A SIMPLE CHEMICAL METHOD FOR THE SYNTHESIS OF CATECHOL ESTROGENS

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ABSTRACT

The preparation of 2-hydroxyestrone, 2-hydroxy-estradiol-17β, 4-hydroxyestrone and 4-hydroxyestradiol-17β by a simple one-step chemical reaction, treatment with potassium nitrosodisulfonate, is described. The structures of the products were established by nmr, ultraviolet, infrared and mass spectra as well as from their chemical and chromatographic properties.

INTRODUCTION

Many investigators have shown that catechol estrogens play an important role in the metabolism of estrogens in man [1-8]. Recently KNUPPEN and his colleagues demonstrated that catechol estrogens act as strong inhibitors of the methylation of catechol amines by the enzyme catechol-O-methyltransferase [9-12]. In order to promote the study of the interaction between catechol estrogens and catechol

amines it was necessary to synthesize radioactive catechol estrogens with a high specific activity as well as unlabeled material on a preparative scale.

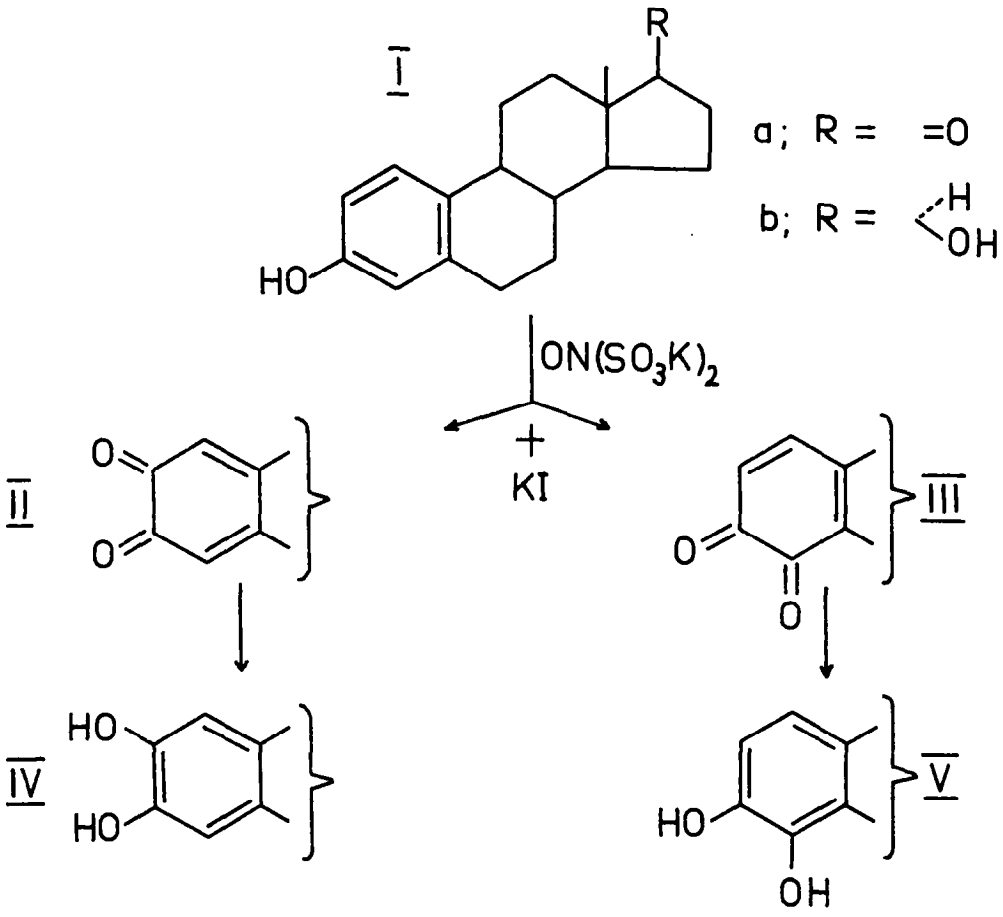
The standard method for preparing 2-hydroxyestrogens as described by FISHMAN et al. [13] and RAO and AXELROD [14] are laborious multistep chemical reactions but large quantities can be prepared, however, highly labeled material cannot be obtained in this way. On the other hand the enzymatic procedures reported [15,16] are easy to perform when only a small amount of 2-hydroxyestrone with relatively low specific activity is required. The chemical methods available for the synthesis of 4-hydroxyestrogens [13,17] give low yields.

The preparation of 2- and 4-hydroxylated estrogens reported here involves a simple one-step chemical reaction: after oxidation of estrone (Ia) or estradiol-17β (Ib) with FREMY's salt (potassium nitrosodisulfonate) to the 2,3- and 3,4-quinone (II,III) subsequent reduction with KI gives the corresponding catechol estrogens (IV,V). In fact, by this method the preparation of highly labeled material as well as of mg- or g-quantities is accomplished.

RESULTS AND DISCUSSION

In the light of a well known method for the preparation of ortho- and para-quinones by oxidation of the corresponding monophenolic compounds with potassium nitrosodisulfonate

(FREMY's salt), first described by TEUBER and coworkers [18, 19] (for a review see [20]), the synthesis of catechol estrogens according to the following scheme seemed possible: oxidation of the monophenolic estrogen (I) with FREMY's salt to the o-quinones (II and III) and subsequent reduction to the catechol estrogens (IV and V).



Pilot experiments showed that the second part of the reaction was easy to perform with the o-quinone IIb, prepared by oxidation of authentic 2-hydroxyestradiol-17β with $NaIO_4$ [21,22]. This o-quinone was nearly quantitatively reduced by

KI in acidic media to the starting material (IV) again. However, as was already mentioned by TEUBER [23], the standard method for the oxidation of monophenols to the o- and p-quinones with FREMY's salt in M/12 NaH_2PO_4-solutions was not successful for estrone. In view of these results, it was necessary to search for acceptable conditions for the first part of the reaction with estrone.

Formation of the o-quinones (II and III) was not detected when the oxidation of estrone with FREMY's salt was carried out in alkaline solutions or when the reaction conditions of TEUBER were employed; moreover, the reaction did not proceed in mineral acid (10% H_2SO_4 in water) because of the extreme instability of FREMY's salt under these conditions. However, the o-quinones were formed readily when a solution of the estrogen (I) in acetone was treated with FREMY's salt dissolved in 10% or 50% acetic acid at room temperature.

The process of oxidation was studied in detail, using [4-^{14}C]estrone, in order to achieve maximum yields of the catechol estrogens. By systematic variation of the concentration of the acetic acid, of the reaction temperature, of the organic solvent for dissolving the estrogens, of the amount of FREMY's salt, and of the reaction time the conditions described in the experimental part were found to be optimal. As demonstrated in Fig. 1 the maximum yield of 2-hydroxyestrone (IVa) was obtained when estrone was allowed to react with an excess of FREMY's salt for 30 min, whereas

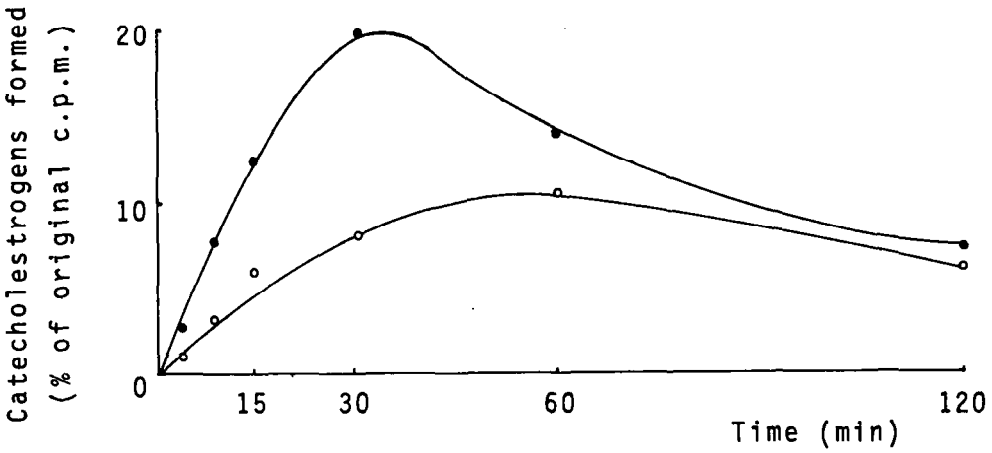


Fig. 1. Time curves of the oxidation of [4-^{14}C]estrone to 2-hydroxyestrone (●——●) and 4-hydroxyestrone (○——○) with FREMY's salt. 0.4 µCi [4-^{14}C]estrone diluted with 850 µg estrone was dissolved in a mixture of 0.5 ml acetone and 0.8 ml 10% acetic acid, oxidized with an excess of FREMY's salt and subsequently reduced with KI, as described in the experimental section.

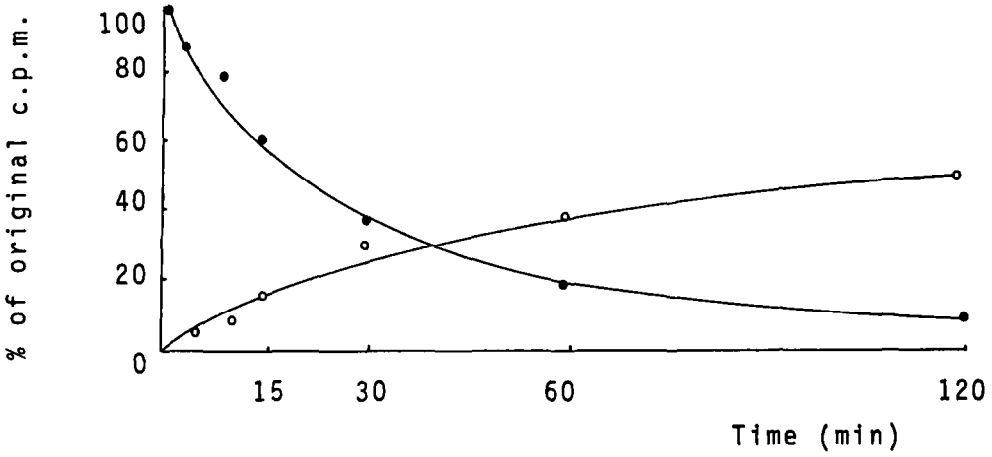


Fig. 2. Time curves of the recovery of [4-^{14}C]estrone (●——●) and of the radioactivity not extracted from the aqueous phase (○——○) during the course of the reaction of estrone with FREMY's salt. Reaction conditions were the same as those described for Fig. 1.

the maximum yield of 4-hydroxyestrone (Va) occurred later.

The amount of radioactivity which was not extracted by chloroform from the aqueous reaction solution increased with the duration of treatment with FREMY's salt (Fig. 2), indicating that the o-quinones (II and III) and possibly estrone were decomposed in the course of the reaction to highly polar compounds.

This method was applied to the preparation of radioactive 2- and 4-hydroxyestrone with a high specific activity on a microgram scale. The separation of 2- and 4-hydroxyestrone thus formed and the unchanged estrone was achieved by chromatography on formamide impregnated paper with monochlorobenzene-ethyl acetate; to avoid oxidative decomposition of the labile catechol estrogens the papers were impregnated with ascorbic acid as described by GELBKE and KNUPPEN [24]. This procedure allowed the purification of highly labeled catechol estrogens without dilution with carrier material. Catechol estrogens synthesized on a preparative scale were separated by using a column of silicagel loaded with ascorbic acid.

In a similar way 2- and 4-hydroxyestradiol-17β (IVb and Vb) were obtained from estradiol-17β (Ib) in good yields; the separation of the products was carried out by paper chromatography or on silicagel columns under the protection of ascorbic acid.

Proofs of the structures of the synthesized compounds IV and V were obtained by their chromatographic and spectral

data, especially nmr, infrared and mass spectra.

Preliminary experiments have also shown that the method described herein can also be applied to the syntheses of related catechol estrogens, for example 2- and 4-hydroxyestradiol-17α and 2- and 4-hydroxyestriol.

EXPERIMENTAL

Estrone, estradiol-17β and 2-hydroxyestradiol-17β were gifts from Schering AG, Berlin, Germany. 4-Hydroxyestrone was kindly supplied by Prof. E. HECKER, Heidelberg, Germany. [4-^{14}C]estrone (specific radioactivity 45 mCi/mmole), [4-^{14}C]estradiol-17β (specific radioactivity 58 mCi/mmole), [6,7-$^{3}H_2$]estrone (specific radioactivity 40 Ci/mmole) and [6,7-$^{3}H_2$]estradiol-17β (specific radioactivity 40 Ci/mmole) were obtained from The Radiochemical Centre, Amersham, Bucks., and purified by paper chromatography.

Potassium nitrosodisulfonate (FREMY's salt) was prepared as described by ZIMMER et al. [20]. The standard solution of ascorbic acid contained 15 g of ascorbic acid and 4 ml of glacial acetic acid in 400 ml of methanol.

Paper chromatography and silicagel column chromatography were carried out on stationary phases loaded with ascorbic acid as described previously [24]; this prevents oxidative decomposition of catechol estrogens. Paper refers to Schleicher and Schüll 2043 b Mgl and silicagel to Kieselgel 60 (0.063-0.2; E. Merck, Darmstadt, Germany).

Ultraviolet spectra of aqueous or ethanolic solutions were recorded with a Shimadzu double beam spectrophotometer UV-200. Infrared spectra, using pressed KBr-discs, were recorded with a Beckman Model IR 8 spectrophotometer. Nuclear magnetic resonance spectra were recorded with a Varian HA-100 spectrometer with tetramethylsilane as internal standard in pentadeuteropyridine solution. Mass spectra

were obtained by using a gas chromatography-mass spectrometer LKB 9 000.

Radioactivity was counted either directly from the paper by a paper-strip scanner (Radiochromatogram Scanner, Berthold, Model LB 280) or by liquid scintillation spectrometry (Intertechnique liquid scintillation spectrometer, Model SL 36).

The melting points were determined with a microscope hot-stage and are uncorrected.

Preparation of 2-hydroxyestrone (IVa) and 4-hydroxyestrone (Va)

Estrone (1.5 g) was dissolved in 500 ml of acetone and then 800 ml of 10% acetic acid in water (v/v) were added. After the addition of 5 g of potassium nitrosodisulfonate the mixture was shaken for 15 min at room temperature. A second portion of potassium nitrosodisulfonate (5 g) was added and shaking was continued for another 15 min. The yellow quinones formed (IIa, IIIa) were extracted from the solution three times with chloroform and the combined organic extracts were washed successively twice with N-HCl and twice with water. After the addition of 100 ml of acetic acid an excess of KI (ca. 3 g) was added. The mixture was then shaken for approximately 2 min during which the solution turned deep yellow; iodine formed during the reaction was reduced by the addition of sufficient 0.1 N-sodium thiosulfate solution (ca. 40 ml) until the solution was nearly colourless. 200 ml of water was added and the chloroform layer was separated. The aqueous phase was further extracted twice with dichloromethane-ethyl acetate (1:1, v/v) and the combined organic extracts were washed successively twice with N-HCl and water, dried over sodium sulfate and evaporated in vacuo.

The yellow residue containing estrone, 2- and 4-hydroxyestrone was dissolved in a mixture of chloroform-methanol-acetic acid (96:3:1, by vol.) and subjected to

column chromatography (200 x 2.5 cm) on ascorbic acid-impregnated silica gel [24]. Elution was carried out with the same solvent system and 20 ml fractions were collected. TLC showed that fractions 15-33 contained estrone, fractions 40-50 contained 4-hydroxyestrone, and fractions 51-67 contained 2-hydroxyestrone. The combined fractions were evaporated and the residues were partitioned between ether and water to remove ascorbic acid and its decomposition products.

2-Hydroxyestrone was crystallized from ethyl acetate containing 2% of acetic acid yielding 310 mg of crystals with m.p. 193-196°; nmr: δ 7.0 (s[1], C-4H), δ 7.2 (s[1], C-1H), δ 2.8 ([2], C-6H_2), δ 0.8 (s[3], C-18H_3), the resonances of the protons in the region between δ 2.5 and δ 1.0 corresponded completely to those of estrone. Infrared-, ultraviolet-, and mass spectra were in accordance with those of authentic 2-hydroxyestrone.

4-Hydroxyestrone yielded 170 mg after crystallisation from ethyl acetate containing 2% of acetic acid; m.p. 268-271°; nmr: δ 6.8 (d[1], C-2H), δ 7.1 (d[1], C-1H), δ 0.8 (s[3], C-18H_3), the resonances of the protons in the region between δ 2.5 and δ 1.0 corresponded completely to those of estrone. Infrared-, ultraviolet-, and mass spectra were in accordance with those of authentic 4-hydroxyestrone.

Preparation of 2-hydroxyestradiol-17β (IVb) and 4-hydroxyestradiol-17β (Vb)

Estradiol-17β (1.5 g) was subjected to the same procedure as above described for estrone. Estradiol-17β, 2- and 4-hydroxyestradiol-17β, obtained after reduction with KI, were separated by column chromatography (75 x 2.5 cm) on ascorbic acid-impregnated silicagel. Elution was carried out with n-hexane-chloroform-acetic acid (4:4:1, by vol.) and 20 ml fractions were collected. Fractions 15-45 contained estradiol-17β, fractions 48-61 contained 4-hydroxy-

estradiol-17β and fractions 63-90 contained 2-hydroxy-estradiol-17β.

2-Hydroxyestradiol-17β was crystallized from ethyl acetate containing 2% of acetic acid yielding 340 mg of crystals with m.p. 186-190°; nmr: δ 7.0 (s[1], C-4H), δ 7.25 (s[1], C-1H), δ 3.9 (t[1], C-17αH), δ 2.8 ([2], C-6H_2), δ 1.0 (s[3], C-18H_3), the resonances of the protons in the region between δ 2.5 and δ 1.0 corresponded completely to those of estradiol-17β. Infrared-, ultraviolet-, and mass spectra showed no difference from those of authentic 2-hydroxyestradiol-17β.

4-Hydroxyestradiol-17β was crystallized from aqueous methanol containing 0.5% acetic acid yielding 160 mg of crystals with m.p. 214-216°; nmr: δ 6.8 (d[1], C-2H), δ 7.1• (d[1], C-1H), δ 3.9 (t[1], C-17αH), δ 1.0 (s[3], C-18H_3), the resonances of the protons in the region between δ 2.5 and δ 1.0 corresponded completely to those of estradiol-17β. Further evidence of the identity of this product as 4-hydroxyestradiol-17β was provided by infrared-, ultraviolet-, and mass spectra.

Preparation of [4-^{14}C]2-hydroxyestrone and [4-^{14}C]4-hydroxyestrone

[4-^{14}C]Estrone (300 μg, 50 μCi) was subjected to the same procedure as 1.5 g of estrone, except that only 1/1000 of the above described amounts of solvents and reagents was employed. After the reduction with KI the final solution containing estrone, 2-hydroxyestrone and 4-hydroxyestrone was protected against autoxidation by adding 1 ml of the standard ascorbic acid solution and subsequently brought to dryness in vacuo at room temperature without previous drying over sodium sulfate. Residual water was evaporated by azeotropic distillation with 1 ml of benzene-ethanol (1:1, v/v). Paper chromatography on 16 cm wide strips of paper in the system formamide-chlorobenzene-ethyl acetate

(3:1, v/v)-ascorbic acid served for the separation of estrone (R_F = 0.88), 2-hydroxyestrone (R_F = 0.52) and 4-hydroxyestrone (R_F = 0.66). Elution of the radioactive steroids from the chromatograms was carried out under the protection of ascorbic acid as described by GELBKE and KNUPPEN [24]. 2-Hydroxyestrone and 4-hydroxyestrone were further purified by chromatography on 8 cm wide papers in the system water-acetic acid-1,2-dichloroethane-methylcyclohexane (60:140:75:125, by vol.)-ascorbic acid and, or by column chromatography on ascorbic acid-impregnated silicagel (7 g, 21 x 1.1 cm diameter) in the system n-hexane-chloroform-acetic acid (4:4:1, by vol.)-ascorbic acid [24].

The radiochemical purity of the purified catechol estrogens was more than 95% and their identity was checked in several paper- and thin layer-chromatographic systems. The yields of radioactivity amounted to 15% for 2-hydroxyestrone, 7% for 4-hydroxyestrone and 40% for the recovered estrone. The catechol estrogens thus prepared were stored at -20° in methanolic solutions containing 2% of acetic acid and 20% of the standard ascorbic acid solution.

Preparation of [6,7-$^3H_2$]2-hydroxyestrone and [6,7-$^3H_2$]4-hydroxyestrone

[6,7-$^3H_2$]Estrone (7 µg, 1 mCi) was subjected to exactly the same procedure as described above for 50 µCi of [4-^{14}C]estrone. Paper chromatographic separation and purification of the products was performed on 4 cm wide strips of paper and silicagel column chromatography on 12 x 0.6 cm columns. The yields and the radiochemical purity of the tritiated catechol estrogens were of the same order as described above for the corresponding [4-^{14}C]-compounds.

Preparation of [4-^{14}C]2-hydroxyestradiol-17β, [4-^{14}C]4-hydroxyestradiol-17β, [6,7-$^{3}H_2$]2-hydroxyestradiol-17β and [6,7-$^{3}H_2$]4-hydroxyestradiol-17β

The 2- and 4-substituted catechol estrogens derived from 7 µg of [6,7-$^{3}H_2$]estradiol-17β and 230 µg of [4-^{14}C] estradiol-17β were prepared by exactly the same procedure as described above for [6,7-$^{3}H_2$]- and [4-^{14}C]estrone. Paper chromatography on formamide-ascorbic acid-impregnated papers with chlorobenzene-ethyl acetate (3:1, v/v) served for the separation of estradiol-17β (R_F = 0.80), 2-hydroxyestradiol-17β (R_F = 0.36) and 4-hydroxyestradiol-17β (R_F = 0.50). 2-Hydroxyestradiol-17β and 4-hydroxyestradiol-17β were further purified by paper chromatography in the system methanol-water-benzene (55:45:100, by vol.)-ascorbic acid and, or by column chromatography on ascorbic acid-impregnated silicagel in the system acetic acid-chloroform-cyclohexane (1:2:2, by vol.)-ascorbic acid. Yields and radiochemical purity corresponded to the analogous estrone preparations.

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Nomenclature list

2-Hydroxyestrone	2,3-dihydroxy-1,3,5(10)-estratrien-17-one
4-Hydroxyestrone	3,4-dihydroxy-1,3,5(10)-estratrien-17-one
2-Hydroxyestradiol-17β	estra-1,3,5(10)-triene-2,3,17β-triol
4-Hydroxyestradiol-17β	estra-1,3,5(10)-triene-3,4,17β-triol
Estrone	3-hydroxy-1,3,5(10)-estratrien-17-one
Estradiol-17β	estra-1,3,5(10)-triene-3,17β-diol
FREMY's salt	potassium nitrosodisulfonate