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15β-Hydroxysteroids (Part V).* Steroids of the human perinatal period: The synthesis of 3β, 15β, 17α-trihydroxy-5-pregnen-20-one from 15β, 17α-dihydroxy-4-pregnen-3,20-dione

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A simple three-step synthetic method is reported on the conversion of Δ^4-3-ketosteroids to the corresponding 3β-hydroxy-Δ^5-steroid analogues. 17α-Hydroxy-4-pregnen-3,20-dione ***(10a)*** *was used as a model to develop a method for the synthesis of 3β,17α-dihydroxy-5-pregnen-20-one* ***(16)****. The major problem being the synthesis of 3,17α-diacetoxy-3,5-pregnadien-20-one* ***(14)*** *was solved by acetylating using a mixture of acetic anhydride and perchloric acid. The conversion of 15β,17α-dihydroxy-4-pregnen-3,20-dione* ***(8)****, product of* Penicillium citrinum *fermentation, to the desired 3β,15β,17α-trihydroxy-5-pregnen-20-one* ***(1)****, is described using a modification of this method. Reaction of* ***8*** *with acetic anhydride and perchloric acid in ethyl acetate gave 3,15β,17α-triacetoxy-3,5-pregnadien-20-one* ***(17)*** *which on reduction with sodium borohydride gave 5-pregnen-3β,15β,17α, 20(S + R)-tetrols* ***(18a and 18b)****; however, reduction of* ***17*** *with a mixture of sodium borohydride and potassium bicarbonate gave after basic hydrolysis with methanolic sodium hydroxide the desired product 3β,15β,17α-trihydroxy-5-pregnen-20-one* ***(1)*** *in good yield (54%). (Steroids* **61**:18–21, 1996)

Keywords: 15β-hydroxysteroids; Δ^4-3-ketosteroids; 3β-hydroxy-Δ^5-steroids; synthesis

Introduction

Steroids of the human perinatal and neonate are characterised by the 3β-hydroxy-Δ^5-steroid configuration and hydroxylations at C-15 and C-16. Identification of such steroids has been slow forthcoming due to the unavailability of simple chemical means for their synthesis. Enzyme-mediated steroid transformations have provided an alternative to laborious chemical synthesis. Cholesterol oxidases from *Nocardia erythropolis* and 3β-hydroxysteroid oxidases from *Brevibacterium sterolicum* are two of the enzyme systems employed in the conversion of Δ^5-3β-hydroxysteroids to their corresponding Δ^4-3-ketones in good yield[1] and in the identification of steroids of the human neonate.[2] Similarly, microbial fermentations employed in the synthesis of 15β,17α-dihydroxy-4-pregnen-3,20-dione (**8**) from *Penicillium citrinum* proved useful in the identification and characterization of 3β,15β,17α-trihydroxy-5-pregnen-20-one (**1**), 3α,15β,17α-trihydroxy-5β-pregnan-20-one (**2**),[2] and more recently of 3α,15β,17α-trihydroxy-5α-pregnan-20-one (**3**), 3β,15β,17α-trihydroxy-5α-pregnan-20-one (**4**), 3β,15β,17α-trihydroxy-5β-pregnan-20-one (**5**), 5α-pregnane-3α,15β,17α,20α-tetrol (**6**) and 5β-pregnane-3α,15β,17α-20α-tetrol (**7**)[3] (Figure 1). A direct synthesis of 3β-hydroxy-Δ^5-steroids is restricted in that enzyme and microbial steroid conversions favor Δ^4-3-ketosteroids.

The significant roles of **1** and **2** in the monitoring of

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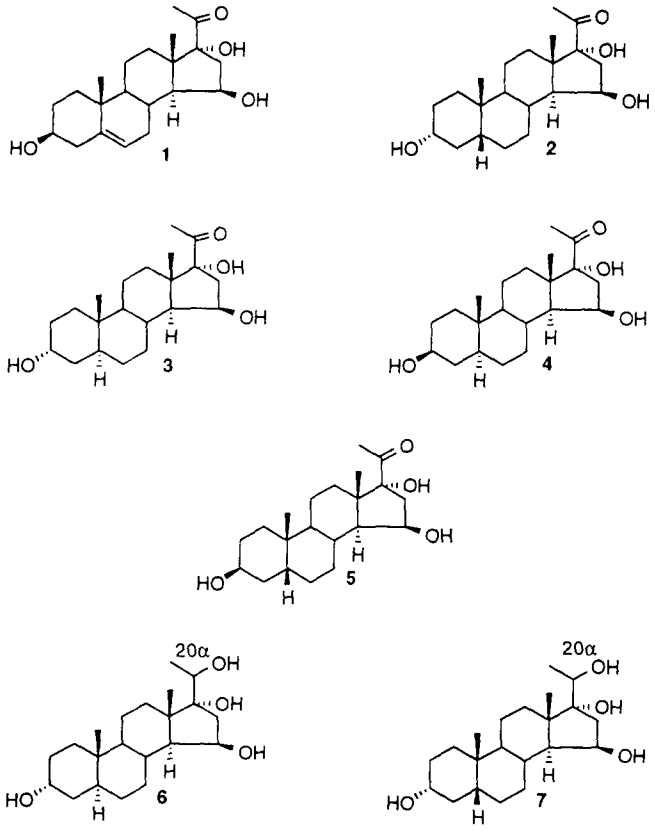

Figure 1 The structure of compounds **1–7**.

healthy newborn infants and in recognition of congenital adrenal hyperplasia (CAH) was demonstrated early in our studies[2] and supported more recently by the identification and characterization of compounds **3–7** in a newborn infant affected with CAH due to 21-hydroxylase deficiency.[3]

The availability of **8** from microbiological transformations[2] and by chemical synthesis from 3β-hydroxy-5-androsten-17-one **(9)**,[4] identified this as an important intermediate in the synthesis 15β-hydroxy-C-21-steroids. However, its conversion to the 3β-hydroxy-Δ^5-steroid analog has proved difficult. In this investigation, we report a simple procedure for the conversion of 17α-hydroxy-4-pregnen-3,20-dione **(10a)** to 3β,17α-dihydroxy-5-pregnen-20-one **(16)** and its application to the conversion of **8** to **1** in a 3-step chemical procedure and in good yield.

Experimental

The starting material 15β,17α-dihydroxy-4-pregnen-3,20-dione, was obtained as a product of microbial transformation of 17α-hydroxyprogesterone from *P. citrinum*[2] and the structural identity of which was confirmed by gas chromatography (GC) and GC-mass spectroscopy (MS)[2] and NMR[4] data.

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 NMRs were recorded at 200 MHz using a Bruker AC-200F spectrometer using TMS as internal reference and deuterochloroform as solvent. $W_{1/2}$ 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 and interpreted using a Shimadzu CR-4A chromatopac utilising Eurekasoft analytical software (Eurekanalytical, Camperdown, Australia), results are expressed as methylene units (MU).[6] 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 d at 70 eV. CIMS was 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 were run on a Finnigan MAT-90. Silica gel H (Merck, type 60) was used for chromatography.[7] Steroid derivatization for GC and GC-MS analyses were as reported earlier.[2]

3,17α-Diacetoxy-3,5-pregnadien-20-one (14)

To a slurry of 17α-hydroxy-4-pregnen-3,20-one (**10a**; 1 g, 3.03 mmol) in ethyl acetate (80 mL), acetic anhydride (10 mL) was added with a solution of ethyl acetate (40 mL) and perchloric acid (40 μL, 70%). After 6 min the organic phase was washed with saturated aqueous sodium bicarbonate, water and dried (Na_2SO_4). Evaporation to dryness and crystallization from methanol gave 1.15 g (92%) of **14:** m.p. 180–182°C (lit.[8] m.p. 179–184°C); λ_{max} 234 nm (Log ε = 4.30); ^{1}H NMR δ 5.80 (1 H, $W_{1/2}$ = 5 Hz, H-4), 5.39 (1 H, $W_{1/2}$ = 10 Hz, H-6), 2.13 (3 H, s, H-3 OAc), 2.11 (3 H, s, H-21), 2.05 (3 H, s, H-17 OAc), 1.00 (3 H, s, H-19), 0.67 (3 H, s, H-18).

17α-Acetoxy-3β-hydroxy-5-pregnen-20-one (15)

a) To a solution of **14** (110 mg, 0.265 mmol) in ethanol (20 mL), sodium borohydride (20 mg) was added. After stirring overnight at room temperature, formic acid was added dropwise until the evolution of gas ceased. The solution was then evaporated to dryness and the residue extracted with ethyl acetate. The organic phase was washed with water twice, dried (Na_2SO_4), evaporated to dryness, and recrystallized from methanol to give 89 mg (89%) of **15:** m.p. 224–226°C (lit.[9] m.p. 225–227°C); ^{1}H NMR δ 5.40 (1 H, $W_{1/2}$ = 10 Hz, H-6), 3.56 (1 H, $W_{1/2}$ = 26 Hz, H-3), 2.11 (3 H, s, H-21), 2.05 (3 H, s, H-17 OAc), 1.04 (3 H, s, H-19), 0.68 (3 H, s, H-18).

b) To a solution of **14** (20 mg, 4.38×10^{-2} mmol) in methanol (10 mL), potassium bicarbonate (100 μL; 1.2 M) and sodium borohydride (2 mg) was added. After 2 h at room temperature, the excess reagent was destroyed with acetic acid and the mixture evaporated to dryness. The residue was extracted with ethyl acetate and the organic phase was washed with water, dried (Na_2SO_4) and evaporated to dryness. Recrystallization of the residue from methanol gave 16 mg (88%) of **15** identical to that above: m.p. 224–226°C (lit.[9] m.p. 225–227°C).

17α-Acetoxy-4-pregnen-3,20-dione (10b)

To a solution of **14** (10 mg, 2.41×10^{-2} mmol) in methanol (4 mL), aqueous potassium bicarbonate (100 μL, 1.5 M) was added. The mixture was stirred at room temperature for 2 h and evaporated to dryness. The residue extracted with ethyl acetate and the organic phase was washed with water, dried (Na_2SO_4) and evaporated to dryness. Crystallization from dichloromethane/methanol gave 8 mg (88%) of **10b:** m.p. 241–243°C (lit.[10] m.p. 243–244.5°C); ^{1}H NMR δ 5.80 (1 H, $W_{1/2}$ = 3 Hz, H-4), 2.11 (3 H, s, H-21), 2.04 (3 H, s, H-17 OAc), 1.20 (3 H, s, H-19), 0.68 (3 H, s, H-18).

3β,17α-Dihydroxy-5-pregnen-20-one (16)

To a solution of **15** (130 mg, 0.348 mmol) in methanol (10 mL), aqueous sodium hydroxide (1 M, 2 mL) was added. The mixture

was refluxed for 3 h and evaporated to dryness after cooling. The residue extracted with ethyl acetate and the organic phase washed with water, dried (Na_2SO_4), and evaporated to dryness to give after recrystallization from aqueous methanol 110 mg (96%) of **16:** m.p. 264–267°C (mixed m.p. 264–266°C) (lit.[11] m.p. 265–267°C).

3,15β,17α-Triacetoxy-3,5-pregnadien-20-one (17)

To a slurry of **8**[4] (50 mg, 0.145 mmol) in ethyl acetate (5 mL), acetic anhydride (625 µL) was added together with 250 µL aliquot of a solution of perchloric acid (20 µL, 70%) in ethyl acetate (20 mL). After 6 min at room temperature, a saturated aqueous solution of sodium bicarbonate (2 mL) was added and the mixture was diluted with ethyl acetate (50 mL). The organic phase was washed with sodium bicarbonate (saturated), water, dried (Na_2SO_4), and evaporated to give 55 mg (81%) of **17** as a gum: ^{1}H NMR δ 5.80 (1 H, $W_{1/2}$ = 5 Hz, H-4), 5.39 (1 H, $W_{1/2}$ = 10 Hz, H-6), 5.15 (1 H, $W_{1/2}$ = 15 Hz, H-15), 2.14 (3 H, s, H-3 OAc), 2.11 (3 H, s, H-21), 2.05 (3 H, s, H-17 OAc), 2.03 (3 H, s, H-15 OAc); λ_{max} = 234 (log 4.25).

GC and GC/EIMS (as MOTMS) MU = 32.37; m/z (%); 472 (14) [M^+], 430 (16), 412 (14), 370 (100), 354 (9), 311 (1), 295 (17), 267 (11), 225 (52), 209 (10); GC-CIMS/CH_4 m/z (%); 473 (11) [M+1]$^+$, 431 (13), 413 (10), 371 (38), 353 (56), 311 (16), 295 (10).

5-Pregnene-3β,15β,17α,20 (R + S)-tetrol (18a, 18b)

Sodium borohydride (2 mg) was added to a solution of **17** (30 mg, 6.35×10^{-2} mmol) in ethanol (10 mL). The reaction mixture was stirred at room temperature for 7 h then the excess reagent was destroyed with acetic acid and the mixture evaporated to dryness. The residue was extracted with ethyl acetate and the organic phase washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in aqueous ethanol (10 mL, 90%) and sodium hydroxide (1 mL, 2 M) was added. The solution was refluxed for 20 min, evaporated to dryness, extracted with ethyl acetate, and the organic phase was washed with water, dried (Na_2SO_4), and evaporated to dryness to give **18a** and **18b** (as a mixture of two isomers). Chromatography of the residue on silica gel and eluting with ethyl acetate/hexane (3:1, v/v) gave after recrystallization from acetone/hexane 18 mg (78%) of **18a:** m.p. 212–214°C; ^{1}H NMR δ 5.36 (1 H, $W_{1/2}$ = 10 Hz, H-6), 4.28 (1 H, $W_{1/2}$ = 12 Hz, H-15), 4.11 (1 H, q *J* = 6 Hz, H-20), 3.50 (1 H, $W_{1/2}$ = 26 Hz, H-3), 1.16 (3 H, d *J* = 6 Hz, H-21), 1.06 (3 H, s, H-18), 1.05 (3 H, s, H-19); SP-EIMS m/z (%); 350 (6) [M^+], 332 (7), 305 (100), 287 (25), 271 (16), 269 (20), 251 (11), 215 (20), 211 (16), 200 (15); SP-CIMS/CH_4 m/z (%); 349 (12) [M-1]$^+$, 333 (92), 315 (100), 287 (37), 269 (13); Calculated for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 72.28; H, 9.82. GC: (as tetraTMS; molecular weight = 638) MU = 29.71; GC-EIMS m/z (%); 638 (1) [M^+], 521 (100), 431 (72), 391 (22), 333 (23), 251 (13), 191 (40), 147 (35), 117 (78).

Further elution gave after recrystallization from acetone/hexane 4 mg (17%) of **18b:** m.p. 223°C; ^{1}H NMR δ 5.36 (1 H, m $W_{1/2}$ = 10 Hz, H-5), 4.28 (1 H, m $W_{1/2}$ = 15 Hz, H-15), 3.91 (1 H, q *J* = 6 Hz, H-20), 3.50 (1 H, m $W_{1/2}$ = 26 Hz, H-3), 1.19 (3 H, d *J* = 6 Hz, H-21), 1.05 (3 H, s, H-19), 0.99 (3 H, s, H-18); SP-EIMS m/z (%); 350 (ND), 305 (100), 287 (6), 269 (5), 211 (16); SP-CIMS/CH_4 m/z (%); 349 (13) [M-1]$^+$, 333 (100), 315 (83), 297 (14), 271 (13), 245 (3); Calculated for $C_{21}H_{34}O_4 \cdot H_2O$: C, 68.44; H, 9.84. Found: C, 68.43; H, 9.90.

GC: (as tetraTMS; molecular weight = 638) MU = 29.84; GC-EI/MS m/z (%); 638 (1) [M^+], 521 (100), 431 (72), 391 (22), 333 (23), 251 (13), 191 (40), 147 (35), 117 (78);

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

To a solution of **17** (20 mg, 4.24×10^{-5} mol) in ethanol (10 mL), potassium bicarbonate (100 µL: 1.5 M) and sodium borohydride (2 mg) were added. The mixture was stirred at room temperature for 2 h. Excess reagent was then destroyed with acetic acid and the mixture evaporated to dryness. The residue was extracted with ethyl acetate and the organic phase washed with water, dried (Na_2SO_4), and evaporated to dryness. GC and GC-MS analysis of the residue identified 15β,17α-diacetoxy-3β-hydroxy-5-pregnen-20-one **(19)** as the major product of synthesis: GC (as TMS) MU = 31.45; GC-EIMS m/z (%); 504 (1), 458 (1), 444 (18), 384 (7), 369 (14), 294 (64), 279 (38), 251 (100), 237 (25), 224 (30), 209 (59), 157 (11); GC-CIMS/CH_4 m/z (%), 503 (3), 489 (22), 445 (93), 385 (100), 355 (57), 295 (41).

To the crude residue dissolved in methanol (60 mL), sodium hydroxide (2 M, 1 mL) was added and refluxed for 6 h. After neutralization with hydrochloric acid (1 M, 4 mL), the mixture was evaporated to low volume and diluted with ethyl acetate. The organic phase was washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography of the residue on silica gel eluting with dichloromethane/acetone (2:1, v/v) gave after crystallization from acetone 10 mg (66%) of **1:** m.p. 258–260°C; ^{1}H NMR δ 5.37 (1 H, m $W_{1/2}$ = 10 Hz, H-6), 4.36 (1 H, m $W_{1/2}$ = 14 Hz, H-15), 3.50 (1 H, m $W_{1/2}$ = 24 Hz, H-3), 2.28 (3 H, s, H-21), 1.04 (3 H, s, H-19), 0.94 (3 H, s, H-18); SP-CIMS/CH_4 m/z (%); 349 (6) [M+1]$^+$, 347 (21), 332 (37), 331 (100), 313 (93), 295 (57), 271 (9), 253 (5). SP-CIMS/NH_3 m/z (%); 366 (100) [M+18]$^+$, 348 (39), 331 (49), 313 (10), 295 (3); high resolution MS expected 348.2300; found = 348.231.

GC and GC-EIMS (as MOTMS) MU = 29.11; m/z (%); 593 (26) [M^+], 562 (76), 503 (10), 472 (38), 362 (19), 258 (100), 231 (12), 188 (31), 129 (29).

Results and discussion

We have shown that **8** can be obtained readily by either microbiological hydroxylation[2] or chemical synthesis.[4] More recently the synthesis of **2** was achieved from **8** by chemical transformation of 15β,17α,21-trihydroxy-4-pregnen-3,20-dione **(12a),** the latter a product of microbial conversion from 17α,21-dihydroxy-4-pregnen-3,20-dione **(11)** by *Bacillus megatirium* (Figure 2; data submitted for publication). As such, **8** became an invaluable intermediate in the synthesis of 15β-hydroxylated steroids. However, deconjugation of the C-4 olefin followed by reduction of the C-3 ketone to yield 3β-hydroxy-Δ^5-C-21-steroids proved difficult.

Initial attempts at the preparation of 3,17α-diacetoxy-3,5-pregnadien-20-one **(14)** by hot acid acetylation resulted in poor yields due to D-homo rearrangement.[12,13] On the other hand, acetylation of 17α-hydroxy-4-pregnen-3,20-dione **(10a)** using acetic anhydride with a trace of perchloric acid at room temperature gave **14**[14,16] in high yield (92%). Reduction of **14** with sodium borohydride in ethanol gave 17α-acetoxy-3β-hydroxy-5-pregnen-20-one **(15)** in good yield (89%) which on subsequent hydrolysis with aqueous sodium hydroxide in methanol 3β,17α-dihydroxy-5-pregnen-20-one **(16)** also in good yield (96%).

Acetylation of **8** with acetic anhydride and perchloric acid gave 3,15β,17α-triacetoxy-3,5-pregnadien-20-one **(17)** in high yield (79%) which on reduction with sodium borohydride gave, after basic hydrolysis, 5-pregnen-3β,15β,17α,20*R*-tetrol **(18a)** and 5-pregnene-

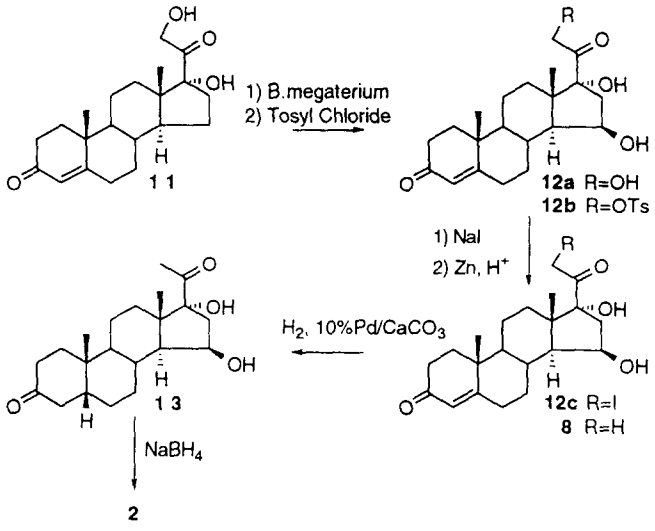

Figure 2 The synthesis of 3α,15β,17α-trihydroxy-5β-pregnan-20-one.

3β,15β,17α,20S-tetrol (**18b;** ratio 4.5:1; the stereochemistry at C-20 in **18a** and **18b** was determined on the basis of GC retention times and by microbiological oxidation using an enzyme specific for one isomer. Unambiguous assignment requires a more thorough NMR study; Figure 3). It was evident that the rate of reduction of the C-20 ketone was similar to the rate of the reduction of the C-3,5-dienyl-3-acetate.

Addition of a weak base thereby increasing the rate of the hydrolysis reaction enhanced the selectivity of the reduction. Reaction of **14** with 1.5 M aqueous potassium bicarbonate gave 17α-acetoxy-4-pregnen-3,20-dione **(10b)** in good yield (89%). Reduction of **14** with sodium borohydride and 1.5 M aqueous potassium bicarbonate gave **15** in 2 h. In contrast, the reduction of **14** without the addition of base required 16 h to go to completion. Reaction of **17** with

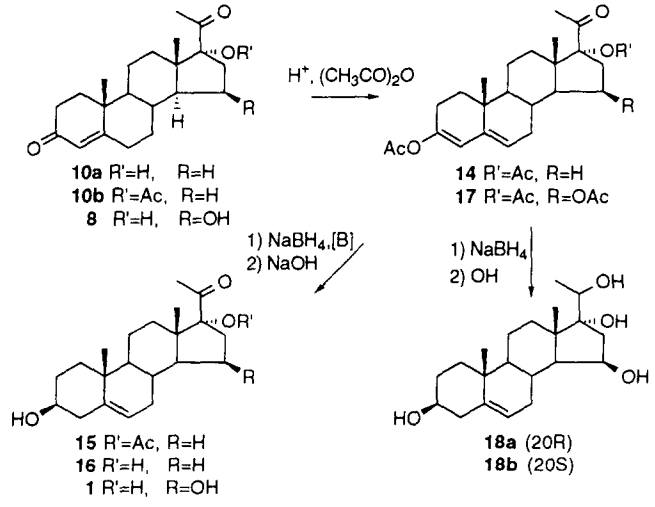

Figure 3 The synthesis of 3β,15β,17α-trihydroxy-5-pregnen-20-one.

sodium borohydride and 1.5 M aqueous potassium bicarbonate gave after basic hydrolysis gave **1** in good yield 68% (Figure 3).

Microbiological 15β-hydroxylations are widely accepted as the most commercially viable methods of production. However these are confined to Δ^4-3-ketosteroids. The significance of our results is that the method employed here would allow the conversion of such steroid products to their corresponding 3β-hydroxy-Δ^5-steroids rapidly and in good yields.

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