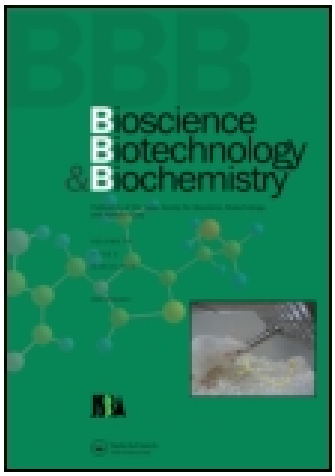

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Syntheses of (14β,17α)-14-Hydroxy- and (14β,17α)-2,14-Dihydroxyestradiols and Their Activities

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Syntheses of (14β,17α)-14-Hydroxy- and (14β,17α)-2,14-Dihydroxyestradiols and Their Activities

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Structure 1 is proposed for the Inagami-Tamura endogenous digitalis-like factor (EDLF), and (14β,17α)-14-hydroxy- and (14β,17α)-2,14-dihydroxyestradiols (2 and 3) were synthesized as models for studies on 1. The latter compound was remarkably potent in inducing a contractile response in isolated rat aorta and guinea pig left atrium.

Key words: endogenous digitalis-like factor; 14β-hydroxyestrogens; hypertension; vasocontraction; cardiotonic

As reviewed in the other paper submitted to this journal,[4] EDLF is an unidentified substance which elevates the intracellular Ca^{2+} concentration through the inhibition of Na/K ATPase and, as a result, contracts cells.[1] Many researchers have tried to isolate the factor and obtained some highly active material, although its amount was too small to determine the structure. In 1987, Inagami and co-workers reported the isolation of EDLF with a molecular weight of 336 from bovine adrenals.[2] On the basis of this information and the other evidence previously described in the paper submitted to this journal,[4] we deduced the structure of this compound to be **1**. However, the positions and stereochemistry of the residual hydroxyl groups still remained undefined. We thus decided to synthesize some of the plausible compounds and evaluate them to confirm the probability of the proposed structure and to elucidate it. We synthesized compounds **A** and **B** and evaluated their contractile effects on isolated rat aorta and guinea pig left atrium (paper submitted to this journal).[4] While both of the compounds induced a contractile response, their potency was unsatisfactory in both concentration and strength. Thus, we judged that the proposed structure was probable and that the structures of compounds **A** and **B** were satisfactory *sine qua non*, although lacking something that would qualify for EDLF; some of the sub-structures of **A** and **B**, possibly involving wrong locations for the undefined hydroxyl groups, might have inhibited the potency. We thus decided first to examine whether the presence of the 2-hydroxyl group was essential to the potency. We report here the syntheses and evaluation of two 14β-hydroxyestradiols (**2** and **3**) as models for studies on **1**.

In order to synthesize **2**, transposition of the conjugate double bond in enone **4**[3] was performed to afford unconjugate enone **5** in 42.8% yield, accompanied by 14β-isomer **4′** (14.8%) of **4**. Epoxidation of **5** with *m*-CPBA gave a complex mixture from which β-epoxide **6**, α-epoxide **7**, 14β-hydroxyenone **8**, and hydroxylactone **9** were isolated by silica gel column chromatography. Although 14α-hydroxyenone **10** was not isolated, the formation of **10** was later confirmed by thin-layer co-chromatography with authentic **10**. β-Epoxide **6** was structurally correlated with **8** by an alkaline treatment. The structure of **9** was tentatively determined from ^{1}H-NMR data, indicating the presence of an allylic oxymethine proton signal with a medium coupling constant at 4.33 ppm, and from FDMS spectral data, showing peaks at *m/z* 372 ($M^+ - H_2O$) as well as at 390 (M^+). The formation of **9** seemed to have resulted from the epoxide ring opening of β-epoxide **6** and

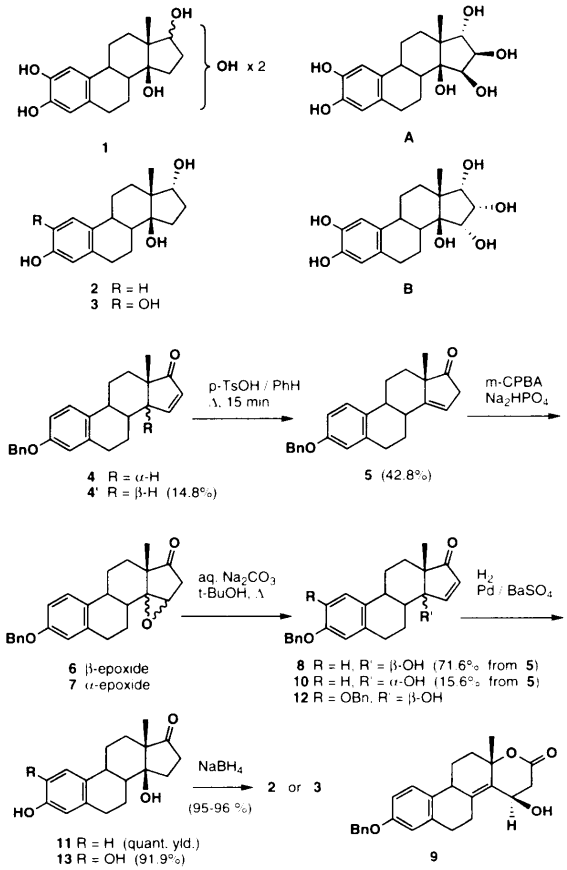

Fig. 1. Syntheses of 14β-Hydroxyestradiols.

Abbreviations: EDLF, endogenous digitalis-like factor; RIA, radio-immunoassay; *m*-CPBA, *m*-chloroperoxybenzoic acid; EtOAc, ethyl acetate; *p*-TsOH, *p*-toluenesulfonic acid; TID, theoretical ion distribution; FDMS, field desorption mass spectrometry; FABMS: fast atom bombardment mass spectrometry.

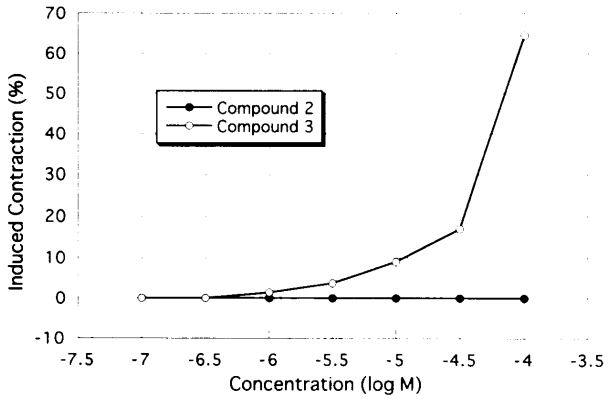

Fig. 2. Contractile Response of Isolated Rat Aorta Induced by **2** and **3**.

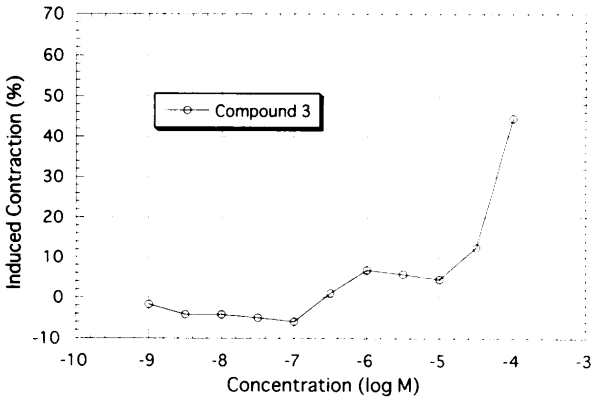

Fig. 3. Contractile Response of Isolated Guinea Pig Left Atrium Induced by **3**.

subsequent Baeyer–Villiger oxidation assisted by a newly resulting neighboring hydroxyl group. To avoid complexity, the crude epoxidation products were, without any purification in the other series of the work, treated with aq. Na_2CO_3 in refluxing *t*-butanol to afford **8** and **10** in 74.8% and 15.6% yields, respectively. Catalytic hydrogenation of **8** gave 14β-hydroxyestrone **11** in quantitative yield, and subsequent reduction of **11** with $NaBH_4$ afforded **2** in 96.0% yield.

Compound **12**[3,4] (see the other paper submitted to this journal)[4] was catalytically reduced as just described for **11** to afford 2,14β-dihydroxyestrone **13** in 91.9% yield, which was then reduced with $NaBH_4$ to afford **3** in 95.0% yield.

The compound **3** induced an intense dose-dependent contractile response in isolated rat aorta,[5] in contrast to **2** which showed no effect on the same tissue, as shown in Fig. 2. Compound **3** also induced an intense contractile effect on isolated guinea pig left atrium,[6] these results being shown in Fig. 3.

It was thus concluded that the presence of the 2-hydroxyl group is essential for inducing a potent contractile response and, thus, is integral to the proposed EDLF structure.

Experimental

All melting point (mp) values are uncorrected. IR spectra were determined with a JEOL Diamond-20 FT-IR spectrophotometer, and ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JEOL JNM-A500 FT NMR spectrometer. Chemical shifts are expressed in ppm relative to the chloroform signal at 7.24 ppm for ^{1}H-NMR and at 77.0 ppm for ^{13}C-NMR as an internal standard, unless otherwise noted. Mass spectra were measured with a JEOL JMS-SX/SX 102A tandem mass spectrometer, and specific rotation values were determined with a JASCO DIP-140 digital polarimeter. Elemental analyses were carried out with a Perkin-Elmer 240C elemental analyzer.

3-Benzyloxy-1,3,5(10),14-estratetraen-17-one (**5**) *and (14β)-3-benzyloxy-1,3,5(10),15-estratetraen-17-one* (**4'**). A solution of **4** (535 mg, 1.49 mmol) and *p*-TsOH (120 mg) in benzene (25 ml) was stirred at refluxing temperature for 15 min. The reaction mixture was then washed with aq. $NaHCO_3$, dried over anhyd. K_2CO_3, and evaporated to afford a yellowish gum (463 mg). This gum was dissolved in $CHCl_3$, and the solution was loaded into a column (20 mm ϕ × 300 mm) of silica gel (46.3 g) and eluted with hexane EtOAc (10 : 1). Desired product **5** (229 mg, 42.8% yield) was obtained. Recrystallization of **5** from hexane EtOAc gave colorless crystals, mp 118–119 °C; $[\alpha]_D^{27}$ +347.0° (*c* 0.47, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 2945, 2927, 2912, 2885, 2854, 2837, 1743, 1614, 1576, 1498, 1466, 1452, 1377, 1286, 1279, 1257. NMR δ_H ($CDCl_3$): 1.15 (s, 3H), 1.45 (dt, *J* = 4.0 and 13.0 Hz, 1H), 1.60 (ddt, *J* = 3.0, 11.0, and 13.5 Hz, 1H), 1.71 (ddd, *J* = 6.0, 12.0, and 24.0 Hz, 1H), 1.90 (dt, *J* = 3.0 and 13.5 Hz, 1H), 2.13 (ddt, *J* = 2.0, 6.0, and 13.0 Hz, 1H), 2.18 (ddd, *J* = 2.0, 3.5, and 11.5 Hz, 1H), 2.23 (ddd, *J* = 2.5, 11.5, and 24.0 Hz, 1H), 2.39 (ddd, *J* = 3.0, 7.0, and 13.0 Hz, 1H), 2.89 (dt, *J* = 2.0 and 23.0 Hz, 1H), 2.90 (ddd, *J* = 2.0, 6.0, and 17.0 Hz, 1H), 2.96 (ddd, *J* = 6.0, 12.0, and 17.0 Hz, 1H), 3.04 (ddd, *J* = 2.0, 4.0, and 23.0 Hz, 1H), 5.03 (s, 2H), 5.59 (dd, *J* = 2.0 and 4.0 Hz, 1H), 6.73 (d, *J* = 3.0 Hz, 1H), 6.79 (dd, *J* = 3.0 and 8.5 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.41 (d, *J* = 7.5 Hz, 2H). NMR δ_C ($CDCl_3$): 20.0, 24.8, 26.5, 29.7, 33.2, 38.9, 41.6, 44.7, 51.0, 69.9, 112.6, 113.2, 114.9, 126.7, 127.4 (×2), 127.9, 128.6 (×2), 131.8, 137.1, 137.8, 152.4, 156.9, 222.5. Elemental analysis. Found: C, 84.04; H, 7.36%. Calcd. for $C_{25}H_{26}O_2$: C, 83.76; H, 7.31%. Positive FABMS *m/z*: 358.1962 (observed); 358.1933 (TID for $C_{25}H_{26}O_2$). Other product **4'**, a 14β-isomer of **4**, was obtained from the polar fractions (79 mg, 14.8% yield). Recrystallization from hexane EtOAc gave colorless crystals, mp 108.5–109.5 °C; $[\alpha]_D^{20}$ +387.9° (*c* 0.79, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 2927, 2866, 1701, 1603, 1581, 1500, 1454, 1227. NMR δ_H ($CDCl_3$): 1.15 (s, 3H), 1.42 (dddd, *J* = 5.5, 8.0, 10.0, and 13.5 Hz, 1H), 1.59 (ddd, *J* = 6.0, 7.5, and 14.0, 1H), 1.69 (ddd, *J* = 5.0, 12.5, and 25.0 Hz, 1H), 1.79 (ddd, *J* = 5.5, 8.0, and 14.0 Hz, 1H), 1.94 (m, 1H), 2.17 (dt, *J* = 6.0 and 13.5 Hz, 1H), 2.27 (dt, *J* = 6.5 and 10.5 Hz, 1H), 2.83 (m, 2H), 2.91 (ddd, *J* = 5.0, 12.0, and 17.0 Hz, 1H), 5.01 (s, 2H), 6.20 (dd, *J* = 2.0 and 6.0 Hz, 1H), 6.68 (d, *J* = 2.5 Hz, 1H), 6.76 (dd, *J* = 2.5 and 8.5 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 7.30 (t, *J* = 7.0 Hz, 1H), 7.36 (t, *J* = 7.0 Hz, 2H), 7.40 (d, *J* = 7.0 Hz, 2H), 7.60 (dd, *J* = 2.5 and 6.0 Hz, 1H). NMR δ_C ($CDCl_3$): 22.1, 27.5, 27.6, 30.6, 31.4, 35.7, 38.1, 47.8, 54.7, 70.1, 113.0, 114.5, 127.4, 127.9 (×2), 128.5 (×2), 132.8 (×2), the other 132.8, 137.2, 137.4, 156.7, 162.0, 214.6. Positive FABMS *m/z*: 358.1970 (observed); 358.1933 (TID for $C_{25}H_{26}O_2$).

(14β,15β)-3-Benzyloxy-14,15-oxido-1,3,5(10)-estratrien-17-one (**6**), *(14α,15α)-3-benzyloxy-14,15-oxido-1,3,5(10)-estratrien-17-one* (**7**), *(14β)-3-benzyloxy-14-hydroxy-1,3,5(10),15-estratetraen-17-one* (**8**), and *(15β)-3-benzyloxy-15-hydroxy-18-oxa-D-homo-1,3,5(10),8(14)-estratetraen-17-one* (**9**). To a stirred solution of **5** (229 mg, 0.64 mmol) in dry CH_2Cl_2 (15 ml), *m*-CPBA (331 mg, 1.92 mmol) and Na_2HPO_4 (284 mg, 2.0 mmol) were added. The mixture was stirred at room temperature for 3.5 h, successively washed with 1 N NaOH and water, dried over anhyd. Na_2SO_4, and evaporated to afford a pale yellow gum (277 mg). Chromatography of the gum in a column (16 mm ϕ × 265 mm) of silica gel (27.7 g), using a gradient solvent system of hexane EtOAc (10 : 1 7 : 3), afforded **6** (14 mg), **7** (26 mg), a mixture of **8** and its 14α-isomer **10** later described (54 mg), **8** (31 mg) and **9** (7 mg). NMR δ_H ($CDCl_3$) of **6** (downfield from tetramethylsilane as an internal standard): 1.17 (s, 3H), 1.28 (m, 1H), 1.53 (m, 2H), 1.79 (dd, *J* = 3.0 and 10.0 Hz, 1H), 1.98 (m, 1H), 2.08 (br.t, *J* = 11.5 Hz, 1H), 2.40 (m, 1H), 2.55 (dd, *J* = 1.0 and 19.0 Hz, 1H), 2.57 (br.t, *J* = 9.5 Hz, 1H), 2.72 (dd, *J* = 2.0 and 19.0 Hz, 1H), 2.85 (d, *J* = 4.0 Hz, 1H), 2.87 (dt, *J* = 1.0 and 5.0 Hz, 1H), 3.73 (d, *J* = 1.0 Hz, 1H), 5.04 (s, 2H), 6.74 (d, *J* = 3.0 Hz, 1H), 6.81 (dd, *J* = 3.0 and 9.0 Hz, 1H), 7.21 (d, *J* = 9.0 Hz, 1H), 7.32 (t, *J* = 7.0 Hz, 1H), 7.38 (t, *J* = 7.0 Hz, 2H), 7.42 (d, *J* = 7.0 Hz, 2H). NMR δ_C ($CDCl_3$) of **6**: 14.2, 21.4, 25.8, 29.5, 32.7, 37.0, 38.9, 43.1, 49.4, 54.3, 70.0, 70.5, 112.8, 114.9, 126.6, 127.4 (×2), 127.9, 128.6 (×2), 131.0, 137.1, 137.8, 157.1, 216.3. FDMS *m/z* of **6**: 374 (M^+). NMR δ_H ($CDCl_3$) of **7** (downfield from tetramethylsilane as an internal standard): 1.15 (s, 3H), 1.50 1.66 (m, 3H), 1.78 (dt, *J* = 4.0 and 13.5 Hz, 1H), 1.92 (ddd, *J* = 3.0, 3.5, and 13.5 Hz, 1H), 2.17 (dt, *J* = 3.5 and 11.0 Hz, 1H), 2.45 (ddd, *J* = 4.0, 7.0, and 14.0 Hz, 1H), 2.52 (d, *J* = 18.0 Hz, 1H), 2.69 (dt, *J* = 4.0 and 11.5 Hz, 1H), 2.71 (dd, *J* = 1.0 and

18.0 Hz, 1H), 2.80 2.90 (m, 2H), 3.93 (s, 1H), 5.04 (s, 2H), 6.73 (d, J=3.0 Hz, 1H), 6.81 (dd, J=3.0 and 8.5 Hz, 1H), 7.23 (d, J=8.5 Hz, 1H), 7.32 (t, J=7.0 Hz, 1H), 7.38 (t, J=7.0 Hz, 2H), 7.42 (d, J=7.0 Hz, 2H). NMR δ_C ($CDCl_3$) of **7**: 18.4, 21.4, 25.6, 27.8, 29.1, 35.1, 39.8, 40.5, 48.7, 56.0, 69.9, 71.1, 112.5, 114.8, 126.5, 127.4 (×2), 127.9, 128.5 (×2), 132.0, 137.2, 137.6, 157.0, 214.2. FDMS m/z of **7**: 374 (M^+). Physical data for **8** and **10** are described later. NMR δ_H ($CDCl_3$) of **9** (downfield from tetramethylsilane as an internal standard): 1.16 (s, 3H), 1.56 (ddd, J=9.0, 11.5, and 14.5 Hz, 1H), 1.74 (ddd, J=4.0, 13.0, and 13.0 Hz, 1H), 1.79 (dd, J=8.0 and 14.0 Hz, 1H), 1.81 (br.s, 1H), 1.93 (dd, J=7.5 and 14.0 Hz, 1H), 2.08 (ddd, J=4.0, 7.0, and 12.5 Hz, 1H), 2.16 (ddd, J=8.0, 11.5, and 14.5 Hz, 1H), 2.21 (dd, J=4.0 and 13.0 Hz, 1H), 2.54 (d, J=18.5 Hz, 1H), 2.61 (dd, J=4.0 and 18.5 Hz, 1H), 2.74 (ddd, J=4.0, 13.0, and 16.0 Hz, 1H), 2.89 (ddd, J=3.0, 4.0, and 16.0 Hz, 1H), 4.33 (d, J=4.0 Hz, 1H), 5.04 (s, 2H), 6.74 (d, J=2.5 Hz, 1H), 6.85 (dd, J=2.5 and 8.5 Hz, 1H), 7.27 (d, J=8.5 Hz, 1H), 7.31 (t, J=7.5 Hz, 1H), 7.34 (t, J=7.5 Hz, 2H), 7.41 (d, J=7.5 Hz, 2H). FDMS m/z of **9**: 390 (M^+) and 372 ($M^+ - H_2O$).

(14β)-3-Benzyloxy-14-hydroxy-1,3,5(10),15-estratetraen-17-one (**8**), *and (14α)-3-benzyloxy-14-hydroxy-1,3,5(10),15-estratetraen-17-one* (**10**). A solution of **5** (147 mg, 0.41 mmol), *m*-CPBA (115 mg, 0.67 mmol) and anhyd. Na_2CO_3 (212 mg, 2.0 mmol) in dry CH_2Cl_2 (10 ml) was stirred at room temperature for 3.5 h. The reaction mixture was diluted with $CHCl_3$ and washed with 1 N NaOH (10 ml). The aq. layer was extracted twice with $CHCl_3$. The combined organic layers were washed with water, dried over anhyd. Na_2SO_4, and evaporated to afford a pale yellow gum (192 mg). To this gum, *t*-butanol (10 ml) and 20% aq. Na_2CO_3 (15 ml) were added, and the mixture was stirred at refluxing temperature in an argon atmosphere for 1 h. After cooling, the mixture was partitioned between ether and water, and the organic layer was washed with water, dried over anhyd. Na_2SO_4 and evaporated to afford a colorless semi-crystalline residue (175 mg). Chromatographic separation in a column (16 mmϕ × 300 mm) of silica gel (30 g), eluting with a gradient solvent system of hexane–EtOAc (10 : 1 7 : 2) afforded less polar **10** (24 mg, 16% yield) as colorless crystals, as well as **8** (110 mg, 71.6% yield). Recrystallization of **8** from hexane acetone gave colorless crystals, mp 185 186.5 C; $[\alpha]_D^{27}$ +224.2° (c 0.465, $CHCl_3$). IR ν_{max}(KBr) cm^{-1}: 3305, 3061, 2970, 2931, 2866, 2841, 1682, 1606, 1502, 1454, 1421, 1387, 1373, 1340, 1309. NMR δ_H ($CDCl_3$): 1.10 (s, 3H), 1.41 (m, 2H), 1.67 (dd, J=8.0 and 14.0 Hz, 1H), 1.72 (s, 1H), 1.86 (ddd, J=5.5, 7.0, and 14.0 Hz, 1H), 1.88 (dt, J=2.5 and 12.0 Hz, 1H), 2.21 (m, 2H), 2.39 (ddt, J=2.5, 5.0, and 10.0 Hz, 1H), 2.81 (m, 2H), 5.00 (s, 2H), 6.26 (d, J=6.0 Hz, 1H), 6.66 (d, J=2.5 Hz, 1H), 6.75 (dd, J=2.5 and 8.5 Hz, 1H), 7.02 (d, J=8.5 Hz, 1H), 7.30 (t, J=7.5 Hz, 1H), 7.35 (t, J=7.5 Hz, 2H), 7.37 (d, J=6.0 Hz, 1H), 7.39 (d, J=7.5 Hz, 2H). NMR δ_C ($CDCl_3$): 19.9, 23.7, 27.3, 29.1, 30.5, 34.9, 45.0, 52.8, 69.9, 83.4, 113.2, 114.3, 127.4 (×2), 127.9, 128.1, 128.5 (×2), 132.6, 133.0, 137.1, 137.2, 156.6, 160.1, 211.3. Elemental analysis. Found: C, 80.65; H, 7.24%. Calcd. for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00%. FDMS m/z: 374 (M^+). Positive FABMS m/z: 374.1894 (observed); 374.1882 (TID for $C_{25}H_{26}O_3$). Recrystallization of **10** from hexane EtOAc gave colorless crystals, mp 167 169 C; $[\alpha]_D^{27}$ +60.0 (c 0.37, $CHCl_3$). IR ν_{max}(KBr) cm^{-1}: 3558, 3466, 2933, 2864, 1718, 1703, 1603, 1500, 1456, 1380, 1309. NMR δ_H ($CDCl_3$): 1.13 (s, 3H), 1.34 (s, 1H), 1.53 (unresolved, 1H), 1.65 (ddd, J= 5.0, 13.0, and 26.0 Hz, 1H), 1.76 (ddd, J=2.0, 5.0, and 13.0 Hz, 1H), 1.84 (m, 1H), 1.98 (ddd, J=3.0, 6.0, and 11.5 Hz, 1H), 2.01 (dt, J=5.0 and 8.0 Hz, 1H), 2.23 (dt, J=5.0 and 13.0 Hz, 1H), 2.36 (ddt, J=2.0, 5.0, and 13.0 Hz, 1H), 2.92 (dd, J=4.0 and 9.0 Hz, 1H), 3.05 (dt, J=6.0 and 11.5 Hz, 1H), 5.02 (s, 2H), 6.14 (d, J=6.0 Hz, 1H), 6.72 (d, J=3.0 Hz, 1H), 6.79 (dd, J=3.0 and 8.5 Hz, 1H), 7.19 (d, J=8.5 Hz, 1H), 7.30 (t, J=7.5 Hz, 1H), 7.36 (t, J=7.5 Hz, 2H), 7.41 (d, J=7.5 Hz, 2H), 7.73 (d, J=6.0 Hz, 1H). NMR δ_C ($CDCl_3$): 22.7, 23.6, 24.1, 24.6, 29.6, 36.6, 37.9, 54.6, 70.0, 82.2, 112.4, 114.8, 126.3, 127.4 (×2), 127.9, 128.5 (×2), 133.6, 133.7, 137.3 (×2), 156.8, 157.9, 211.8. Elemental analysis. Found: C, 80.61; H, 7.29%. Calcd. for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00%. Positive FABMS m/z: 375.1914 (observed); 375.1960 (TID for $C_{25}H_{27}O_3$). M^+ as well as $M^+ + 1$ was observed: 374.1861 (observed); 374.1882 (TID for $C_{25}H_{26}O_3$).

Transformation of **6** *to* **8**. A suspension of **6** (14 mg) and 20% aq. Na_2CO_3 solution (1.5 ml) in *t*-butanol (1 ml) was stirred at refluxing temperature in an argon atmosphere for 1 h. After cooling, the mixture was partitioned between ether and water, and the organic layer was washed with water, dried over anhyd. Na_2SO_4 and evaporated to afford colorless crystals (13 mg, 92.9% yield). Recrystallization from acetone-hexane gave colorless crystals (9 mg) of **8**.

(14β)-14-Hydroxyestrone (**11**). A suspension of **8** (110 mg, 0.29 mmol) and 5% Pd/$BaSO_4$ (27 mg) in EtOAc (15 ml) was stirred in an hydrogen atmosphere for 19 h. The mixture was filtered through Celite® No. 503, and the filter cake was washed with EtOAc. The combined filtrate and washings were evaporated to afford colorless crystals (96 mg, quantitative yield). Recrystallization from $CHCl_3$ MeOH gave colorless crystals, mp 226 229 C; $[\alpha]_D^{20}$ +92.7° (c 0.5, EtOAc). IR ν_{max}(KBr) cm^{-1}: 3491, 3346, 3012, 2962, 2922, 2858, 2845, 1716, 1618, 1585, 1498, 1466, 1444, 1437, 1396, 1369, 1358. NMR δ_H ($CDCl_3$–CD_3OD, 4 : 1): 0.92 (s, 3H), 1.22 (ddd, J=4.5, 12.5, and 25.0 Hz, 1H), 1.34 (m, 3H), 1.45 (dt, J=1.0 and 11.5 Hz, 1H), 1.73 (dt, J=5.5 and 13.5 Hz, 1H), 2.05 (t, J=10.0 Hz, 1H), 2.08 (t, J=10.0 Hz, 1H), 2.15 (ddd, J=3.0, 6.5, and 13.0 Hz, 1H), 2.29 (d, J=5.5 Hz, 1H), 2.31 (d, J=6.0 Hz, 1H), 2.41 (dt, J=3.0 and 11.5 Hz, 1H), 2.71 (m, 2H), 3.20 (s, 1.7H), 3.49 (s, 0.2 H), 6.42 (d, J=2.5 Hz, 1H), 6.48 (dd, J=2.5 and 8.5 Hz, 1H), 6.96 (d, J=8.5 Hz, 1H), 8.60 (s, 0.03H). NMR δ_C (d_6-acetone; standardized by acetone signal at 29.8 ppm): 13.7, 22.9, 26.6, 27.3, 31.0, 32.6, 33.6, 40.6, 45.6, 54.2, 81.5, 113.8, 115.9, 127.6, 131.4, 138.4, 156.1, 220.8. Elemental analysis. Found: C, 75.24; H, 7.96%. Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.74%. FDMS m/z: 286 (M^+). Negative FABMS m/z: 285.1476 (observed); 285.1491 (TID for $C_{18}H_{21}O_3$).

(14β,17α)-14-Hydroxyestradiol (**2**). To a stirred solution of **11** (60 mg, 0.21 mmol) in EtOH (5 ml), $NaBH_4$ (16 mg, 0.42 mmol) was added. After 5 h, an additional 8 mg (0.2 mmol) of $NaBH_4$ was added and stirring was continued for 24 h, before the reaction mixture was evaporated. The residue was dissolved in water, acidified with 1 N HCl, and extracted twice with EtOAc. The combined extracts were washed twice with brine, dried over anhyd. $MgSO_4$, and evaporated to afford colorless crystals (58 mg, 96% yield). Recrystallization from $CHCl_3$ MeOH gave colorless tetragonal crystals, mp 216.5 218 C; $[\alpha]_D^{23}$ +40.4 (c 0.5, EtOH). IR ν_{max}(KBr) cm^{-1}: 3523, 3394, 3250 (sh), 3140, 3022, 2995, 2970, 2960, 2945, 2918, 2873, 2854, 1616, 1579, 1495, 1456, 1437, 1394, 1379, 1342. NMR δ_H ($CDCl_3$–CD_3OD, 4 : 1): 0.88 (s, 3H), 1.10 1.36 (m, 5H), 1.44 (m, 2H), 1.94 (ddd, J=5.5, 13.0, and 14.0 Hz, 1H), 1.98 (ddt, J=2.0, 5.0, and 10.0 Hz, 1H), 2.04 (ddt, J=5.5, 9.5, and 13.0 Hz, 1H), 2.12 (ddd, J=3.0, 6.5, and 12.5 Hz, 1H), 2.27 (dt, J=3.0 and 11.5 Hz, 1H), 2.66 (m, 2H), 3.21 (s, 0.5H), 3.46 (s, 0.1H), 4.08 (t, J=8.5 Hz, 1H), 6.41 (d, J=2.5 Hz, 1H), 6.49 (dd, J=2.5 and 8.5 Hz, 1H), 6.99 (d, J=8.5 Hz, 1H), 8.51 (s, 0.05H). NMR δ_C (d_6-acetone; standardized by acetone signal at 29.8 ppm): 17.3, 23.4, 27.1, 29.4, 29.7, 30.0, 31.3, 41.0, 46.2, 48.2, 80.9, 83.4, 113.8, 116.0, 127.7, 132.3, 138.7, 160.0. Elemental analysis. Found: C, 73.54; H, 8.88%. Calcd. for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39%. FDMS m/z: 288 (M^+). Negative FABMS m/z: 287.1625 (observed); 287.1647 (TID for $C_{18}H_{23}O_3$).

(14β)-2,14-Dihydroxyestrone (**13**). To a solution of **12**[3,4] (114 mg, 0.24 mmol) in EtOAc (12 ml), a suspension of 5% Pd/$BaSO_4$ (30 mg) in EtOAc (2.5 ml) was added. The mixture was stirred in a hydrogen atmosphere for 24 h and then filtered, the filter cake being washed with EtOAc. The filtrate and washings were combined and evaporated to afford a colorless gum (91 mg). Chromatography in a column of silica gel (12.0 g), eluting with hexane–EtOAc (1 : 1), gave a colorless gum (68 mg, 92% yield). Crystallization from $CHCl_3$ MeOH gave colorless crystals, mp 145 C (sinter at 137 C); $[\alpha]_D^{23}$ +97.4 (c 1.0, EtOH). IR ν_{max}(KBr) cm^{-1}: 3475 (sh), 3421, 3280 (sh), 3034, 2978, 2947, 2927, 2862, 2841, 1722, 1620, 1603, 1512, 1446, 1362, 1319. NMR δ_H ($CDCl_3$–CD_3OD, 4 : 1): 0.92 (s, 3H), 1.21 (ddd, J=5.0, 12.0, and 24.0 Hz, 1H), 1.30 (ddt, J=7.5, 10.0, and 12.0 Hz, 1H), 1.34 (m, 2H), 1.43 (dt, J=2.0 and 11.5 Hz, 1H), 1.72 (dt, J=5.5 and 14.0 Hz, 1H), 2.07 (m, 2H), 2.30 (d, J=5.5 Hz, 1H), 2.32 (d, J=6.0 Hz, 1H), 2.37 (dt, J=3.0 and 11.0 Hz, 1H), 2.63 (m, 2H), 3.22 (s, 0.3H), 6.42 (s, 1H), 6.62 (s, 1H), 7.63 (s, 0.08H), 7.81 (s, 0.08H). NMR δ_C ($CDCl_3$–CD_3OD, 4 : 1): 12.7, 22.0, 25.5, 26.3, 29.2, 32.0, 33.1, 39.7, 44.1, 53.7, 81.4, 112.3, 115.1, 127.8, 130.6, 142.3, 142.4, 223.2. FDMS m/z: 302 (M^+). Negative FABMS m/z: 301.1413 (observed); 301.1440 (TID for $C_{18}H_{21}O_4$).

(14β,17α)-2,14-Dihydroxyestradiol (**3**). To a solution of **13** (20 mg, 0.07 mmol) in EtOH (2.5 ml), $NaBH_4$ (30 mg, a large mol excess) was added. After 3.5 h, the mixture was evaporated. The residue thus obtained was dissolved in water, acidified with 1 N HCl, and extracted twice with EtOAc. The combined extracts were washed three times with brine, dried over anhyd. Na_2SO_4, and evaporated to afford colorless crystals (19 mg, 95% yield). Recrystallization from $CHCl_3$–MeOH gave colorless crystals,

mp 218 C (sinter at 195 C); $[\alpha]_D^{20}$ +111.2 (c 0.30, MeOH). IR ν_{max}(KBr) cm^{-1}: 3440 (sh), 3317, 2954, 2916, 2858, 1603, 1516, 1450, 1402, 1365, 1331, 1319. NMR δ_H ($CDCl_3$ CD_3OD, 4:1): 0.90 (s, 3H), 1.12 (ddd, J=3.0, 13.0, and 25.0 Hz, 1H), 1.24 (dt, J=3.0 and 13.0 Hz, 1H), 1.31 (dt, J=3.5 and 13.0 Hz, 1H), 1.32–1.39 (m, 2H), 1.39–1.52 (m, 4H), 1.89 (m, 1H), 2.06 (ddd, J=3.0, 6.0, and 13.0 Hz, 1H), 2.30 (t, J=11.0 Hz, 1H), 2.53 (dd, J=4.0 and 16.0 Hz, 1H), 2.63 (ddd, J=6.0, 12.0, and 16.0 Hz, 1H), 3.22 (s, 0.6H), 3.62 (dd, J=8.0 and 9.0 Hz, 1H), 6.40 (s, 1H), 6.67 (s, 1H), 7.60 (s, 0.04H), 7.63 (s, 0.04H). NMR δ_C (d_6-acetone; standardized by d_6-acetone signal at 29.8 ppm): 20.5, 23.0, 27.3, 27.9, 29.0, 30.3, 37.1, 38.7, 43.4, 48.9, 79.1, 82.6, 113.6, 116.1, 128.5, 132.4, 143.6, 143.8.

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