SYNTHESIS OF C-2 AND C-4 DEUTERIUM-LABELED ESTRADIOL-17β

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ABSTRACT

Estradiol-17β labeled with deuterium in the positions 2 or 4 can be prepared from 2-chloromercurio-1,3,5(10)-estratriene-3,17β-diol 3-methyl ether 17-acetate or 4-chloromercurio-1,3,5(10)-estratriene-3,17β-diol, respectively, in refluxing $CH_3COO^2H/^2H_2O$. The same reaction performed on 4-acetoxymercurio-1,3,5(10)-estratriene-3,17β-diol afforded 2,4-dideuterio-estradiol-17β in good yields.

Deuterium-labeled estrogens have been used for the estimation of estrogen production rates in women and this isotope dilution technique has been shown to afford results concordant with those obtained from radioisotope-labeled estrogens (1). Several methods have been reported for the preparation of estrogens labeled with deuterium at the positions 2 and/or 4 either by exchange of C-2 and/or C-4 hydrogens with deuterium in basic (2) and acid conditions (3) or by reduction of 2,4-dibromoestradiol-17β by $Pd/^2H_2$ (4).

In connection with our recent results on the regioselective functionalization of estrogens (5) we had prepared 2-chloromercurio-estrogens (1a and 1b) and decided to use them for the preparation of 2-deuterio-estradiol-17β (2a). Treatment of organomercurials with NaB^2H_4 is a well known method for the introduction of deuterium into a molecule and the process has been established to proceed by a radicalic mechanism (6). Therefore, we tried the reduction of the

chloromercuriated function in compound 1a with NaB^2H_4 in dioxane, the reaction being monitored by ^1H-NMR spectroscopy. To our surprise no incorporation of deuterium was observed in the final product; only 1,3,5(10)-estratriene-3,17β -diol 3-methyl ether 17-acetate (2b) was obtained in good yields. Changing the solvent to acetonitrile did not improve the situation and we interpreted these negative results as due to the fact that the aromatic radical formed reacted faster with the solvent than with the borohydride anion. We did not further investigate this disappointing reaction and took advantage of our previous observation on the reactivity of the 2-chloromercurio derivative 1a. The chloromercurio group was cleaved by refluxing acetic acid, using a dilution which avoided the concurrent formation of a dimer (5), and compound 2b was obtained in 80-90% yield. Repetition of this reaction with $CH_3COO^2H/^2H_2O$ allowed a clean preparation of 2-deuterio-derivative 2c. However, the hydrolysis of the 3-methoxy group was not easy, since under basic conditions (nBuSNa/dimethylformamide (7)) unsatisfactory yields of 2-deuterio-estradiol-17β (2a) were obtained. On the other hand, treatment with pyridinium hydrochloride (8) afforded the desired demethylated product, but which had lost virtually all the deuterium label. We were able to prepare the 2-chloromercurio derivative 1b from the less reactive estradiol-17β diacetate (5), so we used it as a starting material for introducing the deuterium at position 2, using the conditions as mentioned above. 2-^{2}H -1,3,5(10)-Estratriene-3,17β -diol 3,17-diacetate (2d) was prepared in 89% yield and hydrolyzed by potassium carbonate to 2-deuterio-estradiol-17β (2a), which showed

90% deuterium label by mass spectrometry.

For the preparation of the 4-deuterio-estradiol-17β (3a), the most suitable compound appeared to be the 4-acetoxymercurio-estradiol-17β (3b) prepared according to Chin and Warren (9). Treatment of compound 3b with $CH_3COO^2H/^2H_2O$ afforded a mixture of compound 3c and monoacetylated products. We had already observed partial acetylation of 3- and then 17β -hydroxy groups when compound 1a reacted with $Hg(OAc)_2$ in acetic acid (5), but since hydrolysis of the above mixture could afford compound 3a, we made no further efforts to protect the two hydroxy groups before introducing the deuterium label. In fact, hydrolysis with potassium carbonate in methanol/water quantitatively afforded compound (3c), 95% d_2 as shown by mass spectrometry. The formation of a dideuteriated species in the above reaction can be explained by formation of a molecule of $Hg(OAc)_2$ after introduction of the first deuterium atom. The formed $Hg(OAc)_2$ can rapidly attack the less hindered position 2 and this C-Hg bond is again cleaved by $CH_3COO^2H/^2H_2O$ giving rise to a dideuteriated species. Milder reaction conditions did not allow a clean preparation of 4-deuterio-estradiol-17β (3a). Therefore we converted the 4-acetoxymercurio group into the less reactive 4-chloromercurio function by treatment of compound 3b with a saturated solution of NaCl. The isolated 4-chloromercurio-estradiol-17β (3d) was reacted with refluxing $CH_3COO^2H/^2H_2O$ and the resulting mixture of 4-deuterio-estradiol-17β (3a) and monoacetylated products were recovered and acetylated. The ms spectrum of 4-^{2}H-1,3,5(10)-estratriene-3,17β -diol 3,17-diacetate (3e)

indicated 95% deuteriation. Hydrolysis of the diacetate 3e with potassium carbonate quantitatively afforded 4-deuterio-estradiol-17β (3a) in 78% yields from compound 3b.

EXPERIMENTAL

All mps were determined with a Buchi 510 Apparatus and were uncorrected. Ir spectra were recorded on a Perkin Elmer 157 spectrometer for solution in chloroform or Nujol mulls. ^{1}H-NMR spectra were recorded on a Varian XL-100 spectrometer for solutions containing Me_4Si as internal standard and values are given in ppm. Mass spectra were recorded on a Varian 112 S mass spectrometer (direct inlet). The progress of all reactions and column chromatographies (silica gel 230-400 mesh) was monitored by TLC on Merck silica gel HF_{254} plates visualized by spraying with 5% or 10% ethanolic phosphomolybdic acid followed by heating.

2-^{2}H -1,3,5(10)-Estratriene-3,17β -diol 3-methyl ether, 17-acetate (2c). A solution of 2-chloromercurio-1,3,5(10)-estratriene-3,17β-diol 3-methyl ether 17-acetate (1a) (5) (100 mg, 0.178 mmol) in CH_3COO^2H (Fluka, 8 ml) was prepared at room temperature and ^2H_2O (0.1 ml) was added. The solution was refluxed (3 h), monitored by TLC (benzene-ethyl acetate, 8:2), cooled to room temperature, poured into water and the precipitate filtered off by suction. After crystallization from acetone-water, pure compound 2c was obtained (53 mg, 90.5%). Mp 102-104°C (lit.(10) 103-104°C); ^{1}H-NMR (C^2HCl_3): δ 0.80 (s, 3 H, 18-CH_3), 2.05 (s, 3 H, -$COCH_3$), 3.80 (s, 3 H, O-CH_3), 4.80 (m, 1 H, 17αH), 6.70 (s, 1 H, C-4), 7.30 (s, 1 H, C-1). Calculated for $C_{21}H_{27}{}^2HO_3$: C 76.82, H 8.53; found:C 76.60, H 8.38%.

2-^{2}H -1,3,5(10)-Estratriene-3,17β -diol 3,17-diacetate (2d). To a solution of 2-chloromercurio-1,3,5(10)-estratriene-3,17β -diol 3,17-diacetate (1b) (5) (150 mg, 0.254 mmol) in CH_3COO^2H (12 ml), ^2H_2O (0.15 ml) was added and the resulting solution refluxed (48 h). After cooling to room temperature, the reaction mixture was poured into water and the precipitate filtered off. Silica gel column chromatography afforded compound 2d from the fractions eluted with hexane/ethyl acetate (9:1) (66 mg,71%). The fractions eluted with hexane/ethyl acetate (8:2) consisted of 2-^{2}H -estradiol-17β (2a) (15 mg, 18%) which was quantitatively acetylated to compound 2d; the overall yield of labeling with deuterium was 89%. Compound 2d was recrystallized from methanol; mp 123-125°C (lit.(11) 123-125°C); ^{1}H-NMR (C^2HCl_3): δ 0.80 (s, 3 H, 18-CH_3), 2.00 (s, 3 H, -$COCH_3$), 2.20 (s, 3 H, - $COCH_3$), 4.75 (m, 1 H, 17αH), 6.80 (s, 1 H, C-4), 7.30 (s, 1 H, C-1). Calculated for $C_{22}H_{27}{}^2HO_4$:C 73.95, H 7.56; found: C 73.80, H 7.40%.

2-^{2}H -1,3,5(10)-Estratriene-3,17β-diol (2a).
To a solution of compound 2d (75 mg, 0.21 mmol) in methanol (1.5 ml) potassium carbonate (120 mg) and water (0.3 ml) were added. After 24 h under stirring, the formed precipitate was filtered off to afford compound 2a (50 mg, 90%). A sample crystallized from acetone-water had a mp of 175-176°C; ^{1}H-NMR (DMSO-d_6): δ 0.65 (s, 3 H, 18-CH_3), 4.50 (m, 1 H, 17αH), 6.50 (s, 1 H, C-4), 7.10 (s, 1 H, C-1). Ms: m/e 273 (M^+), 214, 173, 161, 147, 134. Calculated for $C_{18}H_{23}{}^{2}HO_2$: C 79.12, H 9.40; found: C 79.00, 9.28%.

2,4-^{2}H -1,3,5(10)-Estratriene-3,17β-diol (3c).
A solution of 4-acetoxymercurio-1,3,5(10)-estratriene-3,17β-diol (3b) (9) (170 mg, 0.32 mmol) in $CH_3COO^{2}H$ (10 ml) was prepared by warming the mixture and $^{2}H_2O$ (0.15 ml) was added. The solution was refluxed (3 h) and, after cooling to room temperature, poured into water. The precipitate was filtered off, dissolved in methanol (3 ml) and treated with potassium carbonate (150 mg) and water (0.2 ml) under stirring (20 h). After usual work-up, compound 3c was obtained (85 mg, 97%). A sample crystallized from acetone, had a mp of 175-176°C; ^{1}H-NMR (DMSO-d_6): δ 0.65 (s, 3 H, 18-CH_3), 4.50 (m, 1 H, 17αH), 7.10 (s, 1 H, C-1). Ms: m/e 274 (M^+), 215, 188, 174, 162, 148, 135. Calculated for $C_{18}H_{22}{}^{2}HO_2$: C 78.83, H 8.02; found: C 78.68, H 7.95%.

4-^{2}H -1,3,5(10)-Estratriene-3,17β-diol 3,17 -diacetate (3e).
Under vigorous stirring, 4-acetoxymercurio-1,3,5(10)-estratriene-3,17β-diol (3b) (9) (500 mg, 0.94 mmol) was added to a saturated solution of NaCl (25 ml) (2 h). The precipitate was filtered off, washed with water and diethyl ether. 4-Chloromercurio-estradiol-17β (3d) was formed (450 mg, 95%). A sample was crystallized from acetone; mp 156-157°C. Calculated for $C_{18}H_{23}HgClO_2$: C 47.69, H 4.55; found: C 47.39, H 4.35%.
A solution of compound 3d (380 mg, 0.75 mmol) in $CH_3COO^{2}H$ (25 ml) and $^{2}H_2O$ (0.2 ml) was refluxed (1 h). After pouring into water and filtration, the precipitate (180 mg) was dried and acetylated with pyridine (0.7 ml) and acetic anhydride (0.7 ml) (12 h).
Usual work-up afforded compound 3e (210 mg, 78%), which had chemico-physical and elemental analysis identical to compound 2d, except for the ^{1}H-NMR spectrum ($C^{2}HCl_3$): δ 0.8 (s, 3 H, 18-CH_3), 2.05 (s, 3 H, -$COCH_3$), 2.25 (s, 3 H, -$COCH_3$), 4.75 (m, 1 H, 17αH), 6.80 (d, J= 8 Hz, C-2), 7.30 (d, J= 8Hz, C-1). Ms: m/e 357 (M^+), 315, 255, 227, 173, 160, 147.

4 -^{2}H- 1,3,5(10)-Estratriene-3,17β-diol (3a)
The above mentioned acetate (3e) (160 mg) was dissolved in methanol (3 ml) and treated with potassium carbonate (150 mg) and water (0.2 ml) under stirring at room temperature (20 h). After usual work-up, compound 3a was obtained (116 mg, 95%), mp 175-176°C; ^{1}H-NMR (DMSO-d_6): δ 0.65 (s, 3 H, 18-CH_3), 4.50 (m, 1 H, 17αH), 6.80 (d, J= 8 Hz, C-2), 7.30 (d, J= 8 Hz, C-1).

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