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Estrogen Antagonisms: Relationship Between Estrogen Antagonistic And Progestational Potencies of Δ^4-3-Oxosteroids. (31604)

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The relationship between estrogen antagonistic and progestational effects of various Δ^4-3-oxosteroids has been the subject of speculation for some time(1,2,3). Discussing various 17-substituted relatives of 19-nortestosterone, Edgren *et al*(1) stated: " . . . the absence of a direct glandular effect of 19-nortestosterone, an antagonist, and conversely, the absence of estrogen antagonistic action of the butyl, which is still an active progestin, suggest that the activities are separated." Some lack of correspondence beween progestational and anti-estrogenic effects was also observed among the derivatives of 17α-acetoxyprogesterone(2). Since these observations were at best suggestive, it seems germane at this point to examine in detail the relationships of these activities in a number of newer materials. Such considerations are crucial at this time because of the recent clinical interest in estrogen antagonists as contraceptives, acting at the level of cervical mucus(4).

Materials and methods. Estrogen antagonistic potencies were determined in a mouse vaginal smear test following the protocol of Edgren(5). Spayed mice received estrone at a standard dose of 0.5 μg per day for 4 days. Vaginal smears were examined on the afternoon of day 5; those showing an estrogenic vaginal response (absence of leukocytes) were returned to the colony and the remaining mice were reexamined on the morning of day 6. Progesterone was employed as a standard, and compounds were compared to progesterone at doses estimated to reduce by 50% the expected response to the estrogen. Normally, in this test, the standard dose of estrone produced 80-100% positive responses in treated mice, and the ED_{50} for progesterone was approximately 400 μg.

Progestational potencies were estimated from a Clauberg test(6). Intact female rabbits, weighing about 1 kg, were primed daily for 6 days with 5 μg of estradiol-17β. Test compound injections were initiated on the day following the final priming injection and continued for 5 days. On the day after the final injection the rabbits were sacrificed and uterine segments were removed from each for histological examination. The uteri were scored according to the McPhail index(7). Progesterone, again the standard, normally produced an average McPhail index of +2 at a daily dose of about 100 μg. Test compounds were compared at the doses estimated to produce a +2 McPhail index.

The present communication will examine simultaneously the progestational and anti-estrogenic potencies of several series of closely related delta-4-3-oxosteroids that have both types of activity and that are potent, or are related to compounds that are potent, in one or the other test.

Results and discussion. Included in the group of compounds chosen for comparison are some of the most potent progestins and estrogen antagonists that have been studied in this laboratory (Table I). 19-Norproges-

TABLE I. Anti-Estrogenic and Progestational Potencies of Various Steroids.

Compound	Relative potency A Anti-estrogenic	B Progestational	Ratio (A/B)
Progesterone derivatives			
Progesterone	100%	100%	1
19-Norprogesterone	1000	800	1.2
Acetylenic nortestosterones			
17α-ethynyl-17-hydroxy-13β-methylgon-4-en-3-one (norethisterone)	730	8.5	85.9
13β-ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one (norgestrel)	14,600*	1830*	8.0
17α-ethynyl-17-hydroxy-13β-n-propylgon-4-en-3-one	470*	220*	2.1
13β-n-butyl-17α-ethynyl-17-hydroxygon-4-en-3-one	120*	34*	3.5
17α-ethynyl-17-hydroxy-13β-methylgon-4-en-3-one, acetate	600*	50	12
13β-ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one, acetate	5800*	3000*	1.9
17α-chloroethynyl-13β-ethyl-17-hydroxygon-4-en-3-one	22,000*	2660*	8.3
17α-chloroethynyl-13β-ethylgon-4-ene-3, 17-diol, 3-acetate	500*	3035*	.2
17α-chloroethynyl-17-hydroxy-13β-methylgona-4,9-dien-3-one	200	2100	.1
17α-chloroethynyl-13β-ethyl-17-hydroxygona-4,9-dien-3-one	4200*	600	7.0
Alkylated nortestosterones			
13β,17α-dimethyl-17-hydroxygon-4-en-3-one	1800	500	3.6
13β-ethyl-17-hydroxy-17α-methylgon-4-en-3-one	10,000*	200*	50
17α-ethyl-17-hydroxy-13β-methylgon-4-en-3-one (norethandrolone)	1900	750	2.5
13β,17α-diethyl-17-hydroxygon-4-en-3-one (norboletheone)	5200*	1000*	5.2
13β,17α-diethyl-17-hydroxygona-4,9-dien-3-one	2400*	1000*	2.4

* Potencies adjusted (doubled) from data obtained from tests carried out with racemates(9).

terone was about 10 times more anti-estrogenic than progesterone, and about 8 times more progestational. Thus removal of the angular methyl group at carbon 10 of progesterone did not appear significantly to alter the ratio of potencies. Among 6-methylated progesterones(2) the ratio varied from 0.02 to 0.35, although, since other methods were employed, the results are not comparable to those reported here.

The progestational and anti-estrogenic potencies of 13β-homologues of norethisterone (17α-ethynyl-17-hydroxy-13β-methylgon-4-en-3-one or 17α-ethynyl-19-nortestosterone) have already been discussed(6). When the chain at carbon 13 of the nucleus was lengthened from methyl to ethyl both anti-estrogenic and progestational potencies were markedly increased, although the shallow slope of the dose-response curve for norethisterone precluded a satisfactory estimate of progestational potency for that material. However, the 13β-ethyl homologue had a markedly lower ratio of these effects. Further lengthening of the chain at carbon 13 beyond ethyl resulted in decreases in potency, which were more evident in the Clauberg test again producing a slightly decreased ratio of anti-estrogenic to progestational potencies.

The acetate of norethisterone was more potent than the parent compound in the Clauberg assay, whereas it was slightly less potent as an anti-estrogen and therefore had a markedly decreased ratio. The acetate of 13β-ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one was less potent than the parent with respect to both parameters; and the ratio was decreased.

In the 13β-ethyl series, replacement of the hydrogen in the acetylenic side chain with chlorine (17α-chloroethynyl-13β-ethyl-17-hydroxygon-4-en-3-one) was accompanied by increases in both anti-estrogenic and progestational potencies; however, this substitution had no effect on the ratio of activities. The 3-acetoxy derivative of this compound, though

more potent than the parent in the Clauberg test, was less potent as an estrone antagonist; thus the ratio was grossly reduced. Incorporation of a double bond at the 9-10 position of 17α-chloroethynyl-13β-ethyl-17-hydroxygon-4-en-3-one resulted in decreases of both activities; however, since the decrease in progestational effect was greater, the ratio was considerably increased. 17α-Chloroethynyl-13β-ethyl-17-hydroxygon-4,9-dien-3-one was less potent in the Clauberg test, but more potent in the anti-estrogenic assay than the lower homologue. Thus the ratio of potencies was decreased.

13β-Ethyl-17-hydroxy-17α-methylgon-4-en-3-one was more anti-estrogenic and less progestational than its lower homologue. 17α-Ethyl-17-hydroxy-13β-methylgon-4-en-3-one (norethandrolone) was less potent in both assays than its 18-homologue (norbolethone), which because of a greater anti-estrogenic potency had a higher ratio. Incorporation of a double bond in the B-ring of norbolethone to form 13β,17α-diethyl-17-hydroxygon-4,9-dien-3-one did not alter progestational potency, whereas the anti-estrogenic potency and the ratio were decreased.

These data fail to indicate any direct correlation of progestational and anti-estrogenic effects. Although both these biological activities are often present in a single molecule, they do not appear to be necessary correlates.

Summary. The progestational (Clauberg) and anti-estrogenic (mouse vaginal smear) potencies of various Δ^4-3-oxosteroids are compared. The ratio anti-estrogenic potency/progestational potency varies from 0.1 to 85, indicating that there is no necessary correlation between these parameters of steroid action.

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Reversible Inhibition of Mitosis in Lymphocyte Cultures by Non-Viable Mycoplasma.* (31605)

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The observation by Nowell(1) that phytohemagglutin (PHA) increased mitosis in lymphocyte cultures has resulted in the application of this technique to many fields of research. Its application in immunology was established when tuberculin purified protein derivative(2) was the first of many antigens (3,4,5) used to stimulate transformation in lymphocytes from sensitized individuals. A logical extension of this basic immunologic phenomenon leads to the idea of stimulating the lymphocytes from rheumatoid arthritis patients with a suspected etiologic agent and of comparing the response to that of lymphocytes from normal controls.

Recently there have been reports of the isolation of mycoplasma (PPLO) from the synovial fluids of patients with rheumatoid arthritis and other diseases(6,7). However, confirmatory evidence is lacking on the etiological role of these organisms. An attempt was made to test the hypothesis that

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