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15β-Hydroxysteroids (Part VII). Steroids of the human perinatal period: The synthesis of steroid markers and their radioactive tracers

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*We report the synthesis of 10 novel steroids obtained from 3β,15β-diacetoxy-17α-hydroxy-5-pregnen-20-one (**1c**) as intermediates in the synthesis of 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-4-pregnen-3-one (**6a**) and its tritiated tracer 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-1,2,6,7-^{3}H-pregn-4-en-3-one (**6d**). The one pot interconversion of intermediate (**6a**) to 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1a**) and 3α,15β,17α-trihydroxy-5β-pregnan-20-one (**2a**) provides a new and efficient approach to the synthesis of diagnostically important metabolites of the human neonate and a possible route in the synthesis of the tritated tracers 3β,15β,17α-trihydroxy-1,2,7-^{3}H-pregn-5-en-20-one (**1d**) and 3α,15β,17α-trihydroxy-1,2,6,7-^{3}H-5β-pregnan-20-one (**2b**) for the development of new immunoassays. We also report in this investigation an alternative route in the synthesis of 15β,17α-dihydroxy-4-pregnen-3,20-dione (**7a**) an intermediate in the synthesis of human 15β-hydroxysteroid metabolites.* (*Steroids* **62**:221–225, 1997) © *1997 by Elsevier Science Inc.*

Keywords: 15β-hydroxysteroids; synthesis; 3β,15β,17α-trihydroxy-5-pregnen-20-one; 3α,15β,17α-trihydroxy-5β-pregnan-20-one; 15β,17α-dihydroxy-4-pregnen-3,20-dione; radioactive tracers

Introduction

The identification of 15β-hydroxyestrone (**3**) and 15β-hydroxyestradiol (**4**), and later of 5-pregnen-3β,15β,17α,20S-tetrol (**5**) in late pregnancy, first demonstrated the occurrence of 15β-hydroxysteroids in humans.[1,2] However it was not until the identification of 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1a**) and 3α,15β,17α-trihydroxy-5β-pregnan-20-one (**2a**) in the human newborn that the significance of 15β-hydroxysteroids as possible markers in recognition of human pathological conditions emerged. These latest steroids have since been shown to be important biochemical markers of the human perinatal period, the former a normal metabolite of fetal and neonatal well-being, and the latter a metabolite pathognomonic of steroid enzymatic deficiencies in congenital adrenal hyperplasia (CAH).[3–7]

As part of a larger investigation into the chemistry and biochemistry of 15β-hydroxylated steroids, we undertook the synthesis of (**1a**) and (**2a**). The aim was to develop new radioimmunoassays to be utilized in newborn screening programs for CAH and for monitoring of fetal well-being in late pregnancy. The choice of the initial target of this investigation, 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1a**) was based on the possible interconversion of (**1a**) to the other major 15β-hydroxy steroids found in humans. The successful completion of the work reported here along with our work published elsewhere[8–13] completes this interconversion.

The synthesis of (**1a**), (**2a**), and 15β,17α-dihydroxy-4-pregnen-3,20-dione (**7a**) from the common intermediate 3β,15β-diacetoxy-17α-hydroxy-5-pregnen-20-one (**1c**) and the successful one-pot conversion of 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-4-pregnen-3-one (**6a**) to (**1a**) and (**2a**) as a possible route for the synthesis of the tritiated tracers 3β,15β,17α-trihydroxy-1,2,7-^{3}H-pregn-5-en-20-one (**1d**) and 3α,15β,17α-trihydroxy-1,2,6,7-^{3}H-5β-pregnan-20-one (**2b**) is presented.

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Experimental

Solvents were laboratory grade or better. Melting points were determined on a Gallenkamp Melting Point Apparatus and are uncorrected. Ultraviolet spectra were determined on a Varian Techtron ultraviolet-visible spectrophotometer. ^{1}H NMR were recorded at 200 MHz using a Bruker AC-200F spectrometer using TMS as internal reference. $W_{1/2}$ refers to the peak width at its half-height. Gas chromatography was performed on a Hewlett Packard 5710A flame ionization gas chromatograph using a glass solid injector[6] and interpreted using a Shimadzu CR-4A chromatopac utilizing Eurekasoft analytical software (Eurekanalytical, P.O. Box 123, Camperdown, NSW 2050, Australia), results are expressed as methylene units (MU).[14] A 30-m capillary column from Heliflex Capillaries (RSL-150 polydimethylsiloxane; ID 0.25 mm) was used with helium as the carrier gas (2.0 mL/min), temperature programming from 197–270°C at 1°C/min with the detector, and injector block temperatures were 300°C and 250°C, respectively. Mass spectra were recorded on a Finnigan MAT TSQ-70 mass spectrometer scanning from 80–800 daltons at 70 eV. Chemical ionization mass spectra (CIMS) were obtained using methane ($CICH_4$) or methane/ammonia ($CINH_3$) as plasma and with a reagent gas pressure of 5–10 torr. High-resolution mass spectra (HRMS) were run on a VG Autospec. Silica gel H (Merck, type 60) was used for chromatography.[15] Steroid derivatisation for gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS) analyses were as reported earlier.[3]

*3β,15β-Diacetoxy-20,20-ethylenedioxy-5-pregnen-17α-ol (**10c**)*

Ethylene glycol (0.4 mL), triethyl orthoformate (0.6 mL), anhydrous toluene-*p*-sulphonic acid (6.8 mg), and 3β-15β-diacetoxy-17α-hydroxy-5-pregnen-20-one (**1c**)[8] (0.2g, 4.63×10^{-4} mole) were heated in an oil bath at 90°C for 1.5 h and later distilled until the pot temperature measured 110°C. A warm solution of methanol (2.3 mL) and pyridine (28 μL) was added followed by water (0.6 mL), and on cooling, the mixture was filtered, washed with methanol, and the crystals dried to give 129 mg (59%) of **10c:** ^{1}H NMR ($CDCl_3$) δ ppm 5.38 (1H, $W_{1/2}$ = 9 Hz, H-6), 5.16 (1H, $W_{1/2}$ = 17 Hz, H-15), 4.62 (1H, $W_{1/2}$ = 33 Hz, H-3), 4.05 (2H, $W_{1/2}$ = 10 Hz, ketal), 3.86 (2H, $W_{1/2}$ = 32 Hz, ketal), 2.03 (3H, s, H-3 OAc), 2.01 (3H, s, H-15 OAc), 1.37 (3H, s, H-21), 1.07 (3H, s, H-19), 1.03 (3H, s, H-18); CIMS/CH4 *m/z* (%) 443 (6), 407 (9), 389 (8), 375 (7), 373 (21), 347 (11), 329 (14), 315 (10), 313 (100), 311 (18), 295 (20), 126 (69). GC and GC-EIMS (as MOTMS; MW = 548) MU = 32.65; *m/z* (rel. int. %); 473 (1%) $[M\text{-}15\text{-}60]^+$, 428 (0.2%) $[M\text{-}60]^+$ 401 (3%), 341 (0.8%), 268 (0.2%), 251 (0.6%), 182 (0.8%), 169 (2%), 157 (3%), 117 (3%), 87 (100%); GC-CIMS/CH_4 *m/z* (%), 548 (0.2%), 489 (1%), 473 (3%), 429 (6%), 399 (8%), 339 (14%), 295 (3%), 235 (2%), 177 (3%), 161 (10%), 115 (2%), 87 (100%).

The filtrate was evaporated; the residue was dissolved in dichloromethane, washed with water, sodium bicarbonate (sat. aq.), water, and then dried (Na_2SO_4). Evaporation and chromatography of the residue on silica gel, eluting with dichloromethane/acetone (12:1) initially gave 59 mg (26%, a total of 85%) of **10c** followed by 11 mg (5%) of 15β-acetoxy-20,20-ethylenedioxy-5-pregnen-3β,17α-diol (**10b**): ^{1}H NMR ($CDCl_3$) δ ppm 5.38 (1H, $W_{1/2}$ = 9 Hz, H-6), 5.16 (1H, $W_{1/2}$ = 17 Hz, H-15), 4.05 (2H, $W_{1/2}$ = 10 Hz, ketal), 3.86 (2H, $W_{1/2}$ = 32 Hz, ketal), 3.56 (1H, $W_{1/2}$ = 33 Hz, H-3), 2.01 (3H, s, H-15 OAc), 1.37 (3H, s, H-21), 1.06 (3H, s, H-19), 1.03 (3H, s, H-18); CIMS/CH_4 *m/z* (%) 435 (2), 433 (8), 417 (9), 389 (20), 375 (66), 373 (25), 357 (100), 339 (12), 331 (15), 315 (48), 313 (42), 297 (41), 295 (20), 154 (18); HR-CIMS/CH_4 found 433.2594, expected (as M-1) 433.2590. GC and GC-EIMS (as MOTMS; MW = 578) MU = 31.92; *m/z* (%); 578 (ND) $[M^+]$, 491 (0.2%) $[M\text{-}87]^+$, 431 (4%) $[M\text{-}87\text{-}60]^+$, 341 (1%), 195 (2%), 169 (2%), 129 (2%), 117 (3%), 87 (100%); GC-CIMS/CH_4 *m/z* (%); 578 (1%), 563 (2%), 519 (1.2%), 503 (3%), 429 (10%), 367 (1%), 339 (6%), 253 (1%), 177 (2%), 161 (12%), 90 (4%), 87 (100%).

Further elution with dichloromethane/acetone gave 4 mg (2%) of 15β-acetoxy-3β,17α-dihydroxy-5-pregnen-20-one (**1b**): mp = 225–227°C; (Lit[11] mp 225–227°C); ^{1}H NMR ($CDCl_3$) δ ppm 5.35 (1H, $W_{1/2}$ = 10Hz, H-6), 5.25 (1H, $W_{1/2}$ = 15Hz, H-15), 3.54 (1H, $W_{1/2}$ = 20Hz, H-3), 2.29 (3H, s, H-21), 2.04 (1H, s, OAc), 1.06 (3H, s, H-19), 0.94 (3H, s, H-18).

*15β-Acetoxy-20,20-ethylenedioxy-5-pregnen-3β,17α-diol (**10b**)*

Potassium carbonate (1 M, 1 mL) was added to a solution of **10c** (80 mg, 1.68×10^{-4} mole) in methanol (50 mL) and stirred for 2 h at room temperature, then evaporated to low volume. The residue was extracted with ethyl acetate, washed with water, dried (Na_2SO_4), and evaporated to dryness to give 70 mg (96%) of **10b** identical to that obtained above.

*15β-Acetoxy-20,20-ethylenedioxy-17α-hydroxy-4-pregnen-3-one (**6a**)*

A solution of **10b** (28 mg, 6.45×10^{-5} mole) in toluene (10 mL) and cyclohexanone (7 mL) was distilled, collecting ~2 mL. Aluminium *tert*-butoxide (20 mg) was added, and the mixture was refluxed for 3.5 h, then evaporated to low volume, extracted with ethyl acetate, and washed with sodium hydroxide (1 M), water, dried (Na_2SO_4), and evaporated. Chromatography on silica gel eluting with dichloromethane gave an oily phase. Further elution with dichloromethane/acetone (12:1) gave 16 mg (57%) of **6a:** CIMS/CH_4 *m/z* (%) 433 (5), 389 (6), 289 (10), 273 (14), 271 (18), 257 (50), 243 (27), 229 (32), 211 (28), 207 (57), 193 (72), 189 (20), 177 (100), 175 (19), 165 (10), 147 (12); HR-CIMS/CH_4 found 433.2584 expected (as MH) 433.2590. GC and GC-EIMS (as MOTMS; MW = 533), MU 32.88; *m/z* (%); 533 (ND) $[M^+]$, 491 (4) $[M\text{-}42]^+$, 446 (96) $[M\text{-}87]^+$, 384 (14), 369 (25), 294 (100), 279 (48), 222 (62), 209 (37), 198 (32), 129 (144).

*15β-Acetoxy-20,20-ethylenedioxy-17α-hydroxy-5β-pregnan-3-one (**6b**)*

A solution of **6a** (9 mg, 2.1×10^{-5} mole) in ethanol (3 mL) was hydrogenated over palladium on calcium carbonate (1 mg, 10%) at 35°C for 3 h. The mixture was centrifuged, decanted, and evaporated to dryness to give 8.5 mg (94%) of a mixture of **6b** and **6c** in a ratio of 4:1. Chromatography on silica gel eluting with dichloromethane, then with dichloromethane/acetone (4:1) gave 6.3mg (70%) of **6b:** GC and GC-EIMS (as MOTMS), MU = 31.96; *m/z* (%), 535 (0.5%) $[M^+]$, 448(4%), 388(1%), 87(100%) and 1.5mg (16%) of **6c:** GC (as MOTMS), MU = 32.14.

*3α,15β,17α-Trihydroxy-5β-pregnan-20-one (**2a**)*

Sodium borohydride (0.5 mg) was added to a solution of **6b** (4 mg, 9.22×10^{-6} mole) in ethanol (2 mL), and after 30 min at room temperature, sodium hydroxide (1 mL, 2 M) was added, and the mixture was heated to 60°C for 10 h. The mixture was acidified with hydrochloric acid (1 M) and after 1 h, evaporated to low volume at 40°C and extracted with ethyl acetate (×3) washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography of the residue on silica gel eluting with dichloromethane/acetone (12:1) gave 2 mg (70%) of **2a:** mp 140–142°C; (Lit[9] mp 140–142°C); ^{1}H NMR ($CDCl_3$) δ ppm 4.5 (1H, m $W_{1/2}$ = 10 Hz, H-15), 3.7 (1H, m $W_{1/2}$ = 24 Hz, H-3), 2.3 (3H, s, H-21), 0.99 (3H, s, H-19), 0.97 (3H, s, H-18).

*15β-Acetoxy-3β, 17α-dihydroxy-5-pregnen-20-one (**1b**)*

Potassium carbonate (2mL, 4M) was added to a solution of **1c** (101 mg, 2.3×10^{-4} mole) in methanol (50 mL). After 2 h at room temperature, the reaction was neutralized with hydrochloric acid (2 M) and evaporated to low volume. The residue was extracted with ethyl acetate, washed with water, dried (Na_2SO_4), and evaporated to dryness to give, after recrystallization from dichloromethane/hexane, 65 mg (71%) of **1b** identical to that prepared above.

*15β-Acetoxy-17α-hydroxy-4-pregnen-3,20-dione (**7b**)*

To a solution of **1b** (9.8 mg, 2.5×10^{-4} mole) in dichloromethane (1 mL), pyridinium chlorochromate (8.8 mg) was added, and the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with anhydrous diethyl ether (5 mL) and decanted. The residue was washed with anhydrous diethyl ether (10 mL), and the combined organic phase was filtered through a short silica gel pad, eluting with diethyl ether. The solution was acidified with hydrochloric acid (2 drops, 1 M), stirred at room temperature for 40 min, washed with aqueous sodium bicarbonate, water, dried (Na_2SO_4), and evaporated to dryness to give 9 mg (92%) of **7b:** ^{1}H NMR ($CDCl_3$) δ ppm 5.75 (1H, $W_{1/2}$ = 3 Hz, H-4), 5.25 (1H, $W_{1/2}$ = 16 Hz, H-15), 2.30 (3H, s, H-21), 2.04 (3H, s, OAc), 1.23 (3H, s, H-19), 1.00 (3H, s, H-18); λ_{max} = 240 (log ε 4.16). GC and GC-EIMS (as MOTMS; MW = 518), MU = 30.94 + 30.99 (E/Z isomers); *m/z* (%); 518 (20%) [M^+], 487 (12%), 458 (5%), 427 (100%), 397 (8%), 355 (8%), 337 (20%), 281 (19%), 258 (39%), 216 (34%), 207 (61%), 170 (57%), 120 (31%), 105 (37%).

*15β,17α-Dihydroxy-4-pregnen-3,20-dione (**7a**)*

Sodium hydroxide (1.5 mL, 2 M) was added to a solution of **7b** (4 mg) in ethanol (10 ml), and the reaction mixture was heated to 50°C for 16 h. The mixture was evaporated to low volume at 40°C, and the residue was dissolved in ethyl acetate, washed with water until neutral, dried (Na_2SO_4), and evaporated to give **7a:** mp 252–254°C; (Lit[16] mp 252–255°C); ^{1}H NMR ($CDCl_3$) δ ppm 5.75 (1H, $W_{1/2}$ = 3 Hz, H-4), 4.41 (1H, $W_{1/2}$ = 16 Hz, H-15), 2.30 (3H, s, H-21), 1.23 (3H, s, H-19), 1.00 (3H, s, H-18).

*15β-Acetoxy-20,20-ethylenedioxy-17α-hydroxy-1,4,6-pregnatrien-3-one (**14**)*

Dichlorodicyanobenzoquinone (100 mg, 3.3 equiv.) was added to a solution of **10b** (63 mg, 1.45×10^{-4} mole) in dioxan (2 mL) and refluxed overnight while stirring. After cooling to room temperature, the reaction mixture was filtered, three drops of pyridine were added, and evaporated to dryness. The residue was extracted with ethyl acetate, washed with sodium bicarbonate (sat. aq.), water, dried (Na_2SO_4) and evaporated to give 50 mg (80%) of **14:** ^{1}H NMR ($CDCl_3$) δ ppm 7.07 (1H, d $J_{1,2}$ = 10 Hz, H-1), 6.27 (1H, dd $J_{1,2\ 2,4}$ = 10Hz, 2Hz, H-2), 6.26 (1H, dd$J_{6,7\ 7,8}$ = 10, 4 Hz, H-7), 6.04 (1H, $J_{2,4}$ = 2 Hz, H-4), 5.96 (1H, dd $J_{6,7\ 6,8}$ = 10, 2 Hz, H-6), 5.31 (1H, $W_{1/2}$ = 17 Hz, H-15), 4.07 (2H, $W_{1/2}$ = 10 Hz, ketal), 3.86 (2H, $W_{1/2}$ = 33 Hz, ketal), 2.07 (3H, s, OAc), 1.37 (3H, s, H-21), 1.25 (3H, s, H-19), 1.14 (3H, s, H-18); λ_{max} 225 (log ε 4.00), 258 (3.98), 300 (4.08); CIMS/CH_4 *m/z* (%) 429 (100), 413 (11), 385 (94), 369 (17), 325 (15), 307 (34), 161 (5); HR-CIMS/CH_4 found 429.2287, expected (as MH) 429.2277. GC and GC-EIMS (underivatized), MU = 33.53, *m/z* (%); 428 (3%) [M^+], 368 (30%), 306 (18%), 280 (44%), 264 (30%), 249 (17%), 237 (26%), 223 (23%), 209 (21%), 195 (21%), 181 (23%), 171 (81%), 165 (40%), 158 (32%), 147 (52%), 145 (46%), 131 (40%), 129 (80%), 128 (100%), 121 (72%), 115 (76%), 105 (43%).

*15β-Acetoxy-20,20-ethylenedioxy-5-pregnen-3β, 17α-diol (**10b**)*

To a solution of **6a** (2 mg, 4.6×10^{-6} mole) in anhydrous dimethylsulphoxide (2 mL), potassium *tert*-butoxide in dimethylsulphoxide (0.5 mL, 0.5 M) was added. The mixture was stirred at room temperature for 2.5 h before being poured quickly into a solution of sodium borohydride (1 mg, 2.6×10^{-5} mole) in methanol (2 mL) and water (0.1 mL). After 10 min, the reaction mixture was extracted with chloroform. The organic phase was washed with dilute hydrochloric acid, sodium carbonate (sat. aq.), brine, dried (Na_2SO_4), and evaporated to dryness. Chromatography on silica gel eluting with ethyl acetate/hexane (1:2) gave 1.5 mg (75%) of **10b,** which was identical to that obtained above.

*3β,15β,17α-Trihydroxy-5-pregnen-20-one (**1a**)*

To a solution of **10b** (1 mg, 2.3×10^{-6} mole) in ethanol (3 mL), sodium hydroxide (0.5 mL, 2 M) was added and the reaction mixture refluxed for 2 h. The mixture was cooled, acidified with hydrochloric acid (1 M), and stirred at room temperature for 1 h. The solution was neutralized with sodium bicarbonate (sat. aq.) and evaporated to low-volume at 40°C. The residue was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4), and evaporated to give 0.7 mg (87%) of **1a:** mp 258–260°C; (Lit[8] mp 258–260°C); ^{1}H NMR ($CDCl_3$) δ ppm 5.37 (1H, m $W_{1/2}$ = 10 Hz, H-6), 4.36 (1H, m $W_{1/2}$ = 14 Hz, H-15), 3.50 (1H, m $W_{1/2}$ = 24 Hz, H-3), 2.28 (3H, s, H-21), 1.04 (3H, s, H-19), 0.94 (3H, s, H-18).

*15β-Acetoxy-20,20-ethylenedioxy-5α-pregnan-3β, 17α-diol (**15a**)*

A solution of **10b** (27 mg, 6.2×10^{-5} mole) in 0.1% diethylamine in ethanol (15 mL) was hydrogenated over rhodium on carbon (1 mg, 5%) at 40°C for 12 h. The reaction mixture was centrifuged, the supernatent decanted and evaporated to dryness to give 26 mg (95%) of a mixture of isomers **15a** and **15b** in a ratio of 20:1: GC and GC-CIMS/CH_4 MU = 32.98 and 32.75; *m/z* (%); 581 (ND) [MH^+], 507 (14) [M-73]$^+$, 493 (100) [M-87]$^+$, 447 (33), 434 (44), 387 (68), 359 (50) 297 (64), 255 (6)

*15β-Acetoxy-20,20-ethylenedioxy-17α-hydroxy-5α-pregnan-3-one (**6c**)*

Pyridinium chlorochromate (14 mg) was added to a solution of the mixture of **15a+b** (14 mg, 3.2×10^{-5} mole) in 2% pyridine/dichloromethane (2 mL). After 2 h at room temperature, anhydrous diethyl ether (4 mL) was added, the mixture was decanted off the black gum, and the residue was washed with anhydrous diethyl ether (5 mL). The combined organic phase was filtered through a short pad of silica gel, eluting with diethyl ether. Evaporation to dryness gave 10 mg (71%) of a mixture of **6c** and **6b** in a ratio of 20:1: GC and GC-EIMS (as MOTMS; MW = 535), MU = 32.14 and 31.96; *m/z* (%); 535 (0.5%) [M^+], 448(6%), 388(2%), 87(100%).

*15β-Acetoxy-20,20-ethylenedioxy-17α-hydroxy-1,2,6,7-^{3}H-preg-4-en-3-one (**6d**)*

15β-Acetoxy-20,20-ethylenedioxy-17α-hydroxy-1,2,6,7-^{3}H-preg-4-en-3-one (**6d**) was prepared by Amersham Inc., using an undisclosed method, by reducing **14** with tritium gas. Isolation was obtained by semipreparative high-performance liquid chromatography (HPLC) using an RID detector and a normal phase column (250 × 10 mm; 5 μ) with ethyl acetate/hexane (1:2; v/v) as the mobile phase at a rate of 3 mL/min. Retention times for **14** and **6d** were 11.8 and 9.5 min, respectively. 15β-Acetoxy-20,20-ethylenedioxy-17α-hydroxy-1,2,6,7-^{3}H-preg-4-en-3-one (**6d**): λ_{max} = 240 (log ε 4.20); specific activity = 75 Ci/mmol; radio-

active purity (HPLC) = 97.3%; GC and GC-CIMS/NH_3 (as MO-TMS), MU = 32.88; *m/z* (%); 439 (3%) $[M+1]^+$, 308(5%), 291(3%), 122(2%), 87(100%).

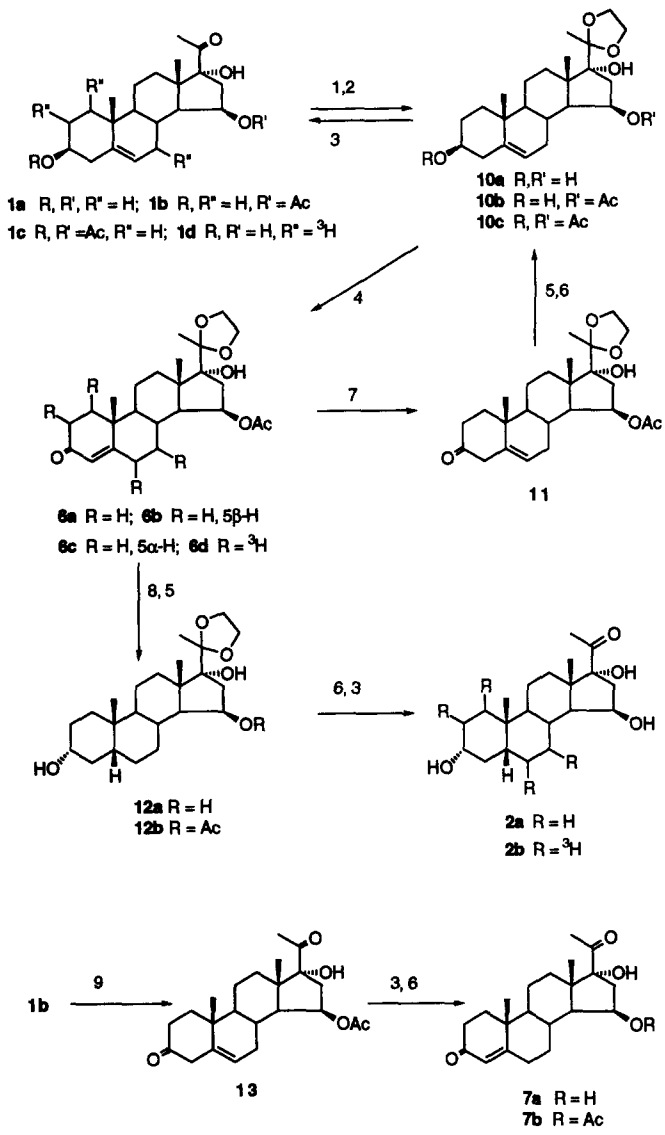
Discussion

In our earlier work,[8–13] we reported the synthesis of a number of 15β-hydroxylated steroids. To complete the investigation into these 15β-hydroxylated steroids, a method for the conversion of 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1a**) to 3α,15β,17α-trihydroxy-5β-pregnan-20-one (**2a**) was required, as well as a method for the synthesis of 15β,17α-dihydroxy-4-pregnen-3,20-dione (**7a**).

The starting material for this investigation was 3β,15β-diacetoxy-17α-hydroxy-5-pregnen-20-one (**1c**), which we synthesized recently as the penultimate step in the synthesis of **1a.**[8] We have previously noted the increased rate of reaction of the C-20 ketone when a 15β-hydroxy group is present[12]; therefore, the first step in the synthesis of **2a** was the protection of the C-20 ketone (Scheme 1). Reaction of **1c** with ethylene glycol, triethyl orthoformate, and acid catalysis gave as the major compound 3β,15β-diacetoxy-20,20-ethylenedioxy-5-pregnen-17α-ol (**10c**) with 15β-acetoxy-3β,17α-dihydroxy-5-pregnen-20-one (**1b**) and 15β-acetoxy-20,20-ethylenedioxy-5-pregnen-3β,17α-diol (**10b**) as side products. Treatment of **10c** with aqueous potassium carbonate resulted in the selective hydrolysis of the C-3 acetate to give **10b.** Early attempts of the synthesis of **6a** by means of oxidation of **10b** with pyridinium chlorochromate[18] failed, because the product obtained, 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-5-pregnen-3-one (**11**) could not reliably be converted under base catalysis to 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-4-pregnen-3-one (**6a**), the more usual acid catalysis being ruled out because of the presence of the C-20 ketal. A more reliable method for the synthesis of **6a** was under Oppenhauer conditions, where the equilibration of the C-5 olefin occurs under the conditions of the oxidation. Oxidation of **10b** using aluminium *tert*-butoxide in cyclohexanone and toluene,[19] gave a 57% yield of **6a.** Hydrogenation of **6a** using 10% palladium on calcium carbonate,[11] using conditions known to favor the synthesis of the 5β-H isomer gave a mixture of 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-5α-pregnan-3-one (**6b**) and 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-5α-pregnan-3-one (**6c**) in a ratio of 4:1. Reduction of **6b** with sodium borohydride gave 15β-acetoxy-20,20-ethylenedioxy-5β-pregnan-3α,17α-diol (**12b**), which after base hydrolysis of using sodium hydroxide to give 20,20-ethylenedioxy-5β-pregnan-3α, 15β,17α-triol (**12a**) was treated with aqueous acid to give **2a** in good yield.

The first step in the synthesis of **7a** was the treatment of **1c** with aqueous potassium carbonate, which resulted in the selective hydrolysis of the C-3 acetate to give 15β-acetoxy-3β,17α-dihydroxy-5-pregnen-20-one **1b.** Oxidation of **1b** with pyridinium chlorochromate[18] gave 15β-acetoxy-17α-hydroxy-5-pregnen-3,20-dione **13,** which under acid catalysis, immediately rearranged to give 15β-acetoxy-17α-hydroxy-4-pregnen-3,20-dione **7b.** Base-catalyzed hydrolysis using aqueous sodium hydroxide readily gave **7a.**

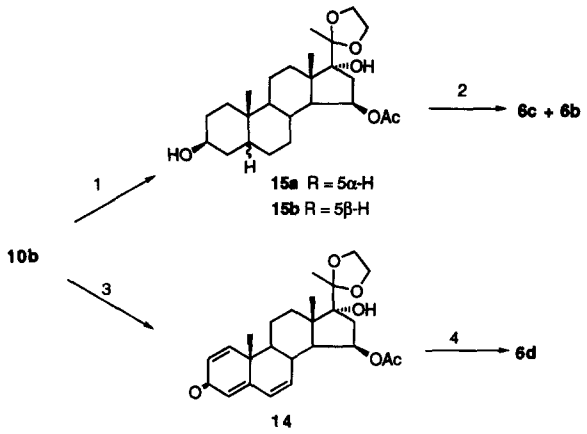
To further our investigations into the biochemistry of 15β-hydroxylated steroids, the synthesis of tritiated tracers



Scheme 1 Reagents: (1) ethylene glycol, triethylorthoformate, H^+; (2) K_2CO_3, aq. EtOH (3) H^+; (4) $Al(t\text{-}BuO)_3$ cyclohexanone, toluene; (5) $NaBH_4$, EtOH; (6) NaOH, EtOH; (7) K^tBuO, DMSO (8) H_2, 10% $Pd/CaCO_3$; (9) pyridinium chlorochromate.

of **1a** and **2a** was desired.[20,21] These compounds may be obtained from a common precursor, the tritiated isomer of **6a,** which itself can be synthesized by tritiation of 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-1,4,6-pregnatrien-3-one **14** (Scheme 2). Oxidation of **10b** with dichlorodicyanobenzoquinone in dioxane gave **14** in good yield.[22]

To complete the development of the use of **6a** as a common intermediate in the synthesis of **2a** and **1a,** the synthesis of **1a** from **6a** was required. Reaction of **6a** with potassium *tert*-butoxide in anhydrous dimethylsulphoxide[23] gave **11,** which was immediately reduced using sodium borohydride to give **10b.** Base catalyzed hydrolysis of **10b** gave 20,20-ethylenedioxy-5-pregnen-3β,15β,17α-triol **10a,** which, on acid treatment, yielded **1a** in good yield. In both cases, the synthesis of **1a** and **2a** from **6a** has been possible without isolation of the intermediates, thus allowing the ready synthesis of the radioactive isomers without any chro-



Scheme 2 Reagents: (1) H2, 5% Rh/C; (2) pyridinium chlorochromate; (3) DDQ; (4) by Amersham Inc.

matographic steps and minimizing the handling of the compounds.

There are two possible products of over-reduction of **14.** They are **6b,** synthesized above and 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-5α-pregnan-3-one **6c.** The synthesis of **6c** was achieved by hydrogenation of **10b** using 5% rhodium on carbon under conditions known to form the 5α-H[11] preferentially to give 15β-acetoxy-20,20-ethylenedioxy-5α-pregnan-3β,17α-diol **15a** and 15β-acetoxy-20,20-ethylenedioxy-5β-pregnan-3β,17α-diol **15b** in a ratio of 20:1. Oxidation of the mixture of **15a** + **b** with pyridinium chlorochromate[18] gave a mixture **6c** and **6b** in a ratio of 20:1.

Tritiation of **14** was followed by chromatographic purification on a normal phase HPLC column eluting with ethyl acetate/hexane (1:2, v/v) by Amersham Inc., to give the target intermediary tracer **6d.** The product received was found to contain four tritium atoms per molecule and gave the expected GC-MS data when compared to the cold analog **6a.**

In conclusion, the successful conversion of **1a** to **2a** and **7a** reported here completes the major part of our investigation into the chemistry of 15β-hydroxylated steroids. Furthermore, by utilization of the chemistry developed here, a simple one-pot conversion of **6a** to **1a** and **2a** has been achieved. This procedure will allow synthesis of the radioactive tracers **1d** and **2b** from 15β-acetoxy-20,20-ethylenedioxy-17α-hydroxy-1,2,6,7-^{3}H-pregn-4-en-3-one **6d** for use in new immunoassays for the estimation of compounds **1a** and **2a** in biological fluids.

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