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Synthesis and structure elucidation of potential 6-oxygenated metabolites of (22*R*)-6α,9α-difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione, and related glucocorticosteroids

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Abstract

(22*R*)-6α,9α-Difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (rofleponide) is a synthetic glucocorticosteroid with high affinity for the rat thymus glucocorticoid receptor and a very high biotransformation rate demonstrated through incubation with a human liver S9 subcellular fraction. Because oxidation in the 6-position is an important metabolic pathway of glucocorticosteroids, the potential 6β-hydroxy and 6-oxo metabolites of rofleponide were synthesized to be used as reference compounds. Three alternative routes were used to reach the 6-hydroxy compound: (a) a one-step procedure involving allylic oxidation of rofleponide by selenium dioxide, (b) selenium dioxide oxidation of the corresponding 1,4-diene followed by selective 1,2-hydrogenation using Wilkinson's catalyst, and (c) autoxidation of a 3-methoxypregna-3,5-diene derivative. All three routes proceeded stereospecifically. Routes (a) and (c) gave approximately the same overall yield of the 6β-hydroxy epimer, whereas the overall yield from route (b) was much lower, primarily because of incomplete 1,2-hydrogenation. The 6-oxo compound was prepared through Pfitzner/Moffat oxidation of the 6-hydroxy compound. The stereochemistry of the 6-hydroxy substituent is discussed on the basis of ^{1}H-NMR spectroscopy and supplementary 2D NOESY experiments.

Keywords: Synthesis; Corticosteroids; Rofleponide 6-hydroxylation; Rofleponide 6-oxogenation; ^{1}H-NMR; 2D NOESY

1. Introduction

(22*R*)-6α,9α-Difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (rofleponide; Fig. 1) is a synthetic glucocorticosteroid suitable for local/topical therapy of diseases such as asthma, rhinitis, and inflammatory bowel disease [1]. The high affinity of rofleponide for the rat thymus glucocorticoid receptor [1] has implied its high anti-inflammatory potency, which has been further confirmed in vivo (unpublished data) in a model for Sephadex bead-induced inflammation and edema formation in the rat lung [2]. The high biotransformation rate of rofleponide has been demonstrated through incubation with human liver S9 subcellular fraction [1]. Rofleponide is expected to undergo metabolism e.g. through enzymatic hepatic oxidation in the 6-position similar to that of cortisol [3] and other endogenous steroids as well as several synthetic glucocorticosteroids [4–9]. Because, as a rule, 6-oxygenated metabolites have considerably lower glucocorticoid activities than the parent compounds [5,10,11], this biotransformation pathway is also expected to result in significant inactivation. Thus, the 6β-hydroxy (**7*R***) and 6-oxo (**12*R***) compounds were needed as reference substances for the proper identification and for the evaluation of glucocorticoid activitiy of the potential metabolites of rofleponide.

The present paper describes several routes for synthesis of (22*R*)-9α-fluoro-6β,11β,21-trihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**7*R***), the syntheses of some structurally related 6-hydroxylated glucocorticosteroids, and the synthesis of (22*R*)-9α-fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,6,20-trione (**12*R***).

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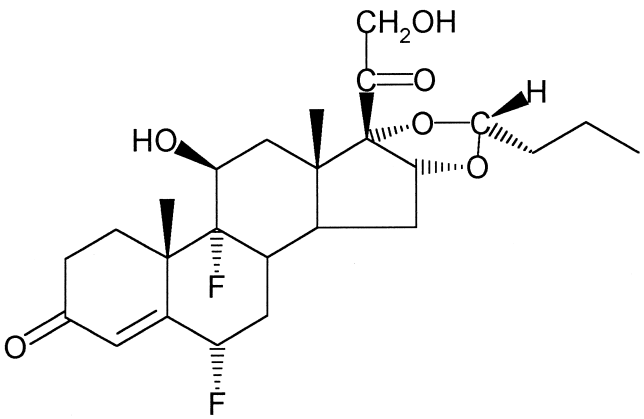


Fig. 1. Rofleponide.

2. Experimental

Triamcinolone(9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione) was purchased from SICOR S.p.A. (Milan, Italy).

^{1}H-NMR spectra were recorded with solutions prepared in $CDCl_3$ at ambient temperature on a 300 MHz Varian VXR-S spectrometer. The chemical shifts are given in δ units (ppm) relative to the internal standard tetramethylsilane; d = doublet, dd = double doublet, m = multiplet, q = quartet, and s = singlet. Supplementary 2D NOESY experiments were recorded on a 500 MHz Varian Unity+ spectrometer using the standard Varian 2D NOESY sequence. A mixing time of 400 ms and 256 increments were used, resulting, after zerofilling in f1, in a 2048 × 1024 2D data matrix. (The abbreviations used are: NOE = Nuclear Overhauser Effect; NOESY = Nuclear Overhauser Spectroscopy; 2D = 2 Dimensional; f1 = frequency dimension 1, indirect frequency dimension in 2D NOESY experiment).

Mass spectra were recorded on a Finnigan 4510 spectrometer with desorption chemical ionization (DCI) using methane as the reagent gas (direct inlet; filament current was increased at a rate of 10 mA/s). Alternatively, the mass spectra were obtained with liquid chromatography thermospray mass spectrometry (TSP-MS) on the same type of spectrometer equipped with a Finnigan thermospray interface. Mobile phase: 0.1M ammonium acetate buffer, pH 5, containing 70% methanol. Temperatures: Ion source 222°C and vaporizer 103°C. Repeller voltage: 90V.

Preparative column chromatography was performed on a Quickfit glass column equipped with adjustable Teflon end pieces. A LKB Uvicord I flow analyzer, working at 254 nm, served as the detection system. The effluent fractions were collected on a LKB 7000 Ultro Rac automatic fraction collector equipped with a LKB 3404 B siphon stand, using a 15 ml siphon. Sephadex LH-20, particle size 25–100 μm (Pharmacia Fine Chemicals, Uppsala, Sweden), was used as the stationary phase. All solvents used as the mobile phase were of *puriss* grade and glass distilled. The ethanol used in the mixed solvent system was 99.5% pure. Alternatively, the preparative column chromatography was carried out as flash chromatography on Merck Silica gel 60 (230–400 mesh) with manual fraction collection. Preparative, centrifugally accelerated, radial TLC was conducted on a Chromatotron apparatus from Harrison Research.

The HPLC-analyses were performed on a liquid chromatograph from Waters Associates equipped with a M6000A pump, a U6K injector system, and a M400 UV detector (240 nm). A column (150 × 4.6 mm I.D.), prepacked with 3 μm Apex octadecylsilane (Jones Chromatography), was used as the stationary phase, unless otherwise stated.

*2.1. 9α-Fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione (**2**)*

A suspension of 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione (**1**, 5.0 g) in absolute ethanol (500 ml) was added to a solution of tris(triphenylphosphine)rhodium chloride (3.0 g) in toluene (1000 ml) and hydrogenated at room temperature and atmospheric pressure for 48 h. The reaction mixture was evaporated to dryness and suspended in methylene chloride (50 ml). The solid was filtered and washed with small portions of methylene chloride, yielding 4.4 g (88%) of **2**. MS (DCI): m/z (relative intensity) 397 (MH^+; 18), 379 ($MH^+ - H_2O$; 15), 377 (MH^+ − HF; 22). ^{1}H-NMR (300 MHz): δ ppm (DMSO-d_6) 0.83 (s, 3H, H-18); 1.49 (s, 3H, H-19); 4.13 (m, 1H, H-11); 4.11 and 4.53 (two q, 2H, H-21); 5.41 (d, 1H, H-16); 5.68 (s, 1H, H-4).

*2.2. (22RS)-9α-Fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**3**)*

Freshly distilled butanal (815 mg) and **2** (2.96 g) were added to a solution of p-toluenesulfonic acid (870 mg) in acetonitrile (40 ml). The reaction mixture was stirred at room temperature under argon protection for 3 h. A saturated aqueous solution of sodium hydrogen carbonate (2 ml) was added to stop the reaction. Methylene chloride (800 ml) was added, and the solution was washed with a saturated aqueous solution of sodium hydrogen carbonate and water to neutral, dried with sodium sulfate, and evaporated. The residue was purified on a Sephadex LH-20 column (75 × 6.3 cm i.d.) using chloroform as the mobile phase. The fraction 2310–3105 ml was collected and evaporated. The residue was precipitated from methylene chloride − petroleum ether yielding 2.66 g (79%) of compound **3**. The purity determined by HPLC-analysis (ethanol/water, 42/58) was 98%, and the 22*R*/22*S* ratio was 60/40. MS (DCI): m/z 451 (MH^+; most abundant ion). ^{1}H-NMR (300 MHz): δ ppm 0.90 and 0.96 (two s, 3H, H-18); 0.92 and 0.94 (two t, 3H, H-25); 1.53 (s, 1H, H-19); 4.38 (m, 1H, H-11); 4.21, 4.27, 4.54 and 4.65 (four q, 2H, H-21); 4.60 and 5.24 (two t, 1H, H-22); 4.92 and 5.20 (two d, 1H, H-16); 5.80 (m, 1H, H-4).

*2.3. (22R)- and (22S)-21-Acetoxy-9α-fluoro-11β-hydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**4R** and **4S**)*

A solution of compound **3** (2.66 g) and acetic anhydride (25 ml) in freshly distilled and dry pyridine (25 ml) was stirred at room temperature for 1 h. The reaction mixture was poured into ice water (150 ml) and extracted with methylene chloride (3 × 100 ml). The extract was serially washed with hydrochloric acid (1M), saturated aqueous sodium hydrogen carbonate, and water. The residue obtained after evaporation of the dried extract was purified by chromatography on a Sephadex LH-20 column (75 × 6.3 cm i.d.) using chloroform as the mobile phase. The fraction 1350–1950 ml was collected, leaving a 22*R*/22*S*-mixture. The C-22 epimers were separated on a Sephadex LH-20 column (76 × 6.3 cm i.d.) with a mixture of heptane/chloroform/ethanol (20/20/1) as the mobile phase.

After evaporation and precipitation from methylene chloride − petroleum ether fraction 1500–1815 ml left 900 mg (31%) of the 22*S*-epimer **4*S***. The purity determined by HPLC (ethanol/water, 50/50) was 97%. MS (DCI): m/z 493 (MH^+; most abundant ion). ^{1}H-NMR (300 MHz): δ ppm 0.94 (t, 3H, H-25); 0.97 (s, 3H, H-18); 1.54 (s, 3H, H-19); 2.18 (s, 3H, C**H**$_3$COO); 4.38 (m, 1H, H-11); 4.78 and 4.94 (two d, 2H, H-21); 5.16 (d, 1H, H-16); 5.24 (t, 1H, H-22); 5.80 (m, 1H, H-4).

After evaporation and precipitation from methylene chloride − petroleum ether fraction 1920–2175 ml left 1180 mg (41%) of the 22*R*-epimer **4*R***. The purity determined by HPLC as above was 98.8%. MS (DCI): m/z 493 (MH^+; most abundant ion). ^{1}H-NMR (300 MHz): δ ppm 0.93 (s, 3H, H-18); 0.94 (t, 3H, H-25); 1.54 (s, 3H, H-19); 2.18 (s, 3H, C**H**$_3$COO); 4.38 (m, 1H, H-11); 4.66 (t, 1H, H-22); 4.76 and 4.92 (two d, 2H, H-21); 4.87 (d, 1H, H-16); 5.80 (m, 1H, H-4).

*2.4. (22R)-21-Acetoxy-9α-fluoro-11β-hydroxy-3-methoxy-16α,17α-propylmethylenedioxypregna-3,5-dien-20-one (**5R**)*

A solution of **4*R*** (848 mg) in methylene chloride (13 ml), dry dioxane (6 ml), trimethyl orthoformate (0.8 ml), and absolute methanol (0.05 ml) was chilled in an ice-water bath. Concentrated sulfuric acid (two drops) was added. After 8 min, the reaction was terminated by the addition of pyridine (eight drops). The reaction mixture was concentrated at reduced pressure to 1/3 of its volume. Water (10 ml) was added, and the mixture was extracted with ethyl ether (4 × 10 ml). The combined extracts were washed with water (3 × 15 ml), dried over sodium sulfate, and evaporated. The residue was purified by flash chromatography on a silica gel column (30 × 2 cm i.d.) with ethyl acetate/heptane (30/70) as the mobile phase. The fraction 100–180 ml was collected and evaporated yielding 610 mg (70%) of **5*R***. The purity determined by HPLC (ethanol/water, 60/40) was 95.6%. ^{1}H-NMR (300 MHz): δ ppm 0.92 (s, 3H, H-18); 0.93 (t, 3H, H-25); 1.28 (s, 3H, H-19); 2.18 (s, 3H, C**H**$_3$COO); 3.58 (s, 3H, OCH_3); 4.40 (m, 1H, H-11); 4.68 (t, 1H, H-22); 4.80 and 4.91 (two d, 2H, H-21); 4.88 (d, 1H, H-16); 5.17 (d, 1H, H-4); 5.22 (t, 1H, H-6).

*2.5. (22R)-21-Acetoxy-9α-fluoro-6β,11β-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**6R**)*

A solution of **5*R*** (600 mg) in ethanol (25 ml) was stirred and exposed to the sun for 10 h. The reaction mixture was evaporated, and the residue purified by flash chromatography on a silica gel column (28 × 2 cm i.d.) using ethyl acetate/heptane (50/50) as the mobile phase, leaving 323 mg (54%) of **6*R***. The purity determined by HPLC (ethanol/water, 45/55) was 98.6%. MS: m/z (relative intensity) 509 (MH^+; 100); 491 ($MH^+ - H_2O$; 10) (MS-FAB). ^{1}H-NMR (300 MHz): δ ppm 0.94 (t, 3H, H-25); 0.94 (s, 3H, H-18); 1.72 (s, 3H, H-19); 2.18 (s, 3H, C**H**$_3$COO); 4.39 (broad m, 2H, H-6 and H-11); 4.66 (t, 1H, H-22); 4.74 and 4.94 (two d, 2H, H-21); 4.87 (d, 1H, H-16); 5.90 (s, 1H, H-4).

*2.6. (22R)-21-Acetoxy-9α-fluoro-6β,11β-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**6R**)*

A stirred suspension of **4*R*** (25 mg) and selenium dioxide (15 mg) in dioxane (5 ml) was heated at 100°C for 2 h. The reaction mixture was chilled to room temperature and filtered through a short column (3 × 2 cm i.d.) of Silica gel 60. The column was washed with dioxane (25 ml). The eluate was evaporated, and the residue purified by flash chromatography on a silica gel column (12 × 2 cm i.d.) with ethyl acetate/heptane (80/20) as the mobile phase. The fractions 25–39 ml (A) and 40–90 ml (B) were collected.

After evaporation, fraction A left 6 mg of solid material, which was shown by ^{1}H-NMR spectroscopy to be the starting material **4*R***. ^{1}H-NMR of the solid obtained after evaporation of fraction B was identical to compound **6*R*** prepared by autoxidation of **5*R***. Yield: 10 mg (39%). The purity determined by HPLC (ethanol/water, 50/50) was 88%.

*2.7. (22R)-9α-Fluoro-6β,11β,21-trihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**7R**)*

A solution of **6*R*** (278 mg) in methanol (30 ml) was agitated with a stream of nitrogen for a few minutes. A solution of 10% aqueous potassium carbonate (2 ml) was added. After stirring for 45 min at room temperature with nitrogen protection, the reaction mixture was neutralized with acetic acid and evaporated. After addition of water (50 ml), the residue was extracted with methylene chloride (4 × 50 ml). The combined extracts were washed with water, dried over sodium sulfate, and evaporated yielding 174 mg

of solid material. This crude product was applied to a short silica gel column (6 × 2 cm i.d.) and eluted with a mixture of ethyl acetate/heptane, 70/30, (100 ml). After evaporation, the residue was further purified by flash chromatography on a silica gel column (28 × 2 cm i.d.) with ethyl acetate/heptane (70/30) as the mobile phase. The fraction 240–400 ml was collected and evaporated yielding 113 mg (44%) of **7*R***. The purity determined by HPLC (10 μm μBondapak C_{18} column, (Waters Associates), 150 × 3.9 mm i.d.; ethanol/water, 35/65) was 98.6%. M.p. 125–129°C. MS: m/z (relative intensity) 467 (MH^+; 100); 449 ($MH^+ - H_2O$; 9) (MS–FAB). ^{1}H-NMR (300 MHz): δ ppm 0.91 (t, 3H, H-25); 0.94 (s, 3H, H-18); 1.72 (s, 3H, H-19); 4.28 and 4.54 (two q, 2H, H-21); 4.40 (m, 2H, H-6 and H-11); 4.61 (t, 1H, H-22); 4.93 (d, 1H, H-16); 5.90 (s, 1H, H-4).

*2.8. (22RS)-9α-Fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**8**)*

The title compound was prepared from 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione (5.0 g) and butanal (1.4 g) and purified as previously described [12]. Yield: 5.0 g (88%).

*2.9. (22R)-9α-Fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**8*R*)*

Compound **8** (3.0 g) was resolved by chromatography on a Sephadex LH-20 column (76 × 6.3 cm i.d.) with a mixture of heptane/chloroform/ethanol (20/20/1) as the mobile phase [13]. The fraction 10 005–10 815 ml was collected and evaporated, yielding 1.32 g of **8*R***. The purity determined by HPLC (ethanol/water, 42/58) was 98%.

*2.10. (22R)-21-Acetoxy-9α-fluoro-11β-hydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**9*R*)*

A solution of acetyl chloride (310 ml) in dioxane (7.5 ml) was added to a solution of compound **8*R*** (450 mg) in freshly distilled and dry pyridine (15 ml), and the reaction mixture stirred at room temperature overnight. The volume was reduced to 5 ml, methylene chloride (25 ml) was added, and the solution was serially washed with hydrochloric acid (1M), aqueous sodium hydrogen carbonate solution (10%), and saturated aqueous sodium chloride solution. The dried organic phase was evaporated, and the residue was purified on a Sephadex LH-20 column (87 × 2.5 cm i.d.) with chloroform as the mobile phase. The fraction 305–350 ml was collected and evaporated. The residue was precipitated from methylene chloride – petroleum ether yielding **9*R*** (319 mg; 65%). The purity determined by HPLC (ethanol/water, 55/45) was 99.1%. MS: m/z 491 (MH^+) (MS-FAB). ^{1}H-NMR (300 MHz): δ ppm 0.92 (t, 3H, H-25); 0.96 (s, 3H, H-18); 1.55 (s, 3H, H-19); 2.19 (s, 3H, C**H**$_3$COO); 4.43 (m, 1H, H-11); 4.65 (t, 1H, H-22); 4.72 and 4.92 (two d, 2H, H-21); 4.87 (d, 1H, H-16); 6.14 (m, 1H, H-4); 6.33 and 6.37 (two d, 1H, H-2); 7.20 (d, 1H, H-1).

*2.11. (22R)-21-Acetoxy-9α-fluoro-6β,11β-dihydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**10*R*)*

A stirred suspension of **9*R*** (286 mg) and selenium dioxide (555 mg) in dioxane (25 ml) was heated at 100°C for 72 h. The reaction mixture was chilled to room temperature and filtered through a short column (5 × 2 cm i.d.) of Silica gel 60. The column was washed with dioxane. The volume of the combined eluates was reduced to a few millilitres, and the residue was suspended in ethyl acetate (50 ml), filtered, and evaporated leaving 330 mg of crude product. This product was purified by flash chromatography on a silica gel column (15 × 2 cm i.d.) with ethyl acetate/heptane (60/40) as the mobile phase. The fractions 15–20 ml (A) and 120–345 ml (B) were collected and evaporated yielding 88 mg of starting material **9*R*** and 130 mg of **10*R***, respectively. The latter product was further purified by flash chromatography on a silica gel column (21 × 2 cm i.d.) with ethyl acetate/heptane (60/40) as the mobile phase. The fraction 180–450 ml was collected and evaporated, yielding 109 mg (37%) of **10*R***. The purity determined by HPLC (ethanol/water, 48/52) was 93.7%. MS: m/z 507 (MH^+; most abundant ion) (MS–FAB). ^{1}H-NMR (300 MHz): δ ppm 0.92 (t, 3H, H-25); 0.97 (s, 3H, H-18); 1.74 (s, 3H, H-19); 2.19 (s, 3H, C**H**$_3$COO); 4.46 (m, 1H, H-11); 4.56 (m, 1H, H-6); 4.65 (t, 1H, H-22); 4.71 and 4.95 (two d, 2H, H-21); 4.86 (d, 1H, H-16); 6.22 (d, 1H, H-4); 6.34 (dd, 1H, H-2); 7.20 (d, 1H, H-1).

*2.12. (22R)-21-Acetoxy-9α-fluoro-6β,11β-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**6*R*)*

A suspension of tris(triphenylphosphine)rhodium chloride (30 mg) in toluene (15 ml) was treated with hydrogen for 30 min. A solution of **10*R*** (25 mg) in absolute ethanol (15 ml) was added, and the hydrogenation was continued at room temperature and atmospheric pressure for 48 h. The reaction mixture was evaporated, and the residue was dissolved in ethyl acetate/heptane (80/20), applied to a short silica gel column (4 × 2 cm i.d.), and eluted with the same solvent mixture. The eluent was evaporated leaving 25 mg of solid material, which by analytical HPLC, was shown to contain two products whose retention volumes corresponded to the starting material (**10*R***) and the desired compound **6*R*** in the ratio 70/30. The presence of the 1,4-diene and the 4-ene in the mixture was confirmed by the following characteristic resonances in ^{1}H-NMR (300 MHz): δ ppm 1.74 (s, 3H, H-19), 4.56 (m, 1H, H-6), 6.22 (d, 1H, H-4), 6.34 (dd, 1H, H-2) and 7.20 (d, 1H, H-1) for **10*R*** and 1.72 (s, 3H, H-19), 4.39 (m, 2H, H-6 and H-11) and 5.90 (s, 1H, H-4) for **6*R***.

*2.13. (22R)-21-Acetoxy-9α-fluoro-11β-hydroxy-16α,17α-propylmethylenedioxpregn-4-ene-3,6,20-trione (**11R**)*

Pyridine (60 μl), trifluoroacetic acid (28 μl), and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulphonate (1.03 g) were added to a solution of **6*R*** (370 mg) in dimethylsulfoxide (1.2 ml) and toluene (2.4 ml). The mixture was stirred for 18 h at room temperature under nitrogen protection, poured into hydrochloric acid (10%, 6 ml), and extracted with methylene chloride four times. The combined extracts were washed with water and saturated aqueous sodium chloride solution, dried over sodium sulfate, and evaporated, leaving 384 mg of solid. This crude product was purified by radial TLC on silica with ethyl acetate/heptane (1/1) as the mobile phase, yielding 258 mg (70%) of **11*R***. The purity determined by HPLC (ethanol/water, 48/52) was 94%. MS: m/z 506 (M^+; most abundant ion) (field desorption due to low stability with other techniques). ^{1}H-NMR (300 MHz): δ ppm 0.94 (s, 3H, H-18); 0.94 (s, 3H, H-25); 1.51 (s, 3H, H-19); 2.20 (s, 3H, C**H**$_3$COO); 4.44–4.52 (broad m, 1H, H-11); 4.67 (t, 1H, H-22); 4.72 and 4.96 (two d, 2H, H-21); 4.88 (d, 1H, H-16); 6.39 (s, 1H, H-4).

*2.14. (22R)-9α-Fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,6,20-trione (**12R**)*

Aqueous potassium carbonate (10%, 0.5 ml) was added to a solution of **11*R*** (247 mg) in methanol (25 ml). The reaction mixture was stirred at room temperature for 20 min under nitrogen protection, neutralized with acetic acid, and extracted with methylene chloride (4 × 15 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated. The crude solid (210 mg) was purified by radial TLC on silica with ethyl acetate/heptane (70/30), yielding 126 mg (56%) of **12*R***. The purity determined by HPLC (ethanol/water, 48/52) was 95.4%. MS (TSP - MS): m/z 465 (MH^+; most abundant ion). ^{1}H-NMR (300 MHz): δ ppm 0.92 (s, 3H, H-18); 0.94 (t, 3H, H-25); 1.51 (s, 3H, H-19); 4.29 and 4.55 (two q, 2H, H-21); 4.44 − 4.54 (broad m, 1H, H-11); 4.62 (t, 1H, H-22); 4.94 (d, 1H, H-16); 6.39 (s, 1H, H-4).

*2.15. (22R)-21-Acetoxy-6α,11β-dihydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**14R**)*

A stirred suspension of (22*R*)-21-acetoxy-11β-hydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**13*R***; 218 mg) and selenium dioxide (150 mg) in dioxane (25 ml) was heated at 100°C for 72 h. The reaction mixture was chilled to room temperature and filtered through a short column (3.5 × 2 cm i.d.) of Silica gel 60. The column was washed with dioxane (30 ml). The filtrate was evaporated, and the residue was dissolved in a mixture of ethyl acetate and heptane (50/50, 50 ml), applied to a dry column of silica gel (13 × 2 cm i.d.), and eluted with ethyl acetate/heptane (50/50). The fractions 25–160 ml (A) and 185–590 ml (B) were collected and evaporated. Fraction A (140 mg) was identified as starting material.

Fraction B yielded 67 mg (30%) of **14*R*** contaminated with 10% of the corresponding 6β-OH derivative (**15*R***), as determined by HPLC (ethanol/water, 48/52). MS of **14*R***: m/z (relative intensity) 489 (MH^+; 100); 488 (M^+; 82) (field desorption due to low stability with other techniques). ^{1}H-NMR (300 MHz) of **14*R***: δ ppm 0.93 (t, 3H, H-25); 0.95 (s, 3H, H-18); 1.43 (s, 3H, H-19); 2.18 (s, 3H, C**H**$_3$COO); 4.51 (m, 1H, H-11); 4.57 (broad m, 1H, H-6); 4.62 (t, 1H, H-22); 4.73 and 4.90 (two d, 2H, H-21); 4.86 (d, 1H, H-16); 6.31 (two d, 1H, H-2); 6.41 (d, 1H, H-4); 7.22 (d, 1H, H-1).

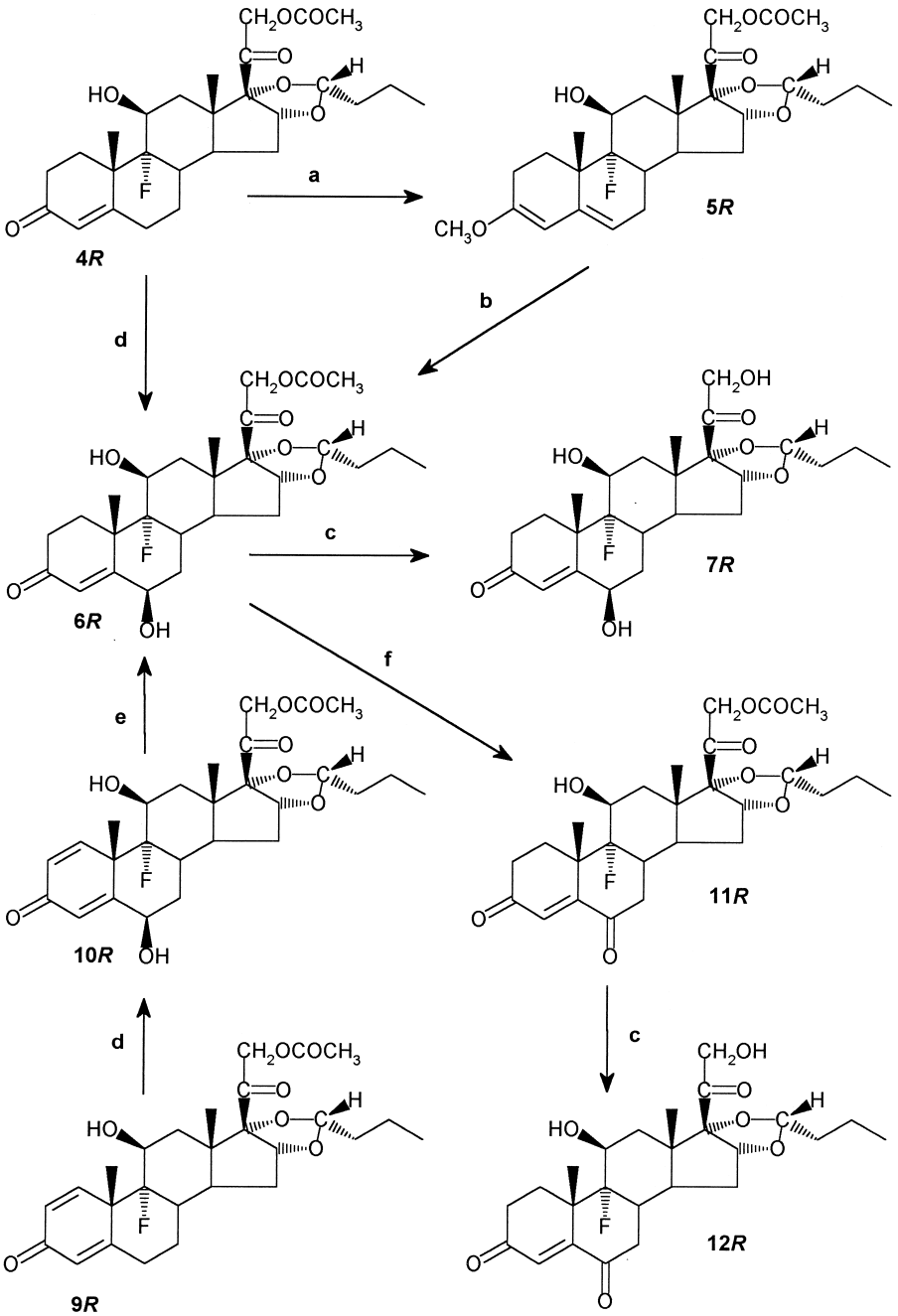
3. Results and discussion

3.1. Chemical synthesis

Autoxidation or peracid oxidation of 3-alkoxy-3,5-dienes followed by 1,2-dehydrogenation and acetalization have been used for 6-hydroxylation of triamcinolone 16α,17α-acetonide [14] and budesonide [15] for example. When such oxidations were applied to the 3-methoxy-3,5-diene derivative of 16α-hydroxyhydrocortisone 16α,21-diacetate (**16**), mixtures of the 6α- and 6β-hydroxy epimers were obtained. The ratio of 6α-/6β-epimers was changed from 43/57 to 84/16 when autoxidation was replaced by peracid oxidation [15]. The 6α- and 6β-hydroxy derivatives were subjected to chromatographic separation and used as starting material in the preparation of 6α- and 6β-hydroxybudesonides [15], respectively. The main objective of the present study was to develop a simple and particularly stereospecific reaction pathway to introduce a 6β-hydroxy substituent into rofleponide.

It is known that olefins are oxidized at the allylic position with selenium dioxide [16]. This procedure was recently successfully applied to hydroxylation of some 1,4-dien-3-one steroids, whereas selenium dioxide oxidation of the corresponding 4-en-3-ones resulted in considerably lower yields [17].

(22*R*)-21-Acetoxy-9α-fluoro-11β-hydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**4*R***) and the corresponding 1,4-diene (**9*R***) were both oxidized with selenium dioxide in dioxane (Scheme 1). The hydroxylations occurred stereospecifically to produce single isomers of the 6β-hydroxy derivatives **6*R*** and **10*R***. The pregn-4-ene **4*R*** was oxidized much more rapidly than the more stable 1,4-diene **9*R*** and to an unexpectedly high yield [17]. Attempts to further improve the yield of **6*R*** through prolongation of the reaction time resulted in complete conversion of **4*R*** but also in degradation, so that no desired product **6*R*** could be detected. An alternative pathway to synthesis of compound **6*R*** was based on selective 1,2-hydrogenation of the 1,4-dien-3-one **10*R***. However, the overall yield of **6*R*** was much lower with this route than by direct selenium dioxide oxi-

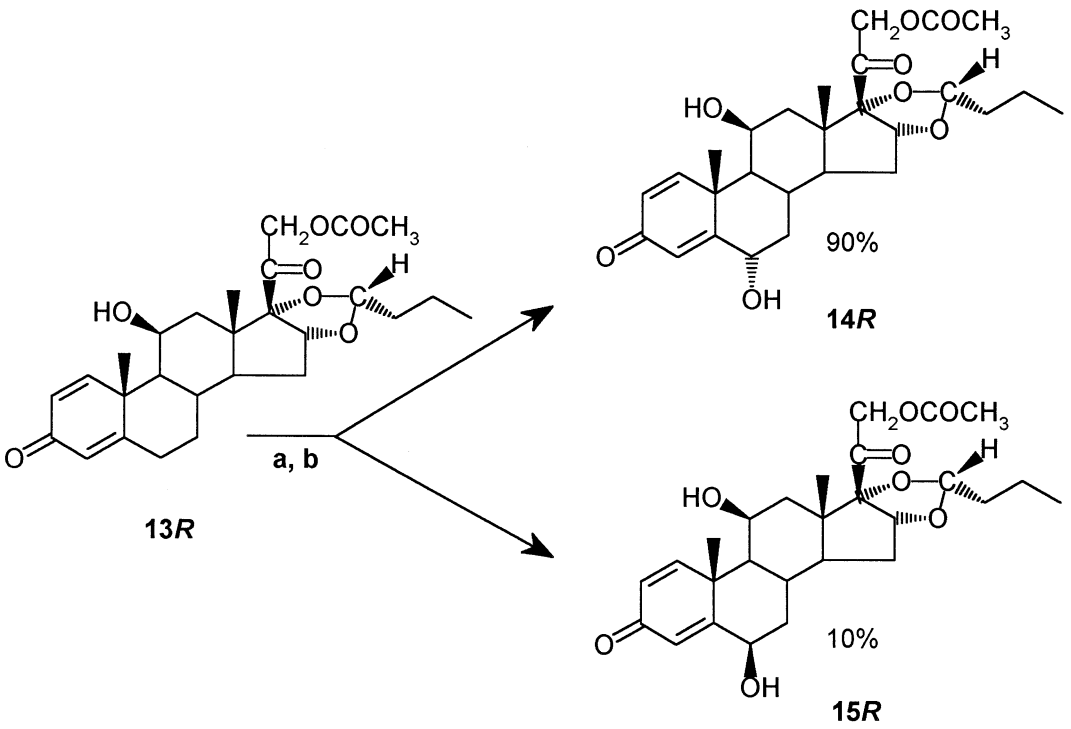


Scheme 1. Synthetic pathways to (22*R*)-9α-fluoro-6β,11β,21-trihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**7*R***) and (22*R*)-9α-fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,6,20-trione (**12*R***). (a) $HC(OCH_3)_3/CH_2Cl_2$/dioxane; (b) autoxidation/EtOH; (c) K_2CO_3/MeOH; (d) SeO_2/dioxane; (e) $[(C_6H_5)_3P]_3RhCl/H_2$; (f) Moffat oxidation.

dation of the pregn-4-en-3-one **4*R*** because of an unexpectedly slow oxidation of **9*R*** and incomplete hydrogenation of **10*R*** with Wilkinson's catalyst.

Therefore, it was of interest to evaluate if allylic oxidation of budesonide 21-acetate with selenium dioxide, followed by ester hydrolysis, could offer a simpler and simultaneously stereospecific synthetic route to 6β-hydroxybudesonide than that previously used [15]. However, such an oxidation applied to the 22*R*-epimer of budesonide 21-acetate (**13*R***) yielded 30% of a mixture consisting of 90% of the 6α-hydroxy (**14*R***) and 10% of the 6β-hydroxy (**15*R***) epimers (Scheme 2). This agrees well with the orientation of the 6-hydroxy group observed on allylic oxidation of prednisolone derivatives [17]. The 9α,11β-halohydrins all produced the 6β-alcohols as the single isomer, whereas the 6α-isomer was formed in the case of prednisolone itself, which has no substituent at the 9-position.

The substitution mode at the 9-position was found to also influence the orientation of the 6-hydroxy group introduced



Scheme 2. Allylic oxidation of the 22*R*-epimer of budesonide 21-acetate (**13*R***). (a) SeO_2/dioxane; (b) separation of the 6α- and 6β-hydroxy epimers in the oxidation product by analytical HPLC.

through autoxidation of the 3-methoxy-3,5-diene derivative of 16α-hydroxycortisol 16α,21-diacetate (**16**) [15] as well as that of compound **5*R***. In the former case, a mixture of the 6α- and 6β-epimers was formed. On the other hand, autoxidation of compound **5*R***, which carries a 9α-fluoro substituent, proceeded stereospecifically to produce the single 6β-hydroxy epimer **6*R*** (Scheme 1). This reaction pathway gave approximately the same overall yield of **6*R*** as selenium dioxide oxidation of **4*R*** did.

Analogous to the autoxidation of steroid Δ^5-enes, e.g. cholest-5-en-3-one [18,19], the autoxidation of steroid $\Delta^{3,5}$-dien-3-ol ethers to 6-hydroxy Δ^4-3-ketones was suggested to proceed via 6-hydroxyperoxides [20]. This was confirmed by the discovery of minor amounts of Δ^4-ene-3,6-diones and Δ^4-ketones as by-products. It is reasonable to assume that 6- hydroxylation of **5** and **16** through autoxidation also proceeds via hydroperoxides. The stereochemistry at the 6-position is probably dependent on the side from which the singlet oxygen attacks the C_5 - C_6 double bond. An attack on this bond from the α-side of the 9α-fluoro substituted compound **5** is less likely than an attack from the β-side attributable to electronic rather than steric reasons [21]. On the other hand, the attack of singlet oxygen from the α-side is equally probable as from the β-side of compound **16**, which lacks a substitution at the 9α-position. This may explain the different stereoselectivity noticed on autoxidation of **5** and **16**.

The 6-hydroxyl group in compound **6*R*** was selectively oxidized using the Pfitzner/Moffatt procedure [22] to yield the 3,6,20-trione 21-acetate **11*R*** (Scheme 1). Removal of the protecting 21-acetate group through hydrolysis with potassium carbonate then gave the corresponding 21-alcohol **12*R***, which is another potential metabolite of rofleponide.

3.2. C-6 stereochemical analysis

^{1}H-NMR analysis has been used to differentiate between the 6α- and 6β-hydroxy epimers of budesonide and its 21-acetate. The chemical shifts of the C-4 and C-19 protons were found to have characteristic features of the α- compared with the β-configuration of the 6-hydroxy substituent (Table 1) [15]. A *cis* 1,3-diaxial interaction with the 6β-hydroxy group gives a chemical shift of the C-19 protons signal at δ 1.68 ppm, compared with δ 1.48 in budesonide. The 6α-hydroxy epimer of budesonide is characterized by a strong (0.43 ppm) downfield shift of the C-4 proton signal caused by a paramagnetic anisotropy effect from the polar 6α-substituent. Analogous to this, the chemical shifts of the C-4 and C-19 protons in the ^{1}H-NMR spectra of compounds **6*R***, **7*R***, and **10*R*** (Table 1) indicate that they are 6β-hydroxy derivatives of the corresponding parent compounds **4*R***, **3**, and **9*R***, respectively. On the other hand, the compound **14*R***, resulting from selenium dioxide oxidation of the 22*R*-epimer of budesonide 21-acetate, seems to have a 6α-hydroxy configuration, according to this analysis (Table 1).

A more direct proof of the stereochemistry at C-6 is derived

Table 1
Partial ^{1}H-NMR spectra[a]

Compound	^{1}H-Assignments	
	4-H	19-CH_3
Budesonide (BUD)[b]	6.12 m	1.48 s
6α-Hydroxy-BUD, epimer 22*R*[b]	6.55 m	1.47 s
6β-Hydroxy-BUD, epimer 22*R*[b]	6.25 m	1.68 s
21-Acetoxy-BUD[b]	6.10 m	1.47 s
21-Acetoxy-6α-hydroxy-BUD, epimer 22R (**14*R***)	6.43 m[b]	1.45 s[b]
21-Acetoxy-6β-hydroxy-BUD, epimer 22R (**15*R***)	6.17 m[b]	1.67 s[b]
3	5.80 m	1.53 s
4*R*	5.80 m	1.54 s
6*R*	5.90 m	1.72 s
7*R*	5.90 m	1.72 s
9*R*	6.14 m	1.55 s
10*R*	6.22 m	1.74 s
14*R*	6.41 m	1.43 s

[a] Chemical shifts are given in ppm relative to the internal standard tetramethylsilane in $CDCl_3$.
[b] From Ref. [15].

from consideration of the NOE's between the C-4 and C-19 protons and the remaining 6-proton of the 6-hydroxy compounds. It has been shown for the 22*R*-epimer of budesonide that the C-4 proton gives a NOE to the equatorial proton at the 6-position (6α-H), but not to the axial proton (6β-H). Simultaneously, there is a strong NOE between the C-19 protons and the axial proton in the 6β-position, but not with the 6-equatorial proton (Gunnar Grönberg, unpublished data).

Analyses of the 2D NOESY spectra of compounds **7*R*** and **10*R*** show a strong NOE between the C-4 proton and the remaining proton in the 6-position, but no NOE between this proton and the C-19 protons. Accordingly, the 6-hydroxy substituent in these compounds has the β-orientation. The same type of analysis performed on the 2D NOESY spectrum of the main component (**14*R***) after 6-hydroxylation of the 22*R*-epimer of budesonide 21-acetate (**13*R***) reveals that there is a strong NOE between the remaining proton in the 6-position and the C-19 protons, but not between the C-4 and C-6 protons. This confirms that selenium dioxide oxidation of **13*R*** predominantly yields the 6α-hydroxy derivative (**14*R***).

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