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STUDIES ON THE STABILITY OF CORTICOSTEROIDS VI. KINETICS OF THE REARRANGEMENT OF BETAMETHASONE-17-VALERATE TO THE 21-VALERATE ESTER IN AQUEOUS SOLUTION *

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SUMMARY

The kinetics of the degradation of betamethasone-17-valerate in aqueous solutions of pH 0.5–8 have been investigated at 60°C using a reversed-phase HPLC procedure for determining remaining steroid and the products of its degradation, betamethasone-21-valerate and betamethasone. The overall degradation was shown to proceed entirely through a rearrangement of the 17-valerate ester to the 21-valerate ester followed by hydrolysis of the latter to yield betamethasone. The acyl group migration from C_{17} to C_{21} was subject to both specific acid and base catalysis as well as to catalysis by water. The pH–rate profile for the rearrangement showed a minimum at pH 3.5.

INTRODUCTION

Clinically used esters of corticosteroids are most commonly C_{21}-esters. Betamethasone is an exception to this since the derivatives most widely used are C_{17}-esters, in particular betamethasone-17-valerate, which has 15 times the activity of the 21-isomer upon topical application (McKenzie and Athinson, 1964).

Corticosteroid-17-α-monoesters are unstable and in the presence of acid or base, they may undergo a rearrangement to the corresponding 21-monoesters (Gardi et al., 1963; Vitali and Gardi, 1972). Recently, Yip and Li Wan Po (1979) studied the stability of betamethasone-17-valerate ointment after dilution by various ointment bases. It appeared that isomerization of the 17-valerate ester to the 21-isomer may take place very facilely in some ointment preparations and that a basic pH favours the acyl migration. A ready iso-

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merization of the 17-valerate has also been described to occur in a propylene glycol solution containing ethanolamine (Li Wan Po and Yip, 1979). The pH does not appear, however, to be the only factor influencing the stability of betamethasone-17-valerate since ointments diluted by Plastibase are highly unstable despite having a pH near that of the stable undiluted ointment (Yip and Li Wan Po, 1979).

Numerous examples of O → O acyl group migrations exist in carbohydrate chemistry and specific acid- and base-catalyzed migrations have been reported; for example, various glycerol-β-monoesters (Lohuizen and Verkade, 1960; Wolfenden et al., 1964). However, no detailed kinetic studies of the isomerization of corticosteroid-17-esters to the corresponding 21-esters in aqueous solutions appear to have been described previously. The purpose of the present investigation was to study the kinetics and mechanism of degradation of betamethasone-17-valerate in aqueous solution over a broad range of pH. Information from such a study may be useful in the formulation and stability prediction of various preparations of the steroid ester.

MATERIALS AND METHODS

Apparatus

For high-performance liquid chromatography (HPLC) a Spectra-Physics Model 3500 B equipped with a variable-wavelength detector (8-μl 1-cm flow cells) and a 10-μl loop injection valve was used. The detector was connected to a Servogor RE 541 potentiometric recorder. A column (10 cm × 4.5 mm i.d.) packed with LiChrosorb RP-8 (5 μm particles) was used. Measurements of pH were done at the temperature of study using a Radiometer Type PHM 26 instrument.

Materials

Samples of betamethasone-17-valerate and betamethasone-21-valerate were kindly provided by Glaxo, Middlesex, England. Betamethasone was purchased from Sigma Chemicals, St. Louis. Solvents and all other chemicals used were of reagent grade.

Analysis of steroids by HPLC

Betamethasone-17-valerate and the degradation products betamethasone-21-valerate and betamethasone were determined by using a reversed-phase HPLC procedure which enabled separation and simultaneous quantitation of the steroids. The column was eluted at ambient temperature with an acetonitrile–water mixture (46 : 54 v/v) at a rate of 1.6 ml min^{-1} and the column effluent was monitored at 238 nm with the detector range setting at 0.01–0.02 absorbance units full scale. The content of steroids in the sample injected (10 μl) into the column was determined by comparing the peak heights with those of standards chromatographed under similar conditions. A chromatogram obtained from a partly degraded solution (pH 0.45) of betamethasone-17-valerate is shown in Fig. 1.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at a constant temperature. The buffers used were phosphate, acetate, citrate and hydrochloric acid solutions. A con-

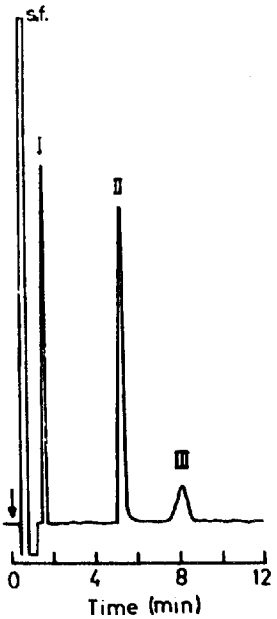


Fig. 1. High-performance liquid chromatogram of a partly degraded solution of betamethasone-17-valerate in 0.5 M hydrochloric acid. Key: I, betamethasone; II, betamethasone-17-valerate; III, betamethasone-21-valerate.

stant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. For the calculation of hydrogen ion and hydroxide ion concentrations from the measured pH at 60°C and $\mu = 0.5$ the following equations were used (Harned and Hamer, 1933):

$$\log[H^+] = 0.15 - pH \quad (1)$$

$$\log[OH^-] = pH - 12.87 \quad (2)$$

The reactions were initiated by adding 500 μl of an ethanolic stock solution of betamethasone-17-valerate (about 10^{-3} M) to 25.00 ml of pre-heated buffer solution in screw-capped test tubes. At appropriate intervals samples were taken and analyzed for remaining 17-ester as well as for 21-ester and free betamethasone by the HPLC assay described above.

RESULTS AND DISCUSSION

Kinetics of betamethasone-17-valerate degradation in aqueous solution

The rates of degradation of betamethasone-17-valerate were measured in aqueous solutions over the pH range 0.45–8.0 at 60°C. At constant pH and temperature the disappearance of the steroid displayed strict first-order kinetics over more than 4 half-lives. Pseudo-first-order rate constants for the overall degradation (k_{obs}) were determined from the slopes of linear plots of the logarithm of residual ester against time and some values are

TABLE 1

OBSERVED PSEUDO-FIRST-ORDER RATE CONSTANTS (k_{obs}) FOR THE OVERALL DEGRADATION OF BETAMETHASONE-17-VALERATE IN VARIOUS BUFFER SOLUTIONS (μ = 0.5; 60°C)

Buffer		pH	$k_{obs} \times 10^2$ (h^{-1})
Hydrochloric acid	0.1 M	1.15	1.5
Hydrochloric acid	0.01 M	2.15	0.35
Citrate	0.1 M	3.39	0.21
Citrate	0.2 M	3.39	0.23
Acetate	0.2 M	4.41	0.40
Acetate	0.1 M	5.48	1.8
Acetate	0.2 M	5.48	1.9
Phosphate	0.05 M	6.47	12
Phosphate	0.1 M	6.47	12
Phosphate	0.2 M	6.47	12
Phosphate	0.05 M	7.50	118
Phosphate	0.1 M	7.98	394

listed in Table 1. The degradation rates were found to be insignificantly affected by variation (0.05–0.2 M) in the concentration of the phosphate, citrate and acetate buffers used to maintain constant pH (cf. Table 1).

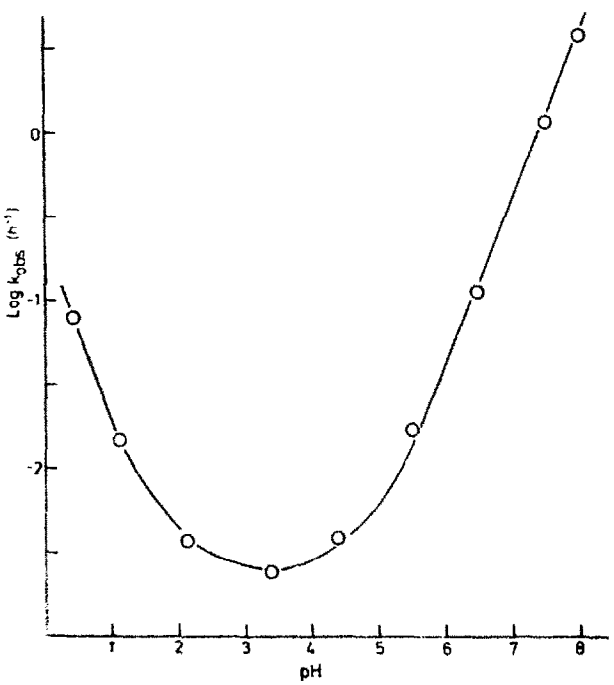


Fig. 2. pH–rate profile for the degradation of betamethasone-17-valerate in aqueous solution at 60°C (μ = 0.5). The points are experimental while the curve is calculated from Eqn. 3.

The effect of pH on the degradation rate at 60°C is shown in Fig. 2 in which log k_{obs} has been plotted against pH. In the ranges pH < 1 and pH > 6 the pH–rate profile shows two straight-line portions with slopes of −1.0 and 1.0, respectively, while an almost pH-independent plateau is observed between pH 2.5 and 4 with a rate minimum occurring at pH 3.5. This profile shape shows that the degradation of betamethasone-17-valerate is specific acid- and base-catalyzed as well as being due to a spontaneous or water-catalyzed reaction. The kinetic data may be described by Eqn. 3:

$$k_{obs} = k_H[H^+] + k_0 + k_{OH}[OH^-] \quad (3)$$

where $[H^+]$ and $[OH^-]$ refer to the hydrogen ion and hydroxide ion concentrations, respectively, k_H and k_{OH} are second-order rate constants for specific acid and base catalysis, respectively, and k_0 is a first-order rate constant for spontaneous degradation. The smooth curve in Fig. 2 was calculated with Eqn. 3 and the following values for the rate constants:

$k_H = 0.15\ M^{-1}\,h^{-1}$

$k_{OH} = 2.6 \times 10^5\ M^{-1}\,h^{-1}$

$k_0 = 2.4 \times 10^{-3}\ h^{-1}$

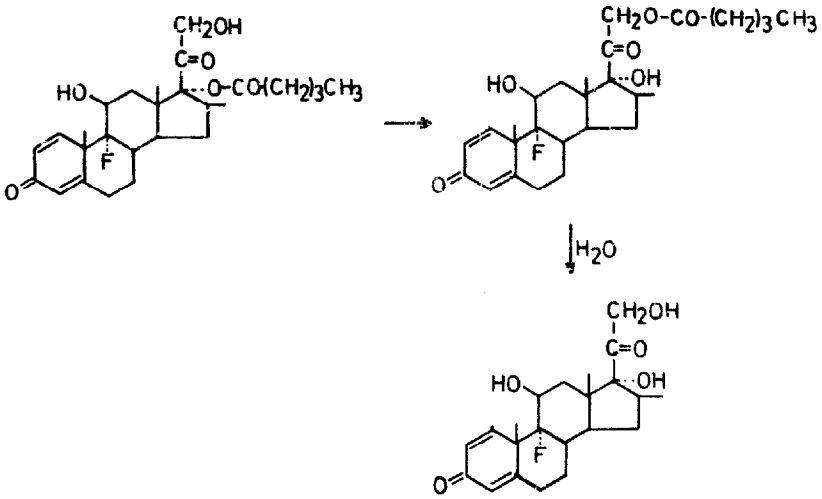
The good agreement observed between the calculated and experimental data demonstrates that the rate expression of Eqn. 3 adequately describes the degradation kinetics.

The effect of temperature on the degradation rate was determined in a 0.1 M phosphate buffer solution (pH 7.5; $\mu = 0.5$) in the range 30–60°C. From an Arrhenius-type plot of k_{OH} vs the reciprocal absolute temperature an apparent activation energy of 32.8 kJ mol^{-1} and a frequency factor of $3.4 \times 10^{10}\ h^{-1}$ were calculated.

Routes and products of degradation

The overall decomposition reactions were found to consist of a reaction sequence involving an initial rearrangement of betamethasone-17-valerate to betamethasone-21-valerate followed by hydrolysis of the latter to betamethasone (Scheme 1). From HPLC analyses of reaction solutions time-courses for these compounds were obtained over the pH range studied. Some examples are shown in Fig. 3. At pH > 4 the decrease in betamethasone-17-valerate was accompanied by the formation of 21-valerate in stoichiometric amounts (cf. Fig. 3B). Only after several reaction half-lives could the formation of free betamethasone be observed. This agreed with the finding obtained in separate experiments that the hydrolysis of the 21-ester to betamethasone at pH 7–8 proceeded much slower than the rearrangement of the 17-ester to the 21-ester, the difference in rate being a factor of 80.

In more acidic solutions the formation of betamethasone occurred simultaneously with the formation of the 21-ester. As can be seen from Fig. 3A a marked initial induction period was observed in the formation of betamethasone, indicating that this product arises from an intermediate (the 21-ester). By treatment of the concentration–time data



Scheme 1

for the 17-valerate and 21-valerate esters in the manner previously described for a combination of parallel and consecutive reactions (Bundgaard, 1980) it was found that the pseudo-first-order rate constants for the formation of 21-valerate were identical (within ±5%) to those for the overall degradation of 17-valerate over the whole pH range studied. Thus, a possible direct hydrolysis of the 17-ester to betamethasone is not significant in comparison with the rearrangement reaction. In addition, measurements of the *initial* rates of 21-valerate formation confirmed that the overall degradation of betamethasone-17-valerate proceeds entirely through rearrangement to the 21-valerate ester in the pH range 0.45–8.0. At pH 0.45 and 1.15 the hydrolysis of 21-valerate was found to proceed

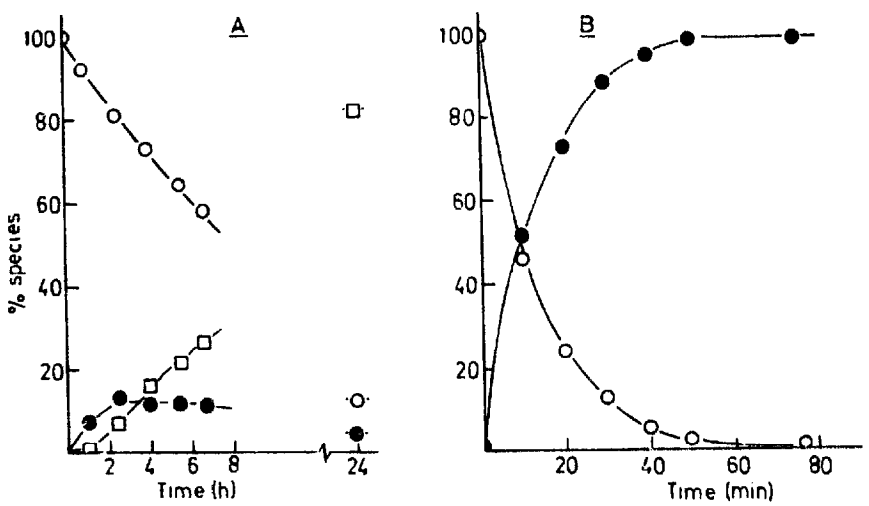
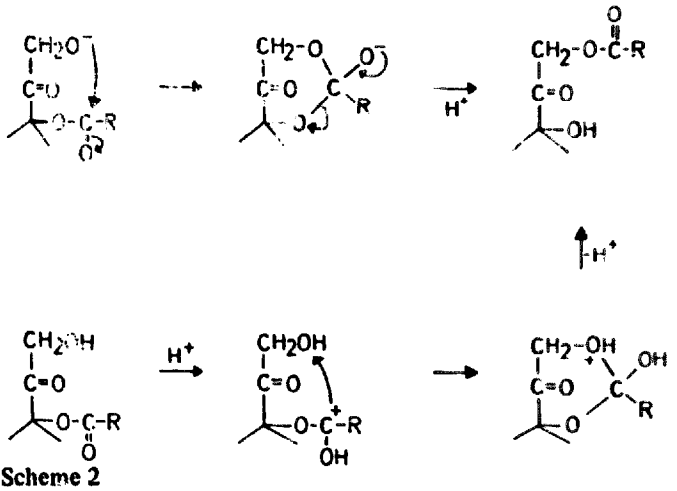


Fig. 3. Time-courses for betamethasone-17-valerate (○), betamethasone-21-valerate (●) and betamethasone (□) in the degradation of betamethasone-17-valerate in 0.5 M hydrochloric acid (A) or 0.1 M phosphate buffer of pH 7.98 (B) at 60°C. The concentrations at various times, expressed as per cent in relation to the initial 17-ester concentration, were determined by HPLC.



Scheme 2

4 times faster than the 17 → 21 acyl group migration.

Possible mechanisms for the rearrangement are depicted in Scheme 2. A cyclic orthoester may be an intermediate (Lohuizen and Verkade, 1960; Gardi et al., 1961), formed by a nucleophilic attack by a C_{21}-alkoxide ion upon the C_{17}-ester carbonyl moiety in the base-catalyzed reaction and by a reaction between the C_{21}-hydroxyl group and a carbonium ion centre at C_{17} in the acid-catalyzed reaction.

In conclusion, the results obtained demonstrate that the degradation of betamethasone-17-valerate in aqueous solution is due to a rearrangement to betamethasone-21-valerate and that this acyl group migration is subject to specific acid- and base-catalysis as well as to a spontaneous or water-catalyzed reaction.

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