5α-Fluoro-17α-ethinyl-19-norandrostane-10β,17β-diol-3-one (VIIb). The boron trifluoride reaction of 200 mg. of the epoxide IIIb was carried out as described above for IIIa and after recrystallization from methanol-benzene there was obtained 160 mg. of the fluorohydrin VIIb, m.p. 247–249°, $[\alpha]_D$ −39° (methanol).

Anal. Calcd. for $C_{20}H_{27}FO_2$: C, 71.83; H, 8.14; F, 5.68. Found: C, 71.71; H, 7.99; F, 5.47.

10β-Hydroxy-17α-ethinyl-19-nortestosterone (IVb). This substance was obtained in about 80% yield when the epoxide IIIb or the fluorohydrin VIIb was heated under reflux for 1 hr. with 5% methanolic potassium hydroxide solution. The analytical sample crystallized from acetone or ethyl acetate and exhibited m.p. 263–264°, $[\alpha]_D$ + 4.5° (methanol), λ_{max}^{EtOH} 236 mμ, log ε 4.16, λ_{max}^{KBr} 2.95, 3.05, and 6.04 μ. The rotatory dispersion curve measured in dioxane solution (*c*, 0.059) was typical[27] of a Δ^4-3-ketosteroid with troughs[27] at $[\alpha]_{378}$ −556° and $[\alpha]_{355}$ −665° and a peak at $[\alpha]_{309}$ −604°.

Anal. Calcd. for $C_{20}H_{28}O_2$: C, 76.40; H, 8.34; O, 15.26. Found: C, 76.22; H, 8.35; O, 15.06.

Dehydration of 10β-hydroxy-19-nortestosterone acetate to estradiol 17-acetate (XI). A current of dry hydrogen chloride was passed for 2 hr. at 5–10° through a solution of 200 mg. of 10β-hydroxy-19-nortestosterone 17-acetate[5] (m.p. 182–183°, $[\alpha]_D$ +70°) in 10 cc. of glacial acetic acid. After diluting with water, extracting with ether, washing until neutral, drying, and evaporating there was left a solid residue which was recrystallized from acetone-hexane to give 145 mg. of estradiol 17-acetate (XI), m.p. 217–218.5°. Identity with an authentic specimen[28] was established by mixture melting point determination and infrared comparison.

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(27) For nomenclature see C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 55 (1957).

(28) C. Djerassi, G. Rosenkranz, J. Romo, S. Kaufmann, and J. Pataki, *J. Am. Chem. Soc.*, 72, 4534 (1950).

[CONTRIBUTION FROM THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, UNIVERSITY OF KANSAS MEDICAL CENTER]

Synthesis of Some 17-Methyl Phenolic Steroids

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17α-Methylestradiol was dehydrated with acid to give a product to which the structure 17-methyl-1,3,5(10),16-estratetraen-3-ol was assigned. The position of the double bond was not established unequivocally. Pd-on-charcoal reduction of the tetraene gave two 17-methyl-1,3,5(10)-estratriene isomers. Neither of the three new compounds was estrogenically active at 5-micrograms in preliminary testing.

Myers *et al.*[1] have stated that "it would be of practical as well as theoretical interest if compounds could be discovered which possess little or no primary hormonal activity, but which still have the ability to modify or regulate endocrine balance." With this general objective in mind several 17-methyl phenolic steroids of the estrane series were prepared; in preliminary testing the substances were estrogenically inactive at a 5 microgram level. One of the compounds has been mentioned in the early literature, but was never properly characterized.

The starting material for their preparation was 17α-methyl estradiol, which has been adequately characterized and tested;[2-4] it is about equal in estrogenic activity to 17β-estradiol. It has been reported[5] that dehydration of 17α-methylestradiol in boiling acetic acid, followed by high vacuum sublimation gives rise to a substance, m.p. 157–159°, having the structure shown in either IIa or III. In our hands treatment of 17α-methylestradiol (I) with either hot acetic acid or hydrochloric acid gave a crystalline mixture which could not be resolved by fractional crystallization. Chromatography on Celite–Mg trisilicate afforded a quantitative separation of unreacted I and a second crystalline substance. The latter when purified melted at 162–162.5°. Julia and Heusser[6] in a somewhat analogous procedure in the androstane series, dehydrated 17-methyl-5-androstene-3β, 17β-diol-3-acetate by mild treatment with phosphorus oxychloride–pyridine and (as the diacetate) by treatment with acetic anhydride–pyridine. In both cases a mixture of 17-methyl and 17-methylene dehydration products were obtained, the latter identified by its characteristic methylene absorption in the infrared at 11.36μ and 6.03μ. Only one product (besides a small amount of unreacted starting material) was found on dehydrating 17α-

(1) T. C. Myers, R. J. Pratt, R. L. Morgan, J. O'Donnell, and E. V. Jensen, *J. Am. Chem. Soc.*, 77, 5655 (1955).

(2) Elsevier's *Encyclopedia of Organic Chemistry*, Series III, Vol. 14, Supplement, Elsevier Publishing Company, New York, N. Y., 1956, p. 1988s. Several foreign patents are mentioned in this reference. Only the melting point of the free compound is given for characterization.

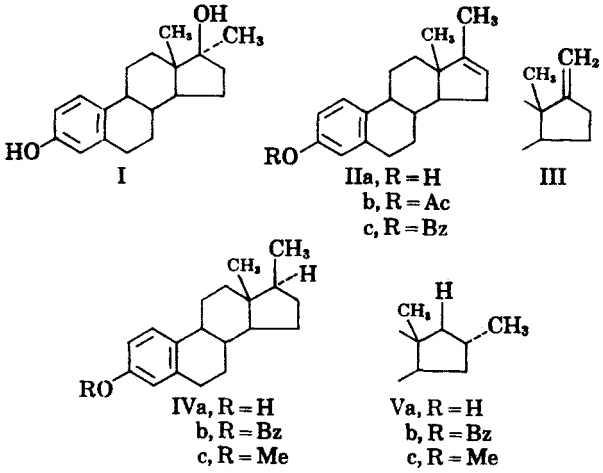
(3) H. H. Inhoffen and G. Zuhlsdorff, *Ber.*, 74, 604 (1941).

(4) B. C. Bocklage, H. J. Nicholas, E. A. Doisy, Jr., W. H. Elliott, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, 202, 27 (1953).

(5) Elsevier's *Encyclopedia of Organic Chemistry*, Series III, Vol. 14, Supplement, Elsevier Publishing Company, New York, N. Y., 1954, p. 1514s. Several foreign patents herein cited give only the m.p. of the free compound. Possible preparation of the compound is mentioned in Ref. 4.

(6) S. A. Julia and H. Heusser, *Helv. Chim. Acta*, 35, 2080 (1952).

methylestradiol with alcoholic HCl. Barring methyl migration such as occurred in the more vigorous experiments of Cohen, Cook, and Hewitt,[7] or unexpected double bond migration (which does not seem very likely), the structure of the dehydration product would appear to be that of IIa. There is no evidence of methylene bands at 11.36μ and 6.03μ in its infrared spectrum. Structure III is therefore ruled out. However the data do not provide unequivocal evidence for the position of the double bond in IIa; further investigation is in progress.



Hydrogenation of IIa in the presence of 5% Pd-on-charcoal gave a mixture of two isomers, resolved as their benzoates. It is a general rule that hydrogenation of steroids with unsaturation at $\Delta^{16,17}$ gives rise largely or almost exclusively to a single 17-epimeride.[8] In keeping with the discussions of Shoppee and similar hydrogenations in the androstane series[9] the dextrorotatory isomer produced in larger quantity has been called 17β-methyl-1,3,5(10), estratrien-3-ol. (IVa). The other isomer, of course, is 17α-methyl-1,3,5(10), estratrien-3-ol(Va). Both compounds exhibited the typical phenolic absorption in the ultraviolet at 280 mμ.

Bioassay. Neither of the 17-methyl steroids herein reported was estrogenically active in 5 microgram quantities when tested in adult ovariectomized mice. The compounds were administered subcutaneously in oil in one injection and assayed by the Allen-Doisy vaginal smear method. When assayed in the same manner 17α-methylestradiol and estradiol-17β produced a response in all mice tested at levels of 0.5 microgram. The compounds are therefore at least ten times less active than the naturally occurring estradiol-17β or the synthetic 17α-methylestradiol. All free compounds and several derivatives are currently being subjected to extensive biological assay.

EXPERIMENTAL

All melting points were determined on a Fisher-Johns melting point block. For the optical rotations the solvent was chloroform. The ultraviolet absorption spectra were determined in 95% ethanol with a Beckman model DU spectrophotometer. The infrared curves were prepared by the KBr disk method on a Baird Associates recording spectrophotometer.

17-Methyl-1,3,5(10),16-estratetraen-3-ol (IIa). A solution of 1.77 g. of 17α-methylestradiol (m.p. 195–196°; $[\alpha]_D^{17°}$ +32.2°), prepared according to Bocklage *et al.*[5] and 100 cc. of *N* ethanolic HCl was refluxed for 24 hr. The slightly colored solution was diluted with H_2O and extracted with ethyl ether. The latter was washed with H_2O, distilled, and the residue dried *in vacuo*. The dried semicrystalline residue could not be resolved by direct crystallization from a wide variety of solvents. It was accordingly dissolved in 15 cc. of warm benzene and transferred to a 50 g. column of 50/50 magnesium trisilicate–Celite (Johns-Manville Analytical Filter Aid) previously washed with petroleum ether (b.p. 30–60°). Eight liters of petroleum ether eluted 1.32 g. (yield about 94%) of solid which on one crystallization from aqueous methanol gave white crystals, m.p. 138–140°. A sample after nine crystallizations from aqueous methanol or acetone gave 0.84 grams of analytically pure IIa, m.p. 162–162.5° as thin micro needles; $[\alpha]_D^{18°}$ +36.6°. λ_{max} 280 mμ ($E_{1cm}^{1\%}$ 80).

Anal. Calcd. for $C_{19}H_{24}O$: C, 85.02; H, 9.01. Found: C, 84.98; H, 9.25.

The infrared spectrum of IIa lacked pronounced peaks at 9.2, 9.6, and 10.28 μ present in 17α-methylestradiol (I). An additional 0.38 grams of IIa, m.p. 159–160° was obtained from the mother liquor of the above crystallizations. There was no evidence of another compound.

Continued elution of the column with 2% ethanol in petroleum ether gave 0.44 grams of product which after two crystallizations from aqueous methanol melted at 192–194°, undepressed on admixture with I.

17-Methyl-1,3,5(10),16-estratetraen-3-ol acetate (IIb). The free phenol (IIa, 0.262 g., m.p. 162°) was acetylated with 3 cc. acetic anhydride and 4 cc. of anhydrous pyridine (room temperature, 24 hr.). Work-up and repeated crystallization (aqueous acetone or methanol) gave minute, glistening needles, m.p. 70–71°; $[\alpha]_D^{19°}$ −65.8°.

Anal. Calcd. for $C_{21}H_{26}O_2$: C, 81.25; H, 8.44. Found: C, 81.27; H, 8.19.

17-Methyl-1,3,5(10),16-estratetraen-3-ol benzoate (IIc). The free phenol (IIa, 0.083 g.) was treated with 20 cc. of 10% aqueous KOH and 1 cc. of benzoyl chloride. Work-up in the usual manner and crystallization to constant melting point from methanol-acetone mixtures gave jagged needles, m.p. 149–150°; $[\alpha]_D^{19°}$ −73.8°.

Anal. Calcd. for $C_{26}H_{28}O_2$: C, 83.84; H, 7.57. Found: C, 83.84; H, 7.56.

Saponification of the acetate or benzoate gave free 17-methyl-1,3,5(10),16-estratetraen-3-ol (IIa), m.p. 162°.

Hydrogenation of 17-methyl-1,3,5(10),16-estratetraen-3-ol. Attempts to selectively hydrogenate the $\Delta^{16,17}$-bond of IIa in the presence of Pt, Ni, or Pd catalyst were unsuccessful; only oils with no characteristic phenolic absorption (280 mμ) were obtained.[10]

IIa (0.884 g.) and 0.8 g. of 5% Pd-on-charcoal in 100 cc. absolute ethanol were shaken under 60 p.s.i. hydrogen for 9

(7) A. Cohen, J. W. Cook, and C. L. Hewitt, *J. Chem. Soc.*, 445 (1935).

(8) C. W. Shoppee, *Nature*, **166**, 107 (1950).

(9) M. Heller and S. Bernstein, *J. Am. Chem. Soc.*, **78**, 1161 (1956).

(10) Probably the "17-methyl octahydrofollicular hormone" (no constants given) of German Patent **643,979** (1937); Schering-Kahlbaum A.-G. According to this reference the substance shows weak androgenic activity in the capon test.

hr. Removal of catalyst and solvent gave an oil from which no crystalline product could be obtained. Elution from a magnesium trisilicate (50/50) column with petroleum ether gave 0.905 g. of light colored oil which likewise could not be crystallized.

Isolation of 17β-methyl-1,3,5(10)-estratrien-3-ol benzoate (IVb). The above oil was treated with 25 cc. 10% aqueous KOH and 5 cc. benzoyl chloride. Work-up gave a light oil which crystallized from methanol as a good crop of jagged needles, m.p. 134–135°. Several crystallizations from acetone gave white rods, m.p. 160–161.5°, unchanged by an additional crystallization from methanol-benzene; $[\alpha]_D^{17°}$ +43.5°.

Anal. Calcd. for $C_{26}H_{30}O_2$: C, 83.38; H, 8.07. Found: C, 83.20; H, 8.01.

17β-Methyl-1,3,5(10)-estratrien-3-ol (IVa). The previous benzoate (IVb, 0.271 g.) was refluxed in 20 cc. of 5% alc. KOH for 1 hr. Work-up and crystallization to constant melting point from aqueous methanol gave micro needles, m.p. 133–135°, not raised by an additional crystallization from aqueous acetone: $[\alpha]_D^{17°}$ +92.5°. λ_{max} 280 mμ ($E_{1\,cm.}^{1\%}$ 80).

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.68. Found: C, 84.20; H, 9.49.

17β-Methyl-1,3,5(10)-estratrien-3-methyl ether (IVc). The free phenol (IVa, 0.125 g.) was treated with 1 cc. of dimethyl sulfate and 20 cc. of 10% aqueous KOH. Work-up gave a colorless oil which could not be crystallized. Sublimation at 70° on a cold finger at 2.5×10^{-3} mm. pressure gave a colorless oil; $[\alpha]_D^{18°}$ +53.1°. λ_{max} 280 mμ.

Anal. Calcd. for $C_{20}H_{28}O$: C, 84.45; H, 9.92. Found: C, 84.36; H, 9.70.

Acetylation of IVa gave an oil which could not be crystallized and was not analyzed. Saponification of this oil gave IVa, m.p. 130°.

Isolation of 17α-methyl-1,3,5(10)-estratrien-3-ol benzoate (Vb). Following the removal of as much IVb as possible by crystallization from methanol, the mother liquor was freed of solvent, leaving an oily deposit. This was saponified, but the resulting oil could not be crystallized, even after chromatography on alumina. It was rebenzoylated (benzoyl chloride in aqueous alkali) and the crude product in methanol gave a small deposit which was filtered and discarded. The filtrate was free of solvent and the residue was dissolved in aqueous acetone. Eventually 0.243 g. of needles deposited. Crystallization to constant melting point from aqueous acetone-methanol gave brilliant needles, m.p. 118–120°; $[\alpha]_D^{17°}$ −50.7°.

Anal. Calcd. for $C_{26}H_{30}O_2$: C, 83.38; H, 8.07. Found: C, 83.37; H, 8.07.

17α-Methyl-1,3,5(10)-estratrien-3-ol (Va). Saponification of the benzoate Vb (0.130 g.) gave small, pearly scales. Crystallization to constant melting point gave white crystals, m.p. 129–129.5°; $[\alpha]_D^{17°}$ −79.1°. λ_{max} 280 mμ ($E_{1\,cm.}^{1\%}$ 81).

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.68. Found: C, 84.49; H, 9.51.

17α-Methyl-1,3,5(10)-estratrien-3-methyl ether (Vc). The free phenol Va (0.020 g.) was treated with 20 cc. 10% aqueous KOH and 1 cc. dimethyl sulfate. Work-up gave an oil which could not be crystallized. Sublimation at 70° under 2.5×10^{-3} mm. Hg (cold finger apparatus) gave 0.015 g. of colorless oil which could not be crystallized.

Anal. Calcd. for $C_{20}H_{28}O$: C, 84.45; H, 9.92. Found: C, 84.40; H, 9.96.

Acetylation of Va (pyridine, acetic anhydride, room temp.) gave a non-crystallizable oil which was not analyzed. This oil on saponification gave free V, m.p. 126–128°.

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KANSAS CITY, KANSAS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MICHIGAN]

Synthesis of Potential Anticancer Agents. I. Nitrogen Mustards Derived from *p*-[*N*,*N*-Bis(2-chloroethyl)amino]benzaldehyde[1]

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An improved synthesis of *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzaldehyde (benzaldehyde mustard) is described. Nitrogen mustard derivatives of cinchophen and barbituric acid have been prepared. Condensation products of benzaldehyde mustard and active methyl derivatives of selected heterocyclic compounds have been prepared. Representative benzylidene acyl hydrazides have been prepared from benzaldehyde mustard.

Compounds containing the β,β′-bischloroethylamino grouping, otherwise known as nitrogen mustards, have frequently displayed selective action against neoplastic cells as compared to normal cells.[2] The concept of a pharmacologically active substance being composed of an active moeity and a carrier moeity was first put forward by Ing.[3] The genesis of the present study was based on this concept.

Considerable information is at hand concerning the absorption and fate of the drug, cinchophen (2-phenylquinoline-4-carboxylic acid)[4] so that it seemed reasonable to expect that cinchophen might act as a carrier molecule to direct a mustard grouping to some effective locus of action. A logical

(1) This work was supported by Research Grant CY-2961 from the National Cancer Institute of the Public Health Service.

(2) The entire field has been reviewed in the monograph *Comparative Clinical and Biological Effects of Alkylating Agents*, Annals of the New York Academy of Sciences, Vol. 68, Art. 3 (April 24, 1958).

(3) H. R. Ing, *Trans. Faraday Soc.*, **39**, 372 (1943); see also ref. 2, p. 1238.

(4) L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics*, 2nd ed., The Macmillan Co., New York, 1955, p. 301.