

STEROIDS, XXIII.

Synthesis of 2- and 4-Hydroxy and 2,4-Dihydroxy Derivatives of Estrone and Estradiol

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The following hydroxy derivatives of estrone and estradiol were prepared during this work¹: 2,3-dihydroxyestrone, 2,3-dihydroxyestradiol, 3,4-dihydroxyestrone, 3,4-dihydroxyestradiol, 2,3,4-trihydroxyestrone and 2,3,4-trihydroxyestradiol. The syntheses were performed *via* the corresponding mono and dinitro derivatives, and all intermediates were isolated and described.

A number of authors have dealt with the direct synthesis of the group of compounds mentioned in the title [1—4], while others have strived to synthesize the monomethoxy derivatives [5] in order to identify the methoxy intermediates occurring among natural estrogens [6—7].

As regards the hydroxylated estrogens accumulating during pregnancy, many authors attribute importance exclusively to the compounds mentioned, but primarily to 2-hydroxyestrone [8].

In the case of estrogens bound to protein, and possibly to glutathion, on the other hand, the important intermediate role of these compounds [9—11] is assumed.

Valuable results have been obtained recently on the isolation of this group of compounds, and on the study of their properties and role in the intermediate metabolism [12, 13].

The synthesis of these compounds has already been dealt with for purposes of identification of the derivatives obtained in previous non-specific enzymatic hydroxylation experiments [14].

¹ Abbreviations used: 2-nitroestrone means 2-nitro-3-hydroxy-1,3,5(10)-triene-17-one; 2-aminoestrone and 2-hydroxyestrone mean the 2-amino and 2-hydroxy derivatives of this same compound; similarly, 2-nitroestradiol means 2-nitro-3,17 β -dihydroxyestra-1,3,5(10)-triene, while 2-aminoestradiol and 2-hydroxyestradiol mean the corresponding 2-amino and 2-hydroxy derivatives, and so on.

*Experimental*²

2- and 4-nitroestrones and 2,4-dinitroestrone were prepared and purified as described by WERBIN and HOLOWAY [1]. The reduction of mono- and dinitroestrones was carried out with sodium borohydride to the corresponding estradiols, just as for the mono- and diamino and the mono- and dihydroxy substituted derivatives, by the method of PATTON [4].

In the identification an effort was made to attain the chemical and physico-chemical properties for the compounds described in the literature, and also to obtain materials which were chromatographically (TLC) completely pure by running them in a number of well-known solvent pairs. Separations were performed under the usual conditions, on silica gel adsorbent prepared according to Stahl (Merck or Reanal) in an ascending system.³

2-Nitroestrone (I) [1]. M.p.: 178–80 °C (Lit.: 183.5–184 or 155 °C). Anal.: Calc.: C₁₈H₂₁O₄N C 68.55 H 6.71 N 4.44; Found: C 68.65 H 6.70 N 4.41. $[\alpha]_D = +30 \pm 1^\circ$ (c=1.0; CHCl₃). ν_{\max}^{KBr} 3350 (OH), 1750, 1640 (CO), 1530 (Ar-NO₂), 880, 760, 650 (C-NO₂) cm⁻¹. (The IR spectra were taken with a UNICAM SP 1000 spectrophotometer in all cases.)

2-Nitroestradiol (II) [4]. The sodium borohydride reduction of I gave the diol. M.p.: 162–3 °C (Lit.: 167–8 °C). Anal.: Calc.: C₁₈H₂₃O₄N C 68.11 H 7.30 N 4.41; Found: C 68.15 H 7.23 N 4.48. $[\alpha]_D = +160 \pm 2^\circ$ (c=1.0; CHCl₃). ν_{\max}^{KBr} 3490, 3300, 2950 (OH), 1630, 1525 (Ar-NO₂), 965, 990, 760 (C-NO₂) cm⁻¹.

2-Aminoestrone (III). It was reduced to amine by the method of KRAYCHY and GALLAGHER [15] with an aqueous solution of alkaline sodium hydrosulphite (Na₂S₂O₄). The yield was very good. M.p.: 192–93 °C (Lit.: 220 °C). Anal.: Calc.: C₁₈H₂₃O₂N C 75.75 H 8.14 N 4.91; Found: C 75.80 H 8.18 N 4.67. $[\alpha]_D = -40 \pm 2^\circ$ (c=0.5; CHCl₃). ν_{\max}^{KBr} 3500, 3390, 3350 (NH₂, OH), 1730 (CO), 1290 (NH₂) cm⁻¹.

2-Aminoestradiol (IV). Sodium borohydride reduction of an alkaline methanolic solution of III led to IV. M.p.: 188–90 °C. Anal.: Calc.: C₁₈H₂₅O₂N C 75.23 H 8.76 N 4.87; Found: C 75.30 H 8.80 N 4.72. $[\alpha]_D = -130 \pm 2^\circ$ (c=0.1; CHCl₃). ν_{\max}^{KBr} 3390, 3320, 2950 (NH₂, OH), 1290 1220 (NH₂) cm⁻¹.

2-Hydroxyestrone (V). Prepared from III by diazotation and boiling the diazo compound with urea (by a combination of the methods of NIEDERL and VOGEL [2] and KRAYCHY and GALLAGHER [15]). The 2-hydroxyestrone formed was purified from the by-products by preparative thick layer chromatography on a silica gel adsorbent mentioned before. The end-product of the above reaction was dissolved in CHCl₃ and transferred in a band to a 500 μ thick adsorbent layer. The plate was run in a 1:2:2 mixture of acetone—cyclohexane—chloroform. The identified band, with the appropriate R_f value, was scraped off together with the adsorbent. The adsorbent containing the substance was repeatedly extracted with acetone—chloroform (1:2). The adsorbent was removed by filtration on a G5 glass filter and the solvent

² Melting points are uncorrected.

³ We wish to publish the detailed TLC properties of the compounds in the near future elsewhere.

was evaporated in N_2 atmosphere. (As formation of quinone was observed, it is very important to avoid contact of the material with air as far as possible.) The product thus isolated was pure by TLC. M.p.: 116—18 °C (Lit.: 200 °C). Anal.: Calc.: $C_{18}H_{22}O_3$ C 75.52 H 7.74; Found: C 75.60 H 7.80. $[\alpha]_D = +190 \pm 2^\circ$ ($c=1.0$; EtOH). ν_{\max}^{KBr} 3350 (OH), 1730, 1710 (CO), 1610, 1290 (Ar) cm^{-1} .

2-Hydroxyestradiol (VI). Prepared by the previously mentioned reduction of V. M.p.: 194—96 °C. Anal.: Calc.: $C_{18}H_{24}O_3$ C 75.00 H 8.39; Found: C 75.05 H 8.43. $[\alpha]_D = +110 \pm 2^\circ$ ($c=0.1$; EtOH). ν_{\max}^{KBr} 3400 (OH), 1510, 1450, 1260 (Ar) cm^{-1} .

4-Nitroestrone (VII). M.p.: 277—78 °C (Lit.: 279—80 °C or 258 °C). Anal.: Calc.: $C_{18}H_{21}O_4N$ (as for I); Found: C 68.60 H 6.78 N 4.41. $[\alpha]_D = -299 \pm 2^\circ$ ($c=0.3$; $CHCl_3$). ν_{\max}^{KBr} 3350 (OH), 1730 (CO), 1530 (Ar- NO_2), 1030, 825, 800 (C- NO_2) cm^{-1} .

4-Nitroestradiol (VIII). Prepared from VII by the method used for II. M.p.: 192—93 °C (Lit.: 220 °C). Anal.: Calc.: (as for II); Found: C 68.08 H 7.20 N 4.72. $[\alpha]_D = +250 \pm 2^\circ$ ($c=0.1$; $CHCl_3$). ν_{\max}^{KBr} 3200, 2950 (OH), 1530 (Ar- NO_2), 1050, 825 (C- NO_2) cm^{-1} .

4-Aminoestrone (IX). Prepared from VII, analogously as for III. M.p.: 250—52 °C. Anal.: Calc.: (as for III); Found: C 75.70 H 8.09 N 4.67. $[\alpha]_D = +50 \pm 1^\circ$ ($c=0.1$; dimethylsulphoxide)

4-Aminoestradiol (X). Prepared from IX as for III and IV. M.p.: 266—68 °C. Anal.: Calc.: (as for IV); Found: C 75.20 H 8.72 N 4.72. $[\alpha]_D = -90 \pm 2^\circ$ ($c=0.1$; dimethylsulphoxide). ν_{\max}^{KBr} 3390, 3320, 2950 (OH, NH_2), 1620, 1300, 800 (NH_2) cm^{-1} .

4-Hydroxyestrone (XI). Prepared from IX by the combined method given for V. M.p.: 233—35 °C. Anal.: Calc.: (as for V); Found: C 75.58 H 7.78. $[\alpha]_D = +100 \pm 2^\circ$ ($c=0.1$; EtOH). ν_{\max}^{KBr} 3400, 3350 (OH), 1740 (CO), 1290 (Ar) cm^{-1} .

4-Hydroxyestradiol (XII). Prepared from XI as for VI. M.p.: 168—70 °C. Anal.: Calc.: (as for VI); Found: C 75.04 H 8.40. $[\alpha]_D = +140 \pm 2^\circ$ ($c=0.1$; EtOH). ν_{\max}^{KBr} 3400 (OH), 1510, 1250 (Ar) cm^{-1} .

2,4-Dinitroestrone (XIII). Prepared as described by WERBIN and HOLOWAY [1]. M.p.: 185—87.5 °C. Anal.: Calc.: $C_{18}H_{20}O_6N_2$ C 59.99 H 5.59 N 7.78; Found: C 60.05 H 5.62 N 8.10. $[\alpha]_D = +120 \pm 2^\circ$ ($c=1.0$; $CHCl_3$). ν_{\max}^{KBr} 3350, 3320 (OH), 1750 (CO), 1730, 1535 (Ar- NO_2), 1320 (NO_2), 750 (C- NO_2) cm^{-1} .

2,4-Dinitroestradiol (XIV). Prepared from XIII similarly as previously. M.p.: 263—65 °C. Anal.: Calc.: $C_{18}H_{22}O_6N_2$ C 59.11 H 6.12 N 7.78; Found: C 59.20 H 6.18 N 8.10. $[\alpha]_D = +100 \pm 2^\circ$ ($c=0.1$; $CHCl_3$). ν_{\max}^{KBr} 3220, 2940 (OH), 1320 (NO_2), 800, 745 (C- NO_2) cm^{-1} .

2,4-Diaminoestrone (XV). Prepared as above, by reduction, from XIII. M.p.: 207—9 °C. Anal.: Calc.: $C_{18}H_{24}O_2N_2$ C 71.97 H 8.05 N 9.03; Found: C 80.02 H 8.10 N 9.40. $[\alpha]_D = +200 \pm 2^\circ$ ($c=0.1$; MeOH). ν_{\max}^{KBr} 3390, 3350 (NH_2 , OH), 1745 (CO), 1615, 1290 (NH_2) cm^{-1} .

2,4-Diaminoestradiol (XVI). Prepared from XV by further reduction. M.p.: 198—200 °C. Anal.: Calc.: $C_{18}H_{26}O_2N_2$ C 71.52 H 8.66 N 9.26; Found: C 71.57

H 8.60 N 9.40. $[\alpha]_D = +160 \pm 2^\circ$ ($c=0.1$; dimethylsulphoxide). ν_{\max}^{KBr} 3380, 3320 (NH_2 , OH), 1290 (NH_2) cm^{-1} .

2,4-Dihydroxyestrone (XVII). Prepared from XV in accordance with the above. M.p.: 228—30 °C. Anal.: Calc.: $\text{C}_{18}\text{H}_{22}\text{O}_4$ C 71.51 H 7.33; Found: C 71.48 H 7.30. $[\alpha]_D = +128 \pm 2^\circ$ ($c=0.1$; EtOH). ν_{\max}^{KBr} 3420, 2940 (OH), 1740 (CO), 1610, 1290 (Ar) cm^{-1} .

2,4-Dihydroestradiol (XVIII). Prepared from XVII by sodium borohydride reduction. M.p.: 223—25 °C. Anal.: Calc.: $\text{C}_{18}\text{H}_{24}\text{O}_4$ C 71.04 H 7.95; Found: C 71.10 H 7.90. $[\alpha]_D = +300 \pm 2^\circ$ ($c=0.1$; EtOH). ν_{\max}^{KBr} 3420, 2940 (OH), 1380, 1260 (Ar) cm^{-1} .

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СТЕРОИДЫ XXIII. СИНТЕЗ 2- И 4-ГИДРОКСИ-, 2,4-ГИДРОКСИ ЭСТРОНА И ЭСТРАДИОЛА

К. Ковач, Э. Ракоңцаи, Б. Маткович

Синтезированы, через соответствующие нитросоединения, все С-2 и С-4 однозамещенные гидроксипроизводные эстро́на и эстрадио́ла. Продукты всех ступеней синтеза выделены и характеризованы.