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Short communication

Estimation and characterisation of budesonide tablets impurities

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Abstract

Budesonide is the 16α,17α-acetal of 16α-hydroxyprednisolone with *n*-butyraldehyde, endowed with anti-inflammatory activity. In a sample of budesonide tablets, kept for 3 years at 25 °C and 60% RH unknown impurities, not reported in European Pharmacopoiea, were present. Their identification was achieved by means of chemical and spectroscopic methods.

Keywords: Budesonide; Anti-inflammatory drug; Impurities profile

1. Introduction

Budesonide **1** is a synthetic glucocorticosteroid with anti-inflammatory activity, widely used for the treatment of asthma [1] and inflammatory bowel diseases [2,3]. Structurally it is the 16α,17α-acetal of 16α-hydroxyprednisolone with *n*-butyraldehyde. In the course of the acetal ring formation a new stereocenter is introduced leading to a mixture of 22*R* and 22*S* epimers endowed with similar pharmacological effects [4]. According to European Pharmacopoeia, the diastereomeric ratio has to be within the range of 60-49/40-51 [5]. Quantification of budesonide, with the simultaneous separation of epimers and impurities was achieved, in general, by HPLC analyses and a few methods are reported in the literature [4,6–9]. We planned to identify either the process related impurities either the degradation products present, in excess of 0.10%, in a stressed sample of budesonide. In the first part of the work we planned to estimate, through a HPLC analysis, the presence of impurities described in European Pharmacopeia and collected in Scheme 1. The presence of other unidentified compounds, required a deeper study, realized at first by a LC–MS analysis followed by the synthesis of the suggested impurities, in order to obtain reference standards. This second part of the work allowed to establish that also the 17-carboxylic acid from side chain degradation **9** and the 11-keto derivative of budesonide **10** were present in the examined sample. Finally in order to identify a last impurity, not easily detectable since present in very little amount, the isolation of a pure sample, by means of preparative HPLC, was required. Treatment of budesonide sample with *m*-chloroperbenzoic acid for 8 days improved the content of this impurity from 0.2 to 8%, shortening, then, the chromatographic procedure of purification.

2. Materials and methods

Budesonide and Ph. Eur. impurities **2–7** were supplied by Industriale Chimica S.r.l. All reagents and solvents were from Sigma–Aldrich, Milan (Italy).

Budesonide tablets were exposed to environmental conditions of 25 °C and 60% RH for 3 years.

2.1. Instruments

2.1.1. HPLC

The HPLC system consisted of an Agilent 1100-Series liquid chromatograph, equipped with auto-injector, DAD detector, and a Chemstation software, installed on a PC, for data collecting and processing. A Discovery (Supelco) C18 column (125 mm × 4.6 mm i.d., 5 μm particle) was employed; acetonitrile–phosphate buffer (pH 3.2, 28.6 mM) (30:70, v/v)

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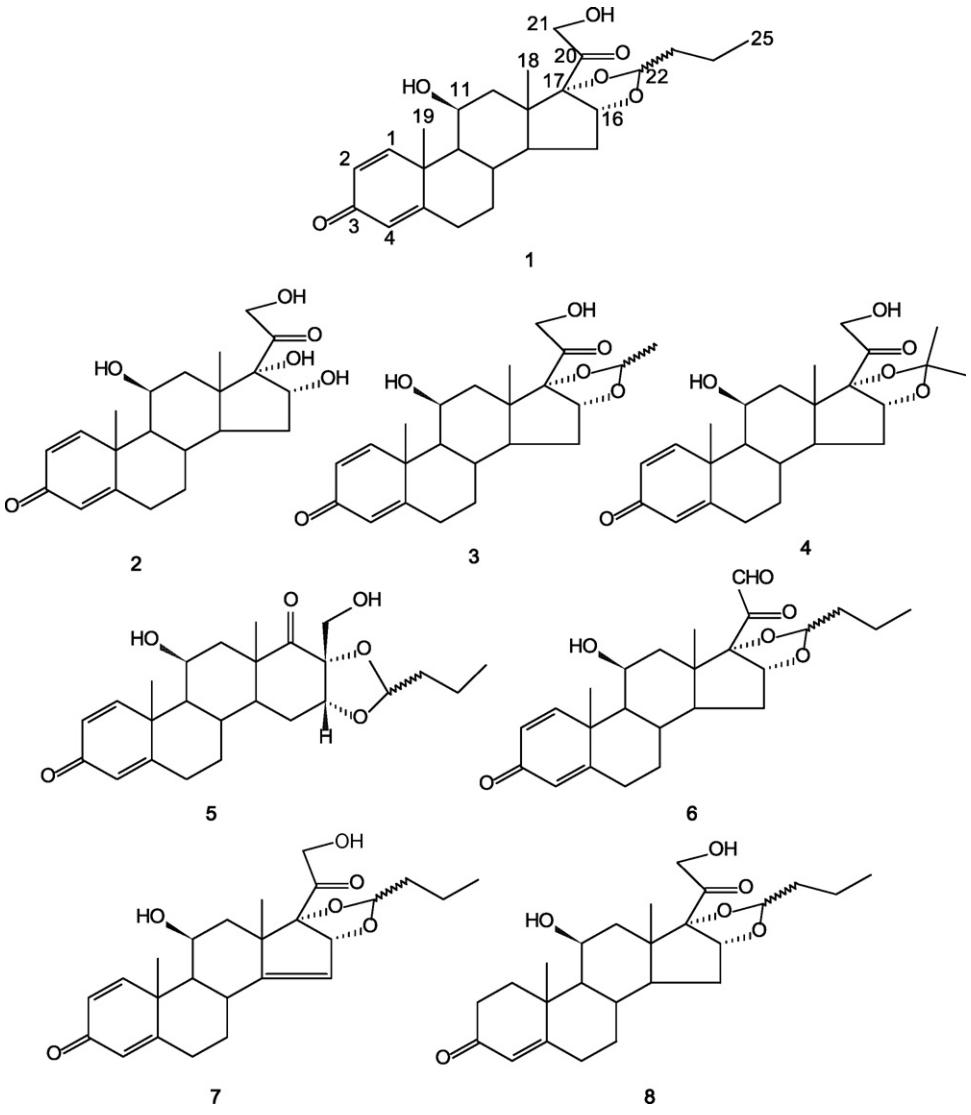


Scheme 1.

was used at a flow rate of 1.5 ml/min, at room temperature. Detector wavelength was set at 240 nm.

The preparative HPLC system consisted of a Waters HPLC apparatus mod. Delta PREP600E equipped with a manual injector (loop 1 ml), an online preparative degasser and a FCIII Waters fraction collector. The detector was a Diode Array (Waters mod. 2996, MA, USA) operating at 260 nm. The column employed was a Symmetry Shield RP 18 (Waters, MA, USA), 150 mm × 19 mm i.d. (5 μm particle size); mobile phase was a mixture of acetonitrile–water (35:65, v/v) eluted at 8 ml/min.

2.2. Mass spectrometry

A Discovery (Supelco) C18 column (125 mm × 4.6 mm i.d., 5 μm particle) was employed; acetonitrile-formic acid (pH 3.8, 0.14 mM) (30:70, v/v) was used as mobile phase at a flow rate of 1.5 ml/min, at room temperature. This method was adapted from the method developed for HPLC–UV assay of budesonide. The optimized source parameters were as follows: source voltage 0.98 kV; sheath gas flow rate 50; capillary voltage −15.00 v; capillary temp 250 °C. Data acquisition and analysis were accomplished with Xcalibur software 1.1. The HPLC apparatus comprised Thermo Finningan MAT P 4000 series pump and vacuum degasser. Analysis was carried out using LCQ^{DECA} ion trap mass analyser (TermoQuest, San Jose, USA) with an electrospray ionization ESI in negative ion mode interface.

2.3. NMR spectroscopy

All NMR spectra were recorded in $CDCl_3$ solutions with a Bruker AVANCE500 spectrometer. Chemical shifts are reported on the δ (ppm) scale from TMS.

2.4. FTIR spectroscopy

FT-IR spectra were collected by using a PerkinElmer (MA, USA) FT-IR Spectrometer "Spectrum One" in a spectral region

Table 1
Impurities in budesonide tablets exposed to environmental conditions of 25 °C and 60% RH for 3 years

Impurity	Retention time (min)	Description	Impurity % respect to budesonide
2	1.7	11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione (Ph. Eur. Budesonide Impurity A)	0.018
3	4.4	16α,17-[(1*RS*)-Ethylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione (Ph. Eur. Budesonide Impurity B)	0.055
4	5.7	16α,17-[1-Methylethylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione (Ph. Eur. Budesonide Impurity F)	0.043
5	5.3	16α,17-[(1*RS*)-butylidenebis(oxy)]-11β-hydroxy-17-(hydroxymethyl)-*D*-homoandrosta-1,4-diene-3,17a-dione (Ph. Eur. Budesonide Impurity C)	0.19
6	10–10.6	16α,17-[(1*RS*)-butylidenebis(oxy)]-11β-hydroxy-3,20-dioxopregna-1,4-dien-21-al (Ph. Eur. Budesonide Impurity D)	0.12
7	13.5	16α,17-[(1*RS*)-butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4,14-triene-3,20-dione (Ph. Eur. Budesonide Impurity E)	Not found
8	19–21	16α,17-[(1*RS*)-butylidenebis(oxy)]-11β,21-dihydroxypregn-4-ene-3,20-dione (Ph. Eur. Budesonide Impurity G)	Not found
9	12.6	17-Carboxyderivative	0.33
10	14.3	11-Ketoderivative	0.20
11	20	11-Hydroxy,16 *n*-butanoyloxy-androstan-1,4-dien-3,17 dione	0.21

between 4000 and 600 cm^{-1}. Samples were analysed by transmittance technique with 16 scansions and 4 cm^{-1} resolution on NaCl tablets.

3. Results and discussion

The presence of known impurities was tested by a HPLC analysis, according to the method described in European Pharmacopoeia [5] using the commercially available reference standards, in the case of compounds **2**–**7**. Compounds **8** was, instead, synthesized in our laboratory, in 63% yields, by means of a selective triphenylphosphine rhodium chloride–catalyzed hydrogenation of the 1,2 double bond of budesonide, 21-acetate in dry acetone, [10] followed by a carefully basic hydrolysis (potassium carbonate in methanol at 0 °C) [11].

The results of HPLC analysis are summarized in Table 1 and the representative chromatogram is reported (Fig. 1).

Impurities **2**–**4** were present in a very little extent (<0.10%) while compounds **7** and **8** were not detectable and only the D-homoderivative **5** and the 21-aldehyde **6** exceeded the 0.1%. In order to achieve additional information about the unidentified peaks on the chromatogram (respectively at 12.6 min and at 14.3 min) a LC–MS analysis was performed. In the spectrum of more polar compound (R_t 12.6 min) the peak at 415 *m/z* was well detectable; additionally we observed that this by-product was not detected using a neutral mobile phase. The mass spectrum of compound with R_t 14.3 min showed the molecular peak at 427 *m/z* that can be explained by loss of two hydrogen from budesonide. The structure of 17-carboxylic acid **9** and 11-ketone **10** (Scheme 2) were, respectively, proposed and in order to verify

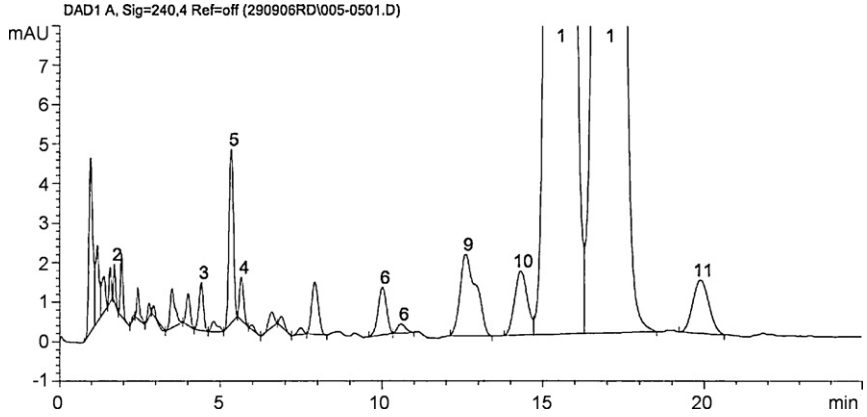
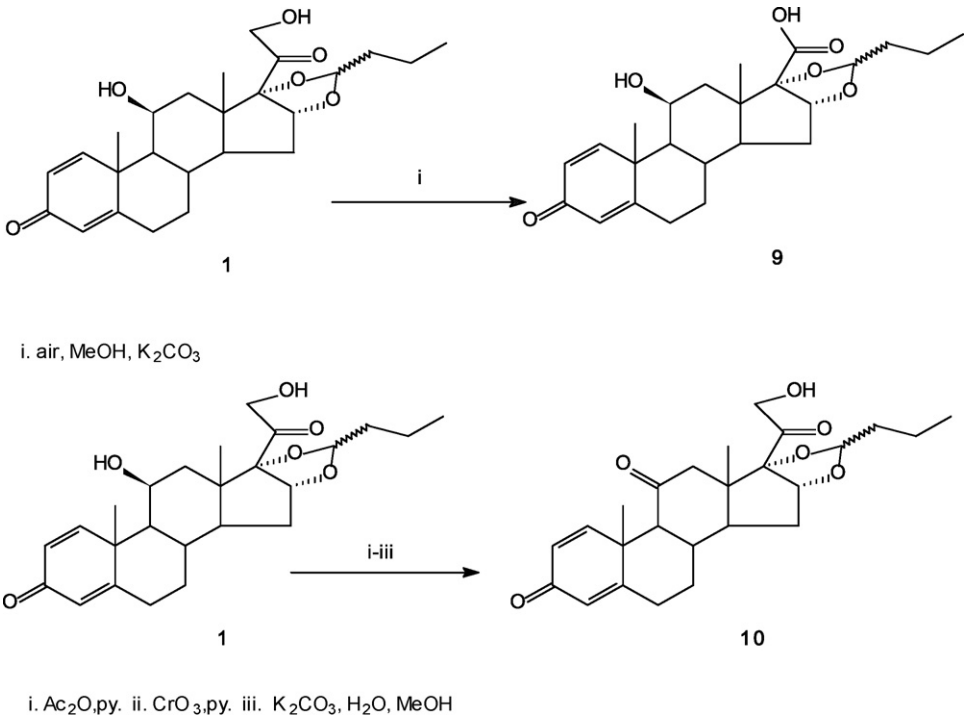


Fig. 1. Representative chromatogram of a sample of budesonide tablets stored at 25 °C and 60% RH for 3 years; peaks identified as **1** correspond to budesonide epimers; for the identification of peaks from **2** to **11** see Table 1.



Scheme 2.

the correctness of this hypothesis we planned to prepare **9** and **10**. The 17-carboxylic acid **9** was obtained, in 82% yields, from budesonide according to a reported method, usually employed for degradation of corticosteroid side chain, *i.e.*, by bubbling air in a methanol solution of **1** containing potassium carbonate [12]. In the case of compound **10**, before oxidation of 11-alcohol, the primary 21-hydroxy group was suitably protected as acetate. Oxidation with chromium trioxide in pyridine [13], respective of acetal function, afforded the 11-carbonylic function. Removal of protecting group of 21-alcohol was achieved, as above, by potassium carbonate in methanol [11] affording compound **10** in 64% overall yields. ^{1}H NMR spectra of the above compounds **9** and **10** were in agreement with the proposed structures (see Table 2); the availability of authentic samples of these two potential impurities allowed to confirm the identification of peaks at 12.6 min and at 14.3 min present on 0.33 and 0.20% amount, respectively (see Table 1).

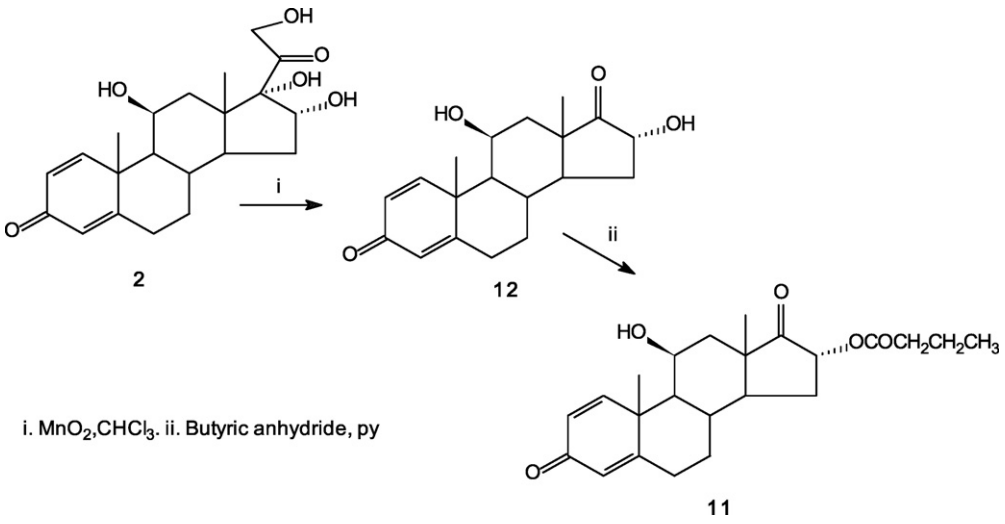
Table 2
^{1}H NMR selected chemical shift data of compound **1**, **9** and **10**

^{1}H	(22*R*,*S*)-budesonide **1**	17-Carboxyderivative **9**	11-Ketoderivative **10**
1	7.27 (two d)	7.32 (two d)	7.67 (two d)
2	6.30 (two dd)	6.33 (two dd)	6.25 (two dd)
4	6.05 (m)	6.08 (m)	6.13 (m)
11	4.52 (m)	4.52 (m)	–
16	4.92 and 5.18 (two d)	5.00 and 5.24 (two d)	4.94 and 5.34 (two d)
18	0.94 and 1.02 (two s)	1.11 and 1.15 (two s)	0.67 and 0.73 (two s)
19	1.48 (s)	1.48 and 1.49 (two s)	1.47 (s)
21	4.18–4.68 (four d)	–	4.14–4.69 (four d)
22	4.58 and 5.20 (two t)	4.80 and 5.23 (two t)	4.65 and 5.34 (two t)
25	0.93 and 0.94 (two t)	0.92 and 0.94 (two t)	0.96 and 0.98 (two t)

We were not able to achieve sufficient information about the peak at 20.0 min, present in the examined sample in 0.21%, the isolation of a small amount of purified compound, from preparative HPLC, being the ultimate method to elucidate its structure. In order to avoid a time consuming separation procedure, we tried to improve the content of this impurity in the sample: the treatment with *m*-chloroperbenzoic acid (0.2 M in chloroform) afforded, after 8 days, the desired result, as shown by HPLC analysis. The mixture containing the unknown compound in 8% amount, was submitted to isolation by preparative HPLC. The mixture (110 mg) was dissolved in the mobile phase (11 ml) and, after 11 cycles of separation, 8.1 mg of impurity (R_t 40.0 min) were isolated. The MS spectrum of pure compound showed a molecule peak at 385 *m*/*z*, indicating the loss of a group with molecular weight 44 from budesonide. In the IR spectrum three signals due to carbonylic groups were observed, instead of the two present in budesonide. ^{1}H NMR spectrum (Table 3) showed the absence of protons at positions 21, at 4.18–4.68 ppm in the spectrum of budesonide, the presence of C_3 alkyl chain and a signal at 5.49 ppm diagnostic of protons of an esterified alcohol.

Table 3
Selected ^{1}H NMR chemical shift data of compounds **11** and **12**

^{1}H	Compound **12**	Compound **11**
1	7.25 (d)	7.25 (d)
2	6.29 (dd)	6.30 (dd)
4	6.05 (m)	6.05 (m)
11	4.53 (m)	4.54 (m)
16	4.45 (d)	5.49 (d)
18	1.32 (s)	1.32 (s)
19	1.50 (s)	1.51 (s)
25	–	0.97 (t)



Scheme 3.

The collected data suggested, for the investigated compound, the structure **11** (Scheme 3).

In order to unambiguously verify this hypothesis, starting from 16-hydroxyprednisolone **2**, we prepared a sample of 11β,16α-dihydroxy-androstan-1,4-dien-3,17-dione, 16-butanoate **11**. A manganese dioxide oxidation in chloroform [14], [15], at room temperature, afforded the 16-hydroxy,17-keto derivative **12** that was selectively esterified by butyric anhydride in pyridine giving the desired compound **11** (Scheme 3). Retention time matching with the authentic sample on HPLC chromatogram, in addition to identical chemical–physical data, provided final evidence to identify the unknown impurity. Its presence in the budesonide sample can be explained through an oxidative degradation of side chain, involving also the 16α,17α-acetal. A similar reaction was reported in literature in the case of an analogue compound bearing a 16α,17α-dimethylketal [16].

4. Conclusions

Following the traditional strategies [17] we were able to achieve a complete impurity profile of budesonide, verifying the presence of reported products and elucidating the structures of hitherto not described impurities. Compound **2** is the starting material of the last step of budesonide preparation and also compounds **3** and **4** are formed on this step. Formation of compound **5** can be explained with an acidic rearrangement of budesonide. **11**-Ketoderivative **10** could be considered either as product of degradation of budesonide than as a side-product of the synthesis. An oxidative process is the common synthetic pathway for compound **6**, **9** and **11**: the aldehyde **6** is oxidized to the 17-carboxylic acid **9** in agreement with reported data about the corticosteroid side chain degradation [18]. Finally, the loss of C-20, followed by a rearrangement of 16α,17α-acetal leads to butanoate **11** [16].

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