

EPALONS: SYNTHESIS OF 3 α ,7 α -DIHYDROXY-5 α -PREGNAN-20-ONE*Hana CHODOUNSKA¹ and Alexander KASAL²*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic,
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Dedicated to Dr Jan Fajkos on the occasion of his 75th birthday.

3 α ,7 α -Dihydroxy-5 α -pregnan-20-one (**12**) was prepared from (20*R*)-pregn-5-ene-3 β ,20-diyi 3-acetate 20-benzoate (**1**). Δ^5 -Olefin was oxidized in an allylic position and hydrogenated. Inversion of a configuration at carbon C-3 was carried out by solvolysis in *N,N*-dimethylformamide of 3 β -tosylate in the presence of sodium nitrite. For stereoselective reduction of the 7-oxo group, L-Selectride® was used.

Key words: Steroids; Epalon; GABA_A-Modulator; 7 α -Hydroxylase; Solvolysis; L-Selectride®, 3 α ,5 α -Tetrahydroprogesterone.

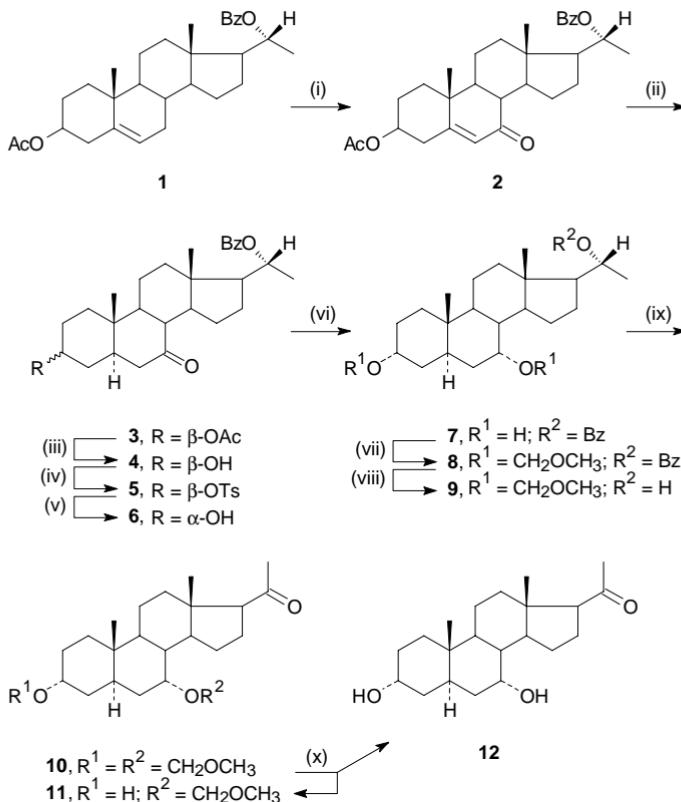
7 α -Hydroxy steroids are common in body fluids of vertebrates; however, the mechanisms of their formation differ in various types of tissue. Further, 7 α -hydroxylations in the liver² and brain³ have different functions: while the former affords highly polar bile acids operative in digestion, the latter leads to compounds used in the brain itself. 7 α -Hydroxylase has been long known in the cholesterol and 3 β -hydroxyandrost-5-en-17-one (DHEA) metabolism, however, 7 α -hydroxylation of progesterone and its metabolites has only recently been described³, though one such metabolite of progesterone – the title compound **12** (see Scheme 1) – had been isolated from the urine of a monkey⁴. As its presence in man is very probable, an authentic sample of compound **12** was needed. Its synthesis is presented in this paper.

The starting compound is 20-oxo-pregn-5-en-3 β -yl acetate. Its oxo group was reduced, the prevailing (20*R*)-20-hydroxy isomer was isolated by crystallization and converted to benzoate⁵ **1**. For allylic oxidation of this olefin, sodium dichromate⁶ was used because it gives better yields than other reagents even at a low excess of the oxidant;

* Part CCCXCVIII in the series On Steroids; Part CCCXCVII see ref.¹.

e.g. pyridinium chlorochromate⁷ oxidation was much slower even though a large excess of the oxidant is used.

Hydrogenation of unsaturated ketone **2** was carried out with palladium on calcium carbonate, the major product being the required 5α isomer **3**. Inversion of the C-3 configuration to 3α , characteristic of epalons⁸, was done by solvolysis of tosylate **5** in the presence of sodium nitrite⁹. The 7-oxo group in compound **6** was reduced¹⁰ to a single derivative **7** with L-Selectride®. ¹H NMR spectroscopy confirmed the axial configuration of both hydroxy groups (see Experimental).



Bz = benzoyl

- (i) $\text{Na}_2\text{Cr}_2\text{O}_7$, AcOH , Ac_2O , NaOAc ; (ii) H_2 , Pd/CaCO_3 ; (iii) HClO_4 , aqueous acetone;
 (iv) TsOCl , pyridine; (v) NaNO_2 , DMF ; (vi) L-Selectride®, H_2O_2 ;
 (vii) $\text{CH}_3\text{OCH}_2\text{Cl}$, *N,N*-diisopropylethylamine; (viii) KOH , EtOH ;
 (ix) pyridinium chlorochromate/ Al_2O_3 , benzene; (x) HCl , MeOH

Scheme 1

Before the deprotection of the C-20 functionality, hydroxy groups in positions 3 α and 7 α were protected by silylation. However, only low yields of *tert*-butyldimethylsilyl ethers were obtained, apparently due to steric hindrance of the 7 α -hydroxyl group. Compound **7** could be easily converted¹¹ into bis(methoxymethoxy) derivative **8**. ¹H NMR spectrum of compound **8** contained a two-proton signal of an AB system at δ 4.66 (due to limited rotation of the 7 α -methoxymethoxy group) and a two-proton singlet of the 3 α -CH₂OCH₃ group at δ 4.64.

Alkaline hydrolysis of compound **8** yielded alcohol **9** which was subsequently oxidised with pyridinium chlorochromate on aluminium oxide¹² to ketone **10** and deprotected to yield the title compound **12**. Its ¹H NMR spectrum is identical with that of the progesterone metabolite in a marmoset female¹³.

A by-product with the methoxymethoxy group in the 7 α -position (compound **11**) was isolated and will also be used in endocrinological studies.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured at 25 °C on a Perkin-Elmer 141 MC polarimeter; $[\alpha]_D$ values are given in ° (10^{-1} rad cm² g⁻¹). Infrared spectra (wavenumbers in cm⁻¹) were recorded on a Bruker IFS 88 spectrometer in chloroform. ¹H NMR spectra were measured on a Varian UNITY-200 FT NMR spectrometer (200.04 MHz) in deuterio-chloroform. Chemical shifts in ppm (δ -scale) were referenced to internal tetramethylsilane. Coupling constants (*J*) and width of multiplets (*W*) are given in Hz. Thin layer chromatography (ICN Silica G, TLC-60 A) was used for checking the purity of individual intermediates. Column chromatography was carried out on silica gel for thin layer chromatography (Silpearl, Kavalier, Czech Republic) using a slight overpressure. Whenever aqueous solutions of hydrochloric acid, potassium hydrogen carbonate and potassium carbonate are used, their concentration is always 5%.

(20*R*)-7-Oxopregn-5-ene-3 β ,20-diy 3-Acetate 20-Benzoate (**2**)

A solution of sodium dichromate dihydrate (3.6 g, 12.08 mmol) and anhydrous sodium acetate (16 g) in acetic acid (400 ml) and acetic anhydride (80 ml) was allowed to stand for 18 h at room temperature. A warm (40 °C) solution of (20*R*)-pregn-5-ene-3 β ,20-diy 3-acetate 20-benzoate⁵ (**1**; 8.0 g, 17.2 mmol) in benzene (32 ml) was added to the solution and the mixture was kept at room temperature. After 55 h, an excess reagent was reduced by methanol (40 ml). After additional 4 h, the mixture was diluted with water (200 ml), a precipitate formed was filtered off, washed with water and dried in air. Chromatography on a column of silica gel (250 g, ligroin-ether 4 : 1) afforded compound **2** (5.6 g, 68%), m.p. 163–165 °C, $[\alpha]_D$ -137 (*c* 1.4). IR spectrum: 1 730 (OAc); 1 709, 1 669 (C=O); 1 634 (C=C); 1 603, 1 585 (arom); 1 275 (C–O). ¹H NMR spectrum: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 5.72 d, 1 H, *J* = 1.2 (H-6); 5.16 dq, 1 H, *J*(17,20) = 10.1, *J*(20,21) = 6.2 (H-20); 4.71 m, 1 H, *W* = 32 (H-3); 2.05 s, 3 H (acetate); 1.27 d, 3 H, *J* = 6.1 (3 × H-21); 1.05 s, 3 H (3 × H-19); 0.66 s, 3 H (3 × H-18). For C₃₀H₃₈O₅ (478.6) calculated: 75.28% C, 8.00% H; found: 75.06% C, 7.91% H.

(20*R*)-7-Oxo-5 α -pregnane-3 β ,20-diyi 3-Acetate 20-Benzoate (**3**)

A solution of ketone **2** (3.0 g, 6.46 mmol) in ethyl acetate (45 ml) and ethanol (9 ml) was stirred in the presence of palladium on calcium carbonate (5%, 400 mg) under slight hydrogen overpressure at room temperature. After 2 h, the catalyst was filtered off and the filtrate was evaporated in a vacuum. Chromatography of the residue (2.8 g, 92%) on a column of silica gel (ligroin-ether 9 : 1) yielded ketone **3** (1.2 g, 40%), m.p. 95–98 °C and 157–159 °C (benzene-ether), $[\alpha]_D$ −60 (c 1.8). IR spectrum: 1 728, 1 706 (C=O); 1 603 (arom); 1 272 (C–O). ^1H NMR spectrum: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 5.16 dq, 1 H, J (17,20) ≈ 10, J (20,21) = 6.2 (H-20); 4.67 m, 1 H, W = 32 (H-3); 2.05 s, 3 H (acetate); 1.27 d, 3 H, J = 6.0 (3 × H-21); 1.05 s, 3 H (3 × H-19); 0.66 s, 3 H (3 × H-18). For $\text{C}_{30}\text{H}_{40}\text{O}_5$ (480.7) calculated: 74.97% C, 8.39% H; found: 74.96% C, 8.49% H.

(20*R*)-3 β -Hydroxy-7-oxo-5 α -pregnan-20-yl Benzoate (**4**)

A solution of ketone **3** (1.2 g, 2.5 mmol) in a mixture of methanol (20 ml), chloroform (2.5 ml) and hydrochloric acid (37%, 1.5 ml) was allowed to stand at room temperature. After 4 h, it was diluted with water (50 ml) and neutralized with a solution of sodium hydrogen carbonate. Solvents were partially evaporated under a reduced pressure and the product **4** (1.05 g, 96%) was filtered off under suction and dried, m.p. 199–200 °C (chloroform-methanol), $[\alpha]_D$ −76 (c 1.0). IR spectrum: 3 611 (O–H); 1 704 (C=O); 1 603 (arom); 1 284, 1 277 (C–O). ^1H NMR spectrum: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 5.14 dq, 1 H, J (17,20) ≈ 10, J (20,21) = 6.0 (H-20); 3.60 m, 1 H, W = 32 (H-3), 1.27 d, 3 H, J = 6.2 (3 × H-21); 1.02 s, 3 H (3 × H-19); 0.66 s, 3 H (3 × H-18). For $\text{C}_{28}\text{H}_{38}\text{O}_4$ (438.6) calculated: 76.68% C, 8.73% H; found: 76.52% C, 8.84% H

(20*R*)-7-Oxo-5 α -pregnane-3 β ,20-diyi 3-Tosylate 20-Benzoate (**5**)

4-Toluenesulfonyl chloride (1.7 g, 8.92 mmol) was added to a solution of **4** (1.0 g, 2.28 mmol) in pyridine (40 ml) cooled with an ice bath. The mixture was allowed to stand overnight at room temperature, then poured onto ice and left in a refrigerator for 2 h. The precipitate was filtered off, washed with water and dried at 20 °C. The product **5** (1.2 g, 89%), m.p. 181–188 °C (dec., ligroin), $[\alpha]_D$ −51 (c 1.4). IR spectrum: 1 728, 1 706 (C=O); 1 603 (arom); 1 365, 1 179 (tosylate); 1 276 (C–O). ^1H NMR spectrum: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.77 d, 2 H, J = 8.5 (H-2 and H-6, tosylate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 7.35 d, 2 H, J = 8.5 (H-3 and H-5, tosylate); 5.16 dq, 1 H, J (17,20) = 10.1, J (20,21) = 6.1 (H-20); 4.34 m, 1 H, W = 32 (H-3); 2.45 s, 3 H (CH_3 , tosylate); 1.27 d, 3 H, J = 5.8 (3 × H-21); 1.03 s, 3 H (3 × H-19); 0.66 s, 3 H (3 × H-18). For $\text{C}_{35}\text{H}_{44}\text{O}_6\text{S}$ (592.8) calculated: 70.92% C, 7.48% H, 5.41% S; found: 70.62% C, 7.69% H, 5.64% S.

(20*R*)-3 α -Hydroxy-7-oxo-5 α -pregnan-20-yl Benzoate (**6**)

Tosylate **5** (1.2 g, 2.02 mmol) was added under stirring to a hot (120 °C) solution of sodium nitrite (10.0 g, 114 mmol) in DMF (100 ml) and then the mixture was heated to 140 °C. After 4 h, the mixture was cooled, water (100 ml) was added and the precipitate was filtered off and washed with water. It was dissolved in ether and washed with the solution of hydrochloric acid (30 ml), sodium hydrogen carbonate (30 ml) and dried over anhydrous magnesium sulfate. The residue after evaporation was purified by chromatography on a column of silica gel (ligroin-ether 4 : 1) which afforded compound **6** (640 mg, 72%), m.p. 224–226 °C (ether), $[\alpha]_D$ −41 (c 1.4). IR spectrum: 3 615 (O–H); 1 728, 1 706 (C=O); 1 603 (arom); 1 276 (C–O). ^1H NMR spectrum: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 5.14 dq, 1 H, J (17,20) ≈ 10, J (20,21) = 6.0

(H-20); 4.04 quintet, 1 H, J = 2.6 (H-3); 1.27 d, 3 H, J = 6.1 (3 \times H-21); 1.03 s, 3 H (3 \times H-19); 0.66 s, 3 H (3 \times H-18). For $C_{28}H_{38}O_4$ (438.4) calculated: 76.92% C, 8.78% H; found: 76.62% C, 8.69% H.

(20*R*)-3 α ,7 α -Dihydroxy-5 α -pregnan-20-yl Benzoate (7)

To a cooled (-78 °C) solution of **6** (320 mg, 0.73 mmol) in tetrahydrofuran (5 ml) L-Selectride® (1 M solution in tetrahydrofuran, 1.0 ml) was added under stirring in an argon atmosphere. After 4 h stirring at -78 °C, 6 M sodium hydroxide (1.25 ml) was added and the mixture was left to reach the room temperature. The mixture was diluted with water and extracted with ether, the extract was washed with the solution of hydrochloric acid and sodium hydrogen carbonate and water. After drying with magnesium sulfate and evaporation, compound **7** crystallized from methanol (285 mg, 89%), m.p. 108–109 °C, $[\alpha]_D$ -27 (c 0.9). IR spectrum: 3 615 (O–H); 1 706 (C=O); 1 603 (arom); 1 276 (C–O). 1 H NMR spectrum: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 5.14 dq, 1 H, J (17,20) = 10.1, J (20,21) = 6.1 (H-20); 4.04 quintet, 1 H, J = 2.7 (H-3); 3.83 q, 1 H, J = 2.1 (H-7); 1.27 d, 3 H, J = 6.1 (3 \times H-21); 1.03 s, 3 H (3 \times H-19); 0.66 s, 3 H (3 \times H-18). For $C_{28}H_{40}O_4$ (440.6) calculated: 76.33% C, 9.15% H; found: 76.52% C, 8.99% H.

(20*R*)-3 α ,7 α -Bis(methoxymethoxy)-5 α -pregnan-20-yl Benzoate (8)

To a solution of **7** (150 mg, 0.35 mmol) in dichloromethane (5 ml), *N,N*-diisopropylethylamine (0.36 ml, 2.1 mmol) and methoxymethyl chloride (0.16 ml, 2.1 mmol) were added and the mixture was stirred under argon at room temperature. After 30 h, ether (30 ml) was added and the organic layer was washed with aqueous citric acid (5%) and the solution of sodium hydrogen carbonate, and dried with magnesium sulfate. The solvents were evaporated and the product crystallized from ether. Compound **8** (130 mg, 72%), m.p. 69–72 °C (ether), $[\alpha]_D$ -57 (c 0.2). IR spectrum: 1 706 (C=O); 1 603 (arom); 1 284 (C–O); 1 144, 1 041, 1 097, 912 (C–O–C–O–C). 1 H NMR spectrum: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 5.14 dq, 1 H, J (17,20) \approx 10, J (20,21) \approx 6 (H-20); 4.69 and 4.62 AB system, 2 H, J (AB) = 6.7 (C(7)-OCH₂O); 4.64 s, 2 H (C(3)-OCH₂O); 3.83 quintet, 1 H, J = 2.5 (H-3); 3.61 q, 1 H, J = 2 (H-7); 3.38 s, 3 H (CH₃O); 3.36 s, 3 H (CH₃O); 1.27 d, 3 H, J = 6.2 (3 \times H-21); 1.03 s, 3 H (3 \times H-19); 0.66 s, 3 H (3 \times H-18). For $C_{32}H_{48}O_6$ (528.7) calculated: 72.69% C, 9.15% H; found: 72.47% C, 9.31% H.

(20*R*)-3 α ,7 α -Bis(methoxymethoxy)-5 α -pregnan-20-ol (9)

A solution of **8** (95 mg, 0.18 mmol) and sodium hydroxide (0.6 g, 15 mmol) in ethanol (20 ml) was heated on a water bath for 6 h. After dilution with water, compound **9** (73 mg, 96%) was isolated by filtration, m.p. 97–99 °C (methanol), $[\alpha]_D$ -52 (c 0.3). IR spectrum: 3 611 (O–H); 1 144, 1 041, 1 097, 909 (C–O–C–O–C). 1 H NMR spectrum: 4.70 and 4.63 AB system, 2 H, J (AB) = 6.7 (C(7)-OCH₂O); 4.64 s, 2 H (C(3)-OCH₂O); 3.83 quintet, 1 H, J = 2.5 (H-3); 3.73 dq, 1 H, J (17,20) = 10.1, J (20,21) = 6.0 (H-20); 3.61 q, 1 H, J = 2.2 (H-7); 3.39 s, 3 H (CH₃O); 3.36 s, 3 H (CH₃O); 1.14 d, 3 H, J = 6.0 (3 \times H-21); 0.81 s, 3 H (3 \times H-19); 0.75 s, 3 H (3 \times H-18). For $C_{25}H_{44}O_5$ (424.6) calculated: 70.72% C, 10.44% H; found: 70.95% C, 10.31% H.

3 α ,7 α -Bis(methoxymethoxy)-5 α -pregnan-20-one (10)

A solution of hydroxy derivative **9** (60 mg, 0.14 mmol) in benzene (5 ml) was stirred with pyridinium chlorochromate on aluminium oxide¹² (200 mg, 0.2 mmol) at room temperature. After 8 h, inorganic solids were filtered off and the filtrate was evaporated. Crystallization from ether afforded 57 mg

(95%) of compound **10**, m.p. 69–72 °C (ether–ligroin), $[\alpha]_D +5$ (*c* 0.3). IR spectrum: 1 699 (C=O); 1 144, 1 095, 1 043, 912 (C–O–C–O–C). ^1H NMR spectrum: 4.70 and 4.62 AB system, 2 H, J (AB) = 6.7 (C(7)-OCH₂O); 4.64 s, 2 H (C(3)-OCH₂O); 3.83 quintet, 1 H, J = 2.5 (H-3); 3.61 q, 1 H, J = 2 (H-7); 3.38 s, 3 H (CH₃O); 3.36 s, 3 H (CH₃O); 2.11 s, 3 H (3 \times H-21); 0.79 s, 3 H (3 \times H-19); 0.60 s, 3 H (3 \times H-18). For C₂₅H₄₂O₅ (422.6) calculated: 71.05% C, 10.02% H; found: 70.84% C, 9.92% H.

3 α ,7 α -Dihydroxy-5 α -pregnan-20-one (12) and 3 α -Hydroxy-7 α -(methoxymethoxy)-5 α -pregnan-20-one (11)

The protected diol **10** (50 mg, 0.12 mmol) in a mixture of benzene (10 ml) and methanol (10 ml) was treated with 2 M hydrochloric acid (0.8 ml) at 40 °C. After 6 h, ether (50 ml) was added and the solution was washed with the potassium hydrogen carbonate solution and water. The dried extract (anhydrous magnesium sulfate) was evaporated and purified by chromatography on a thin layer of silica gel (ligroin–ether 4 : 1). Compound **12** (32 mg, 66%) has a m.p. 148–150 °C (ether), $[\alpha]_D +38$ (*c* 0.7). IR spectrum: 3 615, 3 440 (O–H); 1 698 (C=O); 1 003, 1 029 (C–OH). ^1H NMR spectrum: 4.06 quintet, 1 H, J = 3 (H-3); 3.85 q, 1 H, J = 3 (H-7); 2.56 t, 1 H, J = 8.7 (H-17); 2.11 s, 3 H (3 \times H-21); 0.78 s, 3 H (3 \times H-19); 0.61 s, 3 H (3 \times H-18). For C₂₁H₃₄O₃ (334.5) calculated: 75.41% C, 10.25% H; found: 75.54% C, 10.12% H.

The less polar product from the above chromatography – compound **11** (14 mg, 32%) – has a m.p. 101–104 °C (ether–ligroin), $[\alpha]_D +31$ (*c* 0.3). IR spectrum: 3 615 (O–H); 1 699 (C=O); 1 148, 1 040, 1 094, 913 (C–O–C–O–C). ^1H NMR spectrum: 4.70 and 4.62 AB system, 2 H, J (AB) = 6.8 (C(7)-OCH₂O); 4.06 quintet, 1 H, J = 2.5 (H-3); 3.61 q, 1 H, J = 2 (H-7); 3.38 s, 3 H (CH₃O); 2.12 s, 3 H (3 \times H-21); 0.79 s, 3 H (3 \times H-19); 0.60 s, 3 H (3 \times H-18). For C₂₃H₃₈O₄ (378.6) calculated: 72.98% C, 10.12% H; found: 72.84% C, 9.99% H.

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