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COMMUNICATIONS

The degradation of triamcinolone acetonide in aqueous solution: influence of the cyclic ketal function

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The stability of 21-hydroxycorticosteroids in aqueous systems has been widely studied (e.g. Amin & Bryan 1973; Chulski & Forist 1958; Dekker & Beijnen 1980; Hansen & Bundgaard 1979; Oesterling & Guttman 1964). Oxidative and non-oxidative reactions occur at the C17 side chain, the primary site of corticosteroid decomposition.

Recent work by Dekker & Beijnen (1981, 1982) has indicated that substitution on neighbouring sites to the C17 side chain can affect the mechanisms and rate of decomposition. As there appears to be no information in the literature on the stability of cyclic corticosteroid ketals such as triamcinolone acetonide (I) the decomposition of this compound in aqueous buffers has been examined. Comparison with data generated under similar conditions for triamcinolone (II) would allow determination of the effect of the cyclic ketal function on steroid stability. Preformulation studies of this type also enable more rational formulation of water-containing systems such as creams and lotions to achieve maximum stability of the active in such a preparation.

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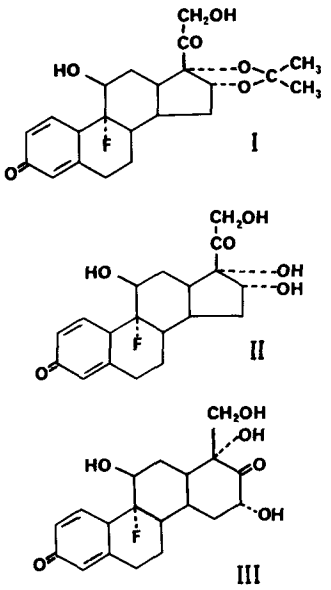

Materials and methods

Triamcinolone acetonide and triamcinolone were of > 99% purity. Buffer salts were of Analar quality (BDH, England) and solvents for h.p.l.c. were HPLC grade (Rathburn Chemicals, Scotland). Water was distilled from an all glass still.

H.p.l.c. analysis of triamcinolone acetonide was carried out using a reversed-phase column (10 cm × 0·5 cm 5 μm Hyperspheres ODS) which was eluted with a mixture of methanol–water (54:46, v/v) and the eluate was monitored with a u.v. detector set at 240 nm. For the analysis of triamcinolone a 20 cm × 0·5 cm 5 μm. Hyperspheres ODS column was used and the eluting solvent was a mixture of methanol: pH 7·0 (0·0067 M) phosphate buffer (42:58 v/v).

Buffer solution (20 μl) containing steroid was injected on to the column with a rotary valve injector. Quantitation of steroid was accomplished by comparison of sample and standard solution peak areas.

For the kinetic experiments 8 ml of buffer in a vial was spiked with a methanolic solution of triamcinolone acetonide or triamcinolone (0·65 mg ml^{-1}) to give a final steroid concentration of approximately 25 μg ml^{-1}. Buffers employed are given in Table 1. All buffers contained 0·05% w/v disodium edetate to retard metal-ion catalysed oxidation and were adjusted to constant ionic strength by addition of a calculated amount of potassium chloride. Headspace was sufficiently large to allow excess oxygen throughout the course of decomposition studies.

The sealed vials were stored in an oven at 50 ± 1·0 °C and samples were removed at intervals for h.p.l.c. analysis.

Results and discussion

Both assay procedures were confirmed as stability-indicating as the peak on the chromatogram due to the

Table 1. Rate constant for degradation of triamcinolone acetonide in aqueous buffers.

Buffer	pH	Rate constt ($days^{-1}$)
0·1 M Hydrochloric acid	1·40	0·161
1·0 M Hydrochloric acid	2·30	$9·67 \times 10^{-3}$
McIlvaine citrate phosphate	3·14	$3·04 \times 10^{-3}$
	3·64	$2·65 \times 10^{-3}$
	4·20	$2·12 \times 10^{-3}$
	4·76	$4·41 \times 10^{-3}$
	5·34	$8·94 \times 10^{-3}$
	5·94	$1·95 \times 10^{-2}$
	6·51	$2·90 \times 10^{-2}$
	7·20	$6·45 \times 10^{-2}$
Sodium borate-sodium	9·13	$7·58 \times 10^{-2}$
hydroxide	9·66	0·204
	10·09	0·498
	10·29	0·827
0·1 M Sodium hydroxide	11·17	2·13

parent steroid completely disappeared during degradation and disappearance of steroid from all buffer solutions could be described by first order kinetics. These observations imply that no stable degradation product with the same retention time as the parent steroid was formed. Products formed were almost exclusively due to degradation of the C-17 side chain, although the pattern of products formed was different for the two steroids (Timmins & Gray, unpublished observations).

Rate constants were calculated from the slopes of the first order regression lines for triamcinolone acetonide and are given in Table 1. A plot of logarithm of the rate constant versus pH (Fig. 1) is very similar to that for hydrocortisone displaying specific acid catalysis in the region pH 1–3 and specific base catalysis in the regions pH 4–7 and 9–12. The pH of maximum stability is approximately pH 4 and a pH-independent region occurs at pH 7–9. In constructing the pH-rate profile any catalytic effect due to the buffers used was neglected. Published work (Hansen & Bundgaard 1979) indicates that any catalytic effect of the chosen buffers would be minor, the significantly catalytic acetate buffer being avoided.

The shape of this pH-rate profile was explained for hydrocortisone in terms of enolization and ionization of the enol (Hansen & Bundgaard 1979). The role of enolization in the degradation of steroids has been further investigated by Johnson (1982) using cloprednol as a model compound. It was shown that in the neutral to alkaline region enolization is not a rate-determining step whereas enolization must occur before product formation in the acid region.

Specific rate constants were calculated from the extremes of pH and at the pH independent region in accord with the argument proposed by Hansen & Bundgaard (1979). In the acid region K_H for the reaction:

$$\text{steroid} + H^+ \rightarrow \text{product}$$

is given by: $K_{obs} = K_H (H^+)$

and found to be 0·17 mol^{-1} h^{-1}. In the alkaline region K_{OH} for the reaction:

$$\text{steroid enolate} + OH^- \rightarrow \text{products},$$

given by: $K_{obs} = K_{OH} (OH^-)$

was found to be 59·2 mol^{-1} h^{-1}. For the pH independent region pH 7–9 the specific rate constant, $K^1_{H_2O}$ for the reaction:

$$\text{steroid enolate} + H_2O \rightarrow \text{products}$$

was found to be $4·84 \times 10^{-5}$ mol^{-1} h^{-1}.

The curve in Fig. 1 was calculated from the contribution of the individual reactions to the overall decomposition rate. In the region pH 6–9 the equation:

$$K = \frac{K_e}{[H^+] + K_e} (K_{OH}[OH^-] + K^1_{H_2O}[H_2O])$$

modified from Hansen & Bundgaard (1979) was used, where K_e, the enol ionization constant, was estimated as $10^{-5·5}$ from the rate data obtained.

Included in Fig. 1 are experimental points for triamcinolone, the rate constants again being determined from the slopes of first order regression lines. No inflection corresponding to that attributed to enolization and enol ionization in triamcinolone acetonide is present. The pH of maximum stability is about pH 3·5 and above that a straight line of slope 1·0 can be drawn through the points above pH 4, implying specific base catalysis.

It would appear that enolization and ionization of the enol is not implicated in an explanation of the pH-rate profile for degradation of triamcinolone in neutral to

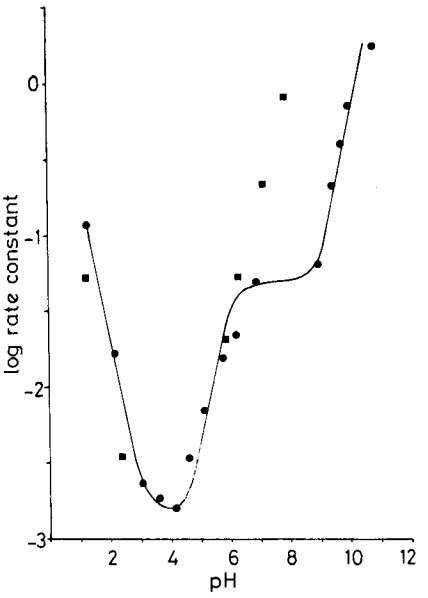

FIG. 1. pH-rate profile for the degradation of triamcinolone acetonide and triamcinolone in aqueous solution. ● triamcinolone acetonide experimental points; ■ triamcinolone experimental points.

basic solution. One of the major degradation products of triamcinolone in basic media is the D-homosteroid III (Timmins & Gray, unpublished observations) and this reaction is therefore not dependent on an enolization step. D-homosteroids were not observed as major degradation products of triamcinolone acetonide.

In summary, it may be seen that in neutral and basic media the presence of the acetonide function stabilizes the steroid ketal in comparison with the parent 16α-hydroxysteroid, preventing hydroxide ion-catalysed decomposition to the D-homosteroid. Below pH 7 the shape of the pH-rate profile for the steroid ketal is almost identical to that for the parent 16α-hydroxy steroid. The shape of the pH-rate profile for the steroid ketal can be explained in terms of enol formation ionization of the enol whereas this has little influence on the decomposition of the parent 16α-hydroxysteroid. The decomposition of the steroid ketal thus parallels that of steroids possessing no C16 hydroxyl such as hydrocortisone.

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Insulin suppository: enhanced rectal absorption of insulin using an enamine derivative as a new promoter

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The rectal administration of insulin suppositories containing the surfactant, polyoxyethylene-9-laurylether (BL-9-EX, final concentration of 3% w/w), to diabetic dogs and diabetic patients has been reported to be effective in lowering the plasma glucose concentration with a dose as low as 2 u kg^{-1} without impairment to the rectal mucosae (Shichiri et al 1979; Yamasaki et al 1981a; Yamasaki et al 1981b). However, the use of non-ionic ether type surfactants at a concentration of 0·1% resulted in transient damage to nasal mucosae (Hirai et al 1981). Rectal absorption of antibiotics was found to be enhanced by enamine derivatives (Murakami et al 1981). One such derivative DL-phenylalanine-ethylacetoacetate, synthesized by reacting sodium phenylalaninate and ethylacetoacetate (β-diketone), is hydrolysed to phenylalanine and ethylacetoacetate during absorption. Ethylacetoacetate is widely used as a food flavouring additive in many countries. Thus, it is considered to be one of the most suitable materials in view of its low toxicity. We report the effectiveness of DL-phenylalanine-ethylacetoacetate, as a promoter for rectal absorption of insulin.

Insulin suppositories were prepared by mixing crystalline pork insulin, 5% DL-phenylalanine-ethylacetoacetate, and Witepsol H-15 as a base.

Insulin (12 u g^{-1}) suppositories so prepared were given at a dose of 1 or 2 u kg^{-1} to 5 normal dogs (10·0 ± 1·0 kg) which were fasted for 18 h previously. The results were compared with those from insulin suppositories containing BL-9-EX (3% w/w) as described by Shichiri et al (1979). The suppositories at 1 or 2 u kg^{-1} were administered also to five depancreatized dogs (10·0 ± 1·1 kg) made hyperglycaemic by with-

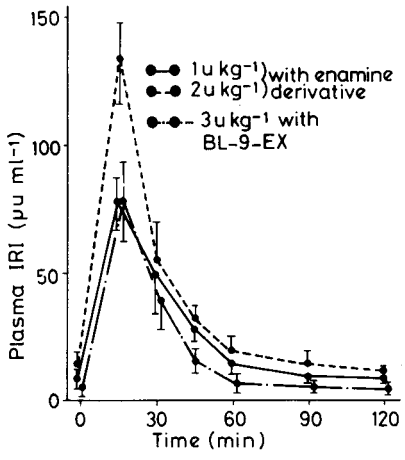

FIG. 1. Plasma insulin concentrations after rectal administration of insulin suppositories (1 u kg^{-1} and 2 u kg^{-1}) containing DL-phenylalanine-ethylacetoacetate, and that (3 u kg^{-1}) with polyoxyethylene-9-laurylether in normal dogs. Data are expressed as mean ± s.e.m. (n = 5).

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