Biological Activity of Certain 4-Methyl Ring A Aromatic Steroids.* (27640)

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Five 4-methyl ring A aromatic steroids recently described by Caspi *et al.*(1) were studied for steroid hormonal activities (Fig. 1). One additional steroid, androst-3,5-dien-17β-ol, is included because of the remote possibility that it may be a contaminant of 4-methylestra-1,3,5(10)-trien-17β-ol (II).

Material and methods. Estrogenic activity was assessed by the method of Rubin *et al.* (2) which involved injection or oral administration of the test compounds to immature female mice for 3 consecutive days and the end-point of uterine weight. The anti-estrogenic activity was measured by essentially the same method and consisted in stimulation of the mouse uterus with estrone and simultaneous administration of the inhibitor at a second site(3,4).

Androgenicity was tested in the day-old white Leghorn cockerel by daily inunction of the test compound in absolute ethanol for 7 consecutive days(5). Anti-androgenicity was assessed by inunction of the test compound to the testosterone stimulated chick's comb (6) and by a mammalian assay involving the testosterone stimulated castrated rat(7).

The seminal vesicle, prostate and levator ani served as the test indicators. Pituitary inhibitor activity was evaluated in the castrated male rat in parabiosis with a normal female and ovarian weight was the end-point. Androgenicity was simultaneously evaluated on the basis of weight of the seminal vesicle, prostate and levator ani of the castrated rat(8). Parallel tests were employed in some instances using the spayed and intact female rat in parabiosis. Again ovarian weight served as the indicator of pituitary inhibition while the uterine stimulation of the spayed partner indicated estrogenicity.

Results and conclusions. 4-Methyl-19-norcholesta-1,3,5(10)-triene (I) was inactive in the following tests: as an inhibitor of 0.4 μg estrone at doses of 100, 400 and 2000 μg, as an inhibitor of the testosterone stimulated

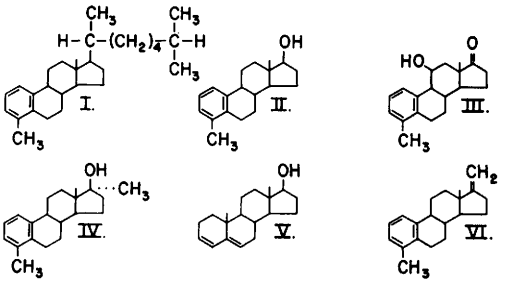

FIG. 1. I. 4-Methyl-19-norcholest-1,3,5(10)-triene. II. 4-Methylestra-1,3,5(10)-trien-17β-ol. III. 11β-Hydroxy-4-methylestra-1,3,5(10)-trien-17-one. IV. 4,17α-Dimethylestra-1,3,5(10)-trien-17β-ol. V. Androst-3,5-dien-17β-ol. VI. 4-Methylestra-1,3,5(10)-trien-17-methylene.

TABLE I. Chick Androgenic Activity of Compounds II, III and IV, V.

Exp. No.	Material administered	Total dose, μg	No. of chicks	Mean comb ratio ± S.E.
A	0	0	16	.27 ± .011
	Testosterone	1	10	.30 ± .014
		3	10	.34 ± .023
		9	9	.45 ± .040
		27	10	.65 ± .057
	II	1	10	.28 ± .013
		5	10	.29 ± .017
		25	10	.31 ± .011
		125	10	.36 ± .037
B	0	0	10	.35 ± .028
	Testosterone	1	10	.36 ± .015
		3	10	.46 ± .031
		9	10	.71 ± .056
		27	8	.80 ± .081
	III	100	10	.45 ± .026
		600	10	.33 ± .024
	IV	10	10	.33 ± .022
		100	10	.37 ± .022
		600	4	.31 ± .018
C	0	0	10	.35 ± .016
	Testosterone	1	10	.39 ± .022
		3	9	.46 ± .035
		9	10	.50 ± .034
		27	9	.73 ± .067
	III	100	15	.38 ± .022
	V	15	9	.37 ± .025
		30	9	.35 ± .026
		60	10	.49 ± .036
		120	10	.50 ± .052

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TABLE II. Uterotrophic Activity of Compounds II, IV, V and VI.

Exp. No. (route of admin.)	Material admin.	Total dose, μg	No. of mice	Mean uterine ratio ± S.E.
D	0	0	10	1.06 ±.084
(subcut. inj.)	Estrone	.1	10	2.66 ±.117
		.3	10	5.30 ±.242
	II	1	10	1.06 ±.075
		10	10	.90 ±.037
		100	10	1.31 ±.086
		500	6	3.02 ±.276
E	0	0	5	1.05 ±.12
(gavage)	Estrone	.8	5	1.39 ±.13
		6.4	5	4.05 ±.39
	II	100	5	1.47 ±.16
		500	5	2.56 ±.19
		2000	5	3.68 ±.40
F	0	0	10	.95 ±.064
(subcut. inj.)	Estrone	.05	10	2.39 ±.303
		.1	10	3.18 ±.168
		.2	10	4.62 ±.147
		.4	10	5.24 ±.207
	IV	100	10	1.24 ±.059
		200	10	1.27 ±.051
		400	10	1.50 ±.081
		800	10	2.18 ±.121
		1600	10	3.78 ±.415
G	0	0	10	.84 ±.062
(subcut. inj.)	Estrone	.05	10	1.82 ±.150
		.1	10	3.23 ±.409
		.2	10	4.30 ±.280
		.4	10	5.19 ±.432
	VI	100	9	.92 ±.068
		1000	5	.98 ±.050
H	0	0	10	1.20 ±.094
(subcut. inj.)	Estrone	.05	10	2.02 ±.153
		.1	10	3.29 ±.092
		.2	10	5.23 ±.278
		.4	10	5.48 ±.234
	V	15	10	1.15 ±.045
		30	10	1.37 ±.113
		60	10	1.16 ±.078
		120	10	1.26 ±.085

chick's comb at doses of 0.5, 1 and 3.0 mg and as an inhibitor of testosterone stimulated seminal vesicle and prostate at doses of 2, 10 and 50 mg.

4-Methylestra-1,3,5(10)-trien-17β-ol (II) was inactive as an anti-estrogen at the 50, 500 and 2000 μg dose levels, and as an anti-androgen in the testosterone stimulated chick's comb test. Compound II stimulated the chick's comb and over a limited dose range gave a reasonable dose-response curve which was parallel to the standard testosterone. The compound had an estimated relative potency

TABLE III. Activity of Subcutaneously Injected Compound in a Parabiosis Assay (Male Castrate—Intact Female).

				Male-castrate				Intact female	
				Parabiont				Parabiont	
Exp. No.	Material administered	Total dose, mg	No. of pairs	Mean body wt, g	Mean seminal vesicle wt	Mean prostate wt	Mean levator ani wt	Mean body wt, g	Mean ovarian wt, mg ± S.E.
					mg ± S.E.				
I	0	0	5	99	7.0 ± .91	6.9 ± .86	14.3 ± .37	85	107.6 ± 10.8
	Testosterone	.4	5	102	29.6 ± 15.5	58.2 ±14.3	28.2 ± 6.62	85	62.5 ± 12.1
		.8	4	110	72.5 ± 16.4	70.2 ± 6.80	39.7 ± 6.56	87	15.4 ± 2.2
	II	1	4	106	10.3 ± .39	10.4 ± 1.54	20.0 ± 3.91	68	102.3 ± 10.1
		10	5	84	9.0 ± .67	10.8 ± 2.27	14.4 ± 2.27	81	45.3 ± 7.2
J	0	0	4	96	10.1 ± .73	14.0 ± .92	14.1 ± .36	88	120.7 ± 12.7
	Testosterone	.4	3	106	38.2 ± 7.35	49.9 ± 8.1	38.1 ± 8.1	86	42.3 ± 10.4
		.8	4	96	113.4 ± 11.9	125.2 ± 9.0	52.8 ±10.6	89	21.4 ± .92
	II	5	3	87	11.4 ± 1.9	13.7 ± 4.6	13.7 ± 3.4	82	62.1 ± 33.5

TABLE IV. Activity of Subcutaneously Injected Compounds III and IV in a Parabiosis Assay (Spayed Female — Intact Female).

Material admin.	Total dose, mg	No. of pairs	Spayed female: Mean body wt, g	Spayed female: Mean uterine ratio ± S.E.	Female: Mean body wt, g	Female: Mean ovarian ratio ± S.E.
0	0	5	84	38.4 ± 7.741	78	131.3 ± 30.455
Estrone	.0005	6	95	50.2 ± 5.698	74	85.4 ± 13.832
	.001	5	94	41.2 ± 2.727	76	66.1 ± 33.244
	.002	6	88	66.9 ± 7.488	82	46.2 ± 14.050
IV	.5	6	89	41.7 ± 3.090	80	67.7 ± 16.848
	1.0	6	88	52.0 ± 3.909	83	19.7 ± 3.486
	2.0	6	89	139.9 ± 14.173	73	15.1 ± 2.643
	4.0	5	85	147.7 ± 24.558	78	22.0 ± 6.199
III	3.0	5	81	50.5 ± 12.035	74	73.2 ± 19.917

of 1/30th of testosterone (Table I). Compound II, when injected subcutaneously, produced a significant increase in the immature mouse uterus at the total dose of 100 μg, at the 500 μg dose a 3-fold increase in uterine weight was found. The slope was significantly shallower than that of the estrone standard so that no precise relative potency could be calculated, but one may judge compound II to be about 1/5000th as active as the standard. The shallow slope for uterotrophic activity of II is reminiscent of that found for androgens by this test.

Compound II also stimulated the uterus when administered by gavage and exhibited a relatively higher uterotrophic activity by this route. The limited number of observations prohibited proper evaluation of the slopes of estrone and Compound II for parallelism. An estimate of relative potency places Compound II at about 1/200th the activity of estrone.

Table III summarizes the pituitary inhibiting activity of Compound II by a parabiosis assay. At the 1 and 5 mg dose levels significant pituitary inhibition was not found but 10 mg total dose gave a pituitary inhibition equivalent approximately to that of 0.5 mg of testosterone. This means that the compound was about 1/20th as androgenic as testosterone on the basis of lack of seminal vesicle and prostate response.

Since Compound V (androst-3,5-dien-17β-ol) could have been contaminated with Compound II to the extent of 5%, it was of interest to ascertain whether the activity of Compound II could be due to Compound V. The chick androgenicity of Compound V by injection was found to be about 1/10th that of testosterone (Table I) but estrogenic activity could be detected. On the basis of the estrogenic assay method employed, the compound was judged to possess less than 1/2000th the activity of estrone (Table II). It seems unlikely, therefore, that the biological activity of Compound II was due to contaminant Compound V.

Neither Compound III nor IV has any significant androgenic activity on the chick's comb (Table I) at doses up to 600 μg. This means that the compounds, if androgenic at all, are less than 1/200th that of testosterone. Compound IV has an estrogenic activity approximately 1/8000th that of estrone.

Compound IV was a highly effective antipituitary compound in the spayed female-intact female parabiosis assay (Table IV). By this assay, a relative potency of 1/500th that of estrone was found. This would indicate a significant degree of separation of the estrogenic and pituitary inhibitory activity since the compound appeared to be 1/8000th as estrogenic as estrone in the mouse. However, in the spayed parabiotic rat, estrogenic potency seems to be closer to a figure of 1/500th to 1/1000th that of estrone. This latter es-

TABLE V. Anti-estrogen. (Injection)

Test material: Designation	Test material: Total dose, μg	Estrone: Total dose, μg	No. of mice	Mean uterine ratio ± S.E.
0	0	0	10	.98 ± .048
0	0	.4	7	5.11 ± .214
IV	50	.4	9	5.08 ± .141
	500	.4	10	5.16 ± .171
	2000	.4	10	4.92 ± .168

TABLE VI. Relative Potencies of Certain 4-Methyl Aromatic Steroids.

Compound designation	Chick androgenic activity Testosterone=1	Estrogenic activity subcutaneous inj. (mouse) Estrone=1	Estrogenic activity subcutaneous inj. (rat) Estrone=1	Pituitary inhibition (parabiosis) Testosterone=1	Pituitary inhibition (parabiosis) Estrone=1
II	1/30	*Ca* 1/5000		1/20	
III	<1/200				
IV	<1/200	1/8000	1/1000		1/500
V	1/10	1/2000			
VI		<1/20,000			

timate would indicate a pituitary suppressing activity essentially related to the estrogenic potency.

Compound IV did not have any anti-estrogenic activity for total doses from 0.05 to 2 mg. Compound VI was less than 1/20,000th as active as estrone when injected into the immature mouse.

Discussion. The data summarized in Table VI indicate clearly that the 3-deoxy-4-methyl steroids show weak estrogenic potency and in the case of 4-methylestra-1,3,5(10)-trien-17β-ol (II), some androgenicity. The pituitary inhibition is definite for II and IV.

Summary. 4-Methylestra-1,3,5(10)-trien-17β-ol (II) and androst-3,5-dien-17β-ol (V) were 1/30th and 1/10th as androgenic as testosterone in chick's comb inunction assay. Subcutaneous injection of 4,17α-dimethylestra-1,3,5(10)-trien-17β-ol, II and V showed 1/8000th, *ca* 1/5000th and 1/2000th the estrogenic potency of estrone, respectively, by a mouse uterine test. By parabiosis assays in the rat, II was approximately 1/20th as pituitary inhibiting as testosterone, while IV was 1/500th as active as estrone.

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Effects of Endotoxin on Turkey Poults.* (27641)

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During studies on dissecting aneurysm in turkeys, methods were sought for the experimental production of the disease. In discussing experimental aortic dissecting aneurysms in animals, Hall(1) mentions the production of aortic medionecrosis in rabbits by the action of adrenalin and also by the use of the exotoxin of *Corynebacterium diphtheriae.* Endotoxins from Gram-negative bacteria are also known to produce necrosis and hemorrhage in a variety of experimental animals. Clinically, the pathological effects of generalized Gram-negative infections are associated with septic shock, attributed largely to the action of endotoxin(2,3,4). The present paper deals with a qualitative survey of the effects of endotoxin on turkey poults.

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Materials, methods, and results. Experi-