Kinetic Study on Stability of Fluprednisolone Acetate in Aqueous Solution

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The shelf-life of a potential formulation of fluprednisolone acetate was predicted from kinetic measurements. Subsequent assay of samples stored at ambient temperatures confirmed the predicted stability. The degradation of the steroid ester to products devoid of the 17-dihydroxyacetone moiety followed a two-step sequence: ester → alcohol → degradation products. The kinetics of this pathway were interpreted on the basis of consecutive first-order reactions. Quantitative thin-layer chromatography was used to determine the two major components in solution.

ACCELERATED STUDIES on the stability of drugs in solution have been used extensively in attempts to predict the approximate shelf-life of potential pharmaceutical products. Several investigators have reported on the stability of steroids in solution (1–5). It has been shown that although the steroidal A-ring is subject to degradation, it is essentially a photolytic process and may be ignored in stability studies and in finished products, provided adequate protection from light is afforded. The rate of degradation of the 17-dihydroxyacetone moiety of cortical steroids in solution, however, is affected by its immediate environment, *i.e.*, of buffers, oxygen tension, pH of the solution, type of steroid, concentration, etc. Ideally, the effect of each component on the rate of decomposition may be determined independently and subsequently combined to yield an expression capable of predicting the shelf-life of a finished formulation. However, such an approach is quite time consuming. In product development it is often preferred to select what may appear to be a superior formulation based upon certain established criteria, then determine the stability of the active ingredient in this preparation.

Fluprednisolone acetate[1] has been found to possess strong antiinflammatory activity, both systemically and topically. This property made it a suitable candidate for incorporation into ophthalmic preparations. A kinetic study on the stability of fluprednisolone acetate in solution was undertaken to determine whether the steroid was sufficiently stable for consideration in ophthalmic solutions.

Previous reports on steroid esters have suggested that the side chain degradation of these compounds may involve both hydrolysis of the ester linkage and degradation of the dihydroxyacetone side chain (6, 7).

Received May 16, 1963, from Product Research and Development Unit, The Upjohn Co., Kalamazoo, Mich.
Accepted for publication August 22, 1963.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.

[1] Fluprednisolone acetate is 6α-fluoroprednisolone acetate.

It was assumed that partially degraded solutions of fluprednisolone acetate would contain fluprednisolone acetate, fluprednisolone, and some unknown steroid degradation products. The presence of the side chains in fluprednisolone acetate and fluprednisolone may be determined by the tetrazolium assay. Degradation products without the dihydroxyacetone side chain do not react to this test. Thus, the tetrazolium procedure may be used for determination of the total amount of fluprednisolone (acetate + alcohol). However, to meet the objectives of studying both the hydrolysis and the side-chain degradation, it was necessary to determine the relative amounts of each of the steroids. This was done by utilizing thin-layer chromatography in combination with the tetrazolium assay.

EXPERIMENTAL

Preparation of Solution

The solution under study was fluprednisolone acetate, 0.5 mg./ml.; polysorbate 80, 60 mg./ml.; sodium chloride U.S.P., 6.5 mg./ml.; phenylmercuric nitrate N.F., 0.025 mg./ml.; neomycin sulfate, 5.75 mg./ml.; water for injection U.S.P. *q.s. ad.* 1.00 ml. The pH of the solution was 7.20. The solution was filled into 5-ml. ampuls which were sealed and placed in constant temperature oil baths at temperatures ranging from 40 to 70°.

At requisite time intervals, ampuls were removed from the baths and assayed.

Assays

Total Fluprednisolone.—The total fluprednisolone (acetate + alcohol) content was determined by the blue tetrazolium procedure (8).

Reagents.—BLUE TETRAZOLIUM SOLUTION.—Blue tetrazolium (Fisher certified reagent) dissolved in 3A alcohol in a concentration of 5 mg./ml.

TETRAMETHYLAMMONIUM HYDROXIDE SOLUTION.—A dilution of 10% aqueous tetramethylammonium hydroxide with 3A alcohol (1:10).

REFERENCE STANDARD SOLUTIONS.—Solutions of fluprednisolone (reference standard) and fluprednisolone acetate (reference standard) in 3A alcohol in concentrations of 10 mcg./ml.

Procedure.—A 2.00-ml. aliquot of the ophthalmic solution was diluted with 2 ml. of water and extracted

with three 25-ml. portions of chloroform. The chloroform layers were separated, filtered through cotton, and collected in a 100-ml. volumetric flask. The volume was made up to 100.0 ml. with chloroform. A 10.0-ml. aliquot of the chloroform solution was evaporated to dryness *in vacuo*. The dry residue was dissolved in 10.0 ml. of 3A alcohol. Exactly 1.00 ml. of the blue tetrazolium solution was added, followed by 1.00 ml. of tetramethylammonium hydroxide solution. Exactly 45 minutes after the addition of the former solution, 1.00 ml. of glacial acetic acid was added to quench further color formation and to stabilize the color already formed. The absorbance of the solution was measured at 525 $m\mu$ in a Beckman DU spectrophotometer with a reagent blank in the reference cell. Parallel runs on standard solutions of fluprednisolone and fluprednisolone acetate were carried out for each sample assayed.

Separation of Fluprednisolone and Fluprednisolone Acetate by Thin-Layer Chromatography.—Four milliliters of the ophthalmic solution were extracted with three 25-ml. portions of chloroform. The chloroform extracts were combined, evaporated to dryness, and redissolved in ethylene dichloride–methanol (1:1) to a total volume of 1.00 ml. Exactly 25 μl. of this solution was spotted on a thin-layer chromatography plate coated with silica gel G in a thickness of 250 μ. The plate was developed in a mobile phase of chloroform-methanol (9:1 v/v) for 80 minutes and dried at 40° for 5 minutes. (Using this procedure, the approximate R_f values for fluprednisolone and fluprednisolone acetate are 0.12 and 0.50, respectively.) The positions of fluprednisolone and fluprednisolone acetate on the plate were located by viewing the plate under a Mineralight, model SL 2537. The silica gel areas containing the steroids were scraped off the plate separately with a razor blade and collected in small Büchner sintered-glass funnels. The silica gel was then eluted with 3A alcohol and the solution filtered through the glass funnel. The volume of the filtrate was made up to 10.0 ml., and the steroid content was determined by the blue tetrazolium procedure described above.

The absorbance readings for fluprednisolone and fluprednisolone acetate, respectively, indicated the relative proportions of the two steroids in the original sample. By applying this proportionality figure to the total fluprednisolone assay, the absolute amounts of fluprednisolone and fluprednisolone acetate in the sample were calculated. Absorptivity values for the calculations were determined from parallel assays of standard solutions of fluprednisolone and fluprednisolone acetate.

Verification of Assay Procedures.—The validity of the assays was checked on freshly prepared solutions of fluprednisolone acetate and fluprednisolone of known concentrations. The solutions were mixed in various proportions and then assayed by the procedures outlined above. The results are shown in Table I.

TABLE I.—RECOVERY OF FLUPREDNISOLONE AND FLUPREDNISOLONE ACETATE FROM SOLUTIONS OF THE TWO STEROIDS OF KNOWN CONCENTRATION

Steroid in Soln., mmoles/L.		Steroid Recovered, mmoles/L.	
Fluprednisolone	Fluprednisolone Acetate	Fluprednisolone	Fluprednisolone Acetate
0	1.226	0.012	1.218
0.632	0.920	0.642	0.917
1.262	0.613	1.244	0.630
1.893	0.307	1.928	0.287
2.524	0	2.537	0

The results show that the separation of fluprednisolone and fluprednisolone acetate in the assay is quite complete. The accuracy of the determination of the major component is within ±2% of theory.

RESULTS AND DISCUSSION

It has been reported in the literature that the hydrolysis of steroid esters is pH dependent (6, 7). Therefore, in the present study it was necessary to stabilize the pH of the solution in order to eliminate the effect of this variable. Neomycin sulfate provided sufficient buffer effect to maintain the pH within 0.6 unit of the initial pH of 7.20.

The assay procedures described above were applied to the individual samples to determine at each temperature the concentrations of fluprednisolone and fluprednisolone acetate in solution as a function of time. A semilog plot of the concentration of fluprednisolone acetate *versus* time is shown in Fig. 1. The linear relationships indicate that the disappearance of fluprednisolone acetate is first order with respect to drug concentration. The corresponding rate constants, k_1, were calculated and are listed in Table II.

The main objective of the study was to determine the suitability of the fluprednisolone acetate solution as a potential product. For this reason, it was of interest to estimate the rate constant, k_1, at 25°. This was done by extrapolation from the higher temperature data while the study was in progress, and k_1 was determined to be 0.0081 days^{-1}. This estimate was later verified by actual experimentation at 25°, and the identical value was obtained. Using this value for k_1, it was calculated that at room temperature 10% of the fluprednisolone acetate would degrade in 13 days ($t_{0.90}$ = 13 days), a period too short to consider the ophthalmic solution a potential product.

Examination of the assay results showed that the decrease in the concentration of fluprednisolone acetate was accompanied by an increase in the concentration of fluprednisolone which reached a maximum and then decreased (Fig. 2). This pattern suggested that consecutive first-order reactions were involved and that fluprednisolone acetate was hydrolyzed to fluprednisolone which then subsequently decom-

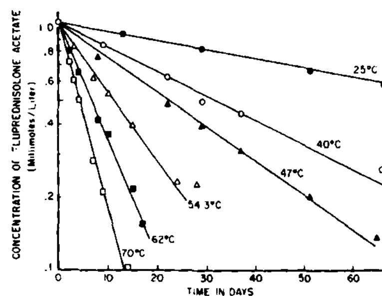


Fig. 1.—First-order plot of the disappearance of fluprednisolone acetate from aqueous solution at various temperatures.

Table II. —Rate Constants[a] for Degradation of Fluprednisolone Acetate in Aqueous Solution

Temp., °C.	k_1, days^{-1}	$\beta_{max.}$	K	k_2, days^{-1}
70	0.186	0.494	0.513	0.0952
62	0.113	0.501	0.497	0.0560
54.3	0.0678	0.493	0.516	0.0350
47	0.0339	0.505	0.495	0.0168
40	0.0250	No maximum	...	...
25	0.0081	No maximum	...	...

[a] These are pseudo first-order rate constants since both reactions are pH dependent.

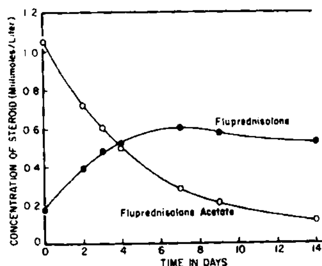
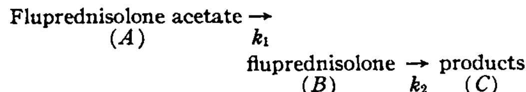


Fig. 2.—Typical plot showing the changes in concentrations of fluprednisolone and fluprednisolone acetate in solution as a function of time (temperature 70°C.).

posed to further degradation products. This is shown schematically in



where k_1 and k_2 are first-order rate constants.

The rate of change in the system may then be expressed by the differential equations

$$\frac{dA}{dt} = -k_1 A \qquad \text{(Eq. 1)}$$

$$\frac{dB}{dt} = k_1 A - k_2 B \qquad \text{(Eq. 2)}$$

$$\frac{dC}{dt} = k_2 B \qquad \text{(Eq. 3)}$$

Equation 1 may be integrated to give

$$A = A_o e^{-k_1 t} \qquad \text{(Eq. 4)}$$

or

$$\log A = -\frac{k_1 t}{2.303} + \log A_o$$

where A_o = initial concentration of A.

Thus, k_1 may be obtained by plotting log A *versus* time as shown in Fig. 1.

Equation 2 may be integrated to give

$$B = \frac{A_o k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \qquad \text{(Eq. 5)}$$

To obtain rate constant, k_2, Eqs. 4 and 5 are transformed using dimensionless parameters and variables as described by Frost and Pearson (9). Let $\alpha = A/A_o$; $\beta = B/A_o$; $q = k_1 t$; and $K = k_2/k_1$ then Eqs. 4 and 5 become

$$\alpha = e^{-q} \qquad \text{(Eq. 6)}$$

$$\beta = \frac{1}{K-1}(e^{-q} - e^{-Kq}) \qquad \text{(Eq. 7)}$$

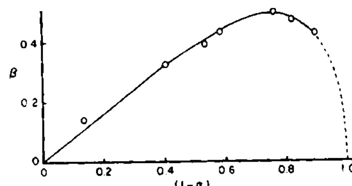


Fig. 3.—Typical plot of β *vs.* (1-α) (temperature 70°C.).

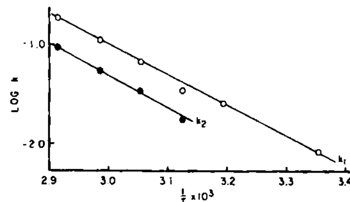


Fig. 4.—Arrhenius plot of the rate constant data ($Ea.k_1$ = 14.2 Kcal./mole; $Ea.k_2$ = 15.2 Kcal./mole).

When β is plotted *versus* $(1 - \alpha)$, a curve is obtained which goes through a maximum, the position of which depends on K. By using Eq. 7 and setting $d\beta/dq = 0$, it is found that $q_{max.} = lnK/(K - 1)$.

The value of β at the maximum is

$$\beta_{max.} = K^{K/(1-K)} \qquad \text{(Eq. 8)}$$

Determination of $\beta_{max.}$ is carried out graphically by plotting β *versus* $(1 - \alpha)$ and reading the value of β at the maximum off the graph. A typical plot of β *versus* $(1 - \alpha)$ is shown in Fig. 3. The value for $\beta_{max.}$ is used for the determination of K from Eq. 8.[2] The rate constant, k_1, was determined from Eq. 4. Thus, it is possible to determine rate constant, k_2, from $k_2 = K \times k_1$.

Values of k_2 and corresponding values of $\beta_{max.}$ and K are listed in Table II. An Arrhenius plot of the rate constant data is shown in Fig. 4.

The fact that the pathway of degradation of fluprednisolone acetate follows a consecutive first-order reaction sequence implies that the 17-dihydroxyacetone side chain is, in effect, protected by substitution at the 21 position. However, in the case of fluprednisolone the acetate ester does not afford adequate protection to assure stability in aqueous, neutral solution.

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[2] Equation 8 may be solved conveniently with an electronic computer. In the absence of a computer, K may be determined graphically. A curve is constructed from Eq. 8 using K as the abscissa and $\beta_{max.}$ as the ordinate, assigning different values for K, and calculating the corresponding values for $\beta_{max.}$. K may then be determined from the graph as the abscissa value which corresponds to the ordinate value of $\beta_{max.}$ as determined from Fig. 3.