FACILE SYNTHESIS OF DEUTERATED ESTROGENS

R.C. Murphy

Department of Pharmacology
University of Colorado School of Medicine
Denver, Colorado 80220

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ABSTRACT

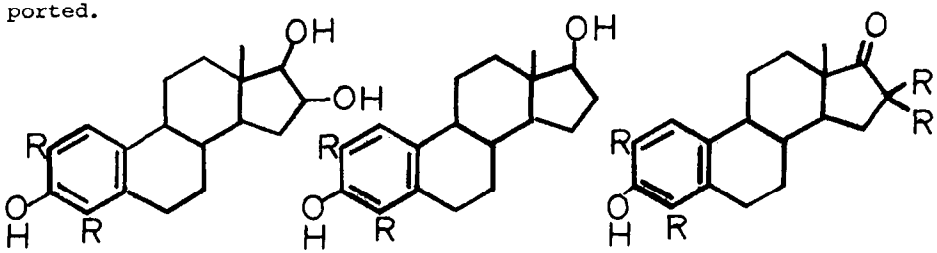
The acid catalyzed exchange of the aromatic protons, ortho to the phenolic hydroxyl moiety in estriol, estradiol, and estrone is described. The acidic protons adjacent to the keto function in estrone are also exchanged leading to tetradeuterioestrone (80.0 atom%). Estriol was converted to dideuterioestriol (81.2 atom%), and estradiol into dideuterioestradiol (90.5 atom%).

One of the recent advances in analytical methods has been the demonstration of the ability to quantitatively measure minute amounts of a wide variety of compounds in biological systems by the use of mass spectrometry and the same compounds labeled with a stable isotope as internal standards. Many examples of the use of mass spectrometry, in particular combined gas chromatography-mass spectrometry, have appeared in the literature in the last few years [1], for example the use of monodeuterioestradiol in the quantitative measurement of estradiol-17β in pregnancy urine [2]. The precision and accuracy obtainable in such mass spectrometric measurements is related to the number of heavy isotopes incorporated into the internal standard molecule. This is due to the reduction of the "background" from the naturally occuring isotopes such as ^{13}C, ^{15}N, and ^{18}O. The natural abundance of ^{13}C is approximately 1.1% for every carbon atom in a molecule, resulting in, for example, 19.8% of all the naturally occurring estriol molecules having at least one ^{13}C in its structure. Therefore it becomes necessary to introduce multiple labels into the molecule, which reduces the "background" for the labeled molecule by the power of the

number of labeling atoms present. Since multiple labels often present a synthetic problem, this paper describes a rather simple procedure for introducing multiple labels into estrone, estradiol, and estriol and which could be easily extended to other biologically important molecules.

Several deuterium labeling procedures for steroids have been reported. Fishman [3] obtained the benzoate ester of 16α, 17α-dideuterio-estradiol-17β by catalytic deuteration of the enol acetate followed by oxidation to 17-keto, then reduction with lithium aluminum deuteride. The introduction of deuterium by hydrogenolysis of a halide has led to the synthesis of monodeuterioestradiol [2,4] and dideuterio-estriol [5]. Base catalyzed exchange of enolizable protons has been used to synthesize progesterone-d_9 [6] testosterone-d_4 [7], and 5α-androstan-11-one-d_3 [8] Various catalytic reduction methods have been used for other deuterated steroids [9-13].

It has been well established that aromatic hydrogens which are ortho or para to the hydroxyl moiety in phenolic compounds can be exchanged for deuterium with acid catalysis [14,15]. This suggested that estrogens such as Ia, IIa, and IIIa could be multiply deuterated in a much easier fashion than by the synthetic methods previously reported.



I a. R=H b. R=D

II a. R=H b. R=D

III a. R=H b. R=D

Figure 1 shows the mass spectrum of estriol and dideuterioestriol (Ib) which was synthesized by such an acid-catalyzed, deuterium exchange. The calculated isotopic purity of this sample [16] is 0.3%-d_0, 7.8%-d_1, 81.2%-d_2, and 10.8%-d_3. Using similar conditions, estrone-d_4 was synthesized and found to be 2.6%-d_0, 0.7%-d_1, 16.6%-d_3, 80.0-d_4. Estradiol-17β was converted into dideuterioestradiol with an isotopic purity of 2.8%-d_0, 6.7%-d_1, and 90.5%-d_2 as seen in Figure 2. The isotopic purity of each product is that which was obtained after only one exchange procedure and theoretically should increase upon further exchange steps. The deuterated estrone and estriol were readily purified by recrystalization; however, the deuteration procedure for estradiol-17β resulted in a trace of estrone and the epimer estradiol-17α which could be easily separated by TLC.

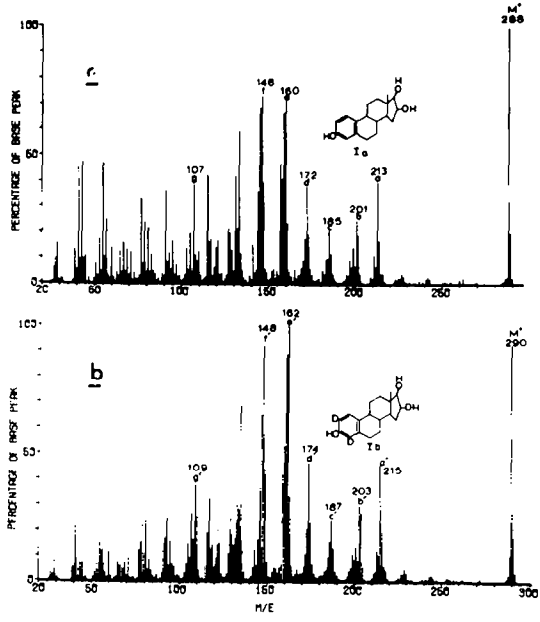


Figure 1. Mass spectrum (70eV) of (a) estriol and (b) 2,4-dideuterioestriol.

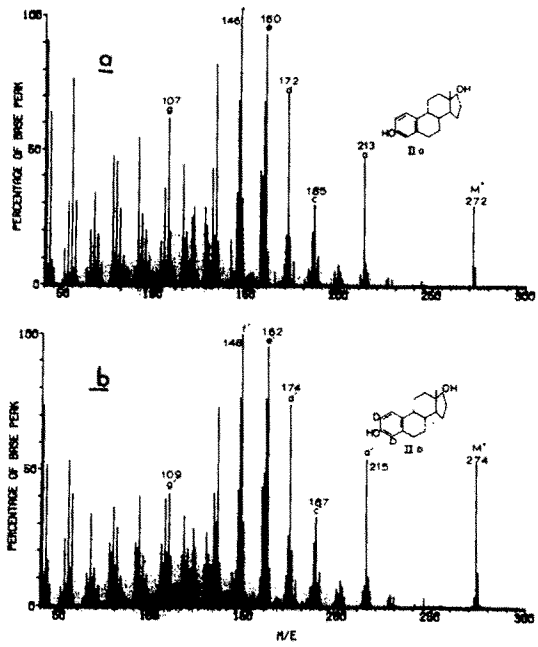
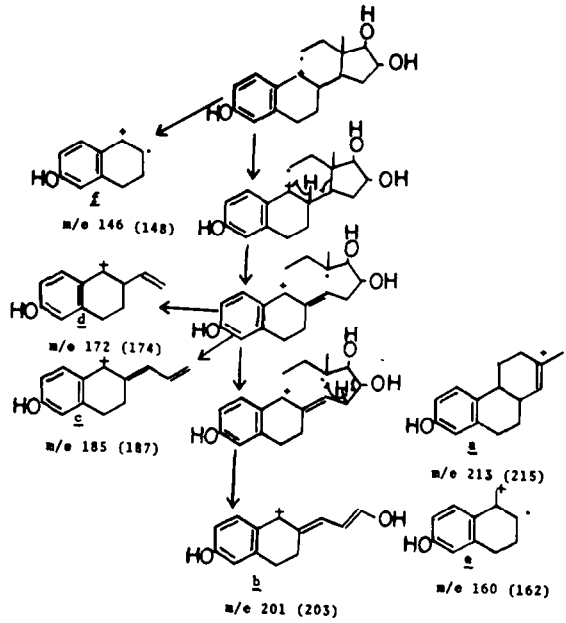


Figure 2. Mass Spectrum (70eV) of (a) estradiol-17β and (b) 2,4-dideuterioestradiol-17β.

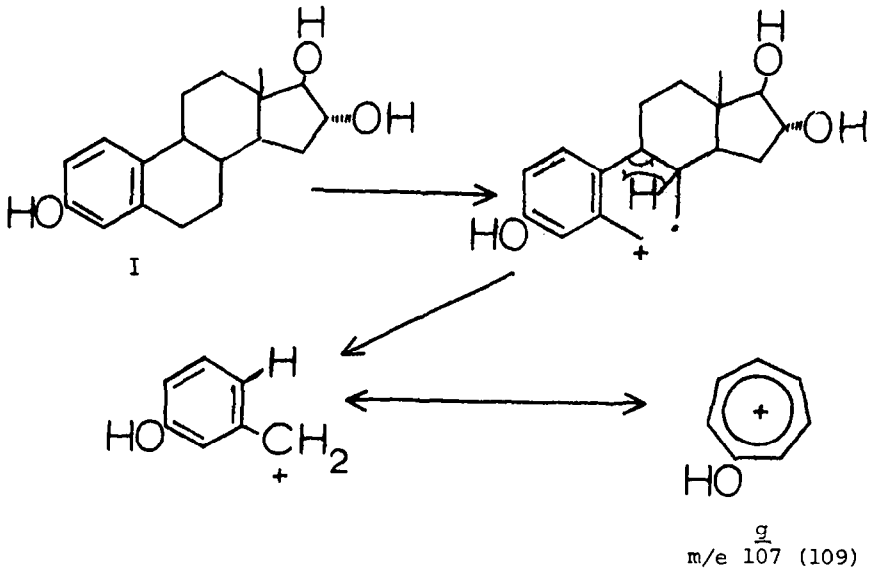
The position of deuterium incorporation in these estrogens is revealed by interpretation of the mass spectral fragmentation. Most of the fragmentation pathways of the methyl ether of estrone as described by Djerassi, et al. [17] are identical for estrone, estradiol, and estriol, since oxygen substitution in the D-ring and methylation of the phenoloic hydroxyl exert very slight mass spectral influence [18]. Many of the fragment ions seem to be initiated by cleavage of benzylic carbon-carbon bonds followed by proton rearrangement. Scheme I below shows the major fragment ions of estriol with the mass spectrum shifts in parentheses for dideuterioestriol. As seen in figure 2 these same major fragment ions occur in estradiol-17β as would be expected, except for ion b (m/e 201) which contains the oxygen atom at C-16 of estriol and is correspondingly absent in estrodiol-17β and dedeuterioestradiol-17β.

Scheme 1



These fragment ions all show an increase in 2 mass units in deuterated estriol and estradiol-17β, which is consistant with the deuterium being in the A-ring. Further evidence comes from inspection of the ion at m/e 107, which is most likely the phenolic tropylium ion of structure g ($C_7H_7O^+$) originating from ring A, with the additional carbon atom from C-6 or C-9. The latter position is less likely since its retention would have to involve cleavage of 2 bonds attached to one carbon atom. A likely mechanism is cleavage of the 6-7 benzylic bond and rearrangement of the C-8 hydrogen to the aromatic ring with cleavage of the 9-10 bond.

Scheme II



g
m/e 107 (109)

It is important to point out a possible limitation in the use of these molecules (or any ortho-deuterated phenol) as biological tracers. Rutschmann, et al. [19] have shown the loss of isotopic label in 2,5, 6-tritritio-L-DOPA to be a first order process with a half-life of 60 hours at 90° in 1N HCl. A long term experiment during which the isotopically labeled steroid would be a part of the biological system, may give erroneous analytical results due to the back-exchange of hydrogen for deuterium and corresponding loss of the internal standard. Therefore, control experiments must be performed to establish to what extent, if any, this phenomenon is occurring.

EXPERIMENTAL SECTION (21)

2,4-dideuterioestriol (Ib).

Estriol (100 mg) was dissolved in dioxane (2 ml) and placed in a bulb equipped with a high vacuum stopcock. This allowed evacuation of the solution with a forepump and removal from the pump without breaking the vacuum above the solution. 0.5 ml of 5.3N DCl (99.8 atom% D, Merck, Sharp, and Dhome) was added to the solution and the bulb evacuated to remove dissolved oxygen. The evacuated bulb was placed in an oil bath at 55°. After 120 hr., the solution was evaporated to dryness with a rotary evaporator at 30°. The residue was dissolved in dioxane/H_2O, evaporated to dryness and again repeated in order to back-exchange the hydroxyl and phenolic protons. The dideuterioestriol (Ib) was crystalized from ethanol. Yield 40 mg. M.p. 278-279°; 0.3%-d_0, 7.8%-d_1, 81.2%-d_2, and 10.8-d_3 by mass spectrometry (16). λ max 280 (shoulder 285) nm, ν max 3500, 3430, 3300-2800, 1600, 1450-1350, 1255, 1225, 1075-1065, 1030 cm^{-1}.

2,4-dideuterioestradiol (IIb).

Estradiol-17β (100 mg) was dissolved in 10 ml dioxane, and transferred to the exchange bulb. 2 ml D_2O (99.87 atom% D, Bio-Rad) and 1.0 ml 5.3N DCl was added to the solution and the bulb evacuated. The bulb was heated to 60° for 72 hrs., then evaporated to dryness. The labile hydrogens were back-exchanged twice with ethanol. The residue was dissolved in 40 ml ethanol, treated with 0.25 g activated carbon powder and filtered. The clear filtrate was evaporated, dissolved in 4 ml 50% ethanol, and cooled to precipitate the dideuterioestradiol. The crude estradiol was purified by preparative TLC following Maugras, et al. (20). Yeild 12 mg. M.p. 173-175°, 2.8%-d_0, 6.7%-d_1, 90.5%-d_2 by mass spectrometry (16). λ max 285 (shoulder 290) nm, ν max 3500-3100, 2910, 2850, 1595, 1570, 1465, 1420, 1380, 1270, 1250, 1055, 1010 cm^{-1}.

2,4,16-tetradeuterioestrone (IIIb).

Estrone (100 mg) was dissolved in 5 ml of dioxane and exchanged with 1.0 ml 5.3N DCl after evacuation. The exchange was run for 90 hrs at 55°. The estrone was isolated by adding 50 ml H_2O and extracting twice with 50 ml chloroform. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was dissolved in 3 ml hot acetone and dideuterioestrone (IIIb) crystalized upon cooling. Yield 35 mg. M.p. 257-260°; 2.6%-d_0, 0.7%-d_1, 16.6%-d_3, 80.0%-d_4 by mass specrommetry (16). λ max 283 nm. ν max 3325, 2940, 2850, 1725, 1610, 1575, 1470, 1355, 1275, 1055 cm^{-1}.

ACKNOWLEDGEMENTS

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21. Melting points are uncorrected. The uv spectra were obtained on a Hitachi 124 double-beam spectrophotometer in chloroform: methanol (2:1) solutions; the IR spectra on a Perkin-Elmer 257 in KBr pellets; the mass specta, on an AEI MS-12 mass spectrometer and Finnigan 3100 mass spectrometer.