The preparation of 2,4-dibromoestra-1,3,5(10),6-tetraene-3,17β-diol diacetate

Zsuzsanna Szendi,* György Dombi,† and Iren Vincze*

**Department of Organic Chemistry, Attila József University, Szeged, Hungary; and †Institute of Pharmaceutical Chemistry, Albert Szent-Györgyi Medical University, Szeged, Hungary*

[2,4,6,7-$^3H_4$]Estradiol is used for the quantitative determination of estradiol receptors. The synthesis of 2,4-dibromoestra-1,3,5(10),6-tetraene-3,17β-diol 3,17-diacetate, the convenient precursor of [2,4,6,7-$^3H_4$]estradiol, is described. 6-Alkoxy compounds were also prepared and investigated. (*Steroids* **56**:392–394, 1991)

Keywords: steroids; estradiol derivatives; 6-alkoxyestradiols; 2,4-dibromoestra-1,3,5(10),6-tetraene-3,17β-diol

Introduction

The quantitative determination of estradiol receptors is important for the evaluation of hormone-dependent tumors.[1] Tritium-labeled estradiol derivatives of high specific activity, such as [2,4,6,7-tetra-^{3}H]estradiol, are frequently used for this purpose. The preparation of this tetratritiated estrogen has been described by Fan et al.[2] The synthesis requires a two-step tritiation, starting from 3-hydroxyestra-1,3,5(10),6-tetraen-17-one (**1**), and two further reaction steps (bromination and acetylation) have to be carried out from tritiated intermediates before the second tritiation.

We propose the use of the 3,17β-diacetoxy-2,4-dibromoestra-1,3,5(10),6-tetraene (**6a**) as a precursor for the one-step labeling process, affording tetratritiated estradiol, and describe its synthesis.

Experimental

Melting points (mp) were determined on a Kofler block and are uncorrected. Optical rotations were measured with a Polamat-A polarimeter. Thin-layer chromatograms were obtained on Kieselgel 60 F_{254} (Merck) layers in the solvent systems: A, benzene/methanol (9 : 1) and B, acetic acid/chloroform (5 : 95). The spots were detected in UV light (254 and 366 nm) and also visualized by spraying with concentrated H_2SO_4 and heating (120 C) for 10 minutes. Infrared (IR) spectra were recorded in KBr pellets on a UNICAM SP 200 instrument. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Spectrospin AC-200 FT NMR instrument (200 and 50.3 MHz for ^{1}H and ^{13}C spectra, respectively) in $CDCl_3$ solution, unless otherwise stated; chemical shifts are given in δ values using tetramethylsilane as internal standard.

2,4,7α-Tribromo-6β-ethoxyestra-1,3,5(10)-triene-3,17β-diol diacetate (**2a**)

Estra-1,3,5(10),6-tetraene-3,17β-diol (**1**, 2.7 g, 0.01 mol) was dissolved in absolute ethanol (800 ml), and *N*-bromosuccinimide (5.67 g, 0.032 mol) was added. The solution was maintained at 25 C for 3 days, then evaporated under vacuum to 1/10 vol. To the concentrated solution, water (200 ml) was added. After 10 hours at 5 C, the separated crystals were filtered and dried. The crude product (**2**, 5.4 g, 84.7%) was dissolved in pyridine (8 ml), and the solution was stirred with acetic anhydride (5 ml) overnight at 20 C. The mixture was poured into water, acidified with concentrated HCl (pH 3) and the separated oily product was extracted with benzene (3 × 200 ml). The combined pale-yellow benzene extract was washed with water (100 ml), dried (Na_2SO_4), and chromatographed on silica gel (150 g). Elution with benzene (100 ml fractions) gave **2a** in fractions 8-23. The solvent was removed under reduced pressure. The recovered steroid (3.82 g, 60%) melted at 99 to 102 C; R_f = 0.71 (ss A); $[\alpha]_D$ = +15° (c = 0.5, ethanol); IR: 1.780, 1.740, 1.245, 1.180, 1.065, 1.040 cm^{-1}; ^{1}H NMR: 0.87 (s, 3 H, 18-H_3), 2.07

Address reprint requests to Dr. Iren Vincze at the Department of Organic Chemistry, Attila József University, Dóm tér 8, Szeged, H-6720 Hungary.
Received August 20, 1990; accepted January 6, 1991.

(s, 3 H, 17-OAc), 2.4 (s, 3 H, 3-OAc), 1.24 (t, 3 H, H_3 of OEt), 3.81 (q, 2 H, CH_2 of OEt), 4.73 (t, 1 H, 17α-H), 4.65 (s, 1 H, 6α-H), 4.46 (s, 1 H, 7β-H), 7.55 (s, 1 H, 1-H) ppm; ^{13}C NMR: 170.6 (C=O in 3-OAc), 167 (C=O in 17-OAc), 66.5 (CH_2 in OEt), 15.5 (CH_3 in OEt), 129.5 (C-1), 117.5 (C-2), 145.0 (C-3), 122.8 (C-4), 136.5 (C-5), 82.0 (C-6), 38.5 (C-7), 36.4 (C-8), 46.5 (C-9), 140.2 (C-10), 22.0 (C-11), 36.6 (C-12), 43.0 (C-13), 52.5 (C-14), 25.7 (C-15), 27.5 (C-16), 80.0 (C-17), 12.3 (C-18) ppm. Analysis calculated for $C_{24}H_{29}Br_3O_5$ (637.35): C, 45.24; H, 4.59; Br, 37.62. Found: C, 45.41; H, 4.49; Br, 37.16%.

2,4-Dibromo-3,17β-dihydroxyestra-1,3,5(10)-trien-6-one (4)

3,17β-Dihydroxyestra-1,3,5(10)-trien-6-one (**3**, 2.6 g, 9.1 mmol) was dissolved in dry ethanol (600 ml), and *N*-bromosuccinimide (4 g, 22.5 mmol) was added. After standing overnight at 25 C, the solution was evaporated to 100 ml and left to crystallize at 5 C. The separated material was filtered, washed with water, and dried. Recovery was 3.8 g (94.3%): mp 175 to 178 C; after recrystallization from ethanol, mp 183 to 186 C; R_f = 0.34 (ss A); $[\alpha]_D$ = +76° (c = 0.5, ethanol). IR: 3.450, 1.680, 1.060 cm^{-1}; ^1H NMR: 0.79 (s, 3 H, 18-H_3), 3.75 (t, 1 H, 17α-H), 7.59 (s, 1 H, aryl-H) ppm. Analysis calculated for $C_{18}H_{20}Br_2O_3$ (444.17): C, 48.67; H, 4.54; Br, 35.98. Found: C, 48.53; H, 4.55; Br, 35.92%.

2,4-Dibromoestra-1,3,5(10)-triene-3,6α,17β-triol and −3,6β,17β-triol (5)

2,4-Dibromo-3,17β-dihydroxyestra-1,3,5(10)-trien-6-one (**4**, 1 g) was dissolved in methanol (150 ml), and $NaBH_4$ (3.5 g) was added in portions over 1 hour. The suspension was left at 25 C for 2 hours, then was poured into ice-water and acidified with concentrated HCl until pH 3. The separated white solid was filtered and washed with water until neutral. The dried crude product (0.85 g, 85%) melted at 224 to 227 C. After recrystallization from acetone, mp = 232 to 235 C; R_f = 0.24 (ss A), 0.34, and 0.37 (ss B); $[\alpha]_D$ = +17° (c = 0.5, ethanol). IR: 3.450, 1.280, 1.060 cm^{-1}; ^1H NMR ($[^2H_6]$DMSO): 0.7 (s, 3 H, 18-H_3 in the 6α-OH isomer), 0.8 (s, 3 H, 18-H_3 in the 6β-OH isomer), 3.68 (t, 1 H, 17α-H), 4.96 (m, 1 H, 6α-H), 5.04 (m, 1 H, 6β-H), 7.38 (s, 1 H, 1-H) ppm. Analysis calculated for $C_{18}H_{22}Br_2O_3$ (446.19): C, 48.45; H, 4.97; Br, 35.82. Found: C, 48.87; H, 4.97; Br, 35.78%.

2,4-Dibromoestra-1,3,5(10),6-tetraene-3,17β-diol (6)

The mixture of 6-isomeric 2,4-dibromoestra-1,3,5(10)-triene-3,6,17β-triols (**5**, 1.5 g) was dissolved in acetone (30 ml), and concentrated HCl (12 ml) was added. The solution was kept at 45 C until thin-layer chromatography no longer showed any starting material (approximately 9 hours). On cooling (5 C for 10 hours), crystals separated that were collected by filtration, washed with petroleum ether, and dried. Recovery was 0.775 g (54%); an additional 0.39 g was obtained from the mother liquor after evaporation and recrystallization (overall yield, 81%): mp = 224 to 227 C; after recrystallization from acetone, mp = 227 to 229 C; R_f = 0.38 (ss A); $[\alpha]_D$ = −190° (c = 0.5, acetone). IR: 3.500, 3.420, 1.280, 1.040, cm^{-1}; ^1H NMR ($[^2H_6]$DMSO: 0.78 (s, 3 H, 18-H_3), 6.35 (dd, 1 H, 6-H, J = 6.5 and 0.5 Hz), 6.82 (dd, 1 H, 7-H, J = 6.5 and 2.0 Hz), 7.0 (br. s, 1 H, OH), 7.34 (s, 1 H, aryl-H) ppm. Analysis calculated for $C_{18}H_{20}Br_2O_2$ (428.17): C, 50.48; H, 4.7; Br, 37.32. Found: C, 50.59; H, 4.67; Br, 37.4%.

2,4-Dibromoestra-1,3,5(10),6-tetraene-3,17β-diol diacetate (6a)

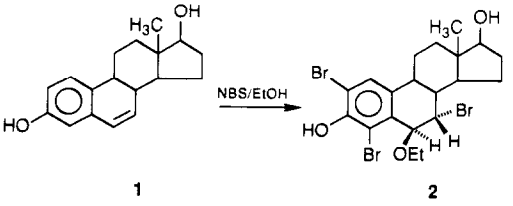
Diol **6** (0.75 g) was acetylated in pyridine (2 ml) with acetic anhydride (2.5 ml) at room temperature overnight. The mixture was poured into water (100 ml, acidified with concentrated HCl), and the separated solid was filtered, washed with water until neutral, and dried. Recovery of 0.84 g (93.4%) **6a** was obtained, mp = 165 to 167 C. After recrystallization from acetone, it melted at 167 to 169 C; R_f = 0.70 (ss A); $[\alpha]_D$ = −155° (c = 0.5, acetone). IR: 1.785, 1.740, 1.250, 1.040 cm^{-1}; ^1H NMR: 0.85 (s, 3 H, 18-H_3), 2.08 (s, 3 H, H_3 in 17-OAc), 2.4 (s, 3 H, H_3 in 3-OAc), 4.72 (t, 1 H, 17α-H), 6.15 (d, 1 H, 6-H), 6.82 (d, 1 H, 7-H), 7.43 (s, 1 H, 1-H) ppm. Analysis calculated for $C_{22}H_{24}Br_2O_4$ (512.24): C, 51.58; H, 4.72; Br, 31.20. Found: C, 51.83; H, 4.79; Br, 31.47%.

2,4-Dibromo-6β-methoxyestra-1,3,5(10)-triene-3,17β-diol (7)

Crude triol **5** (1.07 g) was dissolved in methanol (30 ml), and concentrated HCl (0.5 ml) was added. The temperature of the solution was kept between 45 and 50 C. After 2 hours, it was made alkaline (pH 8) with 2N KOH solution and was evaporated in vacuum to 4 ml. The concentrated solution was treated with ice-water and acidified (pH 3) with concentrated HCl, and the separated product was collected by filtration. It was washed with water until neutral, then dried. Recovery was 0.7 g (69%). After recrystallization from methanol, mp = 120 to 123 C; R_f = 0.38 (ss A); $[\alpha]_D$ = +46° (c = 0.5, ethanol). ^1H NMR ($CDCl_3$-$[^2H_6]$DMSO): 0.78 (s, 3 H, 18-H_3), 3.48 (s, 3 H, OMe), 3.73 (m, 1 H, 17α-H), 7.39 (s, 1 H, 1-H) ppm. Analysis calculated for $C_{19}H_{24}Br_2O_3$ (460.23): C, 49.58; H, 5.25; Br, 34.72. Found: C, 49.47; H, 5.31; Br, 34.68%.

2,4-Dibromo-6β-methoxyestra-1,3,5(10)-triene-3,17β-diol diacetate (7a)

Crude 6-methoxy-diol **7** (3.8 g) was acetylated in pyridine (5 ml) with acetic anhydride (5 ml) at 25 C for 12 hours. The solution was poured into ice-water, and the precipitate was filtered, washed with water until neutral, and dried, yielding 4.3 g (95%): mp = 99 to 102 C; R_f = 0.66 (ss A); $[\alpha]_D$ = +5.1° (c = 0.5, methanol). IR: 1,790 (3-OAc), 1,740 (17-OAc), 1.255 cm^{-1}; ^1H NMR: 0.85 (s, 3 H, 18-H_3), 2.05 (s, 3 H, 17-OAc),



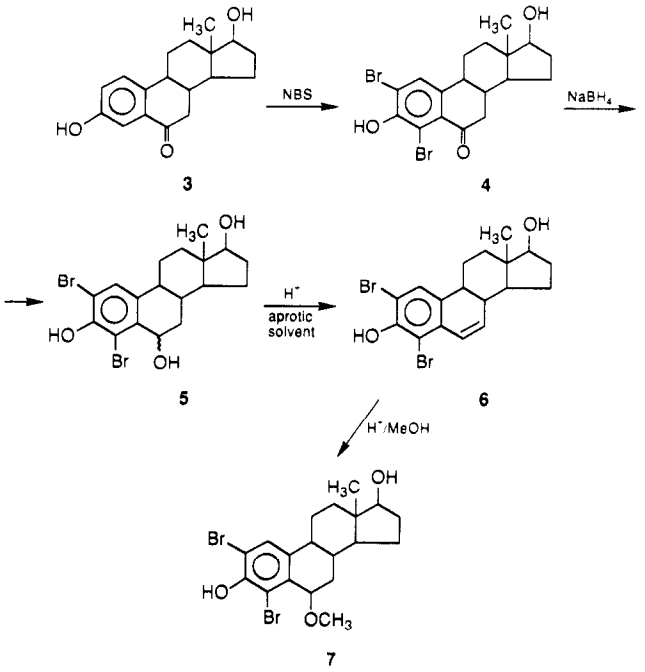
Scheme 1

2.38 (s, 3 H, 3-OAc), 3.47 (s, 3 H, OMe), 4.38 (t, 1 H, 6α-H), 4.72 (t, 1 H, 17α-H), 7.57 (s, 1 H, 1-H) ppm. Analysis calculated for $C_{23}H_{28}Br_2O_5$ (544.30): C, 50.75; H, 5.18; Br, 29.36. Found: C, 50.82; H, 5.19; Br, 29.28%.

Results and discussion

The preparation of 2,4-dibromo-Δ^6-estradiol (**6**) by the direct bromination of estra-1,3,5(10),6-tetraene-3,17β-diol (**1**) is not possible because the olefinic linkage participates in the bromination reaction. The formation of the 6,7-dibromo derivative would be preferred. However, in ethanolic solution at high bromination rates, the ethoxybromo compound **2** forms. It can originate from an intermediate 6α,7α-bromonium ion, opened by the alcoholate. The process results in a compound with *trans* substituents. The position and the steric orientation of the ethoxybromo part were determined from the ^1H and ^{13}C NMR spectra of the diacetate **2a.** The relatively high chemical shift of the hydrogen, geminal to bromine in the proton NMR spectrum (4.46 ppm), and the relatively low shift of the carbon atom bearing the bromine in the ^{13}C spectrum (38.5 ppm) imply crowded substituents,[3] indicating that bromine is located at C-7. The ^1H NMR signals at 4.65 and 4.46 ppm (fused doublets; $J \leq 0.4$ Hz) reveal hydrogens perpendicular to each other and to the neighboring C-8 hydrogen. Since the latter is axial, the C-7 hydrogen must be equatorial and the C-6 hydrogen quasi equatorial. Consequently, the tribromo compound **2** has the 6β-ethoxy-7α-bromo structure (Scheme 1).

The synthesis of 2,4-dibromoestra-1,3,5(10),6-tetraene-3,17β-diol (**6**) used as starting material 6-ketoestradiol (**3**),[4] prepared from 17β-hydroxy-19-norandrost-4-en-3-one 17-acetate by the method of Wiechert and co-workers.[5] Compound **3** was transformed into the 2,4-dibromo derivative (**4**) with a slight excess of *N*-bromosuccinimide. The keto group of **4** was reduced with sodium tetraborohydride, giving rise to the epimeric 6-hydroxy compounds (**5**). The ratio of the isomers, estimated from the ^1H NMR spectrum of the mixture, was 7 : 3, favoring the 6α-hydroxy compound. Both isomers were readily dehydrated to **6,** the 6β derivative reacting more slowly than the α isomer. In methanol solution, an addition reaction occurred, affording the 6β-methoxy compound (**7**). In acetone, dehydration of **5** resulted in the formation of the desired



Scheme 2

2,4-dibromo-6-unsaturated compound **6.** This reaction step has been reported to be relatively inefficient with 6-hydroxyestradiol[6] (Scheme 2).

The diacetate of **6** (**6a**) was transformed by a one-step catalytic tritiation into [2,4,6,7-$^3H_4$]estradiol.[7]

Acknowledgments

Financial support of the Hungarian Academy of Sciences is appreciated. The authors thank G. Bozóki-Bartók and J. Kertész for the elemental analyses and IR spectra.

References

1. Arunachalam T, Longcope C, Caspi E (1979). Iodoestrogens, syntheses, and interaction with uterine receptors. *J. Biol Chem* **254:**5900–5902.
2. Fan Z, Jing P, Lu H, Chu H, Gong J (1983). Preparation of [2,4,6,7-$^3H_4$]-estradiol. *Zhonghua Heyixue Zazhi* **3:**100–102 and *Chem Abstr* **99:**10558ln.
3. Bacca NS, Williams DH (1964). *Applications of NMR spectroscopy in organic chemistry.* Holden-Day, San Francisco, p. 189.
4. Dean PDG, Exley D, Johnson MW (1971). Preparation of 17β-oestradiol-6-(O-carboxymethyl) oxime–bovine serum albumin conjugate. *Steroids* **18:**593–603.
5. Hofmeister H, Laurent H, Wiechert R (1973). Estra-1,3,5(10)-trien-6-ones. *Chem Ber* **106:**723–726.
6. Gill JC, Marples BA, Traynor JR (1989). Improved preparation of 3,17β-dihydroxyoestra-1,3,5(10),6-tetraene. *Synth Comm* **19:**155–158.
7. Volford J, Krómer I, Szendi Z, Vincze I, Bursics L (1988). The preparation of 2,4,6,7-estra-1,3,5(10)-triene-3,17β-diol. Hungarian patent no. 201.958, November 11, 1988.