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6α-Fluoro- and 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-propylmethylene-dioxypregn-4-ene-3,20-dione: Synthesis and evaluation of activity and kinetics of their C-22 epimers

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It is generally accepted that the anti-inflammatory effect of glucocorticosteroids cannot be separated from their adverse effects at the receptor level. However, modification of the pharmacokinetics through structural alterations could provide steroids with a better therapeutic index than those currently used. Thus, new 16α,17α-acetals between butyraldehyde and 6α-fluoro- or 6α,9α-difluoro-16α-hydroxycortisol were synthesized and studied. Acetalization of the corresponding 16α,17α-diols or transacetalization of their 16α,17α-acetonides in dioxane produced mixtures of C-22 epimers, which were resolved by preparative chromatography. Alternatively, an efficient method was used to produce the 22R-epimer stereoselectively through performing the acetalization and transacetalization in a hydrocarbon with an inert material present. The C-22 configuration of (22R)-6α,9α-difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione was unambiguously established by single crystal X-ray diffraction. The present compounds, especially the 22R-epimer just mentioned, bind to the rat thymus glucocorticoid receptor with high potency. The C-22 epimers of the 6α,9α-difluoro derivatives showed a 10-fold higher biotransformation rate than the budesonide 22R-epimer when incubated with human liver S9 subcellular fraction. The high receptor affinity in combination with the high biotransformation rate indicates that (22R)-6α,9α-difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione may be an improved 16α,17α-acetal glucocorticosteroid for therapy of inflammatory diseases, in which the mucous membranes are involved, such as those in the intestinal tract as well in the respiratory tract. (*Steroids* **63**:37–43, 1998) © *1998 by Elsevier Science Inc.*

Keywords: glucocorticosteroid 16α,17α-acetals; synthesis; receptor affinities; biotransformation

Introduction

Since the discovery that the endogenous glucocorticosteroid (GC) cortisone relieved the symptoms of rheumatoid arthritis,[1] GCs have been used in the therapy of many inflammatory and immunologic diseases. A search has been in progress during the subsequent decades for new GCs with reduced adverse effects like osteoporosis and hypothalamic–pituitary–adrenal (HPA) axis blockade. With respect to systemic therapy, only minor progress has been made, due to the fact that both therapeutic and adverse effects are mediated through the same GC receptor.[2] For local/topical GC therapy, modification of the local and systemic pharmacokinetics has been a successful mode. Within dermatology, great advances were already achieved in the 1950s and 60s, since the restricted and slow absorption of GCs through the stratum corneum barrier reduces unwanted systemic activity. For mucous membranes, where the absorption is more complete and rapid, it was later learned that enhanced hepatic first-pass inactivation is a key requirement for achieving topical selectivity.[3] For the therapy of diseases such as asthma, rhinitis, and inflammatory bowel disease, an optimal GC also needs to have a high affinity for the GC receptor, for example, to compensate for the great dilution of the drug on the large airway surfaces. The present paper describes the synthesis and basic pharmacologic effects of new GCs, in which the combination of a few structural modifications favors both higher receptor affinity and enhanced hepatic inactivation rate.

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Received 19 May 1997; accepted 10 September 1997.

Steroids 63:37–43, 1998

655 Avenue of the Americas, New York, NY 10010

0039-128X/98/$19.00
PII S0039-128X(97)00107-4

Experimental

Compounds **5** (6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione) and **6** (6α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3, 20-dione) were purchased from SICOR S.p.A. (Milan, Italy). Dexamethasone and [6,7-^{3}H]-dexamethasone were purchased from Sigma Chemical Company (Sweden) and New England Nuclear Corporation (Boston, Massachusetts, USA), respectively.

^{1}H-NMR spectra were recorded as solutions in $CDCl_3$ at ambient temperature on a Varian VXR-300 spectrometer at 300 MHz. The chemical shifts are given in δ units (ppm) relative to the internal standard tetramethylsilane; d = doublet, dd = doublet of doublets, m = multiplet, q = quartet, dq = doublet of quartets, s = singlet, and t = triplet.

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: 0.1 M ammonium acetate buffer, pH 5, containing 70% methanol. Temperatures: Ion source 222°C and vaporizer 103°C. Repeller voltage: 90 V.

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.

Preparative HPLC was performed on a liquid chromatograph from Waters Associates with a type 590 Programmable HPLC Pump, a type 170 Sample Loader, and a type 484 Tunable Absorbance Detector working at 280 nm. A column (250 × 22.5 mm I.D.), prepacked with Apex Prepsil ODS, 8 μm, (Jones Chromatography Ltd) was used as the stationary phase.

The HPLC analyses were performed on a liquid chromatograph from Waters Associates involving a M 6000A pump, an U6K injector system, and a M 400 UV detector (240 nm). A column (150 × 4.6 mm I.D.), prepacked with 3 μm Apex octadecylsilane (Jones Chromatography Ltd), was used as the stationary phase.

Melting points were determined on a Leitz, Wetzlar, hot stage microscope. Optical rotations were measured with a Perkin Elmer model 241 polarimeter.

*6α,9α-Difluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione (**2**)*

A solution of tris(triphenylphosphine)rhodium chloride (1.13 g) in toluene (400 mL) was hydrogenated at atmospheric pressure for 20 min. A suspension of 6α,9α-difluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione[4] (**1**; 1.0 g) in ethanol (250 mL) was added. The reaction mixture was hydrogenated for 24 h at room temperature and atmospheric pressure. After evaporation, the residue was suspended in chloroform (10 mL) and filtered. The solid product was washed with several small portions of chloroform yielding 0.83 g (83%) of **2**. The purity determined by HPLC analysis (acetonitrile/water, 17:83 v/v) was 95.7%. M.p. 244–254°C. MS: m/z (relative intensity) 415 (MH^+; 14), 397 ($MH^+ - H_2O$; 12), 395 ($MH^+ - HF$; 17). ^{1}H-NMR (300 MHz): δ ppm (CD_3OD) 0.99 (s, 3H, H-18), 1.60 (s, 3H, H-19), 4.32 and 4.69 (dd, 2H, H-21), 4.29 (m, 1H, H-11), 4.97 and 5.00 (dd, 1H, H-16), 5.36 and 5.52 (two m, 1H, H-6), and 6.03 (s, 1H, H-4).

*(22R)- and (22S)-6α,9α-Difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**3a** and **3b**)*

6α,9α-Difluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione (**2;** 1.8 g) was added in small portions to a solution of freshly distilled butanal (470 mg) and perchloric acid (70%; 0.4 mL) in purified and dried dioxane[5] (100 mL) with stirring over 30 min. The reaction mixture was left at ambient temperature with continued stirring for another 5 h. Methylene chloride (600 mL) was added, the solution was washed with aqueous potassium carbonate (10%) and water, and dried over anhydrous sodium sulfate. The crude product obtained after evaporation was purified on a Sephadex LH-20 column (71 × 6.3 cm I.D.), using chloroform as the mobile phase. The fraction 3015–3705 mL was collected and evaporated. The residue (epimeric mixture **3**) was resolved into its C-22-epimers on a Sephadex LH-20 column (76 × 6.3 cm I.D.), using a heptane/chloroform/ethanol mixture (20:20:1 v/v) as the mobile phase. The fractions 8845-9565 mL (A) and 9745-10600 mL (B) were collected and evaporated. The residues were dissolved in methylene chloride and precipitated with petroleum ether (b.p. 40–60°C).

Fraction A gave 337 mg (17%) of the 22S-epimer **3b**. The purity determined by HPLC analysis (acetonitrile/water, 35:65 v/v) was 95.7%; m.p. 231–234°C; $[\alpha]_D^{25} = +84°$ (c = 0.096; CH_2Cl_2). MS: m/z (relative intensity) 469 (MH^+; 100), 397 ($MH^+ - CH_3CH_2CH_2CHO$; 3.6), 379 ($MH^+ - CH_3CH_2CH_2CHO - H_2O$; 12.7). ^{1}H-NMR (300 MHz; $CDCl_3$): δ ppm 0.93 (t, 3H, H-25); 0.96 (s, 3H, H-18); 1.51 (s, 3H, H-19); 4.23 and 4.64 (two q, 2H, H-21); 4.39 (broad m, 1H, H-11); 5.20 (d, 1H, H-16); 5.25 (t, 1H, H-22); 5.14–5.38 (two m, 1H, H-6); 6.14 (s, 1H, H-4).

Fraction B gave 923 mg (45%) of the 22R-epimer **3a**. The purity determined by HPLC analysis (cf. fraction A above) was 98.8%; m.p. 150–156°C; $[\alpha]_D^{25} = +120°$ (c = 0.190; CH_2Cl_2). MS: m/z (relative intensity) 469 (MH^+; 100), 397 ($MH^+ - CH_3CH_2CH_2CHO$; 12.3), 379 ($MH^+ - CH_3CH_2CH_2CHO - H_2O$; 25.0). ^{1}H-NMR (300 MHz; $CDCl_3$); δ ppm 0.89 (s, 3H, H-18); 0.94 (t, 3H, H-25); 1.52 (s, 3H, H-19); 4.26 and 4.53 (two q, 2H, H-21); 4.40 (broad m, 1H, H-11); 4.61 (t, 1H, H-22); 4.92 (d, 1H, H-16); 5.13–5.38 (two m, 1H, H-6); 6.14 (s, 1H, H-4).

*(22R)-6α,9α-Difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**4**)*

A. Compound **4** was prepared in heptane from 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione **(5)** and butanal. The reaction was catalyzed by perchloric acid in the presence of fine sand, as previously described.[6] The purity determined by HPLC analysis (acetonitrile/water, 35:65 v/v) was 98.7%. M.p. 169–172°C; $[\alpha]_D^{25} = +94.5°$ (c = 0.170; CH_2Cl_2). MS: mz (relative intensity) 467 (MH^+; 100). ^{1}H-NMR (300 MHz; $CDCl_3$): δ ppm 0.91 (s, 3H, H-18); 0.91 (t, 3H, H-25); 1.53 (s, 3H, H-19); 4.26 and 4.53 (two q, 2H, H-21); 4.42 (m, 1H, H-11); 4.59 (t, 1H, H-22); 4.92 (d, 1H, H-16); 5.26–5.52 (two m, 1H, H-6); 6.37 and 6.40 (two d, 1H, H-2); 6.44 (d, 1H, H-4); 7.10 and 7.14 (two d, 1H, H-1).

B. Compound **4** was prepared in heptane from 6α,9α-difluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione **(1)** and butanal. The reaction was catalyzed by perchloric acid in the presence of fine sand, as previously described.[6] The purity determined by HPLC analysis (acetonitrile/water, 35:65 v/v) was 98.9%. MS: m/z (relative intensity) 467 (MH^+; 100).

*(22R)-6α,9α-Difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxy-pregn-4-ene-3,20-dione (**3a**)*

A solution of (22R)-6α,9α-difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregna-1,4-diene-3,20-dione (**4**; 4.0 g) and tris(triphenylphosphine)rhodium chloride (0.40 g) in absolute ethanol (150 mL) was hydrogenated at ambient temperature and 50 psi for 68 h. Water (150 mL) was added, and the mixture was filtered through a 0.45 μm Teflon filter. Water (220 mL) was added to the filtrate, and the mixture was stirred for 2 h. The precipitate formed was filtered and dried, leaving 2.94 g (73%) of crude **3a** (A). Most of the ethanol was evaporated from the mother liquor. The residue was extracted with methylene chloride, and the extract was dried and evaporated, leaving 0.97 g of a solid (B), heavily contaminated with catalyst and containing **3a** mixed with 13% of nonhydrogenated **4**.

The crude product A from above was purified on a Sephadex LH-20 column (75 × 6.3 cm I.D.) using chloroform as the mobile phase. The fraction 3600–4200 mL was collected and evaporated, yielding 2.23 g (55%) of **3a**. The purity determined by HPLC analysis (ethanol/water, 48:52 v/v) was 97.3%. MS: m/z (relative intensity) 469 (MH^+; 100).

*6α-Fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (**7**)*

A suspension of tris(triphenylphosphine)rhodium chloride (2.1 g) in toluene (500 mL) was hydrogenated at ambient temperature and atmospheric pressure for 45 min when the catalyst was in solution. A solution of 6α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (**6;** 2.0 g) in absolute ethanol (1000 mL) was added, and the hydrogenation was continued until the ^{1}H-NMR signals for the 1,2-double bond hydrogens had disappeared (65 h). The reaction mixture was subsequently evaporated. The residue was dissolved in chloroform and eluated through a small column packed with Sephadex LH-20 to remove most of the catalyst. After concentration, the residue was further purified by running it twice through a Sephadex LH-20 column (71 × 6.3 cm I.D.), using chloroform as the mobile phase. The fraction 2010–2445 mL was collected and evaporated, yielding 1.51 g (76%) of solid **7**. The purity determined by HPLC analysis (acetonitrile/water, 35:65 v/v) was 94.3%. A 500 mg portion of this product was further purified on a Sephadex LH-20 column with heptane/chloroform/ethanol, 20:20:1 v/v, as the mobile phase, yielding 420 mg of **7** with 99.6% purity (HPLC analysis as above). M.p. 209–219°C; $[\alpha]_D^{25}$ = +133° (c = 0.23; CH_2Cl_2). MS: m/z (relative intensity) 437 (MH^+; 100), 417 ($MH^+ - HF$; 8.4), 379 ($MH^+ - (CH_3)_2CO$; 8.2), 361 ($MH^+ - (CH_3)_2CO - H_2O$; 8.0). ^{1}H-NMR (300 MHz; $CDCl_3$): δ ppm 0.87 (s, 3H, H-18), 1.15 and 1.42 (s and s, resp., 6H, acetonide), 1.46 (s, 3H, H-19); 4.18 and 4.68 (two q, 2H, H-21); 4.49 (m, 1H, H-11); 5.07 (d, 1H, H-16); 5.09–5.31 (broad m, 1H, H-6); 6.03 (m, 1H, H-4).

*(22R)- and (22S)-6α-Fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**8a** and **8b**)*

A. 6α-Fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (**7**; 22 mg) was added to a solution of freshly distilled butanal (11 mg) in conc. hydrochloric acid (0.5 mL) chilled to 0°C. The reaction mixture was left at 0°C with stirring for 2 h. Methylene chloride (25 mL) was added, the mixture was washed with 10% aqueous potassium carbonate and saturated aqueous sodium chloride, and dried over anhydrous sodium sulfate. After evaporation, a quantitative yield of (22RS)-6α-fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione **(8)** was obtained. The purity determined by HPLC analysis (ethanol/water, 42:58 v/v) was 94.7%, and the epimeric ratio 22R/22S was 90/10.

B. 6α-Fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (**7;** 110 mg) was added to a solution of freshly distilled butanal (55 mg) in conc. hydrochloric acid (12 mL) at −15°C. The reaction mixture was stirred at −10°C to −15°C for 2 h. Methylene chloride (25 mL) was added, and the mixture was washed with 10% aqueous potassium carbonate and saturated aqueous sodium chloride to neutralize the reaction. The organic phase was separated, dried over anhydrous magnesium sulfate and evaporated. A quantitative yield of (22RS)-6α-fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione **(8)** was obtained. The purity determined by HPLC analysis (ethanol/water, 42:58 v/v) was 96.8% (1,2% unreacted 16α,17α-acetonide), and the epimeric ratio 22R/22S was 55/45.

The epimeric mixture **8** (110 mg) was resolved by preparative HPLC (mobile phase ethanol/water, 42:58 v/v). Two fractions were collected and evaporated.

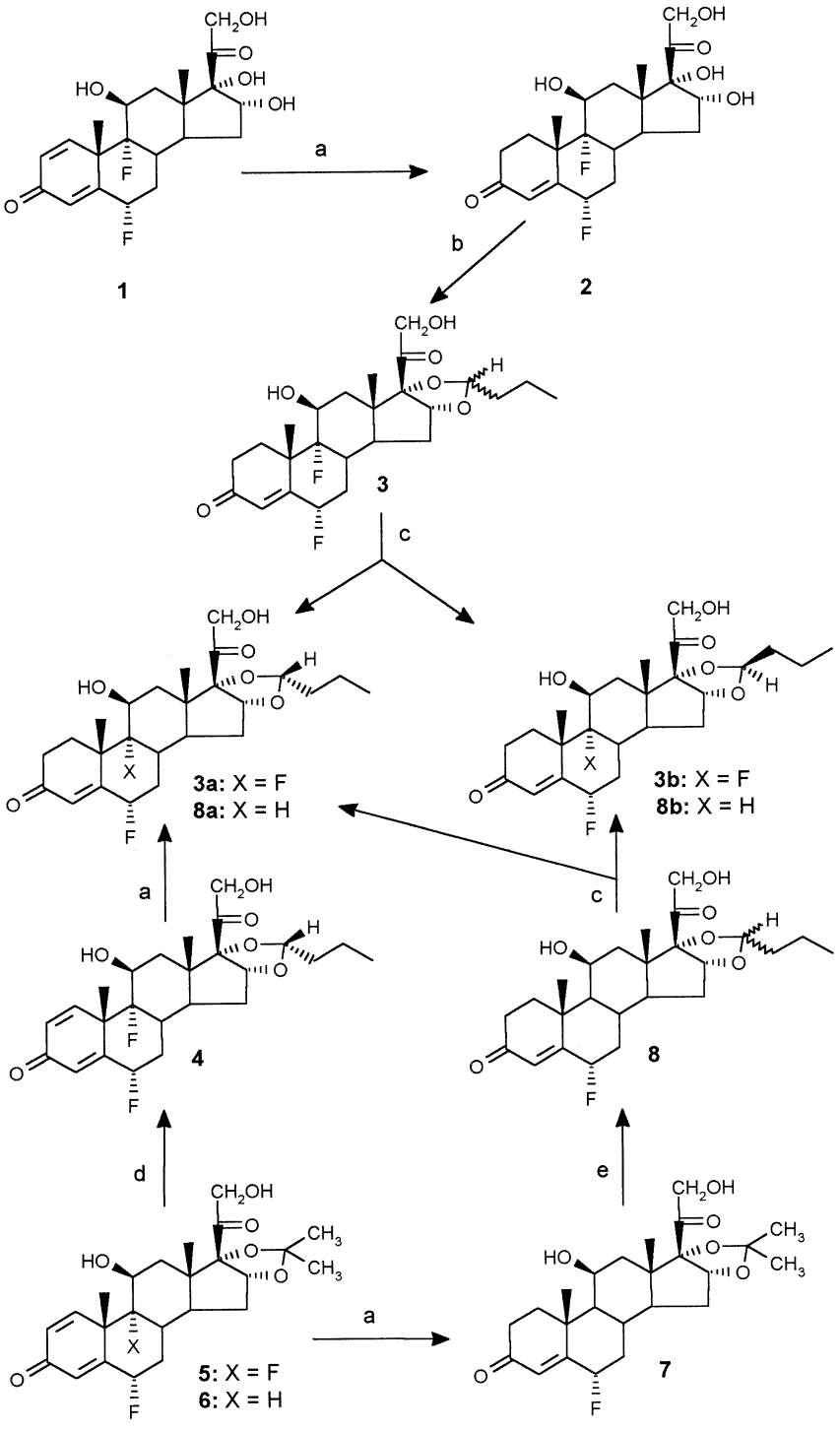
Fraction 730–820 mL gave 44 mg (39%) of the 22R-epimer **8a**. The purity was 99.1% (HPLC analysis as above); m.p. 180–192°C; $[\alpha]_D^{25}$ = +138.9° (c = 0.144; CH_2Cl_2). MS: m/z (relative intensity) 451 (MH^+; 100), 379 ($MH^+ - CH_3CH_2CH_2CHO$; 4), 361 ($MH^+ - CH_3CH_2CH_2CHO - H_2O$; 12). ^{1}H-NMR (300 MHz; $CDCl_3$): δ ppm 0.90 (s, 3H, H-18); 0.94 (t, 3H, H-25); 1.43 (s, 3H, H-19); 4.26 and 4.52 (dd, 2H, H-21); 4.49 (m, 1H, H-11); 4.92 (d, 1H, H-16); 4.57 (t, 1H, H-22); 5.07–5.32 (two m, 1H, H-6); 6.03 (s, 1H, H-4).

Fraction 895–1025 mL gave 30 mg (27%) of the 22S-epimer **8b**. The purity was 96.7% (HPLC analysis); m.p. 168–175°C; $[\alpha]_D^{25}$ = +103.7° (c = 0.26; CH_2Cl_2). MS: m/z (relative intensity) 451 (MH^+; 100), 379 ($MH^+ - CH_3CH_2CH_2CHO$; 4), 361 ($MH^+ - CH_3CH_2CH_2CHO - H_2O$; 12). ^{1}H-NMR (300 MHz; $CDCl_3$): δ ppm 0.93 (t, 3H, H-25); 0.97 (s, 3H, H-18); 1.42 (s, 3H, H-19); 4.22 and 4.63 (dq, 2H, H-21); 4.48 (broad m, 1H, H-11); 5.08–5.32 (two m, 1H, H-6); 5.19 and 5.21 (dd, 1H, H-16); 5.21 (t, 1H, H-22); 6.03 (s, 1H, H-4).

Pharmacological evaluation

GC receptor affinity assay

Thymus glands from male Sprague–Dawley rats, 1–2 months of age, were removed and put into ice-cold saline. The tissue was homogenized in a Potter Elveheim homogenizer with 10 mL of buffer containing 20 mM Tris, pH 7.4; 10% (w/v) glycerol; 1 mM EDTA; 20 mM $NaMoO_4$; and 10 mM β-mercaptoethanol. The homogenate was centrifuged for 15 min at 20,000 × *g*. Portions of the 20,000 × *g* supernatant (230 μL) were incubated for about 24 h at 0°C with 20 μL unlabeled competitor and 50 μL [6,7-^{3}H]-dexamethasone (final concentration ~3 nM). The supernatants were also incubated with a) [6,7-^{3}H]-dexamethasone alone, b) [6,7-^{3}H]dexamethasone plus 1,000 fold excess of unlabeled dexamethasone, and c) [6,7-^{3}H]dexamethasone plus 0.03–300 fold "excess" of competitor. Bound and free steroid were separated by incubating the mixture with 60 μL 2.5% (w/v) charcoal and 0.25% (w/v) dextran T70 suspension in 20 mM Tris, pH 7.4, 1 mM EDTA, and 20 mM $NaMoO_4$ for 10 min at 0°C. Following centrifugation at 500 × *g* for 10 min, 230 μL of the supernatant were counted in 10 mL Insta-Gel in a Packard scintillation spectrophotometer (Packard Instrument Company). Nonspecific binding was determined when 1,000 fold excess of unlabeled



Scheme 1 Synthetic pathways to (22R)- and (22S)-6α,9α-difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**3a** and **3b**, respectively) and (22R)- and (22S)-6α-fluoro-11β,21-dihydroxy-16α ,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**8a** and **8b**, respectively).
a, $[(C_6H_5)_3P]_3RhCl/H_2$;
b, $CH_3CH_2CH_2CHO/HClO_4$/ dioxane;
c, chromatography;
d, $CH_3CH_2CH_2$ $CHO/HClO_4$/heptane;
e, $CH_3CH_2CH_2CHO/HCl$.

dexamethasone was added to [6,7-^{3}H]dexamethasone. The competitors were dissolved in ethanol, and the dilutions were made in homogenization buffer containing 40% ethanol. The final concentration of ethanol in the incubation mixture was 2.5%. The 50% specific binding level for each competitor was normalized by comparison with budesonide, which was assigned a relative binding affinity (RBA) of 1.

Pharmacokinetic evaluation

Biotransformation rate in human liver

To study the biotransformation rate, compounds **3a** and **3b** were separately incubated with human liver S9 subcellular (microsomal plus cytosol) fraction (3 mg protein/mL) at

37°C and in the presence of a NADPH generating system. For comparison, the 22R-epimer of budesonide was also incubated under the same conditions. The incubation concentration of the steroids was 5 μmol/L. Human livers (HL 42, 44, 45, and 48) were obtained from the liver bank at Huddinge Hospital, Huddinge, Sweden.[7] The liver samples were pooled prior to preparation of the S9 fraction. At different incubation time points, the steroids were extracted on Sep-Pak C_{18} cartridges. The unmetabolized steroids were then quantified by liquid chromatography. The procedure and the HPLC system used have been described and discussed in detail previously.[8]

Results and discussion

Chemical synthesis

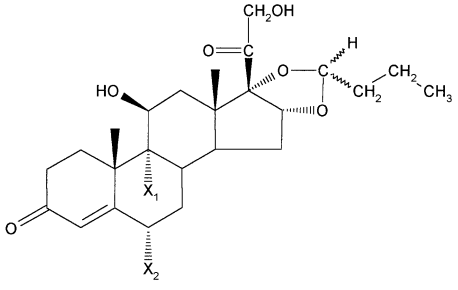
The 1,4-diene-3-ones were converted into 4-ene-3-ones by selective hydrogenation with Wilkinson's catalyst. However, when the lipophilic, non-symmetric 16,17-acetals were hydrogenated, the soluble catalyst was difficult to remove from the product even with chromatography. If the corresponding 16,17-diols were hydrogenated, the low solubility of the product and the high solubility of the catalyst, e.g. in methylene chloride or chloroform, could be utilized to efficiently separate the catalyst from the product. Hydrogenation of the commercially available corresponding 16,17-acetonides, offered an alternative reaction pathway to the 4-ene-3-one non-symmetric 16,17-acetals, due to favorable solubility differences in chlorinated hydrocarbons between the reaction products and trace amounts of the rhodium catalyst.

Acetalization of 16α,17α-diols and transacetalization of 16α,17α-acetonides were the two alternative routes used for preparation of the non-symmetric 16α,17α-acetals (Scheme 1). When a 16α,17α-diol is reacted with an aldehyde in dioxane and catalyzed with perchloric acid, a mixture of the two conceivable C-22 epimers is obtained.[4] The 22R- and 22S-epimers could be separated by preparative chromatography on Sephadex LH-20[9] or by preparative HPLC. Thus, the acetalization of **2** with butanal followed by resolution on Sephadex LH-20 gave the 22R- and 22S-epimers **3a** and **3b**, respectively.

The 22-epimers can be prepared stereoselectively by acetalization of a 16α,17α-diol or by transacetalization of a 16α,17α-acetonide with a hydrocarbon as the reaction solvent, using a method developed at the Astra pilot plant laboratory.[6] The reaction between butanal and the diol **1** in heptane, where the diol is practically insoluble (<1 mg/liter), yielded the 22R-epimer **4** nearly selectively. The same stereoselectivity was reached when the diol **1** was replaced by the 16α,17α-acetonide **5**, yielding **4**. In these heterogeneous reactions, the supposed steroid-catalyst complex forms a big sticky lump. By the addition of small grains of an inert material, e.g., sand, the steroid-catalyst complex is distributed around the grains, which facilitates the stirring and increases the reaction rate.

A less stereoselective transacetalization of the 16α,17α-acetonides with an appropriate aldehyde can be performed in concentrated hydrochloric acid. The ratio between the 22R- and 22S-epimers in the reaction product was highly sensitive to the reaction temperature. Transacetalization of 1,2-dihydroflunisolide **(7)** with butanal gave a 9:1 22R/22S-mixture (**8a**/**8b**) at ~0°C but a nearly 1:1 mixture at −10° to −15°C. The reaction mixtures left crude products of ≥95% purity.

Table 1 Relative binding affinities (RBA) for the rat thymus GC receptor (budesonide assigned the value 1)



Compound	X_1	X_2	22-epimer	RBA ± SEM
Budesonide[1]	H	H	R,S	1
3a	F	F	R	1.68 ± 0.08
3b	F	F	S	1.27 ± 0.06
8a	H	F	R	0.99 ± 0.09
8b	H	F	S	1.02 ± 0.13
Dexamethasone				0.16 ± 0.01

[1]Unsaturated in 1,2-position.

Structure analysis

Structure analysis was performed with ^{1}H-NMR spectroscopy. Characteristic shift differences for the C-16, C-18, and C-22 protons were observed between the C-22 epimers. In (22R)-6α,9α-difluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione **(3a)**, the C-22 protons appeared as a triplet at 4.61 ppm and the C-16 protons as a doublet at 4.92 ppm. The 22S-epimer **3b**, on the other hand, showed a triplet at 5.25 ppm for the C-22 proton which overlaped with the C-16 doublet. There was also a characteristic shift of the C-18 singlet from 0.89 ppm in the 22R-epimer to 0.96 ppm in the 22S-epimer. This was in good agreement with the corresponding signals observed in ^{1}H-NMR of the 22R- and 22S-epimers of budesonide,[10] whose absolute configurations at C-22 have been established with single crystal X-ray diffraction analyses.[11] Similarly, ^{1}H-NMR spectroscopy was used to make an initial differentiation between the 22R- and 22S-epimers of 6α-fluoro-11β,21-dihydroxy-16α,17α-propylmethylenedioxypregn-4-ene-3,20-dione (**8a** and **8b,** respectively). The assignment of the configuration at C-22 of **3a** was confirmed by single crystal X-ray diffraction studies (unpublished data).

Affinities for the cytosolic glucocorticoid receptor

Glucocorticosteroids act by binding to and activating a specific cytosolic receptor. The GC-receptor complex trans-

locates to the nucleus and affects specific DNA-dependent, RNA-mediated protein synthesis. The proteins formed induce the physiological effects seen with GC use.[12,13] Binding of GCs to the GC receptor is a prerequisite for these effects to occur. Therefore, the ability of GCs to bind to their receptors could be a method to compare the biological potency of different GCs in a GC sensitive cell type. A direct structure–activity relationship has been shown between receptor affinity for GCs and their ability to inhibit uridine incorporation into mouse thymocytes.[14] Furthermore, a direct correlation has been shown between the affinity of GCs for their receptor and their anti-inflammatory activity using the ear edema test in the rat.[15] The ligand specificity of the GCs does not vary between man and rat[16] or between tissues of a given species.[17] Because the GC receptor from rat thymus is well characterized, and a correlation to efficacy also is seen there (in the files of Astra Draco), it was selected for determination of receptor affinities in the present study. The full agonistic properties of the steroids have been verified in in vitro and in vivo models (unpublished data).

Ligand binding to the GC receptor is largely hydrophobic in nature.[18] The hydrophobicity of a 16α,17α-acetal substituent is responsible for the increased affinity of budesonide, for instance compared to its parent compound 16α-hydroxyprednisolone,[15] and compensates for the negative influence of the 16α- and 17α-hydroxy substituents in this molecule.[18] The configuration at the C-22 chiral center in non-symmetrical 16,17-acetals, such as that present in budesonide, is essential for the magnitude of the receptor affinity.[15] The 22R-epimer of budesonide had a higher affinity to the receptor than the 22S-epimer. In accordance with this, the two C-22 epimers, **3a** and **3b** had different relative binding affinities to the receptor, and the 22R-epimer **3a** bound more effectively than the 22S-epimer **3b** (Table 1). On the other hand, no differences in RBA were observed between the two C-22 epimers **8a** and **8b**.

Simultaneous fluorination at the 6α- and 9α-positions increased the affinity of the 22R- as well as the 22S-epimers, **3a** and **3b**, respectively, for the GC receptor compared to the corresponding 6α-fluoro substituted 22-epimers. This is contrary to the observations made for the 9α-fluoro and 6α,9α-difluoro substituted 22R-epimers of budesonide.[15]

Since other parameters such as penetration, distribution, and metabolic transformations are of major importance, the results of the receptor affinity studies will be supplemented by in vivo studies to delineate the true pharmacological activities of the individual compounds.

Biotransformation rates

The disappearance of **3a, 3b,** and the budesonide 22R-epimer during incubation with the human liver S9 subcellular fraction is shown in Figure 1. No disappearance occurred during incubation with the denaturated (heated at 65°C) S9 fraction, which suggests that the disappearance was entirely due to enzymatic degradation. The disappearance half lives were calculated as 3.3 (**3a**), 4.8 (**3b**), and 36 min (budesonide 22R-epimer). Thus, the new GC derivatives were biotransformed 10-fold more rapidly in human liver than budesonide was, emphasizing the effect of the 6α,9α-difluoro substitution rather than removal of the 1,2-double bond.

A previous study[8] with 16α,17α-acetal GCs confirms that the biotransformation rate of Δ^4-enes increases only marginally compared to the $\Delta^{1,4}$-dienes, while the rate increases several fold when a 6α,9α-difluoro substitution is

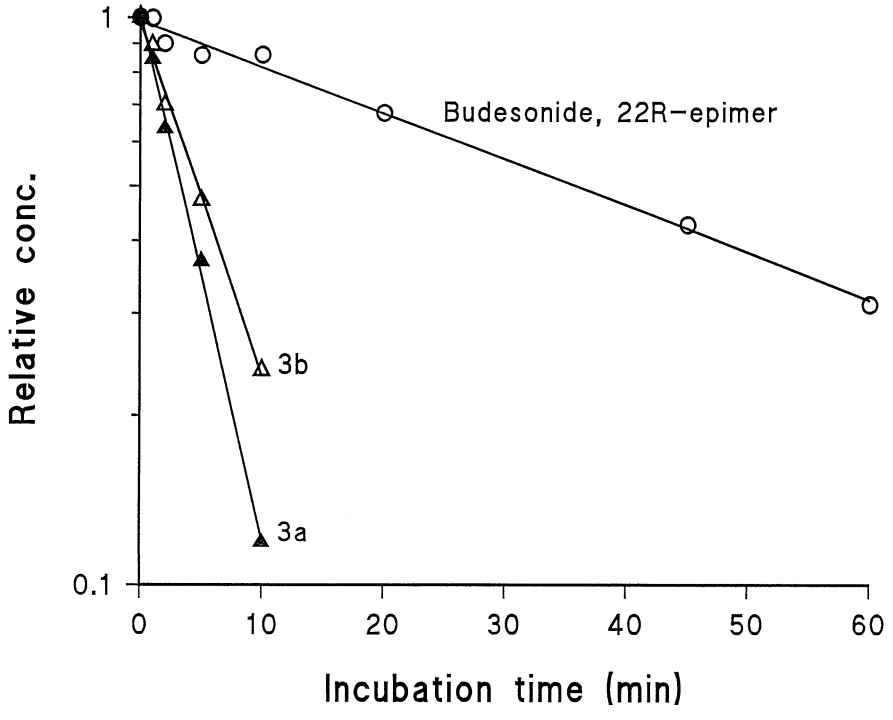


Figure 1 The metabolic disappearance of **3a, 3b**, and the 22R-epimer of budesonide during incubation in human liver S9 subcellular (microsomal + cytosol) fraction.

introduced. The metabolic transformation reactions are principally oxidative, catalyzed by cytochrome P450. They will be published separately.

The rapid metabolism rates of **3a** and **3b** suggest that in vivo these compounds may possess a high hepatic extraction ratio in man, and as a consequence of this, a low bioavailability after oral administration.

Conclusion

In this initial study, 22R- and 22S-epimers of 16α,17α-acetals between butanal and B-ring fluorosubstituted 16α-hydroxycortisols have been synthesized and tested for their affinities to the GC receptor. The human liver biotransformation rates of the two most potent derivatives **3a** and **3b** prompt systematic in vivo studies. A favorable therapeutic index, especially of compound **3a,** is expected from the in vitro results. Thus, compound **3a** should be advantageous in the therapy of inflammatory diseases in which the mucous membranes are involved, such as those in the intestinal and respiratory tracts.

References

1. Hench PS, Kendall EC, Slocumb CH, Polley HF (1949). The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone; compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis. Preliminary report. *Proc Staff Meet Mayo Clin* **24**:181–197.
2. Portner M, Mollmann HJ, Rohdewald P (1988). Glucocorticoid receptors in synovial tissue and relative receptor affinities of glucocorticoid-21-esters. *Pharm Res* **5**:623–627.
3. Morrow Brown H (1980). The introduction and early development of inhaled steroid treatment. In: Mygind N, Clark TJH (eds). *Topical Steroid Treatment for Asthma and Rhinitis.* Baillière Tindall, London, pp. 66–76.
4. Thalén A, Brattsand R, Gruvstad E (1984). Synthesis and pharmacological properties of some 16α,17α-acetals of 16α-hydroxyhydrocortisone, 16α-hydroxyprednisolone and fluorinated 16α-hydroxyprednisolones. *Acta Pharm Suec* **21**:109–124.
5. Hess K, Frahm H (1988). Über darstellung und eigenschaften von reinem dioxan. *Ber* **71**:2627–2636.
6. Jakupovic E, Sourander R, Stenhede U, Svensson P (1987). A method of controlling the epimeric distribution in the preparation of 16,17-acetals or ketals of pregnane derivatives. *European Patent* **262 108**.
7. von Bahr C, Groth CG, Jansson H, Lundgren G, Lind M, Glauman H (1980). Drug metabolism in human liver in vitro: Establishment of a human liver bank. *Clin Pharmacol Therapeutics* **27**:711–725.
8. Andersson P, Lihné M, Thalén A, Ryrfeldt Å (1987). Effects of structural alterations on the biotransformation rate of glucocorticoids in rat and human liver. *Xenobiotica* **17**:35–44.
9. Thalén A, Nylander B (1982). Epimers of budesonide and related corticosteroids. I. Preparative resolution by chromatography on Sephadex LH-20. *Acta Pharm Suec* **19**:247–266.
10. Thalén A (1987). Epimers of budesonide and related corticosteroids. III. Synthesis and structure elucidation by carbon-13 and proton nuclear magnetic resonance spectroscopy. *Acta Pharm Suec* **24**:97–114.
11. Albertsson J, Oskarsson Å, Svensson C (1978). X-ray study of budesonide: Molecular structures and solid solutions of the (22S) and (22R) epimers of 11β,21-dihydroxy-16α,17α-propylmethylenedioxy-1,4-pregnadiene-3,20-dione. *Acta Cryst* **B34**:3027–3036.
12. Feldman D, Funder JW, Edelman IS (1972). Subcellular mechanisms in the action of adrenal steroids. *Am J Med* **53**:545–560.
13. Barnes PJ, Adcock I (1993). Anti-inflammatory action of steroids: molecular mechanisms. *Trends Pharmacol Sci* **14**:436–441.
14. Dausse JP, Duval D, Meyer P (1977). The relationship between glucocorticoid structure and effects upon thymocytes. *Mol Pharmacol* **13**:948–955.
15. Dahlberg E, Thalén A, Brattsand R, Gustafsson J-Å, Johansson U, Roempke K, Saartok T (1984). Correlation between chemical structure, receptor binding, and biological activity of some novel, highly active, 16α,17α-acetal substituted glucocorticoids. *Mol Pharmacol* **25**:70–78.
16. Manz B, Grill H-J, Pollow K (1982). Steroid side-chain modification and receptor affinity: Binding of synthetic derivatives of corticoids to human spleen tumor and rat liver glucocorticoid receptors. *J Steroid Biochem* **17**:335–342.
17. Svec F, Rudis M (1982). Interactions of antiglucocorticoids with glucocorticoid receptors. *J Steroid Biochem* **16**:135–140.
18. Wolff ME, Baxter JD, Kollman PA, Lee DL, Kuntz ID, Bloom E, Matulich DT, Morris J (1978). Nature of steroid-glucocorticoid receptor interactions: Thermodynamic analysis of the binding reaction. *Biochemistry* **17**:3201–3208.