Experimental[5]

Hydroxypyridazones and -pyridazinones.—In a typical experiment a solution of Ia (100 mg.) and hydrazine hydrate (0.1 ml.) in ethanol (2.0 ml.) was heated at reflux for 4 hr. The reaction was diluted with water, and the solid was collected by filtration. When the product did not crystallize on dilution, it was recovered by extraction with ether.

2-Methylpyridazones.—The preparation is exemplified by the following experiment. To a solution of pyridazone IIIa (300 mg.) in potassium hydroxide (30%, 1.0 ml.) and methanol (5.0 ml.) was added dimethyl sulfate (0.5 ml.). The mixture was heated at reflux for 2 hr., then diluted with water. The steroid was taken up in ether, washed with water, then dried. Evaporation of the solvent gave a residue, which crystallized on trituration with solvent.

Oxidation of 17β- or 20β-Hydroxy Steroids.—To a suspension of chromium trioxide (100 mg.) in pyridine (5.0 ml.) was added a solution of a hydroxy steroidal pyridazone or pyridazinone (100 mg.) in pyridine (2.0 ml.). After 3.5 hr., ethyl acetate was added and the solids were removed by filtration with Celite. The filtrate was washed with sodium bicarbonate and water, then dried. Evaporation of the solvents left a residue containing traces of pyridine which were removed by repeated evaporations with ethyl acetate. The product was crystallized directly or purified by thin layer chromatography.

Biological Activity.—Representative new compounds were studied for mouse estrogenic,[6] mouse antiestrogenic,[7] chick androgenic,[8] mouse antiandrogenic,[9] and rat antiuterotrophic[10] activities. The results are listed in Tables II and III. Antiestrogenic activity was detected for five compounds and the tricyclic compound IIa showed an activity by injection equal to that of the reference standard, progesterone. The other four antiestrogens appear to be less active than progesterone.

TABLE III

RELATIVE ANTIESTROGENIC AND ANTIANDROGENIC POTENCY OF THE 3-KETO ACIDS AND PYRIDAZINONES

Compd.	Antiestrogenic activity[a] A	Antiandrogenic activity[a] B	Ratio A/B
Ic	<20	70	<0.3
IIa	100	50	2
IIc	35	100	0.3
IVa	50	70	0.7
IVb	<50	70	<0.7
IVc	50	<20	>2.5

[a] Progesterone = 100.

Five compounds, including two tricyclic compounds, were antiandrogenic in the mouse assay. Compound IIa was judged to be about one-half as active as the standard, progesterone; IIc was about as active as progesterone; and Ic, IVa, and IVb were rated as 70% as active as the standard.

Six compounds were studied in both the antiestrogenic and antiandrogenic assays and the results indicate that the two activities were not correlated (Table III).

(5) Melting points are corrected. Ultraviolet spectra were taken in methanol and infrared spectra in potassium bromide wafers. N.m.r. spectra were determined for chloroform-*d* solutions containing tetramethylsilane as the internal standard on a Varian V4300 B spectrometer. The values are expressed in τ-units.

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Studies on the Aromatization of 10β-Hydroxy-3-keto Steroids[1]

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The rate of aromatization of the A-ring of 17α-ethynylestr-4-ene-10β,17β-diol-3-one (17α-ethynyl-10β-hydroxy-19-nortestosterone) by different concentrations of hydrochloric acid has been studied both in methanol and in water. In methanol the reaction was relatively rapid, and at concentrations of HCl of 0.010 *M* and 0.003 *M*, respectively, the half-life of 17α-ethynyl-10β-hydroxy-19-nortestosterone was 5.0 and 11.0 min. The reaction rate in aqueous solution was very much slower and the acid concentration had to be 3 *M* before measurements could be obtained. The half-life of 17α-ethynyl-10β-hydroxy-19-nortestosterone under these conditions was 55.5 min. It is concluded that the aromatization of this compound under aqueous acid conditions such as those in the human stomach would be slow, although the production of physiologically active amounts of phenolic estrogen during a period of several hours is possible.

The synthetic steroid 17α-ethynylestr-5(10)-en-17β-ol-3-one (norethynodrel) has received considerable use as an inhibitor of ovulation and has been reported[3] to possess estrogenic activity. In a study of the metabolism of this compound after its oral administration to humans and animals we have shown that it is partially converted by gastric juice to 17α-ethynylestr-4-ene-10β,17β-diol-3-one (17α-ethynyl-10β-hydroxy-19-nortestosterone).[4] Since the acid-catalyzed dehydration of 10β-hydroxy and 10β-acetoxy steroids to phenols and their derivatives has been established by several workers[5-9] we have been interested in the extent to which such steroids might be aromatized *in vivo*, in particular in the stomach. The present paper reports a study of the rate of aromatization of estr-4-ene-10β,17β-diol-3-one (10β-hydroxy-19-nortestosterone) and 17α-ethynyl-10β-hydroxy-19-nortestosterone by solutions of HCl at 37°. Also presented is a countercurrent distri-

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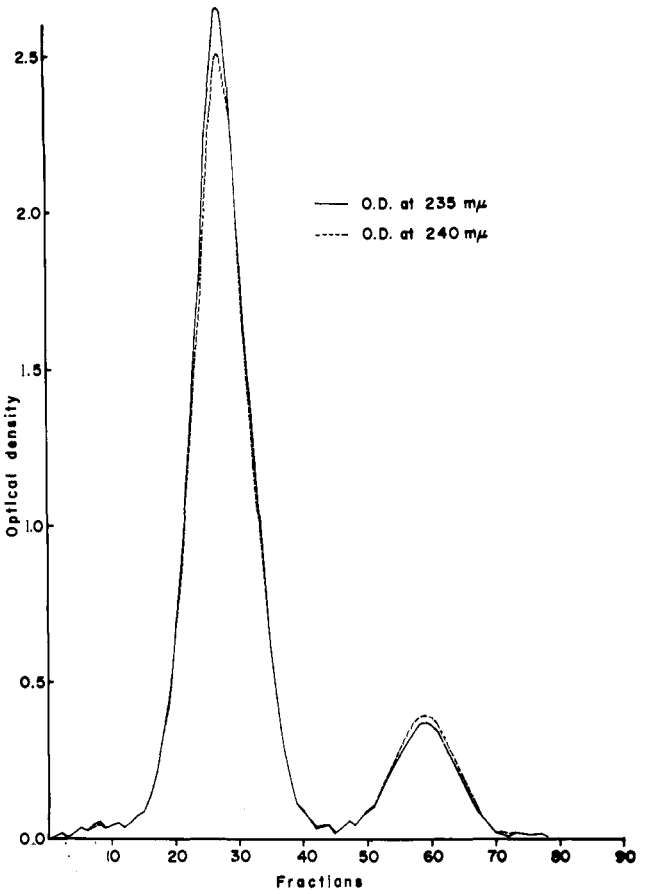

Fig. 1.—Countercurrent distribution of the products of the preparation of 17α-ethynyl-10β-hydroxy-19-nortestosterone. Peak I (K = 0.37) is pure 10β-hydroxy-17α-ethynyl-19-nortestosterone, which absorbs maximally at 235 mμ in ethanol. Peak II (K = 1.47) is 17α-ethynyl-19-nortestosterone which has λ_{max}^{EtOH} 240 mμ. The system was 60% ethyl acetate in hexane and 50% methanol in water, and the number of transfers was 99.

bution system suitable for the facile purification of 10β-hydroxy steroids prepared by the method of Ruelas, *et al.*,[6] in which the 5,10-bond of either estr-5(10)-en-17β-ol-3-one or its 17α-ethynyl analog is epoxidized by monoperphthalic acid in chloroform at low temperature and the epoxide rearranged in methanolic potassium hydroxide. Ruelas[6] reported a crude product in the first reaction of 65%. In our hands, it was not possible to obtain pure epoxide without a considerable decrease in yield and it was found expedient to carry out the two steps of the reaction without attempting a purification of the epoxide. The final reaction mixture was then purified by countercurrent distribution. This procedure offers the advantage of increasing the yield and avoiding the risk of dehydration of the product on chromatography columns.

Experimental

Chemistry.—Estr-5(10)-en-17β-ol-3-one, 17α-ethynylestr-5(10)-en-17β-ol-3-one, and a small reference sample of 17α-ethynylestr-4-ene-10β,17β-diol-3-one were obtained from G. D. Searle and Co., Chicago, Ill., through the courtesy of Dr. Frank Colton.

10β-Hydroxy-19-nortestosterone and 17α-ethynyl-10β-hydroxy-19-nortestosterone were prepared as described by Ruelas.[6] The two steps of the reaction were carried out without attempting a purification of the epoxide. The crude reaction product was then distributed between 60% ethyl acetate in hexane and 50% methanol in water for 99 transfers in a Craig Countercurrent Distribution instrument. The number of equilibration strokes was set for 20 and the settling time for 10 min. The concentration of solute was obtained by determining the optical density at 235 and 240 mμ in methanol of equal aliquots from both upper and lower phases of each tube. In the preparation of both 10β-hydroxy-19-nortestosterone and of the 17α-ethynyl analog, distribution of the crude products gave two peaks with K values of 0.37 and 1.47, respectively. In each case the 10-hydroxy derivatives were found in the first peak while the second peak contained 19-nortestosterone or 17α-ethynyl-19-nortestosterone.

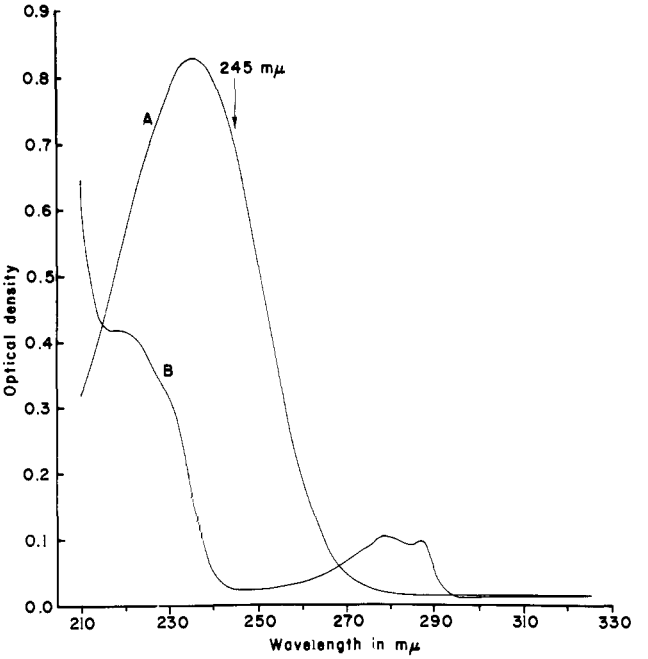

Fig. 2.—Ultraviolet absorption spectra of equimolar quantities (5.466×10^{-5} M) of 10β-hydroxy-19-nortestosterone and estradiol. The arrow (245 mμ) indicates the wave length at which the spectrophotometer was set in order to obtain a record of optical density against time.

The physical constants of the purified products were as follows: 10β-hydroxy-19-nortestosterone, m.p. 208–210°, lit.[6] 208–210°, λ_{max}^{EtOH} 235 mμ (ε 13,270), lit.[6] λ_{max}^{EtOH} 234–236 mμ (ε 13,200); 17α-ethynyl-10β-hydroxy-19-nortestosterone, m.p. 258–260°, lit.[6] 263–264°, λ_{max}^{EtOH} 235 mμ (ε 13,240), lit.[6] λ_{max}^{EtOH} 236 mμ (ε 14,570).[6] These two compounds were pure as judged by chromatography on thin layer silica gel.[10]

Kinetic Experiments.—Measurements of the rates of aromatization were made in the following manner. A solution containing approximately 0.7 mg. of steroid in 1 ml. of methanol was added to a rapidly stirred solution (49 ml.) of HCl in methanol or in water. The addition was carried out at 37°, and the time of addition was taken as zero. A capped cell, previously placed in the thermostated (37°) sample compartment of a Cary Model 14 spectrophotometer was filled with this solution, and a record of optical density *vs.* time was started at an accurately timed interval after zero time. Measurements were made at 252 mμ for aqueous solutions and 245 mμ for methanolic solutions. The spectrophotometer was balanced using a solution of reagent grade hydrochloric acid in the appropriate solvent. The various solutions of methanolic HCl were made by dilution of a stock solution of calculated 2 M acid in methanol. An accurate duplicate of this solution in water was standardized and found to be 2.01 M.

The record was stopped after 6 to 7 half-lives, and the spectrum of the solution was examined. For calculation of the half-life ($t_{1/2}$), ΔD was first obtained from the spectrophotometer chart by subtraction of the final fixed optical density from the values at various time intervals. Log ΔD_0 was determined by plotting log ΔD against time, and extrapolating the resulting straight line to zero. Subtraction of log 2 from log ΔD_0 gave log $\Delta D_{t_{1/2}}$. The half-life ($t_{1/2}$) was then obtained graphically.

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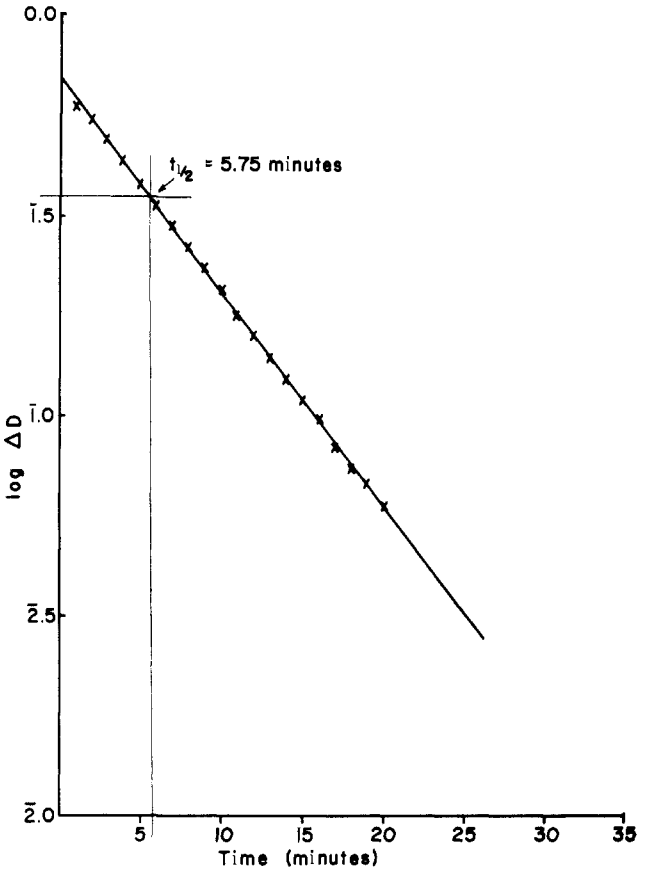

Fig. 3.—Calculation of the half-life ($t_{1/2}$) of 17α-ethynyl-10β-hydroxy-19-nortestosterone in 0.008 M HCl in methanol.

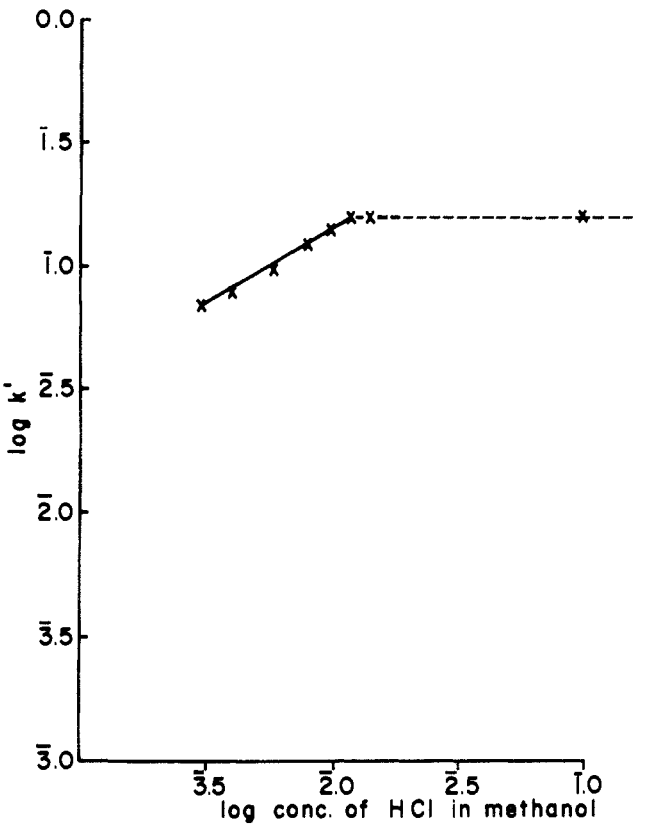

Fig. 4.—Relationship of the rate constant (log k') for the aromatization of 17α-ethynyl-10β-hydroxy-19-nortestosterone to acid concentration (log M) in methanol; see text.

The 10-hydroxy steroids were converted to products which absorbed at 288 mμ in water and at 286 and 278 mμ in methanol. These spectra were assumed to be due, respectively, to the corresponding ring A phenols and their methyl ethers. To confirm the identification the reaction mixture was extracted with ether and the extract chromatographed on thin layer silica gel G plates in a system of 50% ethyl acetate in cyclohexane, using the authentic compounds as standards. The developed chromatogram was heated at 100° and sprayed with a saturated solution of antimony trichloride in chloroform. Coincidence of the extracted material with the standard and similar color reactions in the visible and ultraviolet regions were taken as positive identification.[10]

Results and Discussion

The results of the countercurrent distribution of the crude products of the preparation of 17α-ethynyl-10β-hydroxy-19-nortestosterone are shown in Fig. 1. A curve of similar character was obtained from the products of the preparation of 10β-hydroxy-19-nortestosterone. Optical densities are plotted at 235 and 240 mμ and demonstrate the presence of the 10β-hydroxy Δ^4-3-ketone in the first peak and of the unsubstituted Δ^4-3-ketone in the second. The latter compound undoubtedly resulted from isomerization of the $\Delta^{5(10)}$-en-3-one starting material in either the acidic epoxidation or in the basic rearrangement media.

Pederson, *et al.*,[5] reported the acid-catalyzed dehydration of 10β-hydroxy-19-nortestosterone to estradiol, but did not give details of the reaction conditions. Ruelas, *et al.*,[6] obtained estradiol 17-acetate in 80% yield from 10β-hydroxy-19-nortestosterone by the action of hydrogen chloride in acetic acid at 5–10°. The present investigation of the aromatization of the 10β-hydroxy compounds by acid in aqueous solution indicated that the reaction was very slow at acid concentrations below 3 M. Well-defined spectra of ring A phenols were not observed in the aqueous reaction medium below this concentration of acid in periods of up to 12 hr., and valid quantitation of the reaction rate was not possible.

Recently, Gardi and Pedrali[7,8] found that various 5,10-substituted 3-keto 19-norsteroids gave 3-methoxy derivatives of the corresponding phenols by treatment with acid in methanol. Alvarez[9] confirmed this observation using 10β-acetoxy derivatives as starting compounds. The present work showed that in alcoholic solution, the aromatization of the 5,10-substituted 3-keto steroids proceeded rapidly, and it was possible in this medium to study the rate of reaction at 37° and the effect of acid concentration thereon. The selection of 245 mμ (or 252 mμ for aqueous solutions) for measurement of the disappearance of the 10β-hydroxy steroids is based on the results shown in Fig. 2, in which the spectra of starting material and product (in equimolar quantity) are superimposed. While 245 mμ is only 10 mμ removed from the wave length at which the 10β-hydroxy steroids absorb maximally (ϵ 12,630), it is the point at which the methyl ethers of the corresponding phenols absorb minimally (ϵ 400). Measurements can thus be made at this wave length with little loss of sensitivity and with minimum interference from the product of the reaction.

Fig. 3 shows that the plot of the log of concentration of 17α-ethynyl-10β-hydroxy-19-nortestosterone in methanolic HCl as a function of time is a straight line. However, the reaction is pseudo first order with

TABLE I

CONVERSION OF 10-HYDROXY-3-KETO STEROIDS BY DIFFERENT CONCENTRATIONS OF HCl[a]

Compound	Solvent	Acid concn., *M*	$t_{1/2}$, min.
10β-Hydroxy-19-nortestosterone	MeOH	1.0	4.35
	MeOH	0.012	4.25
	MeOH	0.008	5.75
	MeOH	0.003	10.75
	Water	3.0	66.5
17α-Ethynyl-10β-hydroxy-19-nortestosterone	MeOH	1.0	4.25
	MeOH	0.100	4.5
	MeOH	0.014	4.5
	MeOH	0.012	4.5
	MeOH	0.010	5.0
	MeOH	0.008	5.75
	MeOH	0.006	7.25
	MeOH	0.004	8.75
	MeOH	0.003	11.0
	MeOH	0.002	27.25
	MeOH	0.001	41.00
	Water	3.0	55.5
	MeOH	0.10	26 hr.[b]

[a] Values expressed as half-life of the starting material. [b] This value is of little quantitative significance, but is presented because of its special importance in the discussion.

respect to steroid since it is also dependent on hydrogen ion concentration. Figure 4 is a graph of log k' for the reaction of 17α-ethynyl-10β-hydroxy-19-nortestosterone against the log of the molarity of HCl in methanol. At concentrations of HCl greater than 0.01 *M* in methanol the measured k' values were independent of acid concentration. At the two concentrations of acid studied below 0.003 *M*, the half-life increased rapidly with the decreasing hydrogen ion concentration and the relation of the latter to log k' was not linear. Theories for the mechanism of aromatization have been advanced.[7-9]

Table I compares the rate of aromatization of 10β-hydroxy-19-nortestosterone and its 17α-ethynyl analog in methanol and in water for various concentrations of acid. No difference was observed in the rates for the two compounds. Nes, *et al.*,[11] have reported pronounced effects of 17α-substitution on the rate of isomerization of $\Delta^{5(10)}$- and $\Delta^{5(6)}$-3-keto steroids to their Δ^4-analogs, but the present results indicate that substitution of an ethynyl group for the 17α-hydrogen atom does not affect the acid-catalyzed aromatization of ring A in 10β-hydroxy-Δ^4-3-keto steroids.

The concentration of HCl in the stomach immediately after a meal is about 0.5 *M*.[12] As mentioned above, accurate measurements of the rate of aromatization of 10β-hydroxy-Δ^4-3-keto steroids were not possible at aqueous acid concentrations of this order, but the results in Table I are sufficient to indicate that the rate would be very slow. The major conversion of ingested norethynodrel to phenols would require prior hydroxylation at C-10, with the 10-hydroxy 3-ketone remaining in the stomach for several hours. Since the emptying time of the stomach, although highly variable, is from 3 to 5 hr. after a mixed meal,[13] the conversion of norethynodrel to ethynylestradiol would be small. This speculation takes no account of the possible catalysis of the reaction by other substances in the stomach, and enzymatic aromatization of 10β-hydroxy steroids in other parts of the body is possible. However, the results support the previous observations[12] based on an examination of the urinary and fecal conversion products of norethynodrel, that the per cent conversion of this compound *in vivo* to estrogenic phenols is not large. It must, however, be borne in mind that even a 1% conversion of a 10-mg. dose of norethynodrel to ethynylestradiol would result in a dose of this estrogen (0.1 mg.) which has physiological effects.[14] The aromatization of the 10β-hydroxy derivative in the stomach might therefore explain the low levels of estrogenicity exhibited by orally administered norethynodrel.

Acknowledgment.—The authors are indebted to Mrs. Francoise Gospodarowicz for technical assistance.

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