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Decomposition of Beclomethasone Propionate Esters in Human Plasma

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ABSTRACT: The kinetics of decomposition of beclomethasone dipropionate (BDP), the 17-monopropionate ester (17-BMP), and beclomethasone (BOH) were characterized in whole human plasma (HP), pH 7.1, and in solutions of 1% human serum albumin (HSA), pH 7.4, and 0.067 M phosphate buffer, pH 7.4 ($\mu = 0.17$). A reversed-phase, high-performance liquid chromatography (HPLC) assay enabled simultaneous separation and quantification of beclomethasone propionate esters and six degradation products including three unidentified products, D1–D3, not previously reported. Following incubation of BDP, products were formed in the following sequence, D1, 17-BMP, beclomethasone-21-monopropionate (21-BMP), D3, BOH, and D2. Following incubation of 17-BMP, the same sequence of degradation products was formed with the exception of D1. Following incubation of BOH, only D2 was formed. The decomposition reactions of BDP, 17-BMP, and BOH in HP exhibit pseudo-first-order kinetics. However the degradation reactions of BDP in solutions of 1% HSA and phosphate buffer were found to follow pseudo-zero-order kinetics. At an initial concentration of 40 μg mL^{-1}, the half-lives for BDP, 17-BMP, and BOH in HP were 10.9 ± 0.4, 3.0 ± 0.2 and 24.8 ± 0.2 h, respectively. © 1998 John Wiley & Sons, Ltd.

Key words: kinetics; beclomethasone dipropionate; beclomethasone-17-monopropionate; high-performance liquid chromatography; plasma

Introduction

Beclomethasone dipropionate (BDP) is a synthetic chlorinated corticosteroid diester commonly used by inhalation in the treatment of asthma [1]. Owing to its dipropionate ester functional groups in the side-chain, BDP may undergo hydrolysis in solution to the more polar products (Figure 1), beclomethasone-17-monopropionate (17-BMP), beclomethasone-21-monopropionate (21-BMP), and beclomethasone (BOH).

In early studies [2–6], it was reported that the hydrolytic degradation of BDP is an inactivation step leading to the formation of less potent steroid products. However, in recent reports [7–9], it has been proposed that BDP is actually a poorly active prodrug, which is rapidly hydrolysed to a much more potent glucocorticoid, namely 17-BMP. Therefore, the local anti-inflammatory activity of inhaled BDP may be derived largely from the 17-monopropionate derivative, which is rapidly formed from the parent drug in lung tissue [7,8].

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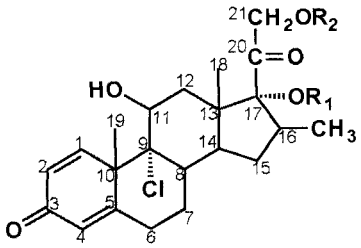
It may be speculated that the rapid formation of 17-BMP, a potent and more hydrophilic degradation product, may lead to extensive absorption of the drug from the lower respiratory tract because it may be cleared more rapidly into the systemic circulation [7,10,11]. Furthermore, more than 80% of the inhaled BDP is swallowed and prone to absorption in the gastrointestinal tract [10]. As a result, substantial systemic effects, such as adrenal suppression, osteoporosis, reduced total bone mass, and mineral density, as well as the inhibition of body growth in children [12], may occur.

Therefore, knowledge of the degradation rates and properties of BDP as well as its degradation products, especially 17-BMP, is of considerable importance to the understanding of factors determining the duration of action in the lung as well as the ratio of local anti-inflammatory action to systemic activity.

The metabolic biotransformation of BDP has been studied and reviewed by many authors [2,3,5,7–9,13]. However, to our knowledge, no study has been reported on the effects of human plasma (HP) esterases and albumin binding on the stability kinetics of BDP, 17-BMP, and BOH. It is known that



	R_1	R_2	m.w.
BDP	C_2H_5CO	C_2H_5CO	521
17-BMP	C_2H_5CO	H	465
21-BMP	H	C_2H_5CO	465
BOH	H	H	409

Figure 1. Structural formulae of beclomethasone dipropionate (BDP), beclomethasone-17-monopropionate (17-BMP), beclomethasone-21-monopropionate (21-BMP), and beclomethasone (BOH)

BDP is appreciably bound (60%) to plasma albumin [14].

The present study reports the stability kinetics of BDP, 17-BMP, and BOH in whole HP, pH 7.1, and in solutions of 1% human serum albumin (HSA), pH 7.4, and 0.067 M phosphate buffer, pH 7.4 ($\mu = 0.17$).

Materials and Methods

Materials and Instrumentation

BDP, BOH, dexamethasone-21-acetate, HSA (fatty acid free, lot 93H9345) and dichloromethane (99.9%, HPLC grade) were purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). Pure reference standards of BDP, 17-BMP, and BOH were kindly donated by Glaxo Australia Pty Ltd. Sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, glacial acetic acid, and ethanol were of analytical reagent grade, whereas methanol and acetonitrile were of ChromAR HPLC grade.

Liquid chromatography was performed on a Beckman System Gold apparatus, comprising a programmable pump (solvent module 126), equipped with a 100 μL external loop injector (autosampler 507), and a variable-wavelength UV detector (module 166), controlled by PC computer software. Separation was achieved using an Alltima C_{18} analytical column (250 mm × 4.6 mm I.D.) from Alltech Associates, Baulkham Hills, NSW, Australia.

Preparation of Media and Standard Solutions

Whole HP, using acid citrate dextrose (ACD) as anticoagulant, pH 7.1 was supplied by the Blood Bank (Sydney, Australia) and stored at −20°C for no longer than 3 months. 1% HSA solution was freshly prepared in Sorensen's phosphate buffer (0.067 M) solution, pH 7.4. Neither sterilization nor aseptic filtration was performed for HP or 1% HSA solution prior to incubation.

Ethanolic stock solutions of BDP, 17-BMP, or BOH (1.0 mg mL^{-1}) were stored, protected from light, at −20°C. Replicate ($n = 6$) standard solutions, in each of the media, were freshly prepared in the concentration range 2–50 μg mL^{-1} and analysed immediately. The internal standard, dexamethasone-21-acetate (IS) was dissolved (40 μg mL^{-1}) in ethanol and stored at −20°C, protected from light. Ratios of the peak areas of BDP, 17-BMP, or BOH to the IS were determined. Standard curves observed for BDP, 17-BMP, and BOH, in HP, 1% HSA, and phosphate buffer, were linear over the range 2–50 μg mL^{-1} ($r > 0.99$, linear regression).

Kinetic Studies

Kinetic experiments were carried out at 37.0 ± 0.1°C, shielded from light in a shaking water bath. Prior to commencement, media were preadjusted to the temperature of study. At appropriate times, 1 mL samples were removed for immediate analysis. The concentrations of undegraded parent drug and degradation products were determined. There was no observable turbidity or change of the sample before and after the incubation study. The samples, in screw-capped tubes, were tightly sealed to minimize microbial contamination during incubation.

Extraction Procedure

One millilitre of working standard solution or sample was spiked with 0.75 mL of a 40 μg mL^{-1} ethanolic solution of IS. Working standard solutions or samples in HP and 1% HSA were extracted with 8 mL dichloromethane for 30 min, using a roller mixer, followed by centrifugation at 2500 rpm for 10 min (25°C). The dichloromethane layer was collected and evaporated to dryness at 30°C under a stream of nitrogen. The dried extract was reconstituted in 1 mL mobile phase, transferred to a polypropylene microcentrifuge tube (1.5 mL, Eppendorf) and centrifuged at 15 000 rpm for 2 min prior to injection onto the HPLC column (injection volume, 100 μL).

Recovery Studies

Recoveries for BDP, 17-BMP, or BOH were assessed at concentrations of 2, 6, 10, 30, and 50 μg mL^{-1} in six replicates. Stock solutions of BDP, 17-BMP, or BOH were added to 1 mL blank HP or 1% HSA and 8 mL dichloromethane simultaneously to give the

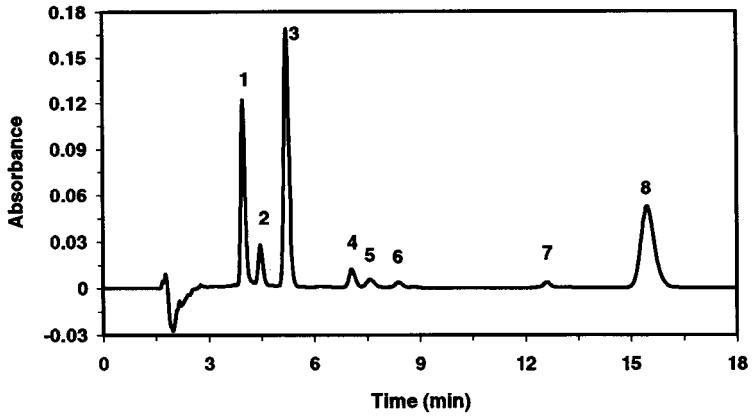


Figure 2. A typical HPLC chromatogram of the degradation of beclomethasone dipropionate (BDP) to its degradation products recorded after 10 days incubation in 0.067 M phosphate buffer, pH 7.4, at 37°C: 1, BOH; 2, D2; 3, dexamethasone-21-acetate (IS); 4, 21-BMP; 5, 17-BMP; 6, D3; 7, D1; 8, BDP. D1–D3 represent unknown species

above concentrations. HP and 1% HSA samples were extracted with an equal volume of dichloromethane as described above in the extraction procedure. Recovery was calculated as the percentage recovered from either HP or 1% HSA relative to the amount determined in the dichloromethane matrix. High recoveries (91.9–99.9%) of BDP, 17-BMP, and BOH were obtained over the range 2–50 μg mL^{-1}, following extraction from HP and 1% HSA, indicating that losses during the extraction were negligible. The coefficients of variation (CVs) calculated during replicate assays varied between 0.07 and 5.51%.

Solubility Determination

Five milligrams of BDP in 5 mL 0.067 M phosphate buffer solutions, pH 7.4, were equilibrated by tumbling continuously in 20 mL screw-capped tubes at 37.0 ± 0.1°C. Solutions were sampled after 3, 4, and 5 day equilibration times and filtered through a 0.20 μm filter (Minisart, Sartorius) prior to analysis. At the concentrations used, BOH and 17-BMP formed homogeneous solutions in all three media.

HPLC Analysis

Isocratic liquid chromatography was performed at ambient temperature and a flow rate of 1.3 mL min^{-1} with detection at 242 nm (λ_{max} of BDP, 17-BMP, and BOH). The mobile phase was a mixture of methanol–water–acetonitrile–glacial acetic acid (650:262:88:1.75, v/v), filtered through a 0.45 μm pore size HVLP filter (Millipore, Sydney, Australia), and degassed under reduced pressure. Separation of beclomethasone propionate esters, their degradation products, and the IS was achieved successfully, free from interfering endogenous substances in HP and 1% HSA. Retention times were 3.9 min for BOH, 4.4 min for D2, 5.2 min for IS, 7.1 min for 21-BMP, 7.7 min for 17-BMP, 8.4 min for D3, 12.7 min for D1, and 15.4 min for BDP (Figure 2).

Injections of an identical volume (100 μL) of a series of working standards were repeated regularly. All experiments were performed in six replicates.

Analysis of Parent Drug and its Degradation Products

Degradation products were identified by comparison of their retention times on HPLC chromatograms as well as their zero-, first-, and second-derivative spectra, using a photodiode array detector (Model SPD-M10A, Shimadzu), with those of pure reference standards. Owing to the lack of pure reference standard of 21-BMP, it was identified by determining its molecular weight and structure, using mass spectrometry (Finnigan TSQ 7000 LCMS/MS) and ^1H nuclear magnetic resonance spectrometry (Varian Gemini 300 MHz), respectively, as well as comparing its UV spectrum with that of 17-BMP. It was found that 21-BMP exhibited a spectrum which is superimposable with that of 17-BMP. Quantification was achieved by interpolating relative peak area ratios on the standard curves.

Data Analysis

The order of the degradation reaction was determined by graphical and half-life methods [15]. All pseudo-zero-order or pseudo-first-order plots reported in this study were linear, showing correlation coefficients greater than 0.99. Degradation rate constants (k) were calculated from the slope of linear regression plots of the concentration against time, when the data exhibited pseudo-zero-order reaction, or the natural logarithm of concentration versus time, when the data followed pseudo-first-order kinetics. The half-life ($t_{1/2}$) values were obtained by substituting k into half-life equations [15], depending on the reaction order. The data were tested for significance of differences by using single-factor analysis of variance (ANOVA) with p values of less than 0.05 taken to be significant.

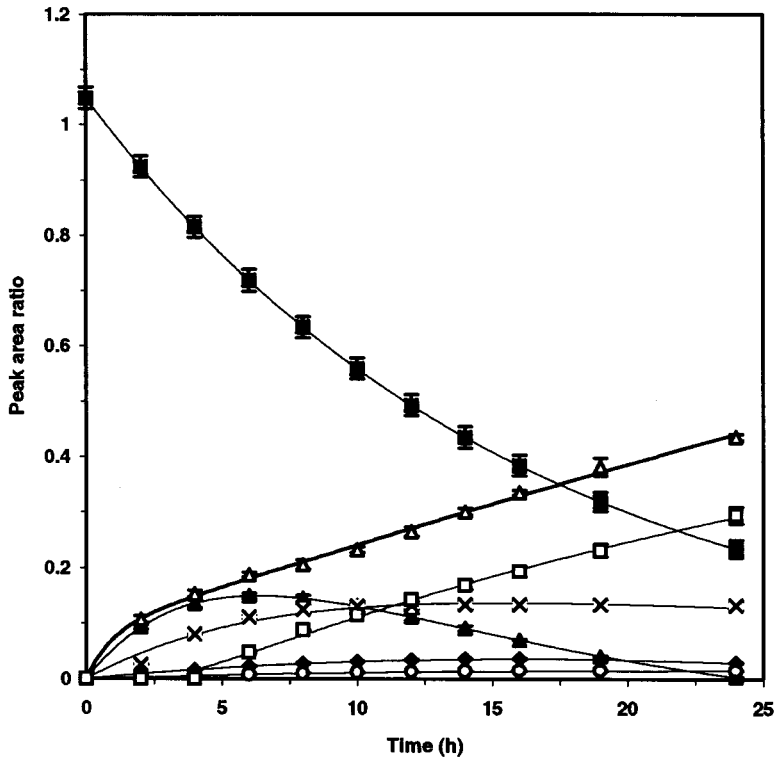


Figure 3. A typical kinetic profile of beclomethasone dipropionate (BDP, 40 μg mL^{-1}) and its degradation products in HP, pH 7.1, at 37°C: ■, BDP; ◆, D1; ▲, 17-BMP; ×, 21-BMP; ○, D3; Δ, BOH; □, D2. D1–D3 represent unknown species. The vertical bars indicate the S.D. of six determinations. When no bars are shown, the S.D. fell within the symbol dimensions

Results and Discussion

Degradation Reactions

Following the incubation of BDP as parent drug at 37.0 ± 0.1°C in pH 7.1 HP, 1% HSA in pH 7.4 phosphate buffer, and pH 7.4 phosphate buffer, 17-BMP, 21-BMP, BOH, and three unknown degradation products, namely, D1–D3, never reported previously, to our knowledge, were formed (Figure 3; Table 1). During the period up to 45 h, BOH was the major degradation product, following the incubation of BDP in HP. However, unknown D2, rather than BOH, was the ultimate major degradation product observed in HP, ranging from 40 to 53% of the parent drug, after longer incubation times (96 h). Thus it would seem that BOH ultimately decomposes to unknown D2. This conclusion was confirmed by the formation of D2 following the incubation of BOH as parent drug in all three media (Table 1).

Following the incubation of 17-BMP as parent drug in all three media, 21-BMP, BOH, and two unknown degradation products, namely D2 and D3, were formed (Figure 4; Table 1). The formation of 21-BMP suggests that 17-BMP undergoes interesterification with the change of the propionate ester group from the 17α- to the 21-position, in agreement with a previous report [7]. Unknown D1, which was not formed following the incubation of 17-BMP as parent drug, and D3 were quite stable in non-enzymatic media (1% HSA and phosphate buffer), indicated by their presence in appreciable amounts (Figure 4). However, they were always present in small amounts in HP, suggesting that they may be unstable (Figure 3; Table 1). In addition, there was a delay in the appearance of D2 (Figures 3 and 4; Table 1), suggesting that it might be the ultimate degradation product. The product, D2, was relatively stable in all three media. Its continued formation was observed with incubation times up to 30 days in solutions in 1% HSA and phosphate buffer.

Degradation Products

Although the three unknown degradation products have not been characterized, the incubation of different compounds, either BDP, 17-BMP, or BOH as parent drugs may suggest routes for the formation of unknowns D1–D3 (Table 1). It is postulated that unknown D1 is an intermediate resulting from the partial degradation of dipropionate esters (BDP) during the formation of monopropionate esters, predominantly 17-BMP. Unknown D1 was not formed following incubation of either 17-BMP or BOH as parent reactants. Unknown D2 was found to be the ultimate product resulting from the degradation of BOH and was formed following incubation of either BDP or 17-BMP as parent reactants. Unknown D3 was formed following incubation of both BDP and 17-BMP, especially after 21-BMP had

Table 1. The formation of degradation products, as a function of time, following incubation of BDP, 17-BMP, or BOH (40 μg mL^{-1}) in HP at 37°C. Experimental data represent the mean ± S.D. of six determinations

Parent drug	Incubation time (h)	Degradation products (percentage)[a]					
		D1	17-BMP	21-BMP	D3	BOH	D2
BDP	3	1.2 ± 0.1	11.3 ± 0.3	6.1 ± 0.2	0.38 ± 0.02	12.4 ± 0.2	0
	6	2.2 ± 0.1	14.2 ± 0.4	9.8 ± 0.1	0.76 ± 0.05	17.2 ± 0.4	4.5 ± 0.1
	9	2.9 ± 0.1	13.2 ± 0.2	11.8 ± 0.2	1.04 ± 0.07	21.6 ± 0.8	9.1 ± 0.1
	12	3.3 ± 0.1	10.7 ± 0.7	12.8 ± 0.2	1.24 ± 0.08	25.8 ± 1.3	13.4 ± 0.2
17-BMP	3	NF[b]	NF	23.6 ± 1.7	0.27 ± 0.02	22.6 ± 0.6	0
	6	NF	NF	29.8 ± 2.0	0.73 ± 0.03	41.1 ± 0.5	3.4 ± 0.1
	9	NF	NF	19.4 ± 1.3	0.88 ± 0.07	57.5 ± 1.0	10.5 ± 0.2
	12	NF	NF	10.8 ± 0.6	0.51 ± 0.04	62.3 ± 1.0	18.4 ± 0.5
BOH	4	NF	NF	NF	NF	NF	3.2 ± 0.2
	8	NF	NF	NF	NF	NF	9.0 ± 0.4
	12	NF	NF	NF	NF	NF	14.9 ± 0.8

[a] Expressed as percentage of parent drug.
[b] Not formed.

been formed in a significant amount. This suggests that it is an intermediate resulting from the partial degradation of 21-BMP, rather than 17-BMP. Unknown D3 was not formed following incubation of BOH. A proposed degradation pathway of BDP in HP is shown in Figure 5.

It was observed that D1 was chromatographically more polar than BDP. Unknown D3 was chromatographically less polar than both 17- and 21-monopropionate esters, whereas D2 was less polar than BOH (Figure 2). From the ultraviolet spectral analysis using a photodiode array detector, it was found that D1–D3 exhibited bathochromic shifts (λ_{max} of the parent compound (242 nm) is increased by 2, 9, and 9 nm, respectively). Further experiments to confirm the type and pathway of degradation reaction by identifying the unknown compounds are being conducted.

Degradation Rate Constants and Reaction Order

In solutions of 1% HSA and phosphate buffer, at concentrations of 20–40 μg mL^{-1}, BDP formed suspensions. The degradation rates of BDP in these solutions were observed to be constant. From the solubility study conducted in 0.067 M phosphate buffer, pH 7.4, it was found that the solubility of BDP was 0.10 ± 0.01 μg mL^{-1} at 37°C (mean ± S.D.; $n = 6$). This is comparable with that of BDP in water (0.12 μg mL^{-1}) reported previously [8]. This indicates that in the buffer systems containing 20–40 μg mL^{-1} BDP, only approximately 0.2–0.5% of the total concentration of BDP was in solution and available for the hydrolysis reaction.

Owing to the presence of the insoluble fraction, the kinetics of degradation of BDP in 1% HSA and phosphate buffer exhibited pseudo-zero-order kinetics, characteristic of suspensions. The solubility of BDP was the limiting factor determining the degradation rate of BDP in non-enzymatic media. Thus the half-lives of BDP in 1 % HSA and phosphate buffer (Table 2) are not comparable with that in HP. Decomposition of BDP in suspension in non-enzymatic media was observed to be very slow (Table 2).

In human plasma, BDP formed solutions. The values of $t_{1/2}$ obtained following the incubation of BDP in HP were essentially independent of concentrations of the parent drug (Table 2), consistent with the existence of pseudo-first-order kinetics. The rapid degradation of BDP in HP can be attributed to relatively high esterase activity [8] in HP. The $t_{1/2}$ of BDP ($C_0 = 20$ μg mL^{-1}) observed in HP was 10.5 h, in contrast to the findings of earlier workers who reported 4.7 h [7]. This discrepancy might be due to differences in the esterase load, the pH of the whole HP, or the type of anticoagulant used during blood collection. The use of CAD as anticoagulant may increase the concentrations of potassium, citrate, lactate, and pyruvate ions as well as reducing the pH of plasma. In the previous work, neither pH of plasma nor anticoagulant was reported [7].

The degradation rates of 17-BMP in solution in non-enzymatic media, either 1% HSA ($\bar{k} = 0.134 \pm 0.006$ h^{-1}) or phosphate buffer ($\bar{k} = 0.188 \pm 0.007$ h^{-1}) were slower ($p < 0.0005$) than that in HP ($\bar{k} = 0.22 \pm 0.01$ h^{-1}). The degradation rate of 17-BMP in 1% HSA was substantially slower still compared with that in phosphate buffer (Table 2), suggesting that binding to serum albumin protects the ester, at least partially, from hydrolysis. The slower degradation rate found in phosphate buffer compared with that in HP is probably the result of the absence of esterases. Values of $t_{1/2}$ for 17-BMP in all three media were independent of the initial concentrations of parent drug (Table 2), consistent with the existence of pseudo-first-order kinetics as demonstrated previously for degradation of 17-BMP in artificial intestinal fluid [7]. The half-lives of 17-BMP ($C_0 = 40$ μg mL^{-1}) observed, in this study, in HP

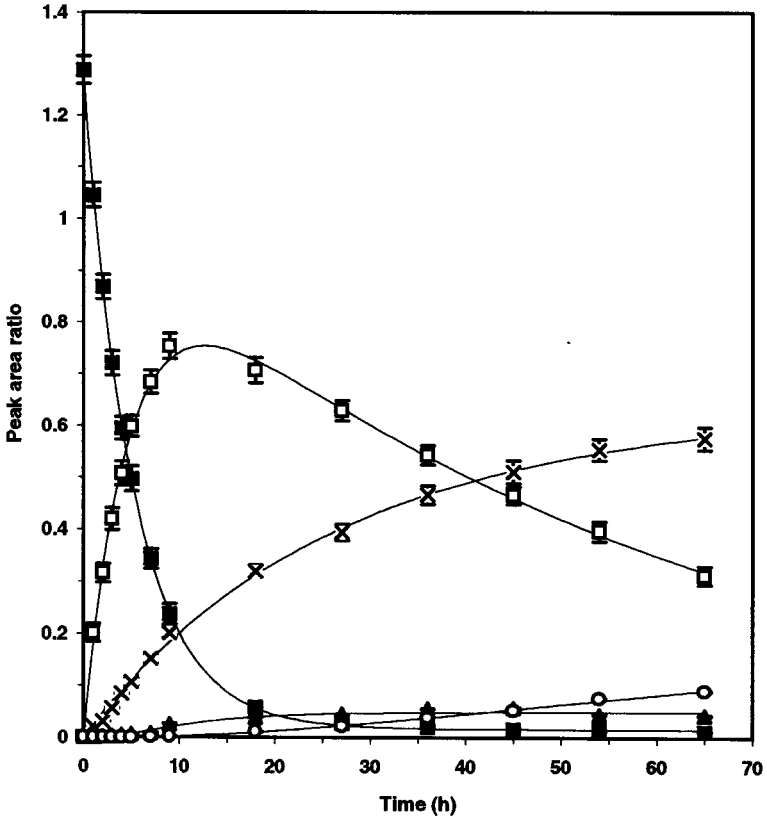


Figure 4. A typical kinetic profile of beclomethasone-17-monopropionate (17-BMP, 40 μg mL^{-1}) and its degradation products in 0.067 M phosphate buffer, pH 7.4, at 37°C: ■, 17-BMP; □, 21-BMP; ×, D3; ▲, BOH; ○, D2. D2 and D3 represent unknown species. The vertical bars indicate the S.D. of six determinations. When no bars are shown, the S.D. fell within the symbol dimensions

and phosphate buffer were 3.0 and 3.7 h, respectively. These values are broadly comparable with the corresponding values of 2.5 and 5.4 h which were reported previously [7].

It was observed that BOH was relatively more stable than 17-BMP and exhibited pseudo-first-order kinetics in all three media. Following the incubation of BOH as parent drug in HP, phosphate buffer, and 1% HSA for 3 h, 8.0, 5.4, and 4.4% of BOH, respectively was converted to D2. The degradation rate of BOH ($\bar{k} = 0.0280 \pm 0.0003\ h^{-1}$) in HP was 1.9 and 1.5 times faster ($p < 0.0005$) than that in 1% HSA ($\bar{k} = 0.0150 \pm 0.0002\ h^{-1}$) and phosphate buffer ($\bar{k} = 0.0185 \pm 0.0004\ h^{-1}$), respectively.

Effects of Esterases and Binding to Albumin on the Degradations of 17-BMP and BOH

There were significant differences in half-lives obtained, following the incubation of either 17-BMP and BOH in three different media (Table 2). It was observed that the half-lives of 17-BMP and BOH were substantially decreased in HP compared with those in phosphate buffer. However, values of $t_{1/2}$ obtained for 17-BMP and BOH in 1% HSA were considerably greater that those in phosphate buffer, presumably as a result of albumin binding. Overall it is expected that the presence of esterases and albumin binding might accelerate and retard, respectively, the degradation reactions of 17-BMP and BOH.

In conclusion, BDP is an important glucocorticoid used in the inhalation therapy of inflammatory pulmonary disease. BDP and its decomposition products, 17-BMP and BOH, undergo rapid, pseudo-first-order decomposition in HP at rates which are faster than those observed in 1% HSA and phosphate buffer. This is attributable to the presence of esterases in HP. Three unknown degradation products, D1–D3, were detected during the decomposition of BDP. These have not been reported previously. Based on the findings of the study, a new decomposition pathway for BDP and its degradation products is proposed, in which unknown D2 is the ultimate degradation product. Owing to the stability of unknown D2 in HP, it is possible that this compound could persist in the body for an appreciable period. Thus, characterization of its molecular structure and its intrinsic glucocorticoid activity is of considerable importance. Further work is needed to characterize the structures of the unknown species, D1–D3, which were separated in this study, and to test them for glucocorticoid activity.

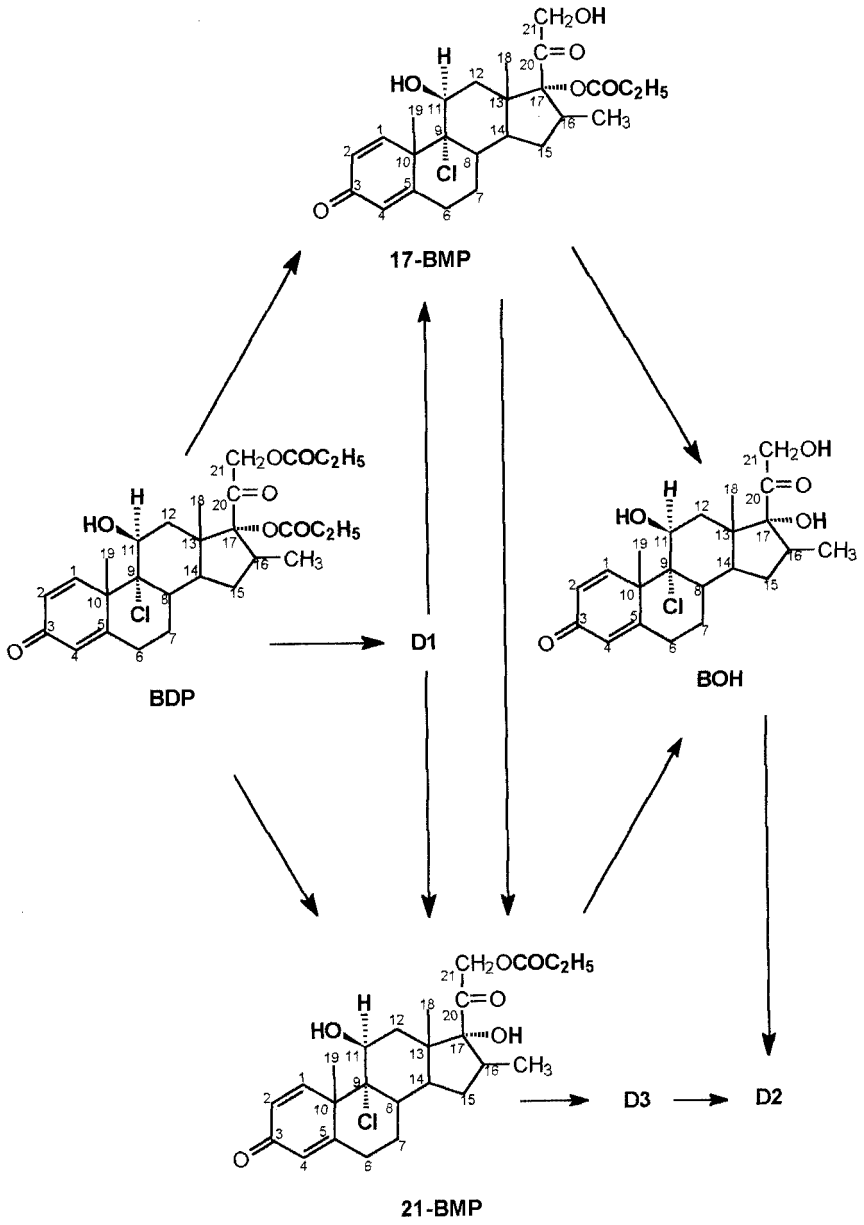


Figure 5. A proposed degradation pathway of beclomethasone dipropionate (BDP) in HP

Table 2. Half-life values (h) for the degradation of BDP, 17-BMP, and BOH with various initial concentrations (C_0) in three different media at 37°C. Experimental data represent the mean ± S.D. of six determinations

Parent drug	C_0 (μg mL^{-1})	HP	Phosphate buffer	1% HSA	p value[a]
BDP	40	10.9 ± 0.4	481 ± 6[b]	788 ± 6[b]	NA[c]
17-BMP	40	3.0 ± 0.2	3.7 ± 0.1	5.3 ± 0.2	<0.0005
BOH	40	24.8 ± 0.2	37.4 ± 0.9	46.1 ± 0.6	<0.0005
BDP	30	11.3 ± 0.2	360 ± 8[b]	618 ± 9[b]	NA
17-BMP	30	3.3 ± 0.1	3.7 ± 0.1	5.1 ± 0.2	<0.0005
BDP	20	10.5 ± 0.4	235 ± 5[b]	427 ± 9[b]	NA
17-BMP	20	3.1 ± 0.1	3.7 ± 0.2	5.0 ± 0.1	<0.0005

[a] Single-factor ANOVA between media.
[b] At concentrations of 20–40 μg mL^{-1}, BDP formed suspensions in 1% HSA and phosphate buffer. Thus half-lives in suspension are not comparable with those in solution.
[c] Non-applicable.

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