[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN AND NO. 1036 FROM THE DEPARTMENT OF CHEMISTRY OF INDIANA UNIVERSITY]

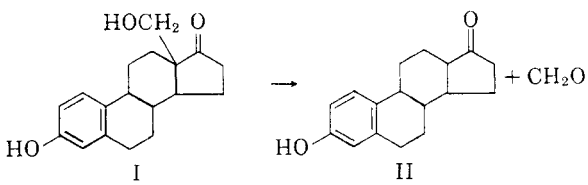
Total Synthesis of *dl*-18-Norestrone[1]

WALTER L. MEYER,[2a] D. D. CAMERON, AND WILLIAM S. JOHNSON[2b]

Received November 6, 1961

The synthesis of *dl*-18-norestrone (II) from the known *dl*-18-nor-D-homoestrone methyl ether (IVβ) is described. The corresponding methyl ether (VIIIβ) was shown to be the racemic form of the methyl ether of the compound obtained by alkaline treatment of 18-hydroxyestrone (I), thus confirming the structure of the latter. In the equilibrium mixture of VIIIβ and its 13-iso epimer, the latter predominates in an approximately 2 to 1 ratio.

Loke, Watson, and Marrian[3] reported the isolation of a new estrone derivative from the ketonic phenolic fraction of the urine of pregnant women. This compound, $C_{18}H_{22}O_3$, was provisionally assigned the structure 18-hydroxyestrone (I) on the basis of a negative reaction with the blue tetrazolium reagent (absence of an α-ketol function), sodium borohydride reduction to an estriol, absence of the absorption at 7.26 μ in the infrared that is characteristic of the angular methyl, and cleavage by alkali to formaldehyde and a new ketone, II, $C_{17}H_{20}O_2$. The alkaline cleavage was considered to be a dealdolization, and the ketonic product II was thus formulated as 18-norestrone, these structures for I and II being the only ones to accommodate the production of formaldehyde by the retrograde aldol cleavage of an hydroxylated C_{18} steroid.



I II

Since intermediates developed in these laboratories in the course of the total synthesis of estrone[4] promised ready conversion to 18-norestrone (II), it was considered that the total synthesis of II would offer the most direct unequivocal proof of its structure, and hence that of its natural precursor I. Further, at that time few 18-nor steroid hormones were known,[5] and in view of the established therapeutic importance of certain 19-nor hormones, it seemed of interest to examine the physiological behavior of representative members of the 18-nor series.[6] The attainment of the synthetic objective, together with several interesting observations on the nature of the products, is the subject of the present paper.

Total synthesis of dl-18-norestrone methyl ether and its C-13 epimer. It was previously reported[4] that aluminum chloride catalyzed cyclization of 5-β-*m*-methoxyphenylethyl-Δ^9-1-octalone, III, produced a mixture of two isolable stereoisomeric forms of the methoxyhydrochysenone IV, designated α (IVα) and β (IVβ).[7] That the β isomer possesses the *trans-anti* (C-9, C-8, C-14) configuration of the natural steroids has been unequivocally demonstrated by its conversion to estrone,[4] which is known to have this stereochemistry,[8] by a sequence of reactions which left the centers of asymmetry in question undisturbed. In the present work, cyclization of III afforded, rather than the mixture of IVα and IVβ previously obtained, only the desired isomer (IVβ) as an isolated product. In fact the yield of this isomer, although poor, was significantly higher than previously obtained, and scrutiny of the phenolic fraction formed concomitantly in the aluminum chloride treatment revealed further amounts of the corresponding phenol. Clearly the nature and quantity of products

(1) A preliminary account of a portion of this work has appeared, K. H. Loke, G. F. Marrian, W. S. Johnson, W. L. Meyer, and D. D. Cameron, *Biochim. Biophys. Acta*, **28**, 214 (1958).

(2)(a) National Science Foundation Postdoctoral Fellow, University of Wisconsin, 1957–58; present address, Department of Chemistry, Indiana University; (b) Present address, Department of Chemistry, Stanford University.

(3) K. H. Loke, E. J. D. Watson, and G. F. Marrian, *Biochim. Biophys. Acta*, **26**, 230 (1957); K. H. Loke, G. F. Marrian, and E. J. D. Watson, *Biochem. J.*, **71**, 43 (1959).

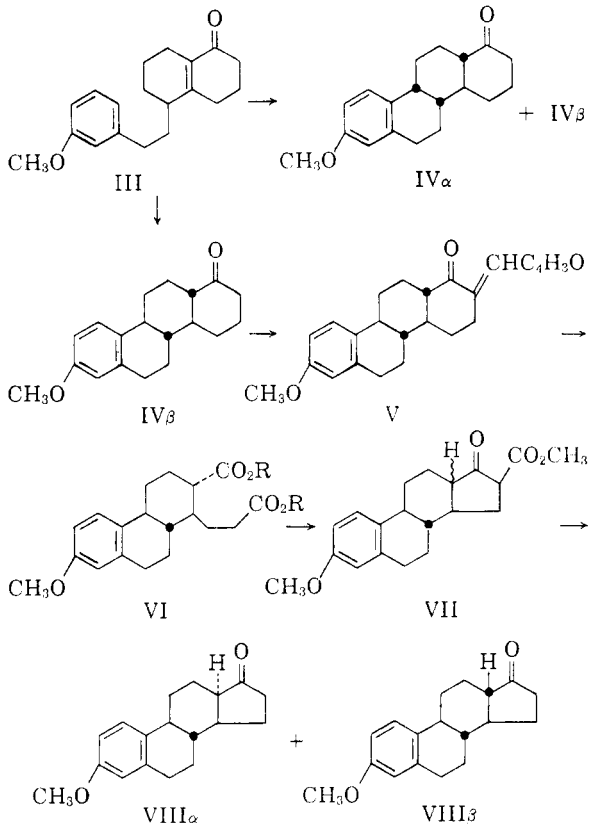
(4) W. S. Johnson, D. K. Banerjee, W. P. Schneider, C. D. Gutsche, W. E. Shelberg, and L. J. Chinn, *J. Am. Chem. Soc.*, **74**, 2832 (1952).

(5) *Cf.* N. A. Nelson and R. B. Garland, *J. Am. Chem. Soc.*, **79**, 6313 (1957).

(6) Since our initial communication, several other partial and total syntheses of 18-norsteroids have been published, *inter alia* (a) W. F. Johns, *J. Am. Chem. Soc.*, **80**, 6456 (1958); (b) G. Stork, H. N. Khastgir, and A. J. Solo, *J. Am. Chem. Soc.*, **80**, 6457 (1958); (c) R. Anliker, M. Müller, M. Perelman, J. Wohlfahrt, and H. Heusser, *Helv. Chim. Acta*, **42**, 1071 (1959); (d) L. Velluz, G. Amiand, R. Heymes, and B. Goffinet, *Compt. rend.*, **250**, 371 (1960); (e) W. S. Johnson and K. V. Yorka, *Tetrahedron Letters*, No. 8, 11 (1960).

(7) Unless otherwise specified, all synthetic compounds herein discussed are racemic. A single enantiomer is depicted in each of the structural formulas. Steroid numbering is used throughout.

(8) W. S. Johnson, I. A. David, H. C. Dehm, R. J. Highet, E. W. Warnhoff, W. D. Wood, and E. T. Jones, *J. Am. Chem. Soc.*, **80**, 661 (1958).



formed in this and related cyclization reactions are highly dependent on the reaction conditions.[8,9]

The contraction of ring D of IVβ was accomplished by the reaction sequence applied in other series.[10,11] Condensation with furfuraldehyde afforded in excellent yield the corresponding furfurylidene ketone V, m.p. 172–173°, λ_{max} 325 mμ. Initial attempts to ozonize this to the dibasic acid VI (R = H) afforded low and variable yields of the desired product. While ozone has been used to carry out this type of conversion in excellent yield in saturated steroid syntheses,[12] results in other aromatic series have often been less favorable,[4,8] apparently because of attack at the activated benzylic position at C-9 with the production of undesirable by-products.[8] In the hope of minimizing such side reactions resulting from an excess of ozone or prolonged exposure to oxygen, the use of a solution of ozone as the reagent was investigated. Indeed, we found that on addition of a saturated methylene chloride solution of the appropriate amount of ozone to the furfurylidene ketone, reaction was nearly instantaneous, and subsequent decomposition of the ozonide with periodic acid afforded reproducibly high yields of the desired *dl*-18-norhomomarrianolic acid methyl ether VI (R = H), m.p. 215–215.5°. The ultraviolet spectrum of this product, λ_{max} 278, 287 mμ, confirmed the presence of the 3,4-disubstituted anisole as the only chromophore.

The dimethyl ester VI (R = CH_3), m.p. 84–84.5°, λ_{max} 278, 287 mμ, prepared by the use of diazomethane, underwent Dieckmann cyclization in nearly quantitative yield on treatment with potassium *t*-butoxide in benzene.[11] Surprisingly, however, attempted hydrolysis and decarboxylation of the resulting keto ester VII with hydrochloric and acetic acids[11] produced little, if any, of the desired 18-norestrone methyl ether (VIII).[13] Only higher-melting materials were obtained, perhaps the products of aldol dimerization of the initially produced norestrone, C-16 and C-17 being considerably less hindered here than with the C_{18} homologs.

As basic hydrolysis of VII would be expected to open ring D, producing VI rather than VIII,[14] we turned our attention to a purely thermal process.[15] Hydrolysis and decarboxylation proceeded smoothly when the keto ester VII was heated briefly at 186° in aqueous triethylene glycol. The neutral product from this reaction, obtained in 85% yield, proved to be a mixture of the two C-13 epimeric *dl*-18-norestrone methyl ethers VIIIα and VIIIβ. This mixture was separated into its components by chromatography on Florisil, affording VIIIα, m.p. 123–124°, λ_{max} 278, 287 mμ, and VIIIβ, m.p. 153–153.5°, λ_{max} 278, 287 mμ in a ratio of about 2 to 1. The assignment of configurations to the epimers is considered in the sequel.

On demethylation with pyridine hydrochloride,[8] the crude mixture of VIIIα and VIIIβ afforded the parent phenol, *dl*-18-norestrone (II). There is no reason to doubt that this product, like the methyl ether, consisted of a mixture of C-13 epimers. However, only one of these, m.p. 225.5–227° dec., was readily isolated from the reaction mixture. Only a sufficient quantity of the pure phenol for melting point comparison with the naturally derived *d*-enantiomorph,[3] m.p. 226–228°, was pre-

(9) A similar observation of the formation of the β-isomer as the sole isolable product in a related cyclization was made by H. C. Dehm, Ph.D. dissertation, University of Wisconsin, 1954. In that and the present work, freshly resublimed aluminum chloride was used to catalyze the cyclization, and it is to this more active catalyst that the results are ascribed. The yield of IVβ in the present work was 9.6%, including that obtained by methylation of the by-product phenol.

(10) W. S. Johnson, *J. Am. Chem. Soc.*, **65**, 1317 (1943).

(11) W. S. Johnson, B. Bannister, and R. Pappo, *J. Am. Chem. Soc.*, **78**, 6331 (1956).

(12) W. S. Johnson, E. R. Rogier, and J. Ackerman, *J. Am. Chem. Soc.*, **78**, 6322 (1956) and subsequent papers in the series.

(13) In our preliminary communication (ref. 1) we reported acidic conversion of VII to VIII, but this experiment could not be repeated.

(14) *Cf.* W. Dieckmann, *Ann.*, **317**, 27 (1901).

(15) Several high-boiling solvents have been utilized for such thermal hydrolyses, *cf.* H. Meerwein, *Ann.*, **398**, 242 (1913); W. S. Johnson, R. Pappo, and W. F. Johns, *J. Am. Chem. Soc.*, **78**, 6339 (1956); W. S. Johnson, B. Bannister, R. Pappo, and J. E. Pike, *J. Am. Chem. Soc.*, **78**, 6354 (1956); and W. L. Meyer and W. R. Vaughan, *J. Org. Chem.*, **22**, 1554 (1957).

pared. All further studies, *i.e.*, comparisons and configurational determinations, were carried out with the methyl ethers.

The infrared spectrum of the 153° synthetic (racemic) isomer was rich in detail and identical with that of the methyl ether[3,16] obtained by methylation of the naturally derived *d*-II. The spectrum of the 124° racemate, on the other hand, differed considerably in detail in the 6–13-μ region. Subsequent to the completion of this work, Johns[6a] described a partial synthesis of the enantiomers of VIIIα and VIIIβ from natural steroids. Infrared spectra of these two substances proved to be identical with those of the 124 and 153° racemates, respectively.[17] Thus the dealdolization product, II, was unequivocally shown to have the assigned structure, and the natural precursor must indeed be 18-hydroxyestrone (I). The possibility of the 13-iso configuration is considered unlikely on biogenetic grounds.

Configurations of the epimeric norestrone ethers. By virtue of the method of synthesis from IVβ, the asymmetric centers at C-9, C-8, and C-14 of VIIIa and VIIIβ must have the same relative configurations, and this must be *trans-anti* (see above). Thus VIIIα can differ from VIIIβ *only* in the relative configuration at C-13, *i.e.*, C/D fusion *cis* (13α-H) and *trans* (13β-H). The greater solubility, lower melting point, and more rapid elution on chromatography of the 124° isomer initially suggested that it corresponded to the 13-iso structure with the C/D *cis* fusion and the carbonyl group axial to ring C. This was readily confirmed by optical rotatory dispersion measurements. The curve for the naturally derived methyl ether[3,16] exhibited a strong positive Cotton effect typical of *trans*-α-hydrindanones of this (17-keto steroid) enantiomeric series. Since it is known that analogous *cis*-α-hydrindanones show a negative Cotton effect,[18] Marrian's[3,16] methyl ether and, in turn, the 153° racemate must be *d*- and *dl*-18-norestrone methyl ether (VIIIβ), respectively. The 124° isomer accordingly is *dl*-13-iso-18-norestrone methyl ether (VIIIα).[18c]

The 13-iso epimer VIIIα is inert to pyridine-borane[6e] under conditions which effect complete reduction of VIIIβ and estrone methyl ether. Thus the generality of this reagent for ascertaining configurations of 17-keto-18-norsteroids is supported and the validity of the earlier structural assignments using this technique[6e] (C/D *trans* reduced, C/D *cis* unattacked) is confirmed.

The vigor of the conditions for their formation suggests that the isolated mixture of VIIIα and VIIIβ may represent nearly the equilibrium ratio of these epimers with respect to the enolizable center C-13. Although no precise quantitative estimation of this ratio was made in the course of this work, the infrared spectrum of the hydrolysis mixture suggested the two isomers to be present in roughly equal amounts. On chromatographic fractionation, about 65% of the separated material was isolated as nearly pure VIIIα and about 35% as nearly pure VIIIβ, although in several intermediate fractions, amounting to 10% of the total, the mixture remained unresolved. Allinger[18c] has measured the position of this equilibrium by means of optical rotatory dispersion, and found the equilibrium mixture to consist of 55% VIIIα and 45% VIIIβ, while Johns,[6a] from rotation measurements, suggested a 70% VIIIα–30% VIIIβ mixture. The fact that only VIIIβ was obtained on methylation of naturally derived II is undoubtedly a result of separating only the less soluble isomer.

EXPERIMENTAL[19]

*dl-17-Furfurylidene-18-nor-*D*-homoestrone methyl ether* (V). A solution of 256 mg. of the methoxyhydrochrysenone IVβ, m.p. 153.5–156.0°, in 51 ml. of methanol was treated with 0.5 ml. of furfuraldehyde and 2.6 ml. of 33% sodum hydroxide solution under a nitrogen atmosphere. After 16 hr. in the dark at room temperature, the crystalline precipitate was filtered and washed with methanol, affording a first crop of 274 mg. (84%), m.p. 156–171°.[20] A second crop of 31 mg., m.p. 162–167°, could readily be obtained by concentration and cooling, bringing the total yield to 305 mg. (93%). After one recrystallization from benzene–petroleum ether, the product formed pale yellow needles, m.p. 172–173°, $\lambda_{max}^{95\%\ C_2H_5OH}$ 325 mμ (log ϵ 4.33), $\lambda_{max}^{CHCl_3}$ 6.02, 6.23, 6.30 μ. Several recrystallizations from the same solvent pair produced the analytical sample, m.p. 170–172°, $\lambda_{max}^{95\%\ C_2H_5OH}$ 325 mμ (log ϵ 4.35).

Anal. Calcd. for $C_{24}H_{26}O_3$: C, 79.52; H, 7.23. Found:[21] C, 79.4; H, 7.3.

dl-18-Norhomomarrianolic acid methyl ether (VI. R = H). Ozonized oxygen, generated by a Wellsbach Model T-23 ozonizer operating at an oxygen pressure of 8 p.s.i., 110 volts, and a flow rate of 0.015 cu. ft./min., was passed through 8.4 ml. of dry methylene chloride at −70° for 10 min. in chamber B of the Rubin[22] ozonization apparatus

(16) We are indebted to Professor G. F. Marrian for the sample of this material.

(17) We wish to express our thanks to Dr. Johns for samples of his products.

(18) (a) C. Djerassi *et al.*, *J. Am. Chem. Soc.*, **78**, 440, 3163, 6362 (1956); (b) J-F. Biellmann, P. Crabbé, and G. Ourisson, *Tetrahedron*, **3**, 303 (1958); (c) N. L. Allinger, R. B. Hermann, and C. Djerassi, *J. Org. Chem.*, **25**, 922 (1960). We are indebted to Professor Carl Djerassi and Dr. E. J. Eisenbraun for the optical rotatory dispersion measurements. The O. R. D. curve is reproduced and discussed in ref. 18c.

(19) Unless otherwise specified, all melting points are corrected for stem exposure.

(20) This material appeared to undergo a polymorphic change during melting, starting as prisms which underwent extensive melting from 156–158°, at which temperature stout needles could be observed in the melt. Cooling from 158° produced recrystallization as needles which remelted 165–171°. In some preparations, only needles were obtained from the reaction, in which cases the crude product had m.p. 169–171°.

(21) Microanalysis by G. Winestock and S. M. Aronovic.

(22) Designed by Dr. M. B. Rubin at the University of Wisconsin. The volume of ozone-saturated methylene chloride was determined as that which is just sufficient to be completely decolorized by 0.208 mmole of 2-furfurylidene-1-decalone in the same apparatus.

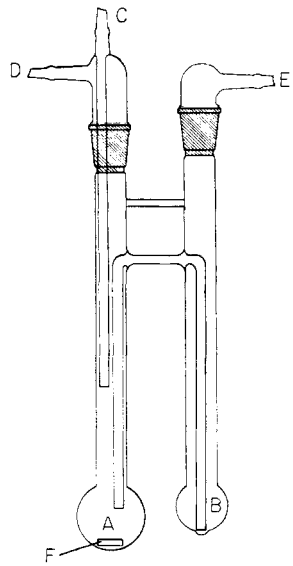


Fig. 1. Ozonization apparatus. A, ozonization chamber; B, chamber for saturation of solvent with ozone; C, sample introduction tube; D, ozone inlet; E, ozone outlet; F, magnetic stirrer

shown in Fig. 1. A solution of 75 mg. (0.208 mmole) of the furfurylidene ketone V in a small volume of methylene chloride was introduced into chamber A, and at −70° with stirring the ozone solution was forced into chamber A by applying a small positive pressure of dry nitrogen to tube E. The blue color of the ozone was completely discharged, a colorless solution resulting. The apparatus was transferred to an ice bath and 3 ml. of 0.33 *M* aqueous periodic acid was added, followed by sufficient acetic acid to produce a homogeneous solution, approximately 11 ml. being required. This mixture was allowed to come to room temperature and remain there for 22–24 hr.

Three such reaction mixtures, pale yellow in color, were combined (0.624 mmole of V) and diluted with 250 ml. of dilute sodium chloride solution and 100 ml. of ethyl acetate. The phases were separated, the organic phase was washed with dilute salt, and the combined salt solutions were extracted with ethyl acetate. The combined organic solutions were dried with sodium sulfate, and the solvent was removed at reduced pressure. The residue was taken up in ethyl acetate, acidic material was thoroughly extracted with excess 5% sodium bicarbonate, these basic extracts were chilled in ice, acidified to Congo Red with 10% hydrochloric acid, and the product was extracted with ethyl acetate. The extracts were washed with saturated brine, dried with sodium sulfate, and the solvent was removed at reduced pressure. The tan oil which remained crystallized readily on trituration with ether, and after being washed three times with small volumes of ether, afforded 93 mg. of pale tan crystals. The ether wash solutions were evaporated and again triturated to afford a second crop of 44 mg. By chromatography of the residue on 600 mg. of silicic acid, a further quantity of nicely crystalline colorless acid could be obtained in the chloroform and 1% methanol in chloroform eluates, bringing the total yield of crude VI (R = H) to 170 mg. (82%) of tiny prisms, m.p. 208–215°. After one recrystallization from benzene–ethyl acetate, 108 mg. (52%) of tiny prisms, m.p. 216–219°, was obtained, $\lambda_{max}^{95\%\ C_2H_5OH}$ 278 mμ (log ε 3.29) and 287 (3.26). An analytical sample, m.p. 215–215.5°, was prepared by several recrystallizations from the same solvent pair.

Anal. Calcd. for $C_{19}H_{24}O_5$: C, 68.66; H, 7.28. Found:[21] C, 68.4; H, 7.4.

A dimethyl ester VI (R = CH_3), m.p. 74–79°, was obtained in quantitative yield on treatment with diazomethane. One recrystallization from methanol raised the m.p. to 77–80°. The analytical sample was recrystallized several times from methanol, m.p. 84.0–84.5°, $\lambda_{max}^{95\%\ C_2H_5OH}$ 278 mμ (log ε 3.33), 287 (3.30), $\lambda_{max}^{CHCl_3}$ 5.84 and 6.24 μ.[23]

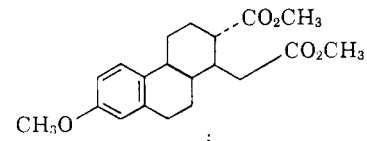
Anal. Calcd. for $C_{21}H_{28}O_5$: C, 69.98; H, 7.83. Found:[24] C, 69.7; H, 7.8.

dl-18-Norestrone methyl ether (VIIIβ) *and dl-13-iso-18-norestrone methyl ether* (VIIIα). The Dieckmann cyclization was carried out by the procedure used in the epiandrosterone series.[11] To a suspension of alcohol-free potassium *t*-butoxide in 20 ml. of dry benzene (prepared from 0.7 g. of potassium by the procedure in ref. 11) in a dry nitrogen atmosphere was added a solution of 225 mg. of the dimethyl ester VI (R = CH_3), m.p. 79–83°, in 25 ml. of dry benzene, and this was followed by an additional 30 ml. of benzene. The pale yellow suspension was stirred at reflux for 4 hr. and then at room temperature for an additional 15 hr. To the mixture was added 1.8 ml. of acetic acid, the resulting white suspension was poured into excess 5% sodium bicarbonate, the layers were separated, and the aqueous solution was extracted twice with ether. The ether solution was washed with saturated brine and dried with sodium sulfate and the solvent was removed at reduced pressure to afford 205 mg. (100%) of oily product which crystallized readily on trituration with methanol, m.p. 96–141°, $\lambda_{max}^{95\%\ C_2H_5OH}$ 278 mμ (log ε 3.30) and 287 (3.27) and $\lambda_{max}^{CHCl_3}$ 5.73, 5.80, 6.22, and 6.35 μ. This material, which was presumed to be a mixture of epimeric keto esters, gave the typical violet color with alcoholic ferric chloride.

A 196-mg. sample of the crude keto ester was suspended in 55 ml. of triethylene glycol containing 2 ml. of water, and the mixture under a nitrogen atmosphere was immersed in an oil bath preheated to 208°. Stirring was initiated, the temperature rapidly fell to 186°, and the suspended material quickly dissolved. The temperature was maintained at 186° for 8 min. after initiation of heating. The mixture was then cooled, diluted with 150 ml. of water, and extracted with chloroform. After washing the chloroform extracts with water, they were dried with sodium sulfate and evaporated to dryness. Chromatography of the oily residue (which gave no color with ferric chloride) on Florisil afforded 137 mg. (85%) of a crystalline mixture of VIIIα and VIIIβ in the 50–95% benzene in petroleum ether eluates.

This mixture was separated into its two components by a second chromatogram on Florisil, VIIIα being eluted in the early 50% benzene in petroleum ether fractions, and VIIIβ being removed in later fractions and finally completely eluted by 10% ether in benzene. From 249 mg. of the crystal-

(23) A substance, m.p. 110–111°, to which this structure was assigned was obtained by esterification of the permanganate oxidation product of the corresponding piperonylidene ketone in the course of the estrone synthesis, ref. 4. The oxidation product was isolated in very low yield, and too little of the 111° material was available for further study at that time. The infrared spectra of VI and the 111° material show slight differences in the fingerprint region, and a mass spectral molecular weight determination supports formulation of the 111° material as the corresponding norester i. This presumably arises from over-oxidation by permanganate as is known to sometimes occur. The mass spectral determination was carried out on a Type 21 C. E. C. mass spectrometer by Dr. H. Budzikiewicz to whom we extend our thanks.



i

(24) Microanalysis by Spang Microanalytical Laboratory, Ann Arbor, Mich.

line mixture there was obtained in this manner 146 mg. which was predominantly VIIIα and 78 mg. which was predominantly VIIIβ.

dl-13-Iso-18-norestrone methyl ether, VIIIα, was further purified by recrystallization from methanol, m.p. 118–124°. The analytical sample was obtained as colorless prisms from methanol, m.p. 123–124°, $\lambda_{max}^{95\%\ C_2H_5OH}$ 278 mμ (log ϵ 3.34), 287 (3.31), $\lambda_{max}^{CHCl_3}$ 5.79, 6.20 μ.

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.94; H, 8.20. Found:[24] C, 79.9; H, 8.2.

dl-18-Norestrone methyl ether, VIIIβ, was recrystallized from methanol, m.p. 142–150°. Repeated recrystallization gave colorless prisms, m.p. 153–153.5°, $\lambda_{max}^{95\%\ C_2H_5OH}$ 278 mμ (log ϵ 3.35), 287 (3.32), $\lambda_{max}^{CHCl_3}$ 5.79, 6.20 μ.

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.94; H, 8.20. Found:[24] C, 79.8; H, 8.25.

The infrared spectrum of the latter isomer (VIIIβ) was rich in detail and identical in all respects with that of a sample of the naturally derived enantiomorph, m.p. 143–147° (uncorr.) supplied by Prof. G. F. Marrian.[3,16] A mixture melting point determined in Prof. Marrian's laboratory was 142–147° (uncorr.).

dl-18-Norestrone (II). The demethylation of the ether mixture of VIIIα and VIIIβ was carried out by the usual procedure.[8] A mixture of 1.0 g. of freshly prepared pyridine hydrochloride and 48.7 mg. of the crude mixture of VIIIα and VIIIβ was heated at 210° under a nitrogen atmosphere for 40 min. After cooling, the mixture was taken up in 5% hydrochloric acid and chloroform. The aqueous phase was thoroughly extracted with chloroform, the organic extracts were washed with sodium bicarbonate and brine, dried with sodium sulfate, and the solvent was removed at reduced pressure. The crude crystalline product was chromatographed on Florisil to yield 21 mg. (42%), m.p. 203–217° dec., which after recrystallization from methanol had m.p. 225.5–227° dec., (stage), 262–263° (sealed capillary[25]). On admixture with the naturally derived *d*-enantiomorph, (m.p. 264–268° cap.[25]), the melting point was 255–260° (sealed capillary,[25] different heating rate). When compared on the hot stage, the melting point of the natural enantiomorph, 226–228°, was undepressed on admixture.[25]

Pyridine–borane reductions.[6e] To a solution of 0.38 mg. of estrone 3-methyl ether in 0.030 ml. of glacial acetic acid was added 0.23 mg. of pyridine borane (Callery Chemical Co.) in 0.005 ml. of acetic acid. The mixture was allowed to stand at room temperature in a nitrogen atmosphere for 3 hr., one drop of 10% hydrochloric acid was added, followed by excess water. The product was isolated by extraction with ether, washing successively with water, 10% potassium carbonate, and water, drying with anhydrous sodium sulfate, and evaporation of the solvent. The crude product was assayed by thin-layer chromatography on silica gel, using 4% ether in benzene as the developing solvent. Spots were located by spraying with a solution of 0.2 ml. of 37% formaldehyde in 10 ml. of concd. sulfuric acid. The product showed a single spot with an R_f identical with that of authentic estradiol-3-methyl ether and considerably different from starting ketone.

Reductions of 0.566 mg. of VIIIα and 0.433 mg. of VIIIβ were caried out in an identical manner, using in each case a total of 0.050 ml. of acetic acid and 0.75 mg. of pyridine borane. Thin-layer chromatographic assay of the products, isolated as described above, showed that VIIIα had been substantially unaltered. The R_f of the major spot was identical with that of VIIIα, although a second very faint spot appeared at a location between that of estrone 3-methyl ether and estradiol 3-methyl ether. This may be indicative of a small amount of reduction. The product from VIIIβ, on the other hand, showed no residual VIIIβ, but only material of R_f nearly the same as that of estradiol 3-methyl ether.

Acknowledgment. We wish to express thanks to the National Science Foundation and the General Electric Co. for supporting this study.

MADISON, WIS.
BLOOMINGTON, IND.

(25) These melting points were determined by Prof. G. F. Marrian.