% Rats ovulating	Ovulations per rat ovulated (av ± SE)	Relative activities
Control	—	100 (10/10)[a]	18.9 ± 2.2	
QA	0.3	100 (10/10)	10.7 ± 1.5	100
	0.9	100 (9/9)	15.1 ± 2.2	
	2.7	85.7 (6/7)	15.8 ± 4.5	
MCPE	0.1	80 (8/10)	12.4 ± 1.8	1000
	0.3	80 (4/5)	8.5 ± 1.8	
	0.9	22.2 (2/9)	16.0 ± 2.0	
ECPE	0.3	90 (9/10)	10.7 ± 2.2	200
	0.9	50 (5/10)	13.2 ± 2.2	
	2.7	66.7 (6/9)	10.5 ± 1.9	

[a] Number ovulated/total treated.

(MTCPE) (10, 11).

It has been proposed that the increased and prolonged androgenic activity exhibited by MCPE following oral administration may be due to fat storage (7). Failure to demonstrate the presence of MCPE in body fat depots by the procedure of Steinetz *et al.* (20) does not exclude such a possibility. In fact, the compound could have been stored in amounts not detectable by such a method and yet sufficient to exert, upon release, a significant biological effect. Furthermore, unlike the other 19-nortestosterone enol ethers, the subcutaneous administration of MCPE was followed by a prolonged stimulation of the ventral prostate altogether similar to that observed following subcutaneous MTCPE (10). Therefore it appears that only the use of isotopically labeled MCPE will clarify this point.

It is apparent that the introduction of different substituents at carbon 17 resulted in significant alterations in the biological properties of 19-nortestosterone-3-cyclopentyl enol ether. These findings indicate, as stated previously (10), that the nature of the parent ketone and not enol etherification *per se* governs the biological behavior of these compounds.

Summary. The biological properties of some 19-nortestosterone-3-cyclopentyl enol ethers have been studied. The 17α-ethyl analogue resulted in decreased estrogenicity, increased progestational and pregnancy maintenance properties as compared to 17α-methyl and 17α-ethynyl derivatives. The methyl analogue, however, exhibited a considerable increase in androgenicity and greater antiovulatory and gonadotropin inhibitory properties when compared to the other compounds. Both the methyl and ethyl substitutions resulted in masculinized female rat fetuses which in the case of the ethyl derivative demonstrated the lack of correlation between this effect and androgenic properties. Unlike the others, the 17α-methyl analogue, exhibited prolonged androgenic activity by either oral or subcutaneous administration. Our findings

TABLE III. Summary of Relative Activities of the Cyclopentyl Enol Ethers.

Compound	Progestational activity	Pregnancy maintenance	Activity				
			Estrogenic	Androgenic	Masculinizing	Antigonadotropic	Antiovulatory
QA	100	No	100	100	100	100	100
MCPE	100	No	157	815	1500	1020	1000
ECPE	200	Yes	10	126	600	170	200

indicate that the nature of the parent ketone and not enol etherification *per se* governs the biological behavior of such compounds.

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