Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 3 October 2007, Revised 29 October 2007, Accepted 1 November 2007 Published online 21 January 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1480

Synthesis of tritium- and deuterium-labeled budesonide

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Tritium-labeled budesonide was prepared by the selective reduction of a double bond in the butenylenedioxy side chain using carrier-free tritium and palladium on carbon as a catalyst in absolute ethanol. Although the reduction gave a mixture of the desired product and the expected byproducts resulting from over reduction of the other double bonds in ring A, the desired tritium-labeled budesonide was easily isolated by reverse phase HPLC and with specific activity of 54 Ci/mmol. [D_8]-budesonide was prepared from 16α-hydroxyprednisolone and D_8-butyraldehyde in 1,4-dioxane in the presence of perchloric acid. The isotopic enrichment was found to be more than 99 atom% D.

Keywords: tritium; deuterium; radiosynthesis; budesonide; 16α-hydroxyprednisolone

Introduction

Budesonide, (22*R*,*S*)-16α,17α-butylenedioxy-11β,21-dihydroxy-pregna-1,4-diene-3,20-dione, is a glucocorticosteroid used in inhalation treatment of asthma, rhinitis, skin, and the treatment of inflammatory bowel disease.[1–4] The mechanism of this anti-inflammatory activity of budesonide and other glucocorticosteroids (GC) is only recently being investigated at the genetic level. Budesonide and other GC play important roles in the expression of key genes involved in the inflammatory response. GC interact with an intracellular GC receptor, which shuttles them into the nucleus and regulates the expression of genes such as those encoding cytokines, cell adhesion molecules, and metalloproteinases known to be critical to both inflammation and the immune response.[5]

Budesonide is a 1:1 epimeric mixture,[1] resulting from an asymmetric 16α, 17α-acetal group, see Scheme 1, with epimer 22*R* having 2–3 times higher topical glucocorticoid potency than epimer 22*S*.[6] (22*R*)-epimer has approximately twice the volume of distribution, clearance, and *in vitro* liver biotransformation rate as the 22*S*-epimer in man.[7] In all reported syntheses of budesonide, a mixture of 1:1 of epimers 22*R* and 22*S* is always isolated.[1] This ratio can be modified by using stronger acids in the coupling of 16α-hydroxyprednisolone to butyraldehyde. For example, the 22*R*-epimer was obtained with 99% epimeric excess when a solution of 70% hydrogen fluoride was used at 0°C.[8] Budesonide followed the general metabolic pathways reported for synthetic glucocorticoids. However, 22*R*- and 22*S*-epimers gave different metabolites, probably due to substrate-selective oxidation of the non-symmetric 16α,17α-acetal substituent.[7] 16α-Hydroxyprednisolone being the major budesonide metabolite both *in vitro*, in rat, in mouse, in human liver preparations, and in plasma after i.v. administration to healthy subjects and arising solely from the 22*R*-epimer.[7,9].

Non-enzymatic hydrolysis of budesonide, i.e. using strong acids over prolonged periods of time, failed to produce 16α-hydroxyprednisolone.

Results and discussion

Tritium-labeled budesonide with specific activities of 43 and 68 Ci/mmol was synthesized before, although no details were given.[10] An abstract described the preparation of tritium-labeled budesonide with 36 Ci/mmol by selective reduction of one of the two double bonds in ring A, and then reintroduction of this double bond.[11] In optimizing the conditions for introducing tritium on budesonide, attempts were made to reduce crotonaldehyde first with deuterium gas to butyraldehyde-$^2H_2$ and then coupling it to 16α-hydroxyprednisolone. The volatility of the product (boiling point of 70–80°C), the low yield of the reduction, and the excess butyraldehyde required for the coupling with 16α-hydroxyprednisolne prevented us from pursuing this method. A similar approach would have been to use an unsaturated butyl hemiketal, which would have a higher boiling point, reduce the unsaturation, and then couple it to 16α-hydroxyprednisolne using the above conditions. Other ideas included the preparation of a budesonide analogue with a terminal unsaturation on the butyl side chain. Reduction of such a compound with tritium gas would be fast and the over reduction observed in ring A will be minimized. However, the presence of acids in the coupling of 3-butenal diethyl acetal for example with 16α-hydroxyprednisolone gave a mixture of products rising from the migration of the terminal double bond. In this article, carrier-free tritium was used to reduce a double bond in the alkyl side chain of carbon 22 using 10% palladium on carbon in absolute ethanol. The precursor: (22*R*,*S*)-16α,17α-(2-butenyl)enedioxy-11β,21-dihydrox-

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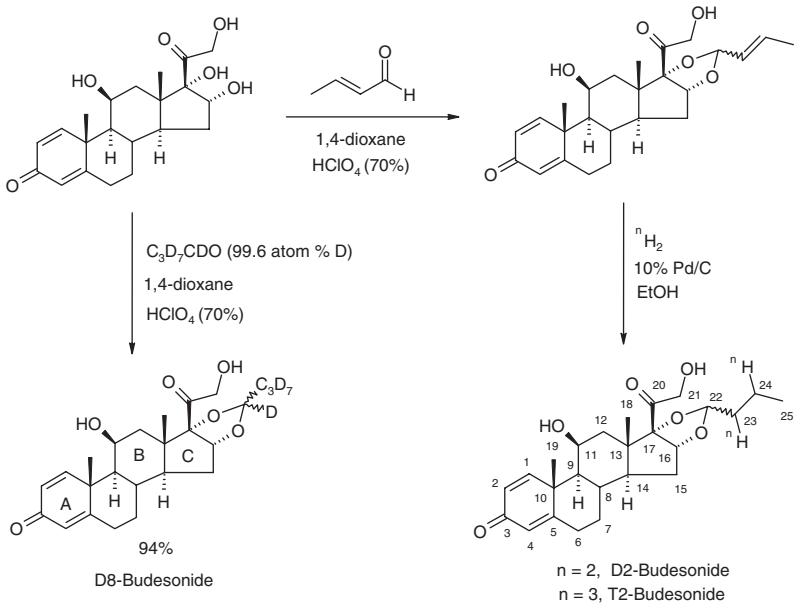

Scheme 1

ypregna-1,4,23-triene-3,20-dione was prepared from reacting 16α-hydroxyprednisolone with crotonaldehyde in 1,4-dioxane and a solution of 70% perchloric acid[1] (Scheme 1). The reduction was first performed using deuterium gas and gave a mixture of deuterium-labeled budesonide and compounds where the double bonds in ring A of the steroid were reduced as well. The tritium reduction was similar to the deuterium and gave the same over-reduced products. The tritium-labeled budesonide was isolated by HPLC, and its specific activity of 54 Ci/mmol was determined by comparison of the UV absorbance with that of a standard analytical sample and liquid scintillation counting of the isolated HPLC peak. Deuterium-labeled budesonide with isotopic enrichment of more than 99% was also prepared as an internal standard in the quantification of human plasma, urine, and a tool to enable real characterization of the pharmacokinetic parameters of budesonide used in the therapy of asthma and other diseases. Deuterium-labeled budesonide and derivatives have been prepared before as well.[12] [$^2H_8$]-Budesonide, [(22*R,S*)-(22,23,23,24,24,25,25,25-$^2H_8$)-16α,17α-butylenedioxy-11β,21-dihydroxypregna-1,4-diene-3,20-dione, for example, was prepared from the [$^2H_8$]-butyraldehyde.[13–15] However, the isotopic composition was only 80% $^2H_8$, 17% $^2H_7$, and 3% $^2H_6$ as determined by mass spectrometry. The high content of $^2H_7$ was explained by an exchange of deuterium atom in the aldehyde group during the oxidation of butanol-$^2H_{10}$ to butanal-$^2H_8$ using sodium dichromate in concentrated sulfuric acid. The yield of this oxidation was very poor too, about 15%.[14] Butyraldehyde-$^2H_8$ with isotopic enrichment of more than 99% was purchased from a commercial supplier and reacted according to the literature[13–15] with 16α-hydroxyprednisolone in 94% yield. Mass spectroscopy showed that the product has more than 99% atom ^2H and only traces of $^2H_7$.

Experimental procedures

Materials and methods

Silica gel TLC was performed from analysis with pre-coated aluminum sheets with fluorescent indicator (EM Separating, Gibbstown, NJ). NMR spectra were recorded with Bruker 400 MHz spectrometer, using $CDCl_3$ as a solvent and TMS peak as a reference. All reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI), except 16α-hydroxyprednisolone (technical) was purchased from SICOR (Milan, Italy) and butyraldehyde-$^2H_8$ was obtained from CDN (Pointe-Claire, Quebec, Canada). Solvents were of HPLC grade. Liquid scintillation counting was carried out on a Beckman LS 5000TA, using Ready Safe cocktail (Beckman, Fullerton, CA).

HPLC

Reverse phase separations were accomplished on a Kromasil C18 column (4.6 × 150 mm) and a gradient mobile phase 30–50% acetonitrile in water in 30 mins, using Rainin HPLC equipped with HPXL solvent delivery system and DYNAMAX absorbance detector. Radio-HPLC was performed using HITACHI L-6200A intelligent pump and Radiomatic Flo-oneBeta (Packard), and LINEAR UVIS 200 set at 240 nm and Ultima flo AP cocktail (Packard). The HPLC column was maintained at 35°C with an Eppendorf CH-30 column heater.

Synthesis

(22R,S)-16α,17α-(2-butenyl)enedioxy-11β,21-dihydroxypregna-1,4,23-triene-3,20-dione

To a solution of crotonaldehyde (400 μL, 4.8 mmol) and 16α-hydroxy-prednisolone (190 mg, 0.5 mmol) in dry 1,4-dioxane (10 mL) were added five drops of 70% $HClO_4$. After stirring for 5 min at room temperature, a solution of 10% K_2CO_3 was added (10 mL) and the organic phase was extracted with CH_2Cl_2. The combined organic solutions were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 340 mg of crude product. Purification by silica gel chromatography using 0–10% MeOH/$CHCl_3$ as eluent gave 320 mg of an oily residue, which was crystallized from CH_2Cl_2:hexane (280 mg) of white crystals, $R_f = 0.7$ in 30% MeOH/$CHCl_3$. HPLC: 14.32 min as a 3:2 mixture of C22-epimers. ^{1}HNMR ($CDCl_3$)(mixture): δ 7.25(d, $J = 10.1$ Hz,

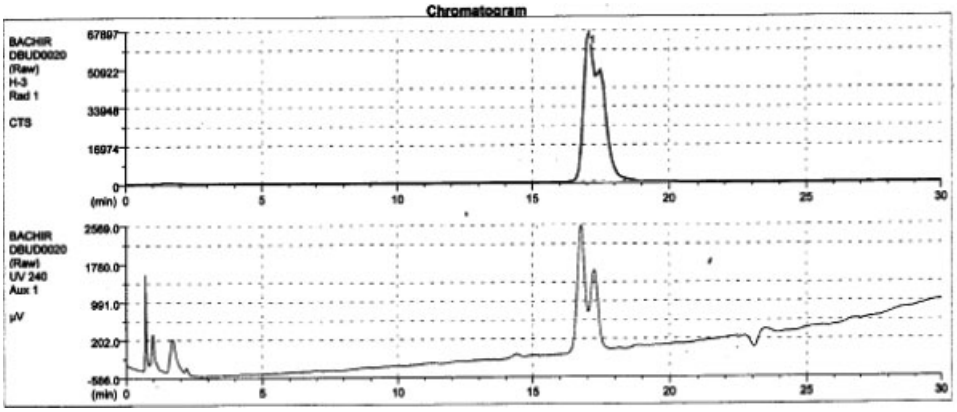

Figure 1. Radio- and UV-chromatograms of tritium-labeled budesonide.

1H), 6.32(d, $J = 10.1$ Hz, 1H), 6.05(s, 1H), 5.95(m, 1H), 5.50(dd, $J = 4.2$, 8.4 Hz), 5.30(dd, $J = 7.58$, 16.86 Hz), 5.22(d, $J = 6.74$ Hz), 4.98(d, $J = 4.2$ Hz), 4.92(d, $J = 6.74$ Hz), 4.6(m), 4.3(m), 3.8(m), 2.6(td, $J = 5.75$, 8.63), 2.48(m), 2.3(m), 1.85(*t*, $J = 8.63$ Hz), 1.78(m), 1.5(s, 3H), 1.1–1.4(m), 1.01(s), 0.98(s). CI-MS: MH^+ (429, 70%), 411(100%).

(22,23-$^2H_2$)-16α,17α-(22R,S)-butylenedioxy-11β,21-dihydroxypregna-1,4-diene-3,20-dione ($^2H_2$-budesonide)

A mixture of the above compound (2.07 mg, 4.83 μmol), 10% Pd/C (0.5 mg), a stirring bar, and absolute ethanol (100 μL) in a 2.2 mL glass vessel, was attached to a deuterium manifold. After degassing the mixture using three cycles of freeze–thaw under argon atmosphere, 4.95 μmol of deuterium gas was then introduced. The mixture was warmed to room temperature and stirred for 2.5 h. The vessel was then removed from the manifold and filtered through a Gillman PTFE 0.4 μm filter and washed with 2.0 mL of ethanol. The product was analyzed by HPLC. Starting material, $R_t = 14.32$ min, 46%; an unidentified byproduct, $R_t = 15.43$ min, 19%; the desired deuterated budesonide, $R_t = 17.93$ min, 22%; and over reduced products, $R_t = 19.29$, 11%, and 22.37 min, 2%.

Synthesis of tritium-labeled budsonide

As seen before in the reduction with deuterium, a mixture of (22*R*,*S*)-16α,17α-(2-butenyl)enedioxy-11β,21-dihydroxypregna-1,4,23-triene-3,20-dione (2.0 mg, 4.67 μmol), 10% Pd/C (0.55 mg), and a stirring bar in absolute ethanol (100 μL) in a 2.2 mL glass vessel was introduced tritium gas (4.4 μmol). The specific activity of tritium used was decay corrected and found to be 54 Ci/mmol. The total activity of tritium in the vessel was 237 mCi. The mixture was stirred for 3 h at room temperature and then transferred to a 20 mL vial and diluted with 1.0 mL of water. The mixture was filtrated through a Sep Pak (a reverse phase C18 cartridge that was washed with 5.0 mL of ethanol and 15 mL of water prior to use) and washed with 10 mL of water to elute any exchangeable tritium. The reaction products were then eluted with acetonitrile and collected in five vials. The first vial was 0.5 mL and the rest each 1.0 mL per vial. The vials were then counted for radioactivity. Most of the radioactivity was in vial 2 (97 mCi) and vial 3 (11 mCi). The aqueous phase contained 60 mCi of labile tritium. A fraction of vial 2 was diluted with acetonitrile and purified by HPLC. A second purification gave the tritium-labeled budesonide of more than 99% radiochemical purity (See Figure 1).

Synthesis of [$^2H_8$]-budesonide

To a mixture of 16α-hydroxyprednisolone (11.7 g, 28 mmol) in dry 1,4-dioxane (435 mL) was added *n*-butyraldehyde-$^2H_8$ (5.0 g, 62.5 mmol) and a solution of 70% $HClO_4$ (1.65 mL). The resulting clear solution was stirred at room temperature for 1 h before it was diluted with CH_2Cl_2 (600 mL) and washed with a solution of 10% K_2CO_3 (200 mL), water, and brine. The organic solution was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 14.4 g of a viscous oil that was dissolved in CH_2Cl_2 and precipitated with hexane. The precipitate was filtered and then purified by flash chromatography to give 13.5 g. The purity of the product was only 92% by HPLC analysis. Hence, the product was crystallized twice from methanol to 98% pure. LC-MS showed only traces of [$^2H_7$]-budesonide. ^1H NMR ($CDCl_3$): δ 7.28(d, $J = 101$ Hz, 1H), 6.31(d, $J = 10.1$ Hz, 1H), 6.05(br s, 1H), 5.2(d, $J = 6.87$ Hz, 0.3 H), 4.92(d, $J = 5.5$ Hz, 0.55H), 4.65(dd, $J = 5.5$, 20.6 Hz, 0.3H), 4.51(m), 4.29(dd, $J = 4.2$, 20.6 Hz, 1H), 4.22(dd, $J = 4.2$, 20.6 Hz, 0.3H), 3.05(t, $J = 6.87$ Hz, 1H), 2.60(td, $J = 4.67$, 14.1 Hz, 1H), 2.38(dd, $J = 4.61$, 14.1 Hz, 1H), 2.1–2.25(m), 1.55–1.80(m), 1.48(s, 3H), 1.01(s, 1.15H), 0.95(s, 1.95H).

Conclusion

Budesonide, a glucocorticosteroid used in the treatment of asthma, labeled with deuterium and tritium was needed to perform metabolism and pharmacokinetics research. The deuterium-labeled budesonide was prepared from 16α-hydroxyprednisoline and [$^2H_8$]-butyraldehyde. Tritium was incorporated by the reduction of a double bond in credesonide side chain. Although the reduction gave a mixture of over reduced products, tritium-labeled budesonide was easily isolated by reverse phase HPLC (Figure 1). The specific activity of this material was 54 Ci/mmol.

Acknowledgement

We thank Dr Pan Peng for the LC-MS analysis of the deuterated budesonide.

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