Colton, F. B., *Endocrinology*, 1959, v65, 265.
2. Edgren, R. A., *Acta Endocrinol.*, 1960, v34, 536.
3. Biggers, J. D., Claringbold, P. J., *J. Endocrinol.*, 1954, v11, 277.
4. Elton, R. L., Edgren, R. A., Calhoun, D. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 175.

Received June 13, 1960. P.S.E.B.M., 1960, v104.

Estrogen Antagonisms: Effects of Several Steroidal Spirolactones on Estrogen-Induced Uterine Growth in Mice. (25944)

RICHARD A. EDGREN AND RICHARD L. ELTON
Division of Biological Research, G. D. Searle and Co., Chicago, Ill.

There has been continuous growth of interest in compounds that antagonize renal action of aldosterone. Pharmacology of the first of these agents to be discovered was summarized by Kagawa, Sturtevant and Van Arman(1), who noted briefly that SC-5233, 3-(3-oxo-17β - hydroxy - 4 - androsten - 17α - yl)propionic acid γ-lactone was inactive as estrone antagonist. Recently, interest has shifted to 19-nor (SC-8109) and 7α-acetylthio (SC-9420) derivatives of SC-5233(2,3). Since Hertz and Tullner(4) were able to demonstrate progestational activity for SC-5233 and SC-8109, it seemed worthwhile to study these materials as estrone antagonists. In addition to these 3 compounds, several other steroidal spirolactones have been studied. Chemistry of these materials has been discussed by Cella and his colleagues(5,6,7).

Materials and methods. Structural formulae for the compounds tested are presented in Fig. 1. Each substance was tested initially at a dose of 1000 μg, against 0.3 μg of estrone, following technic described earlier(8). Briefly, immature mice received test compound mixed with the standard dose of estrone in 3 daily injections, each containing $\frac{1}{3}$ of the total doses indicated in this paper. Twenty-four hrs after final injection mice were sacrificed and cleaned uteri weighed on a torsion balance. Compounds were compared at doses estimated to produce a 50% depression in response expected from estrone alone. Earlier experiments, including certain studies dealing with SC-8109, employed Rockland mice and method of evaluation has been discussed(8). Most studies to be described here employed Carworth mice obtained from Small Animals Farms, Des Plaines, Ill., which responded with greater increment of uterine growth. Three groups of 10 animals each that received only estrone averaged 46.3 mg uterine weight; oil-treated controls averaged 9.4 mg. Thus, dose of material estimated to produce uterine weight response of 28.4 mg, which corresponds to a 50% depression, was employed for comparison, and any material that failed to produce this level was considered inactive. In contrast to earlier studies no material considered as inactive here produced any suggestion of inhibition. Progesterone, standard in this test, produces 50% depression at a dose of about 40 μg. Secondary testing of each compound progressed by decreasing dose at increments of about $\frac{1}{3}$ until activity was lost. Progestational potencies were determined from subcutaneous Clauberg test(8). For purposes of comparison, potencies were based upon doses that produced a +2 response.

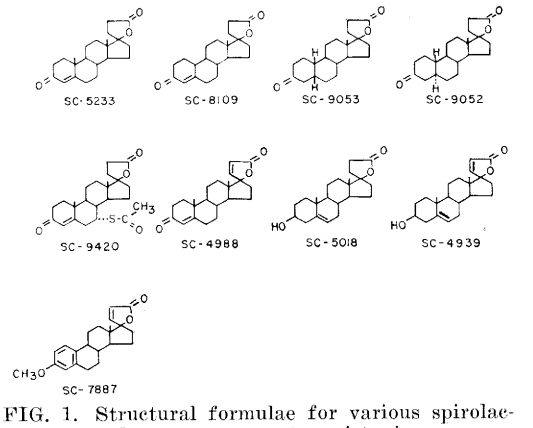

FIG. 1. Structural formulae for various spirolactones tested as estrone antagonists in a mouse uterine growth assay.

TABLE I. Comparison of Estrogen Antagonistic, Progestational and Aldosterone Antagonistic Potencies of a Series of Steroidal Lactones. I = inactive.

Compound	Estrogen antagonist potency	Progestational potency	Aldosterone antagonist*
Progesterone	100	100	1.3
SC-5233	I	100	.22
8109	100	2000	.088
9053	10		>2.4
9052	4		.8
9420	I	I	.33
4988	I	I	>2.4
5018	I	I	"
4939	I	I	"
7887	I		"

* Data from C. M. Kagawa. Figures represent median effective doses.

Results. SC-5233, which might be considered as parent compound in series, was totally inactive as estrone antagonist, confirming Kagawa *et al.*(1), whereas 19-nor-analogue was most potent of series, being about 100% progesterone (Table I). Dihydro-19-nor derivatives were considerably less potent, 5β isomer being about 10% and 5α isomer being approximately 4% progesterone. These structure-activity relationships correlate with those found for relatives of 19-nortestosterone (8) in which 19-nor configuration generally increased potency, and 4,5 dihydro derivatives were less potent than the Δ^4 materials. In dihydro-19-nortestosterone series only isomers with hydrogens at β position at carbons 5 or 10 were active; in present study the 5β material (SC-9053) was more potent than the 5α isomer (SC-9052). 7α-Acetylthio grouping (SC-9420) was accompanied by loss of progestational, but not anti-aldosterone activity. No other structural modification was commensurate with activity.

These studies show no clear correlation between estrogen-antagonistic, progestational or anti-aldosterone activities. SC-5233 has progestational and anti-aldosterone properties, but it is not an estrogen antagonist, whereas its 4,5-dihydro-5β derivative (SC-9053), while maintaining considerable estrogen antagonistic potency, is less than ½ as potent as progesterone as anti-aldosterone. Reverse is true of 7α-acetylthio form (SC-9420) which lacks anti-estrogenic and progestational but retains anti-aldosterone effects. 19-Nor derivative (SC-8109) is relatively potent in all 3 areas.

Summary. A series of steroidal spirolactones, of interest for their aldosterone antagonistic actions, were tested as estrogen antagonists in a mouse uterine growth test. The 2 activities did not appear to be correlated. Progestational activity similarly showed no direct relationships. Structural requirements for estrogen antagonistic potency are discussed. SC-8109 and 2 other 19-nor derivatives of SC-5233, 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone, were estrogen antagonists.

1. Kagawa, C. M., Sturtevant, F. M., Van Arman, C. G., *J. Pharmacol. and Exp. Therap.,* 1959, v126, 123.

2. Kagawa, C. M., Cella, J. A., Van Arman, C. G., *Science,* 1957, v126, 1015.

3. Kagawa, C. M., *Endocrinology,* 1960, v67, 125.

4. Hertz, R., Tullner, W. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 451.

5. Cella, J. A., Kagawa, C. M., *J. Am. Chem. Soc.,* 1957, v79, 4808.

6. Cella, J. A., Brown, E. A., Burtner, R. B., *J. Org. Chem.,* 1959, v24, 743.

7. Cella, J. A., Tweit, R. C., *ibid.,* 1109.

8. Edgren, R. A., Calhoun, D. W., Elton, R. L., Colton, F. B., *Endocrinology,* 1959, v65, 265.

Received June 13, 1960. P.S.E.B.M., 1960, v104.