SOME RING A METHOXY-DERIVATIVES OF ESTRONE AND ESTRADIOL-17β

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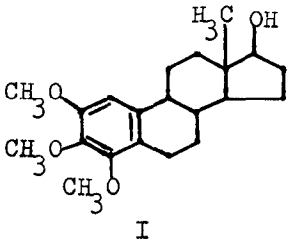
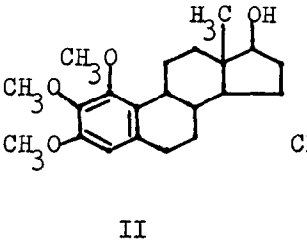
The synthesis of 1,2,3-trimethoxyestra-1,3,5(10)-trien-17β-ol (II) and 1,3,4-trimethoxyestra-1,3,5(10)-trien-17β-ol (III), and related compounds is described.

Analgesic activity has recently been reported[1] for 2,3,4-trimethoxyestra-1,3,5(10)-trien-17β-ol (I). Because of the potential significance of this result, and as a continuation of our interest in oxygenated estrogens[2] we undertook the synthesis of some isomeric trimethoxy-estrogens, namely, 1,2,3-trimethoxyestra-1,3,5(10)-trien-17β-ol (II) and 1,3,4-trimethoxyestra-1,3,5(10)-trien-17β-ol (III). The syntheses



I



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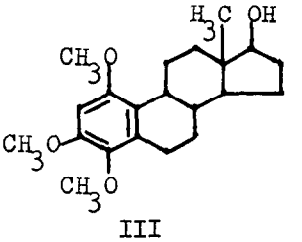


III

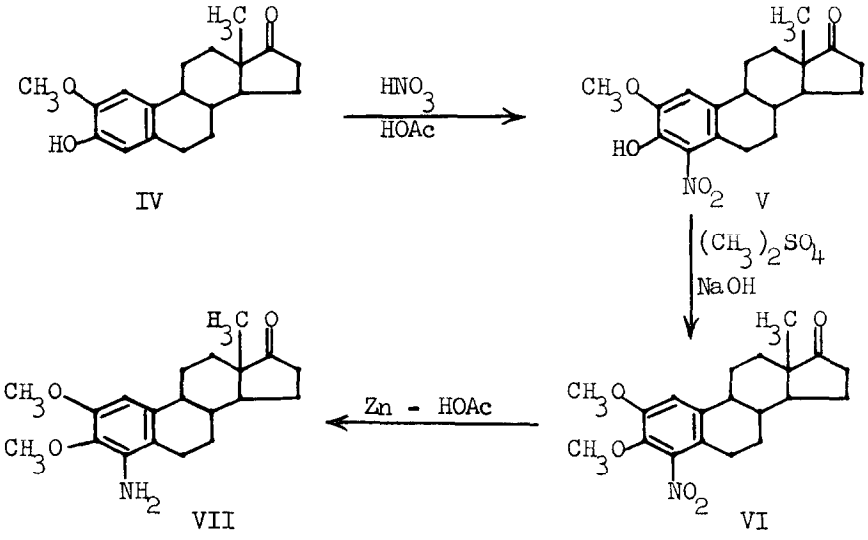
of these compounds and related chemical aspects are reported herein.

Although steric **hindrance** exists between C-1 and C-11,[3] this caused no unusual difficulty in introducing the oxygen function at C-1. In fact this was accomplished by a modifica-

tion of previously reported methods, a different method being used for each isomer.

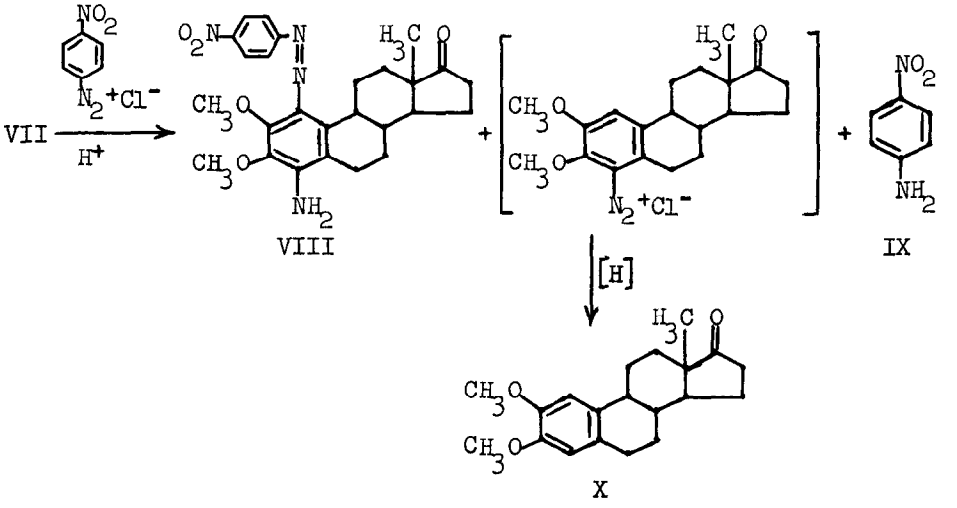
In a previous publication[4] we described the preparation of 1-aminoestrone via a diazo-coupling reaction in which p-nitrobenzenediazonium chloride reacted directly, and in high yield, at the C-1 position of 4-amino-3-methoxyestra-1,3,5(10)-trien-17-one. Fortunately this method was found to be also adaptable to the preparation of the 1,2,3-trimethoxy derivative II, regardless of an added steric hindrance at C-1 created by the presence of an ortho-methoxyl group at C-2.

Nitration of 2-methoxyestrone[5] (IV) with one equivalent of nitric acid in acetic acid gave the 4-nitro derivative V.

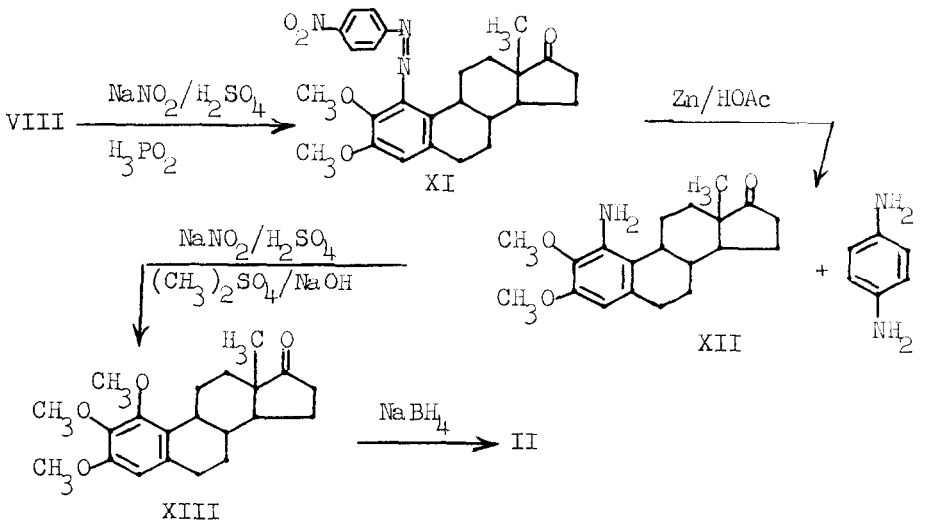


The crude product was methylated with dimethylsulfate-sodium hydroxide to give VI (not purified). Reduction with zinc-acetic acid gave the easily purified 4-amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one (VII) in an overall yield of 51% (based on IV).

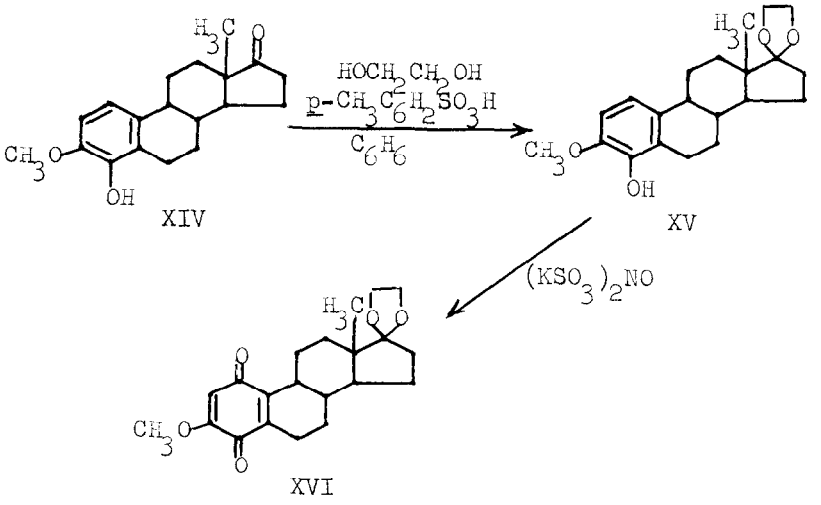
Our initial attempt to couple this amine (VII) with p-nitrobenzene diazonium chloride in aqueous acetic acid resulted in extensive diazo migration,[6] as evidenced by the isolation of p-nitroaniline (IX) and 2,3-dimethoxyestra-1,3,5(10)-trien-17-one (X). Only a poor yield of coupled product (VIII)



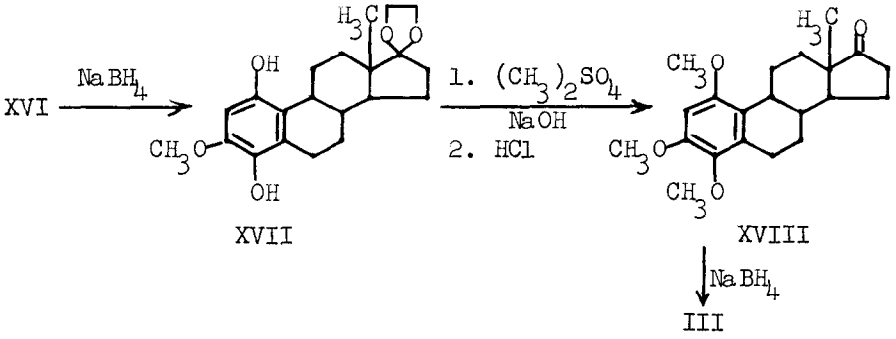
was obtained. However, when the p-nitrobenzene diazonium chloride was first neutralized with sodium bicarbonate solution and the coupling reaction carried out in aqueous acetone, a 60% yield of amino-azo product (VIII) was formed.[6] Removal of the 4-amino group was accomplished by reduction of the 4-diazonium salt with hypophosphorous acid.[4] Subsequent reduction with zinc-acetic acid resulted in cleavage of the azo linkage to give 1-amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one (XII). This was then converted by standard methods into the 1-methoxy derivative (XIII). Finally, reduction of the 17-keto group with sodium borohydride furnished the desired 1,2,3-trimethoxyestra-1,3,5(10)-trien-17β-ol (II).



For the synthesis of the 1,3,4-trimethoxy derivative (III), the para arrangement of the 1,4-methoxyl groups suggested that in this instance the C-1 oxygen function could best be introduced by oxidation of a 4-hydroxyl group to a p-quinone. Thus, when 4-hydroxyestrone-3-methyl ether[7] (XIV) was ketalized and the product (XV) treated with potassium nitrosodisulfonate (Fremy's salt)[8] in aqueous acetone there was obtained a near quantitative yield of 17-ethylenedioxy-3-methoxyestra-2,5(10)-dien-1,4-dione[9] (XVI).



Reduction of the quinone (XVI) with excess sodium borohydride followed by in situ methylation of the resulting hydroquinone (XVII) with dimethylsulfate-sodium hydroxide and hydrolysis of the protective 17-ethylene ketal function gave the 1,3,4-trimethoxyestra-1,3,5(10)-trien-17-one (XVIII) in a 68% overall yield (based on XIV). Borohydride reduction then converted the 17-ketone to a 17β-hydroxyl group (96% yd.).



The following compounds were found[10] inactive as analgesics in the rat tail flick test of D'Amour & Smith:[11] 4-amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one (VII), 1,2,3-trimethoxyestra-1,3,5(10)-trien-17-one (XIII), 1,2,3-trimethoxyestra-1,3,5(10)-trien-17β-ol (II), 1,3,4-trimethoxyestra-1,3,5(10)-trien-17-one (XVIII) and 1,3,4-trimethoxyestra-1,3,5(10)-trien-17β-ol (III).

Experimental

Solutions were dried over anhydrous sodium sulfate and all evaporations were under reduced pressure.

Thin Layer Chromatography - was carried out on glass plates coated with a 0.25 mm layer of silica gel G (Merck, Darmstadt) containing approximately 0.3% Radelin Phosphor GS-115 (United States Radium Corporation). In preparative

work a 0.5 mm layer on 20 x 20 cm plates was used. After development, the chromatograms were visualized by ultraviolet light and by spraying with a 10% phosphomolybdic acid methanol solution. In multiple development the plates were run as usual then dried and re-run in the same solvent system. This process was repeated as required.

Magnesol® - a hydrous magnesium silicate (Food Machinery Chemical Corp.).

Celite® - a diatomaceous silica (Johns-Manville).

Florisil® - a synthetic magnesium silicate adsorbent, 60-100 mesh (Floridin Corp.).

Silica gel - refers to Mallinckrodt SilicAr, CC-7, 100-200 mesh.

Petroleum ether - refers to the fraction boiling at 30-75°.

Melting Points - were determined on a Mel-Temp apparatus in open capillaries and are uncorrected.

Infrared Spectra - were determined in pressed potassium bromide discs on a Perkin-Elmer model 21 spectrophotometer.

Ultraviolet Spectra - were determined in methanol solution on a Cary recording spectrophotometer.

Optical Rotations - were measured at 25° in chloroform solution at concentrations of approx. 0.7-1.0% and with an error of ± 3-3.5° unless otherwise noted.

Nuclear Magnetic Resonance Spectra - were determined on a Varian A-60 spectrometer with tetramethylsilane as internal standard in deuterochloroform solution, unless otherwise noted.

The instrumental spectral analyses were performed by

W. Fulmor and associates and the elemental analyses by

L. Brancone and associates.

4-Amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one (VII). - To a stirred solution of 3.37 g (11.2 millimoles) of 2-methoxyestrone (IV) in 175 ml of glacial acetic acid was added a solution containing 0.71 ml (11.2 millimoles) of concentrated nitric

acid (sp g 1.42; 70.3%) in 25 ml of glacial acetic acid. The resulting colorless solution was stirred overnight at room temperature. After 17 hr the amber colored solution was evaporated at 50-60° during which the solution turned a deep red color. The residue was dissolved in a mixture of 175 ml of methanol and 25 ml of water. The product was methylated over $2\frac{3}{4}$ hr by the alternate addition of 100 ml of 30% potassium hydroxide solution and 33 ml of dimethyl sulfate. The reaction mixture was poured onto 1 liter of ice-water and the resulting precipitate was filtered, washed with water and then partitioned between 10% potassium hydroxide solution and methylene chloride. The methylene chloride solution was washed to neutrality with water and dried overnight over anhydrous sodium sulfate. The dark colored extract was filtered through Magnesol in ca 200 ml of methylene chloride and the adsorbent was washed with an additional 250 ml of methylene chloride. The combined filtrate was evaporated to give 3.2 g of a viscous oil. The latter was dissolved in 60 ml of glacial acetic acid and treated with 6.0 ml of concentrated hydrochloric acid. The resulting solution was heated to reflux, and 6.0 g of zinc dust was added portionwise over 10 min. The reaction mixture was refluxed for 1 hr, cooled, filtered and evaporated. The residue was partitioned between methylene chloride and water. The methylene chloride extract was washed several times with 10% potassium hydroxide solution and with water to neutrality. The dried extract was filtered through Magnesol and evaporated. The crude solid product (2.45 g) was partitioned between ca

200 ml of 5% hydrochloric acid and ca 100 ml of ether. The aqueous solution was extracted several times with ether and then made basic with 20% potassium hydroxide solution. The and the extract was washed with water to neutrality, dried, filtered through Magnesol and evaporated to give 1.9 g (51%) of a crystalline solid, mp 222-225°. A specimen for analysis was dissolved in methylene chloride, filtered through Magnesol and crystallized from methylene chloride-methanol and had mp 226-228°, λ_{max} 278 mμ (ε 1970); $[\alpha]_D$ +154°; ν_{max}^{KBr} 3425, 3344 cm^{-1}; nmr ($CDCl_3$ + d_6-DMSO), 6.35 ppm (s,[1], C-1H), 3.82, 3.80 ppm(s,s,[6], C-2 + C-3 OCH_3), 3.80-3.50 ppm (m,[2], NH_2), 0.9 ppm (s,[3], C-18 CH_3).

Anal. Calcd for $C_{20}H_{27}NO_3$ (329.42): C, 72.92; H, 8.26; N, 4.25. Found: C, 73.09; H, 8.31; N, 4.44.

4-Amino-2,3-dimethoxy-1-(p-nitrophenylazo)estra-1,3,5(10)-trien-17-one (VIII). - A. Preparation under weakly acid conditions: A solution of p-nitrobenzene diazonium chloride in aqueous acetic acid was prepared as follows: - To a solution of 345 mg (2.5 millimoles) of p-nitroaniline in 3.75 ml (9.75 millimoles) of 2.6 N hydrochloric acid plus 2.0 ml of acetic acid at 0° was added a solution of 173 mg (2.5 millimoles) of sodium nitrite in 1.5 ml of water, below the surface of the liquid. The solution was stirred for 2-3 minutes then diluted up to a total volume of 10.0 ml with water and stored at 0°C.

To a solution of 82 mg (0.25 millimoles) of 4-amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one (VII) plus 82 mg (1.0 millimole) of anhydrous sodium acetate in 4 ml of acetic acid

was added 1.0 ml (0.25 millimole) of the previously prepared solution of p-nitrobenzene diazonium chloride. The deep red-brown solution was stirred for 1/2 hr at room temperature, then diluted with 10 ml of water and extracted with methylene chloride. The extract was washed with water, saturated sodium bicarbonate solution and finally with water and then dried and evaporated to give 63.5 mg of a red-brown glass. Preparative TLC (upper phase benzene:acetone:water 5:1:4, developed twice) of the product gave 51 mg of an orange-red glass which was still a mixture. Further purification by TLC using methylene chloride as developing solvent gave 23 mg (67%) of a yellow solid whose infrared spectrum was identical to an authentic sample of p-nitroaniline (IX). Also isolated was 30 mg (25%) of a bright red-brown solid whose infrared spectrum indicated that it was the desired aminoazo coupled product (VIII). This was confirmed in a subsequent preparation (part B below) in which sufficient material was isolated for a positive identification.

The aqueous phases obtained from extraction of the crude product were combined and made strongly basic with a sodium hydroxide (3 g) solution and extracted again with methylene chloride. The extract was washed with water, dried and evaporated to 38 mg of a glass. Preparative TLC (upper phase of benzene:acetone:water 5:1:4) of this material gave 10 mg of colorless solid whose infrared spectrum was identical to an authentic sample of 2,3-dimethoxyestra-1,3,5(10)-trien-17-one (X).

B. - Preparation under weakly basic conditions: A neutralized solution of p-nitrobenzene diazonium chloride was prepared as follows: To a well stirred solution of 1.38 g (10 millimoles) of p-nitroaniline in 8 ml of acetone plus 15 ml of 2.6 N hydrochloric acid at 0° (ice bath), was added a solution of 697 mg (10.1 millimoles) of sodium nitrite in 10 ml of water, below the surface of the liquid. After stirring for 2-3 minutes the solution was neutralized by the portionwise addition of 1.72 g (20.4 millimoles) of a sodium bicarbonate slurry in 20 ml of water.

The resulting mixture (some yellow solid separated) was added, in one portion, to a solution of 3.29 g (10 millimoles) of 4-amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one in 350 ml of acetone at room temperature, and the deep red mixture was stirred for 15 minutes, and then poured into 500 ml of water. The mixture was extracted with methylene chloride and the extract was washed with water, dilute sodium bicarbonate solution, and finally with water, dried and evaporated. The crude product was crystallized from methanol (approx. 50 ml) to give 2.73 g of VIII, greenish black plates, mp 178-179° dec.

An analytical sample was obtained by chromatography of combined impure samples from previous reactions. The crude product (1.17 g) was chromatographed on Florisil (120 g in a 2.7 cm i.d. column) using 10% acetone-petroleum ether (30-75°) as the final eluting solvent to give 560 mg of product. This was crystallized twice from methanol to give 422 mg of shiny, green-black plates, mp 178-179° dec. (inserted in

bath at 175°). λ_{max} (neutral) 283, 440 mμ (ε 11,000, 19,350); λ_{max} (0.1 N HCl) 262, 330, 478 mμ (ε 6450, 4780, 57,000); $[\alpha]_{6907}$ -1290° ± 20 (c 0.3 in $CHCl_3$); ν_{max}^{KBr} 3378, 1733, 1608, 1339 cm^{-1}.

Anal. Calcd for $C_{26}H_{30}N_4O_5$ (478.52): C, 65.26; H, 6.32; N, 11.71. Found: C, 65.37; H, 6.72; N, 11.55.

2,3-Dimethoxy-1-(p-nitrophenylazo)estra-1,3,5(10)-trien-17-one (XI). - A solution of 2.73 g (5.71 millimoles) of 4-amino-2,3-dimethoxy-1-(p-nitrophenylazo)estra-1,3,5(10)-trien-17-one in 30 ml of acetic acid plus 10 ml of 30% (w/w) sulfuric acid was diazotized at 0° by the addition of 394 mg (5.71 millimoles) of sodium nitrite in 2 ml of water below the surface of the liquid. The solution was stirred for 2-3 minutes then 60 ml of 25% aqueous hypophosphorous acid was added and stirring continued at room temperature overnight. The mixture was diluted with water (100 ml) and the solid filtered to give 1.68 g of crude red-brown product.

The aqueous filtrate was extracted with methylene chloride and the extract was washed with water, dilute sodium bicarbonate solution and finally with water and then dried and evaporated to a dark red glass which was starting material by TLC and contained only a trace of product. The recovered starting material in 7 ml of acetic acid plus 3 ml of 30% (w/w) sulfuric acid was diazotized at 0° by the addition of 105 mg (1.52 millimoles) of sodium nitrite in 1 ml of water. The solution was stirred for 2-3 minutes then 12 ml of 25% aqueous hypophosphorous acid was added and stirring continued

overnight at room temperature. The mixture was diluted with 25 ml of water, and the solid was collected by filtration to give an additional 344 mg of crude product.

The total crude product (2.02 g) was chromatographed on Florisil (200 g in 3.6 cm i.d. column) using 7% acetone-petroleum ether as the final eluting solvent. The product thus obtained was crystallized from methanol to give 1.451 g of red-brown solid, mp 150-153°. The filtrate from this crystallization plus impure fractions from the column were evaporated and purified by preparative TLC (two systems; benzene:acetone:water 5:1:4, developed once and cyclohexane:ethyl acetate 65:35, developed twice) to give an additional 130 mg of recovered product. Thus the total yield of product was 1.58 g (60%).

The material recovered by preparative TLC was crystallized once from methanol to give an analytical sample of 99 mg red-brown crystals, mp 165-170°; λ_{max} 278, 340 mμ (ε 14,800, 10,400); $[\alpha]_{6907}$ -675° ± 9.7° (c = 0.31 in $CHCl_3$); ν_{max}^{KBr} 1739, 1527, 1344 cm^{-1}.

Anal. Calcd for $C_{26}H_{29}N_3O_5$ (463.52): C, 67.37; H, 6.31; N, 9.07. Found: C, 67.31; H, 6.66; N, 8.80.

1-Amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one (XII). - To a solution of 1.158 g (2.5 millimoles) of 2,3-dimethoxy-1-(p-nitrophenylazo)estra-1,3,5(10)-trien-17-one (XI) in 30 ml of acetic acid was added 5.8 g (89 millimoles) of zinc dust, portionwise, with stirring over two minutes. During this time the temperature rose from room temperature

to 65°. The mixture was cooled to 45°, then stirred at ambient temperature for 10 minutes and filtered. The residue was washed on the filter with a small amount of acetic acid and the total filtrate was heated on the steam bath for 30 minutes with 1 ml of concentrated hydrochloric acid. The mixture was diluted with 100 ml of water and 5 g of sodium hydroxide was added to neutralize the hydrochloric acid.

The mixture was extracted with benzene and the extract washed with water, saturated sodium bicarbonate solution and finally with water, and dried. Evaporation of the extract gave 900 mg of an oil which was purified by chromatography on Florisil (100 g in 2.7 cm i.d. column) using 15% ethyl acetate-hexane as the final eluting solvent. The product was crystallized from methylene chloride-methanol (5 ml) to give 244 mg of tan crystals, mp 157-158°.

The impure fractions from the column were purified by preparative TLC (developed with lower phase of benzene: acetone:water 5:1:4) to give 155 mg of product which was crystallized twice from methylene chloride-ether to give an analytical sample of 87 mg of colorless crystals, mp 160-162°, λ_{max} 213, 284 mμ (ε 40,000, 1645); $[\alpha]_D$ +237°; ν_{max}^{KBr} 3390, 3333 cm^{-1}; nmr, 6.10 ppm (s,[1], C-4H), 3.78 ppm (s, [6], C-2 + C-3 OCH_3), 3.62 ppm (NH_2), 0.95 ppm (s,[3], C-18 CH_3).

Anal. Calcd for $C_{20}H_{27}NO_3$ (329.42): C, 72.92; H, 8.26; N, 4.25. Found: C, 72.88; H, 8.24; N, 4.59.

Material from filtrates of the above crystallizations

was purified by preparative TLC, as above, to yield an additional 160 mg of pure product. Thus the total yield of product was 491 mg (60%).

1,2,3-Trimethoxyestra-1,3,5(10)-trien-17-one (XIII). - To a stirred solution of 460 mg (1.4 millimoles) of 1-amino-2,3-dimethoxyestra-1,3,5(10)-trien-17-one (XII) in 20 ml of 10% (w/w) sulfuric acid at approximately -10° was added a solution of 100 mg (1.45 millimoles) of sodium nitrite in 2 ml of water below the surface of the liquid. The solution was warmed up to room temperature during ten minutes, and then heated on the steam bath for 15 minutes during which time nitrogen was evolved and a solid precipitated.

The mixture was extracted with methylene chloride and the extract was washed with water, saturated sodium bicarbonate solution and finally with water and then dried, and evaporated to give 447 mg (97%) of dark tan crystals which contained only minor impurities by TLC analysis.

The crude product, without further purification, was methylated as follows. To a mixture of the steroid (445 mg 1.35 millimoles) in 20 ml of methanol was added dropwise, 1.5 ml of 30% aqueous sodium hydroxide and 1.5 ml of dimethyl sulfate at 21-23° over 1 hr such that the mixture was maintained at a basic pH throughout the reaction. After reacting for a further 0.5 hr the mixture was poured into 50 ml of water, and any excess dimethylsulfate was decomposed by the addition of 2-3 ml of concentrated ammonium hydroxide. The mixture was extracted with methylene chloride and the extract was

washed with water, dried over sodium sulfate and evaporated to 498 mg of an oil which still appeared to contain some unreacted starting material by TLC. Accordingly, the total crude product was reacted again as above. On workup, the mixture yielded 453 mg of a glass which contained a small amount of slightly more polar impurities.

The total crude product was purified by preparative TLC (n-hexane:ether 1:1, developed three times), and the product band was divided into an upper half which contained 206 mg of pure, colorless material, mp 98-102°, and a lower (more polar) half which contained 172 mg of a slightly less pure tan material, mp 86-92°. Both fractions were essentially identical by IR analysis. The yield of product was therefore approx. 378 mg (80%).

The pure material was crystallized once from n-hexane to give an analytical sample of 169 mg of colorless needles, mp 103-105°, nmr: 6.44 ppm (s,[1], C-4H), 3.85 ppm (s,[9], C-1 + C-2 + C-3 OCH_3), 0.95 ppm (s,[3], C-18 CH_3); λ_{max} 228 (sh), 280 mμ (ε 9800, 1550); $[\alpha]_D$ +190° ($CHCl_3$); ν_{max}^{KBr} 1742, 1600, 753 cm^{-1}.

Anal. Calcd for $C_{21}H_{28}O_4$ (344.44): C, 73.22; H, 8.19. Found: C, 72.94; H, 8.16.

1,2,3-Trimethoxyestra-1,3,5(10)-trien-17β-ol (II). - To a solution of 60 mg (0.17 millimoles) of 1,2,3-trimethoxyestra-1,3,5(10)-trien-17-one (XIII) in 3 ml of 90% ethanol was added 60 mg (1.59 millimoles) of sodium borohydride and the mixture stirred at room temperature for 0.5 hr. The excess boro-

hydride was then decomposed by the dropwise addition of 1.5 ml of 1 N sulfuric acid with stirring and cooling. The mixture was diluted with water and extracted with benzene. The extract was washed with saturated sodium bicarbonate solution and water, and dried.

Evaporation of the benzene extract gave an oil which crystallized on trituration with n-hexane to give 61 mg of coloroless solid, mp 143-145°. The product was recrystallized from n-hexane-methylene chloride to yield 55 mg (91%) colorless crystals, mp 145-147°; λ_{max} 230 (sh), 280 mμ (ϵ 9500, 1550); $[\alpha]_D$ +123° ± 4.5° ($CHCl_3$); ν_{max}^{KBr} 3484 cm^{-1}.

Anal. Calcd for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.37. Found: C, 72.96; H, 8.68.

17-Ethylenedioxy-3-methoxyestra-1,3,5(10)-trien-4-ol (XV). - A mixture of 1.5 g (5 millimoles) of 4-hydroxy-3-methoxyestra-1,3,5(10)-trien-17-one (XIV), 3 ml of ethylene glycol and 100 mg of p-toluenesulfonic acid in 60 ml of benzene was stirred and refluxed overnight using a water entrainer for the removal of water of reaction.

The mixture was shaken with 50 ml of saturated sodium bicarbonate solution and the organic phase was separated and washed with water, dried, and evaporated to give 1.75 g (100%) of a tan solid, mp 149-156°. The product was used for the next step without further purification.

17-Ethylenedioxy-3-methoxyestra-2,5-diene-1,4-dione (XVI). - To a solution of 2.68 g (10 millimoles) of potassium nitrosodisulfonate plus 2.68 g (32.6 millimoles) of anhydrous sodium

acetate in 200 ml of water plus 50 ml of acetone was added a solution of 1.75 g (5 millimoles) of 17-ethylenedioxy-3-methoxyestra-1,3,5(10)-trien-4-ol (XV) in 150 ml of acetone. The mixture was stirred at room temperature for 1 hr during which time the color changed from purple to yellow, and some solid crystallized out. The solid contained some starting material and was filtered off, dissolved in 100 ml of acetone and added to the filtrate to which there was then added a further 1.34 g (5 millimoles) of potassium nitrosodisulfonate and 1.34 g of sodium acetate.

The mixture was stirred for 0.5 hr at room temperature then diluted with water and extracted with methylene chloride. The extract was washed with water, dried, and evaporated to give 1.75 g of crude product which was crystallized from ether to give 1.55 g (87%) of yellow crystals, mp 225-230°.

The product was used in the next step without further purification.

1,3,4-Trimethoxyestra-1,3,5(10)-trien-17-one (XVIII). - To a solution of 1.58 g (4.4 millimoles) of 17-ethylenedioxy-3-methoxyestra-2,5-diene-1,4-dione in 360 ml of methanol was added 1.0 g of sodium borohydride. The mixture was stirred at room temperature for five minutes and then concentrated *in vacuo* to a volume of 100 ml.

The resulting solution, which was very susceptible to air oxidation, was treated again with a small amount of sodium borohydride until colorless, and then methylated in an atmosphere of nitrogen by the simultaneous addition of 20 ml of

30% sodium hydroxide solution and 20 ml of dimethyl sulfate. The reagents were added dropwise at 25-30° over 2.5 hr such that the mixture was maintained at a basic pH.

After stirring for an additional 0.5 hr the mixture was made strongly acidic by the dropwise addition of 10 ml of concentrated sulfuric acid. The resulting warm, thick mixture was allowed to stand for 15 minutes, and then was poured into 200 ml of water and extracted with methylene chloride. The extract was washed with water, saturated sodium bicarbonate solution and finally with water, and dried and evaporated to give 1.4 g of brown crystalline material which contained only minor impurities by TLC analysis.

The total product was chromatographed on Florisil (140 g in 2.7 cm i. d. column) using 5% ethyl acetate-petroleum ether as the eluting solvent. The product so obtained was crystallized from methylene chloride-n-hexane to give 1.08 g colorless crystals, mp 111-115°. The filtrate from this crystallization plus some less pure fractions from the column were crystallized to give an additional 116 mg of pale tan crystals, mp 111-115°. Hence the total yield of product was 1.19 g (78%). Analytical material was obtained by a further crystallization from methylene chloride-n-hexane to give large colorless crystals, mp 112-115°; λ_{max} 230 (sh), 286 mμ (ϵ 7400, 3100); $[\alpha]_D$ +275°; ν_{max}^{KBr} 899, 759 cm^{-1}; nmr, 6.39 ppm (s,[1], C-2H), 3.85 ppm (s,[3], OCH_3), 3.77, 3.75 ppm (s,s,[6], $2OCH_3$), 0.93 ppm (s,[3], C-18 CH_3).

Anal. Calcd for $C_{21}H_{28}O_4$ (344.44): C, 73.22; H, 8.19. Found: C, 73.08; H, 7.98.

1,3,4-Trimethoxyestra-1,3,5(10)-trien-17β-ol (III). - To a solution of 500 mg (1.45 millimoles) of 1,3,4-trimethoxyestra-1,3,5(10)-trien-17-one (XVIII) in 35 ml of 90% ethanol was added 500 mg of sodium borohydride and the mixture stirred at room temperature for 0.5 hr.

The excess borohydride was decomposed by the dropwise addition of 10 ml of 1 N sulfuric acid with cooling and stirring. The mixture was diluted with 100 ml of water and extracted with methylene chloride. The extract was washed with water, saturated sodium bicarbonate solution, and finally with water, and then dried, filtered through a bed of Celite and evaporated to give 510 mg of a colorless solid, mp 102-107°. Crystallization from methylene chloride-n-hexane afforded 443 mg (88%) of analytical material, mp 105-107°; λ_{max} 230 (sh), 286 mμ (ε 7600, 2940); $[\alpha]_D$ +208°; ν_{max}^{KBr} 3390 cm^{-1}; nmr, 6.39 ppm (s,[1], C-2H), 3.85 ppm (s,[3], OCH_3), 3.77, 3.75 ppm (s,s[6], $2OCH_3$), 1.82 ppm (s,[1], OH), 0.82 ppm (s,[3], C-18 CH_3).

Anal. Calcd for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73. Found: C, 72.68; H, 9.09.

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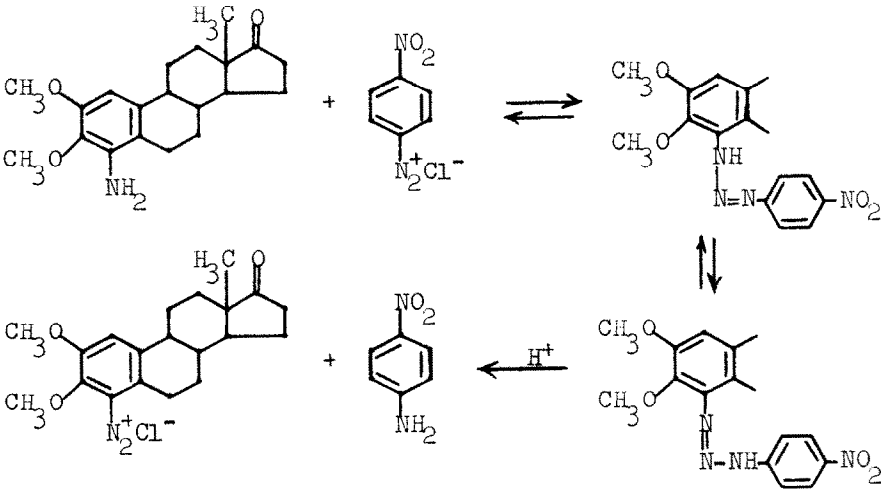

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