[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

# Synthesis of Potential Metabolites of Estradiol[1]

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The synthesis of three isomeric guaiacol derivatives of estrone, 4-hydroxyestrone 3-methyl ether, 2-hydroxyestrone 3-methyl ether and 4-methoxyestrone, is described.

Recent investigations in this Laboratory have resulted in the isolation from human urine and identification by partial synthesis of 2-methoxyestrone, a new metabolite of estradiol.[3] In addition a second synthesis, which permits the preparation of this substance in practical quantity, has been recorded.[4] This paper described the synthesis of the three remaining isomeric estrone guaiacols that were prepared for comparison purposes.

Nitration of estrone with concentrated nitric acid (Chart I) gave 2-nitroestrone (Ia) and 4-nitroestrone (IIa)[5] which could be separated readily by chromatography on alumina. Methylation of the two isomeric nitrophenols followed by reduction of the nitro groups of the methyl ethers Ib and IIb by means of sodium hydrosulfite in alkali, provided 2-aminoestrone 3-methyl ether (IIIb) and 4-aminoestrone 3-methyl ether (IVb). Diazotization and photodecomposition of the diazonium salts[6] afforded 2-hydroxyestrone 3-methyl ether (Vb) and 4-hydroxyestrone 3-methyl ether (VIb), respectively. The synthesis of the remaining two guaiacols followed a similar route with the difference that the methylation step was eliminated and the photodecomposition was carried out in the presence of a large excess of methanol.[7] The diazonium group was directly replaced by methoxy to give 2-methoxyestrone (Va) and 4-methoxyestrone (VIa), the former proving identical in all respects with the natural compound.[3] It is of interest to note that thermal decomposition of the diazonium salts in methanol led to reductive deamination, with estrone the only product isolated. This result is somewhat unexpected in view of the work of Horner and Stöhr[7] who found that thermal decomposition of diazonium salts in methanol gave predominantly ethers rather than the deaminated products, while the reverse was the case on photodecomposition.

Suitable as the above synthetic scheme was in providing a single route to all four compounds, it suffered from the disadvantage of low yields. This was particularly the case with 2-methoxyestrone, where the over-all yield of pure material was less than 1%. Diazotization and photodecomposition of the benzyl ethers IIIc and IIc was carried out in the hope of obtaining better yields of the 2-hydroxy and 4-hydroxy compounds. This approach, however, failed to produce the desired results and other attempts at modification of the final stage of the synthesis also failed to improve the yield.

## Experimental[8]

**2-Nitroestrone and 4-nitroestrone (Ia and IIa)** were prepared and separated using the procedure of Werbin and Holoway.[5]

**4-Nitroestrone 3-Methyl Ether (IIb).**—To a solution of 463 mg. of 4-nitroestrone in 25 ml. of methanol and 15 ml. of 10% potassium hydroxide solution in an atmosphere of nitrogen was added 6 ml. of dimethyl sulfate dropwise with stirring at such a rate that the temperature did not rise above 35°. Stirring was continued until the color of the solution changed from deep red to light yellow. Potassium hydroxide solution (40%) was added as required in 2-ml. portions to keep the mixture alkaline until all of the dimethyl sulfate present had reacted. Additional dimethyl sulfate was then added dropwise with periodic additions of 40% alkali as required until the final mixture remained yellow in color while still basic. A total of 14 ml. of alkali and 7 ml. of dimethyl sulfate was required in addition to the initial quantities. The total reaction time was 4 hours.

The alkaline mixture was cooled in an ice-bath for 0.5 hour and the precipitated product was filtered and washed with several portions of 5% potassium hydroxide solution followed by several portions of water. There was obtained 393 mg. of colorless 4-nitroestrone methyl ether, m.p. 263–265°, 81% yield. An additional 12%, m.p. 237–246°, was obtained by extraction of the filtrate with ether. This compound occurred in more than one polymorphic modification.

A pure sample, obtained by recrystallization from ethanol–benzene, had m.p. 261–261.5°, $[\alpha]^{30}D$ +212°, $\lambda_{max}$ 275.5 mμ (ε 1570), $\lambda_{min}$. 242 mμ (ε 1140), $\lambda_{infl}$ 283 mμ (ε 1450) and 250–257 mμ (ε 1220).

*Anal.* Calcd. for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04; N, 4.25. Found: C, 69.32; H, 6.98; N, 4.31.

**4-Nitroestrone 3-Benzyl Ether (IIc).**—A mixture of 502 mg. of 4-nitroestrone, 1 g. of freshly ignited anhydrous potassium carbonate and 1 ml. of benzyl chloride in 30 ml. of absolute ethanol was heated under reflux for 4 hours. Most of the solvent was removed and the residue was poured into 300 ml. of water. The aqueous suspension was made alkaline with potassium hydroxide and extracted 5 times with chloroform. The combined extracts were washed with water, dried over sodium sulfate and evaporated. The product was twice suspended in 15 ml. of petroleum ether and filtered to remove excess benzyl chloride. Recrystallization from benzene–petroleum ether afforded 484 mg. (75%) of long flat needles, m.p. 195–201°, and 134 mg. (21%), m.p. 190–198°. Further recrystallization gave pure samples melting at 201–202° (hexagonal plates) and at 204–205° (rectangular prisms), $[\alpha]^{25}D$ +165°.

*Anal.* Calcd. for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 3.45. Found: C, 73.96; H, 6.84; N, 3.38.

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(8) The estrone and estradiol used in this work were kindly supplied by Schering Corporation and Chas. Pfizer and Co. Rotations were determined in a 2-dcm. tube and chloroform was the solvent unless otherwise noted. Ultraviolet spectra were measured in ethanol using a Cary recording spectrophotometer and ethanol was the solvent unless otherwise noted. Melting points were determiued on a micro hot-stage apparatus. Analyses were performed by Spang Microanalytical Laboratories.

**4-Aminoestrone 3-Methyl Ether (IVb).**—A solution of 314 mg. of 4-nitroestrone 3-methyl ether in 50 ml. of acetone and 10 ml. of 0.5 *N* sodium hydroxide solution was heated to reflux and 1.0 g. of sodium hydrosulfite was added. The mixture was refluxed with stirring for 35 minutes, and a further 0.8 g. of sodium hydrosulfite and 10 ml. of 0.5 *N* sodium hydroxide solution were added. After refluxing for a further 40 minutes, 25 ml. of water was added and most of the acetone was removed under reduced pressure. The resulting suspension was cooled in an ice-bath for 2 hours and filtered. After washing the product thoroughly with water and drying *in vacuo*, there was obtained 181 mg. of 4-aminoestrone 3-methyl ether as colorless platelets, m.p. 188–191°, yield 64%. Recrystallization from benzene–methanol and from methanol afforded a pure sample, m.p. 190.5–191.5°, $[\alpha]^{28}D$ +149°, $\lambda_{max}$ 287.5 mμ (ε 2820), $\lambda_{min}$. 264.5 mμ (ε 1070).

*Anal.* Calcd. for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68. Found: C, 76.13; H, 8.53; N, 4.71.

**4-Aminoestrone 3-Benzyl Ether (IVc).**—Reduction of 175 mg. of 4-nitroestrone 3-benzyl ether using the conditions described for the methyl ether afforded 106 mg. of 4-aminoestrone 3-benzyl ether as colorless short needles, m.p. 220–224°, yield 66%. Recrystallization from benzene–petroleum ether gave rectangular plates, m.p. 223.5–225.5°, $[\alpha]^{25}D$ +109°.

*Anal.* Calcd. for $C_{25}H_{29}NO_2$: C, 79.96; H, 7.79; N, 3.73. Found: C, 80.08; H, 7.73; N, 3.81.

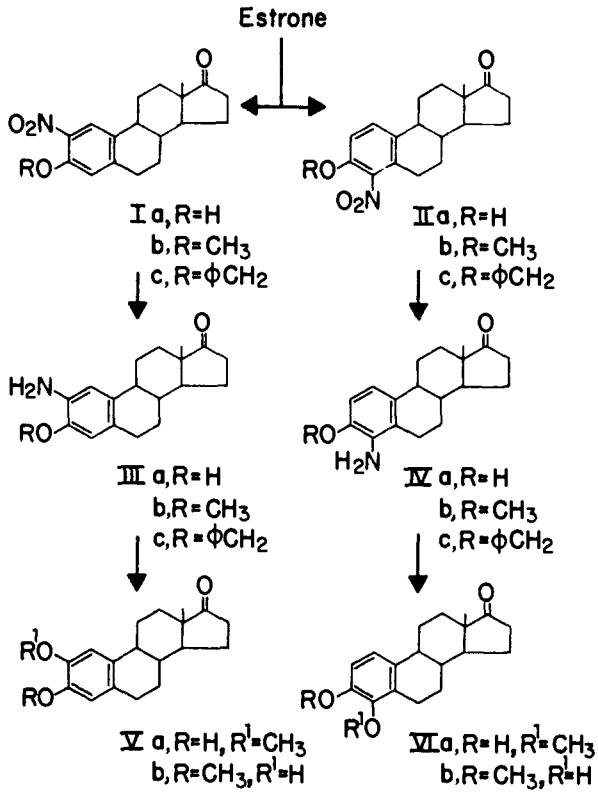
**4-Hydroxyestrone 3-Methyl Ether (VIb).**—A solution of 248 mg. of 4-aminoestrone 3-methyl ether in 6 ml. of 10% sulfuric acid was cooled to 0° and a solution of 150 mg. of sodium nitrite in 1 ml. of water was added dropwise with swirling. The mixture was allowed to stand at 0° for 20 minutes, with frequent agitation. Then 0.5 ml. of 40% aqueous urea solution was added and the solution was kept at 0° for 5 minutes. The diazonium solution was added to 150 ml. of ice-cold water in a beaker. The resulting mixture was irradiated with ultraviolet light at 0° for 6 hours by means of a Hanovia Type 30600 lamp, without filters. The solution was then extracted thoroughly with ether and the combined ether layers were washed with water once and extracted with 5% potassium hydroxide solution 5 times. The ether layer, after washing with water, drying, and evaporation of the solvent, afforded 199 mg. of a yellowish "neutral" solid. This was subsequently found to be chiefly the desired 4-hydroxyestrone 3-methyl ether.

The potassium hydroxide extracts upon acidification and extraction with chloroform yielded 66 mg. of a tan semi-solid oil. Counter-current distribution of this material in the system 70% methanol–30% water–carbon tetrachloride (203 transfers) gave 41 mg. of colorless solid with a distribution coefficient *K* of 0.10. This was combined with the "neutral" fraction from above and recrystallized from acetone and from absolute ethanol. There was obtained 195 mg. (79%) of 4-hydroxyestrone 3-methyl ether as colorless platelets, m.p. 220–226°, and 47 mg. (19%), m.p. 217–223°. A pure sample, obtained by recrystallization from methanol, had m.p. 220–224°, $[\alpha]^{23}D$ +154° (ethanol), $\lambda_{max}$ 277–283 mμ (ε 2110), $\lambda_{min}$. 252 mμ (ε 370).

*Anal.* Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 76.01; H, 8.07.

**2-Nitroestrone 3-Methyl Ether (Ib).**—In the same manner as described for 4-nitroestrone, 1.07 g. of 2-nitroestrone was methylated with a total of 32 ml. of dimethyl sulfate in 50 ml. of methanol and 50 ml. of 10% potassium hydroxide solution. The time required was 6–8 hours and an additional total of 32 ml. of 40% potassium hydroxide solution was added in small portions to keep the mixture basic. The neutral product was collected by filtration and washed thoroughly with dilute potassium hydroxide solution and with water. There was obtained 0.928 g. of 2-nitroestrone 3-methyl ether as light yellow platelets, m.p. 153–155°, yield 83%. Extraction of the filtrate with ether afforded, after recrystallization, a further 0.072 g. (7%), m.p. 150–152°.

A pure sample, recrystallized from methanol, had double m.p. 147° (platelets) and 157.5–159.5° (prisms). Another sample had double m.p. 146.5–148.5° (rectangular platelets) and 154–155.5° (hexagonal plates), $[\alpha]^{24}D$ +146°, $\lambda_{max}$ 272.5 (ε 4510) and 336 mμ (ε 3130), $\lambda_{min}$. 255 (ε 3240) and 304 mμ (ε 2120).




*Anal.* Calcd. for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04; N, 4.25. Found: C, 69.25; H, 7.05; N, 4.15.

**2-Nitroestrone 3-Benzyl Ether (Ic).**—Benzylation of 501 mg. of 2-nitroestrone in the manner described for the 4-nitro isomer afforded 596 mg. of 2-nitroestrone 3-benzyl ether, m.p. 237–241°, yield 93%. Recrystallization from benzene–petroleum ether gave a pure sample, m.p. 239.5–240.5°, $[\alpha]^{25}D$ +124°.

*Anal.* Calcd. for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 3.45. Found: C, 74.03; H, 6.81; N, 3.60.

**2-Aminoestrone 3-Methyl Ether (IIIb).**—Reduction of 302 mg. of 2-nitroestrone 3-methyl ether with sodium hydrosulfite under the conditions described for the 4-nitro isomer afforded 163 mg. (59%) of 2-aminoestrone 3-methyl ether as colorless needles, double m.p. 139–142° and 158–160°. A pure sample, recrystallized from methanol, had m.p. 172.5–174.5° (rhombs); another sample melted at 160.5–162.5°, $[\alpha]^{28.5}D$ +155°, $\lambda_{max}$ 239 (ε 6980) and 295.5 mμ (ε 4480), $\lambda_{min}$. 230 (ε 6470) and 267 mμ (ε 1080).

*Anal.* Calcd. for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68. Found: C, 76.18; H, 8.50; N, 4.42.

**2-Aminoestrone 3-Benzyl Ether (IIIc).**—Reduction of 95 mg. of 2-nitroestrone 3-benzyl ether gave a 64% yield of the corresponding amino derivative, m.p. 225–227°. Recrystallization from a mixture of benzene, ethanol and petroleum ether gave pale yellow needles, m.p. 228–230°, $[\alpha]^{25}D$ +132°.

*Anal.* Calcd. for $C_{25}H_{29}NO_2$: C, 79.96; H, 7.79; N, 3.73. Found: C, 79.81; H, 7.72; N, 3.68.

**2-Hydroxyestrone 3-Methyl Ether (Vb).**—A solution of 255 mg. of 2-aminoestrone 3-methyl ether in 100 ml. of 10% sulfuric acid was diazotized at 0–4° with 291 mg. of sodium nitrite dissolved in 2 ml. of water. After standing at 0° for 15 minutes, the solution was treated with 1 ml. of 40% urea solution and allowed to stand for a further 5 minutes. The resulting mixture was transferred into 250 ml. of ice-cold water and was irradiated with ultraviolet light at 0° for 5 hours with continuous stirring. The solution was extracted with ether and the combined ether layers were washed 5 times with 5% potassium hydroxide solution and then with water. The ether was removed and 87 mg. of crystalline "neutral" material was obtained. This subsequently proved to be the desired phenolic product. Acidification and extraction of the alkaline extracts and subsequent

counter-current distribution in 70% methanol–carbon tetrachloride yielded 79 mg. of colorless solid with $K$ = 0.31. This was combined with the "neutral" product and recrystallized from absolute ethanol. There was obtained 77 mg. (30%) of 2-hydroxyestrone 3-methyl ether as coarse prisms, m.p. 182–185°, and 24 mg. (9%), m.p. 180–184.5°. Further recrystallization gave a pure sample, rhombs, m.p. 182.5–185°, $[\alpha]^{23}D$ +158° (ethanol), $\lambda_{max}$ 287.5 mμ (ε 4200), $\lambda_{min}$. 254 mμ (ε 670), $\lambda_{Infl}$ 310 mμ (ε 290).

*Anal.* Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 76.09; H, 8.04.

Although the distribution coefficient, melting point and ultraviolet spectrum of 2-hydroxyestrone 3-methyl ether were in agreement with those of 2-methoxyestrone 3-methyl ether, the specific rotations and the infrared spectra in potassium bromide dispersion were conclusively different.

**4-Aminoestrone (IVa).**—One gram of sodium hydrosulfite was added to a refluxing solution of 196 mg. of 4-nitroestrone in a mixture of 50 ml. of acetone, 10 ml. of water and 5 ml. of 1 $N$ sodium hydroxide solution. After 30 minutes, 10 ml. of water and 0.7 g. of sodium hydrosulfite were added and refluxing was continued with periodic additions of alkali as before. After another 30 minutes, a final 0.3 g. of sodium hydrosulfite was added and heating was continued until the deep red color of the mixture had changed to light yellow. Following the addition of 25 ml. of water, most of the acetone was removed and the solution was neutralized with dilute acetic acid. After refrigeration for 2 hours a white precipitate was filtered off and washed with water. After drying, there was obtained 179 mg. of 4-aminoestrone, m.p. 254–255° dec., yield quantitative. For analysis, a sample was obtained by recrystallization from benzene–methanol as colorless needles, m.p. 260–262° dec., $[\alpha]^{24}D$ +139° (dioxane), $\lambda_{max}$ 289 mμ (ε 2710), $\lambda_{min}$. 264 mμ (ε 850).

*Anal.* Calcd. for $C_{18}H_{23}NO_2$: C, 75.75; H, 8.12; N, 4.91. Found: C, 75.71; H, 8.23; N, 4.84.

**4-Methoxyestrone (VIa).**—A solution of 928 mg. of 4-aminoestrone in 15 ml. of 40% sulfuric acid was cooled to 0° and treated dropwise with a solution of 707 mg. of sodium nitrite in 15 ml. of water. The mixture was allowed to stand at 0° for 15 minutes and then 3 ml. of 40% aqueous urea solution was added. After 5 minutes at 0° the diazonium solution was added to 50 ml. of ice-cold absolute methanol and the solution was irradiated with ultraviolet light, with cooling and stirring, for 5 hours. Sodium hydroxide solution was added to neutrality and the solution was concentrated to about 100 ml. Water was added and after acidification the mixture was extracted thoroughly with ether. The ether layers were combined and washed with water. Extraction of the ether with 5% sodium bicarbonate solution followed by acidification and extraction with chloroform afforded 136 mg. of a brown oil. Extraction of the ether layer with 5% potassium hydroxide solution several times gave 344 mg. of a brown phenolic fraction. Evaporation of the ether layer gave 326 mg. of a brown neutral material.

The phenolic product was chromatographed on silica gel (100 g.) with petroleum ether containing increasing proportions of ethyl acetate. The desired 4-methoxyestrone was eluted with 1 liter of 25% ethyl acetate–75% petroleum ether. There was obtained 97 mg. of colorless solid, m.p. 185–212°, yield 10%. Recrystallization from ethyl acetate afforded 53 mg. of colorless platelets, m.p. 220–224°. Further recrystallization from ethyl acetate gave a pure sample, m.p. 224–225°, $[\alpha]^{24}D$ +146° (ethanol), $\lambda_{max}$ 278 mμ (ε 1820), $\lambda_{min}$. 249 mμ (ε 490).

*Anal.* Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.96; H, 8.09.

Another sample had double m.p. 215–217° (prisms) and 222–225° (rectangular plates); $K$ = 0.50 in the system 70% methanol–carbon tetrachloride.

**2-Aminoestrone (IIIa).**—Reduction of 2-nitroestrone as described for 4-nitroestrone gave 2-aminoestrone, which was crystallized from dilute methanol as long needles darkening at 220° with no real melting point; $[\alpha]^{24}D$ +178° (ethanol), $\lambda_{max}$ 297 mμ (ε 4190). All manipulations were carried out in an atmosphere of nitrogen to avoid decomposition of the material.

*Anal.* Calcd. for $C_{18}H_{23}O_2N$: C, 75.75; H, 8.14; N, 4.91. Found: C, 75.62; H, 8.04; N, 4.67.

**2-Methoxyestrone (Va).**—The same procedure as used for 4-methoxyestrone gave an over-all yield of 0.8% of 2-methoxyestrone.[3]

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[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND DERMATOLOGY, BOSTON UNIVERSITY SCHOOL OF MEDICINE, AND THE DEPARTMENT OF CHEMISTRY, BOSTON UNIVERSITY]

# D-Glucopyranosiduronates. I. Steroidyl-β-D-glucopyranuronosides[1,2]

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The β-D-glucopyranosiduronic acids of 17β-hydroxy-androst-4-en-3-one, 3β-hydroxy-androst-5-en-17-one, estra-1,3,5(10)-triene-3,17β-diol, 3β-hydroxy-pregn-5-en-20-one and androst-4-ene-3,17-dione as well as the methyl (2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronates of the above steroids and of 3β-hydroxy-5α-androstan-17-one, 3-hydroxy-estra-1,3,5(10)-trien-17-one, 3β,17α,21-trihydroxy-5α-pregnan-20-one, 11,20-dioxo-3α,17α-dihydroxy-5β-pregnan-21-yl-acetate, 17α,21-dihydroxy-pregn-4-ene-3,11,20-trione, 3-hydroxy-pregna-3,5-dien-20-one and cholesta-3,5-dien-3-ol were prepared by the Koenigs–Knorr procedure. In several cases the method of Schapiro was compared with that described by Meystre, *et al.* Proof of structure was obtained from elemental analysis, ultraviolet and infrared absorption spectra, color reactions and enzymatic hydrolysis.

The preparation of methyl-2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate[3] by Goebel and Babers[4] opened the way for the synthesis of steroid glucosiduronates by Schapiro[5] in 1938 and by Huebner and co-workers[6] in 1944, using the Koenigs–Knorr reaction. More recently Schneider, *et al.*,[7]

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(3) For naming glucuronic acid derivatives see *J. Chem. Soc.*, 5108 (1952) and *Chem. Eng. News*, **31**, 1776 (1953).

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