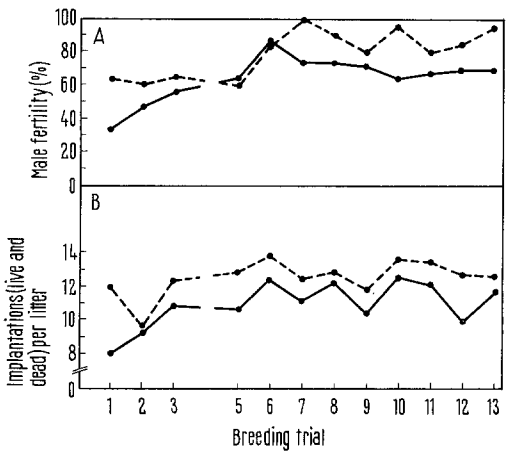
of a dominant mutation effecting the skeleton is remote. Resorption sites are considered a measure of dominant lethal mutation. When females are not treated, a decrease in litter size without a concomitant increase in resorption sites might be due to preimplantational wastage as a result of either decreased ability of the sperm to fertilize or low viability of the zygotes, prevention of mitotic activity, or inability of the blastocyst to implant. A previous cytological study of semen of CHS-treated rats recovered from the vagina after mating indicated no visible change in sperm morphology or motility. Further work is required to elucidate the nature of the decreased litter size and impaired fertility observed following CHS treatment of males[6].




Male fertility (A), and average implantations (live and dead)/litter (B) in CHS-treated (solid lines) and control (broken lines) rats. Data from mating trials, 1–3 (CHS-treated males and females), 5–8 and 12–13 (CHS-treated males only) and 9–11 (neither sex treated) is plotted.

*Résumé.* Des rats mâles traités oralement avec du sulfate de cyclohexylamine (220 mg/kg/jour) ont été accouplés à des femelles traitées ou non avec du sulfate de cyclohexylamine. Dans les 2 cas, il y a eu des descendants moins nombreux que chez les contrôles. Les résultats suggèrent que l'effet est transmis par les mâles traités et est, peut-être, causé par un dommage génétique exprimé avant l'implantation de l'embryon.

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## 11β-Methyl-19-Norsteroids: Novel Progestational Hormones

A continued search for substances which may have advantages over known oral contraceptives had led us to the discovery that in experimental animals certain 11β-methyl-19-norsteroids, as single substances, possess some of the hormonal properties characteristic of mixtures of steroid hormones presently used in human fertility control[1] and lack other undesirable properties. The synthesis and some of the biological properties of 17α-ethynyl-17β-hydroxy-11β-methylestr-4-en-3-one (XII) and 3β,17β-diacetoxy-17α-ethynyl-11β-methylestr-4-ene (XIII) are described presently.

Thus the readily available 17,17-ethylenedioxyestra-1,3,5(10)-triene-3,11β-diol[2] (I) was converted with methyl iodide, methanol, and potassium carbonate to II[3], mp 127–128°, which was oxidized with 8*N* chromic-sulfuric acid in acetone at 0° to the ketone III. Treatment of the crude ketone III with methyl magnesium bromide followed by hydrolysis of the product IV in strong acid yielded 11β-hydroxy-3-methoxy-11α-methylestra 1,3,5,(10)-trien-17-one (V), mp 178–179°, $\lambda_{max}$ 2.72, 5.71, and 6.18 μ, NMR maxima at 67 and 99 (C-11 and C-13 methyls) Hz. Dehydration of V in refluxing benzene containing *p*-toluenesulfonic acid provided 3-methoxy-11-methylestra-1,3,5(10), 9(11)-tetraen-17-one (VI), mp 95° $\lambda_{max}$ 257.5 nm (ε = 18,050) which upon hydrogenation in methanol with Pd-C afforded 3-methoxy-11β-methylestra-1,3,5(10)-trien-17-one (VII), mp 152°, NMR maxima at 51 and 58 (C-11β methyl) and 62 (C-13 methyl) Hz and, in minor amount, 3-methoxy-11α-methyl-9β-estra-1,3,5(10)-trien-17-one (VIII), mp 129–130°, NMR maxima at 75 and 83 (C-11α methyl) and 61 (C-13 methyl) Hz. Reduction of VII with sodium borohydride gave the corresponding alcohol IX, mp 108–110°, which upon reduction with sodium, ammonia, and *t*-butyl alcohol followed by Oppenauer oxidation of the product with aluminum *i*-propoxide, cyclohexanone, and refluxing toluene yielded the ketone X, mp 140–142°. Ethynylation of X with lithium acetylide in tetrahydrofuran followed by hydrolysis of the product XI in strong acid yielded 17α-ethynyl-17β-hydroxy-11β-methylestr-4-en-3-one (XII), mp 222–223°, $\lambda_{max}^{MeOH}$ 241 nm (ε = 17,500), $\nu_{max}^{KBr}$ 2.92, 3.07, and 6.00 μ. Reduction of XII with lithium tri-*t*-butoxyaluminum hydride followed by diacetylation of the product afforded 3β,17β,diacetoxy-17α-ethynyl-11β-methylestr-4-ene (XIII), mp 148–150°.

A buffered hormonal action is characteristic of XII and XIII. In experimental animals, they exhibit potent progestational activities, anti-estrogenic activities, and estrogenic responses in the estrogen deficient state.

Thus, in the Clauberg assay[4] for progestational activity the activities of XII, XIII, and XIV when administered s.c. were 25:25:1, respectively, and when administered orally were 10:10:1, respectively[5]. In the rat vaginal

[1] See G. PINCUS, *The Control of Fertility* (Academic Press, Inc., New York, N.Y. 1965) and V. A. DRILL, *Oral Contraceptives* (McGraw-Hill Book Co., New York, N.Y. 1966).

[2] J. S. BARAN, J. med. Chem. *10*, 1188 (1967).

[3] This substance and others prepared below gave satisfactory analyses. The NMR-spectra were determined in deuterochloroform on a Varian Model A-60 spectrometer at 60 Mc with $Me_4Si$ as an internal standard.

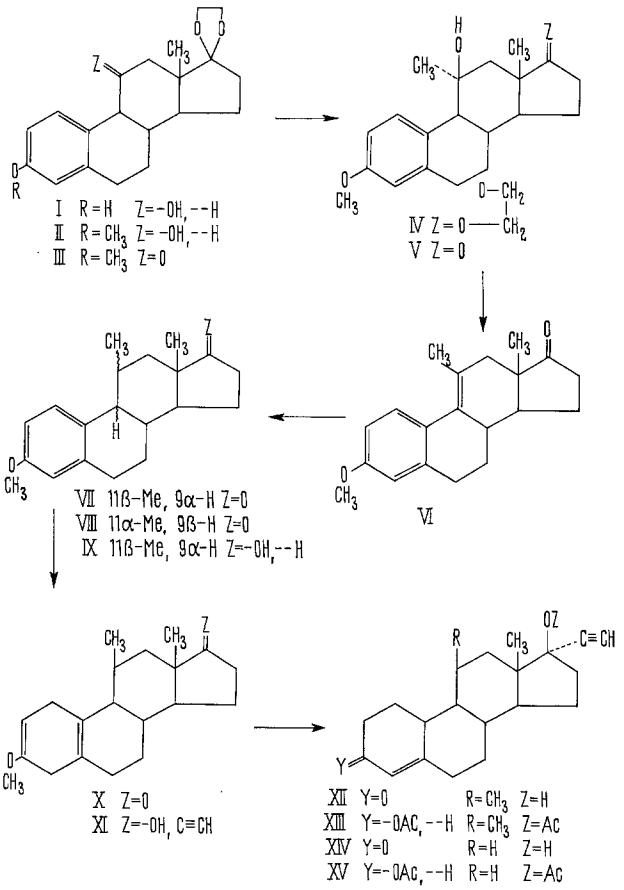
[4] C. W. EMMENS, in *Hormone Assay* (Academic Press, Inc., New York, N.Y. 1950), p. 422.

[5] XIII also exhibits potent anti-estrogenic activity in the immature female mouse treated with estrogen which correlates well with the progestational activity obtained in the Clauberg assay.

smear assay[6] s.c. administered XII and XIII exhibited 16% and 18% the activity of s.c. administered estrone, respectively, and orally administered XII and XIII exhibited 37% and 17% the activity of s.c. administered estrone, respectively. In the ovariectomized rat treated with XIII and XV the inhibition of the increase of pituitary LH content was 140% and 15%, respectively, and of FSH content was 300% and 12%, respectively, that of estrone, when the compounds were administered s.c.

Hepatic excretory function, which can be altered by estrogen therapy in man and may be linked to changes in metabolic function in the liver[7], was assessed following administration of XIII in both the rabbit and rat using the sulforbromophthalein (BSP) test. The rabbit BSP test was performed according to the method of LENNON[8] and the rat BSP test was done using a method similar to that reported by GALLAGHER et al.[9] 17α-Ethynyl estradiol, 17β-estradiol, and sodium estrone 3-sulfate, when administered orally to rats at 0.5, 10 and 5 MPK per day, respectively, caused significant effects on BSP retention. The oral administration of XIII at 10 MPK per day for 4 days in the rabbit and at 20 MPK per day for 10 days in the rat failed to cause significant alteration in BSP retention[10]. In like manner, XV (Ethynodiol diacetate) and progesterone were devoid of effects on BSP retention in rats.

Thus, XIII may be similar to other progestational agents in failing to alter hepatic excretory function and may possess anovulatory properties without some of the metabolic effects which at times can be associated with mixtures of steroid hormones presently used in human fertility control. Because XIII also appears to exert, as a single substance, a buffering type of hormonal action, it may not exhibit some of the physiological effects exerted by higher doses of pure estrogenic or progestational agents.




*Zusammenfassung.* Die Synthese von 3β,17β-Diacetoxy-17α-Äthinyl-11β-Methylestr-4-en (XII) wird beschrieben. Die Substanz besitzt besonders starke Antifertilitätseigenschaften, wie sie sonst nur Mischungen von Hormonen eigen sind, welche für die Humanfertilität benutzt werden, jedoch ohne deren Nebenwirkungen.

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[7] M. N. MÜLLER and A. KAPPAS, J. clin. Invest. *44*, 1214 (1965).
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[9] T. G. GALLAGHER JR., M. M. MÜLLER and A. KAPPAS, Trans. Ass. Am. Physicians *78*, 187 (1965).
[10] It should also be noted that oral administration of XIII at 5 MPK in the rat failed to cause any elevation of serum glutamic oxaloacetic transaminase levels.

## Zur Leberregeneration der Ratte nach Teilhepatektomie während akuter $CCl_4$-Intoxikation[1]

Nach Injektion von $^3$H-Thymidin wurde autoradiographisch der Ablauf der Leberzellproliferation bei gleichzeitiger kompensatorischer und reparativer Regeneration an eben ausgewachsenen Ratten untersucht. Als Reiz für die kompensatorische Regeneration diente dabei eine 2/3 Teilhepatektomie (2/3 TH) und als Stimulus für die reparative Regeneration eine akute $CCl_4$-Intoxikation, wobei wir relativ einheitliche, läppchenzentral gelegene, bis auf die Intermidiärzone übergreifende Nekrosen induzierten. Als Funktion der Zeit nach diesen beiden Eingriffen überprüften wir folgende Faktoren: a) den Proliferationsumfang im Leberepithel gemessen am Prozentsatz DNS synthetisierender Kerne ($^3$H-Index) und in Mitose befindliche Zellen (Mitose-Index), b) den Proliferationsmodus, das heisst die DNS-Syntheserate (relative Neubildung/Zeiteinheit) sowie die Dauer der postsynthetischen Phase $G_2$ und der Mitose, c) den Proliferationsablauf in den Sternzellen. Die Befunde wurden solchen nach alleiniger 2/3 TH[2] konfrontiert.

Untersucht wurden männliche, im Mittel 240 g schwere Sprague-Dawley-Ratten, typische 2/3 TH zu beliebigen Tages- und Nachtzeiten, stets 3 h später $CCl_4$-Injektion i.p. (frisch bereitete 3% Lösung von reinem $CCl_4$ in

[1] Mit Unterstützung der Deutschen Forschungsgemeinschaft.
[2] E. STÖCKER, Verh. dt. Ges. Path. *50*, 53 (1966).
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