Factors Influencing Stability of Prednisolone in Aqueous Solution

By THOMAS O. OESTERLING and DAVID E. GUTTMAN*

An investigation was made of some of the factors which influence the rate of disappearance of prednisolone from aqueous solution. The rate exhibited a marked dependency on buffer concentration. Incorporation of a sequestering agent into the solutions eliminated this dependency and indicated that trace-metal impurities which were present in the buffer reagents were catalytically involved in the degradation. Rate constants were determined over a wide range of pH in borate and phosphate buffers and in the presence and absence of disodium ethylenediaminetetraacetic acid. The rate of the apparent metal-catalyzed reaction was pH independent above pH 7 and below pH 5 and exhibited a first-order dependency on hydroxide-ion in the intermediate range. In the presence of sequestering agent, the rate of reaction was strongly dependent on hydroxide-ion concentration above pH 8 but exhibited only slight dependency at lower pH values.

A NUMBER OF studies has demonstrated that prednisolone and related corticosteroids are susceptible to degradative reactions which involve the 17-dihydroxyacetone side-chain (1–4). Transformations and elimination of the side-chain have been shown to occur both in the presence and absence of oxygen. Autoxidation, however, appears to be the mode of destruction which is most likely to be responsible for stability problems in drug products. The involvement of trace metals in catalyzing the autoxidation is an obvious possibility and has been reported in the literature. For example, Caspi *et al.* (5) effected an oxidative cleavage of the dihydroxyacetone moiety of cortisone with lead tetraacetate to therapeutically inactive products. Similarly, trace amounts of copper are actively involved in the reactivity of the C-17 function. Lewbart and Mattox (6) reported that trace amounts of copper, present in glassware, caused destruction of cortisone and related steroids. The steroidal destruction was prevented by the addition of ethylenediaminetetraacetic acid. Other workers have shown that 20-keto-21-hydroxy steroids, such as prednisolone, are readily oxidized to the corresponding glyoxals (20-keto-21-aldehydes) in the presence of cupric acetate (7–10).

The objectives of this investigation were to quantitate an observation that the trace metal content of chemicals commonly employed to formulate buffers was sufficient to accelerate markedly the destruction of prednisolone in solution and to investigate stability characteristics of the steroid in the presence and absence of a sequestering agent.

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EXPERIMENTAL

Materials.—Prednisolone was crystallized from a hydro-alcoholic solution several times and dried at 110°, m.p. 233–234°, uncorrected. Sequestrene Na_2 [disodium salt of ethylenediaminetetraacetic acid (EDTA)] was obtained from the Alrose Chemical Co. Assay reagents were 2,3,5-triphenyltetrazolium chloride (Mann Research Laboratories, Inc.) and tetramethylammonium hydroxide (Eastman Organic Chemicals) as a 10% aqueous solution. Buffers were prepared with reagent grade chemicals and double-distilled deionized water and were adjusted to the desired pH using a Beckman model G pH meter. Solutions containing sequestering agent were adjusted to the desired pH after the concentration had been made 0.1% with respect to sequesterene Na_2. A constant ionic strength of 0.2 was maintained by adding KCl to the borate systems utilized in studying the effect of buffer concentration on rate of degradation.

Procedure.—A 100-ml. low-actinium volumetric flask containing approximately 98 ml. of appropriate buffer was placed in a Sargent Thermonitor constant temperature bath. After thermal equilibrium had been attained, 25 mg. of prednisolone in 1 ml. of absolute methanol was added, and the contents of the flask were mixed. The solution was made to volume with buffer and again rendered homogeneous. At appropriate intervals 5-ml. samples were withdrawn and added to 5 ml. of 0.2 *N* acetic acid to quench the reaction. Four-milliliter aliquots of each sample were extracted with four successive 10-ml. portions of chloroform. The chloroform was removed *in vacuo*, and the residue was reconstituted with 20 ml. of absolute ethanol. The concentration of undergraded prednisolone in the sample was determined by subjecting 10 ml. of the reconstituted ethanolic solution to a tetrazolium assay (11).

RESULTS AND DISCUSSION

This study of factors which influence the stability of corticosteroids was motivated primarily by an observation that the rate of disappearance of pred-

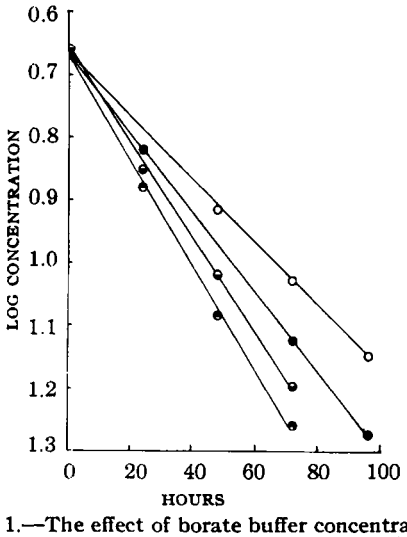

Fig. 1.—The effect of borate buffer concentration on the rate of prednisolone degradation at 35° and ionic strength 0.2. Key: ○, 0.05 *M*; ●, 0.01 *M*; ◒, 0.15 *M*; ◓, 0.20 *M*.

nisolone from buffered aqueous solutions, prepared with double-distilled deionized water, exhibited a dependency on the concentration of buffer, in spite of the fact that pH and ionic strength were maintained constant. This effect is illustrated in Fig. 1, where some representative results are plotted and show that the first-order disappearance of prednisolone occurred much more rapidly from solutions which contained a relatively high concentration of borate buffer than from less concentrated solutions. This effect was noted at a number of different pH values and suggested, at least initially, the possibility that general acid and/or general base catalysis was responsible. A more definitive study, which is illustrated in Fig. 2, ruled out this possibility. This plot summarizes the results of a study in which first-order rate constants were determined as a function of buffer concentration in two sets of systems, which differed only in that one contained 0.1% of the disodium salt of EDTA, while the other contained no sequestering agent. It may be seen that in the presence of EDTA, the observed rate was independent of the concentration of buffer. In its absence the rates were significautly faster, dependent on the concentration of buffer, and the rate constant appeared to be approaching an asymptotic value at high concentrations of buffer. On the basis of this evidence, it was concluded that components of the buffer had no catalytic influence *per se*, but that they served as carriers of trace metal contaminants which accelerated the decomposition.

The influence of pH on the stability of prednisolone in borate buffer in the presence and absence of EDTA is graphically summarized in Fig. 3, where rate constant is plotted as a function of pH. In reference to this plot and Fig. 4, it should be pointed out that there is some uncertainty about the accuracy of the rate constants for systems which did not contain EDTA. In such systems the disappearance of steroid occurred quite rapidly, and the scatter of experimental points in the semilog plots precluded precise graphical determination of rate constants. In contrast, excellent fits were obtained in equivalent plots for systems which contained EDTA.

The shape of the curves indicates that, both in the presence and in the absence of EDTA, the rate of disappearance of prednisolone was first order with respect to hydroxide-ion at higher pH values but less than first order at the lower values studied. A comparison of the two curves shows that the sequestering agent had little effect on rate in systems maintained at the lower pH values. At higher pH values the disappearance of prednisolone occurred much more rapidly from systems which were not protected by sequestering agent. The degree of protection, reflected by the vertical distance between the two curves, increases with an increase in pH. The nature of the curves suggests that a constant difference is approached at higher pH values.

The pH profile was extended to a lower pH region by investigating the stability in phosphate buffers. A higher temperature (70°) was utilized here to increase the rate for the sake of expediency. The results are shown in Fig. 4. The observed influence of pH is rather unusual and indicates interesting changes in the nature of the involvement of hydroxide-ion in the degradative reactions. In the EDTA-containing system, the rate exhibited only a relatively small pH dependency over a fairly wide range of pH 5–8. Above pH 8, the rate is much more dependent on pH; the curve suggests that in this pH region the order of the degradation with respect to hydroxide-ion changes from zero order

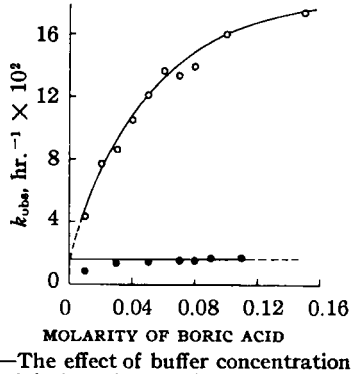

Fig. 2.—The effect of buffer concentration on the rate of prednisolone degradation in the presence and absence of sequestrene Na_2 at 30°, pH 10, and ionic strength 0.2. Key: ○, no EDTA; ●, 0.1% EDTA.

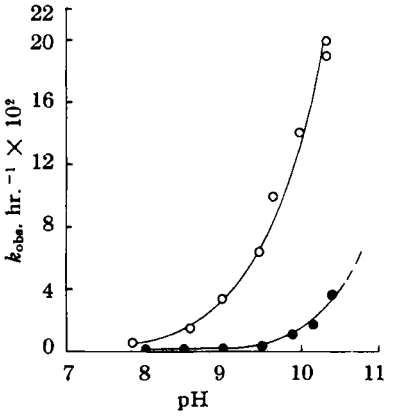

Fig. 3.—The effect of pH on the rate of degradation of prednisolone in the presence and absence of sequestrene Na_2 at 35° in 0.1 *M* borate buffers. Key: ○, no EDTA; ●, 0.1% EDTA.

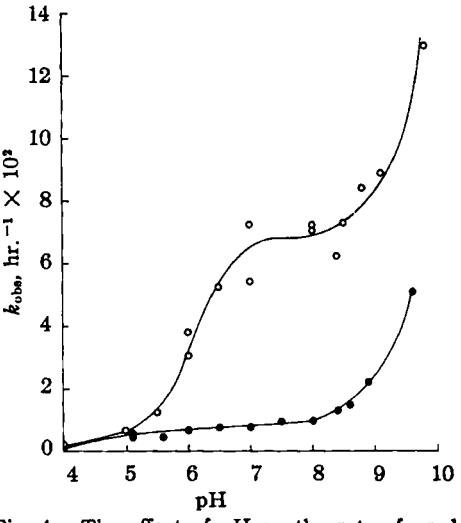

Fig. 4.—The effect of pH on the rate of prednisolone degradation in the presence and absence of sequestrene Na_2 at 70° in 0.1 *M* phosphate buffers. Key: ○, no EDTA; ●, 0.1% EDTA.

to first order. In the absence of EDTA, the order with respect to hydroxide-ion appears to be first at lower pH values, zero at intermediate values, and first at the higher extreme.

An insight into the nature of the metal-catalyzed reaction was obtained by a treatment of the data which are illustrated in Fig. 5. Here rate constants for the metal-catalyzed degradation were plotted as a function of pH. These constants were obtained by taking the difference between rate constants determined in the absence (k_0) and presence (k_s) of EDTA at the same pH. The differences were interpolated from the curves of Fig. 4.

Below pH 5, incorporation of EDTA into the solutions had little effect on the velocity of disappearance of prednisolone. This might have resulted from a reduction in the efficiency of the sequestering agent at lower pH values. Thus, metal catalysis might have been operant in systems which contained EDTA. Alternatively, and possibly more likely, the observed instability in both systems in this pH region was primarily due to reactions which were not dependent on metal catalysis. As the pH was increased, the velocity of the metal-catalyzed reaction increased in a manner which suggested first-order dependency on hydroxide-ion. With subsequent increases in pH, the order changed from first to zero, so that at higher pH values the rate of the metal-catalyzed reaction was essentially independent of pH.

It is interesting to note that the influence of pH on the metal-catalyzed reaction in phosphate buffer was somewhat different from that in borate buffer. At the lower pH values investigated in borate buffer (*i.e.*, below approximately 8.5), only small differences in rate constants were obtained between solutions which contained EDTA and those which did not. In contrast, the differences were quite large in phosphate systems throughout this pH range. No clear-cut explanation can be offered to account for this effect. The different behavior might be due to the difference in temperature between the two studies. Differences in the nature and concentration of trace metals also might be a causative factor. Further experimentation is required to elucidate this observation.

It has been reported that at least three products are formed by at least three parallel pseudo first-order reactions when 17-dihydroxyacetone steroids are subjected to an aqueous alkaline environment (11). The presence of trace metals in such systems increases the complexity of the degradation, and the precise role of the metals is unknown. The metals may function by catalyzing any number of the previously reported reactions, or they may increase the observed rate constant by providing a new degradative route for the steroid. The results of this study lend evidence to the postulation that trace metals are involved primarily in the oxygen-dependent degradative route of the steroid. This evidence manifests itself in that the hydroxide-ion dependency of the metal-catalyzed reaction of this study is similar to the hydroxide-ion dependency of the oxygen-dependent reaction described by Guttman and Meister (11). On the other hand, the fact that products of both the oxygen-dependent and oxygen-independent reactions have been isolated from the metal-catalyzed degradation of 17-dihydroxyacetone steroids (5, 12) suggests that catalytic amounts of metals are involved in more than one of the degradative reactions. It is possible that the metals function as catalysts for all of the reactions or are involved in an initial step resulting in the formation of an intermediate which then decomposes to the reported products.

Lewbart and Mattox (13) reported that the glyoxals obtained by cupric acetate oxidation of 17-dihydroxyacetone steroids are unstable and undergo rearrangement both in the presence of alcoholic cupric acetate and aqueous alkali. The cupric acetate catalyzed rearrangements in alcoholic media are many times slower than those in basic aqueous systems and are futher retarded by the addition of water. Our study is in agreement with these results. The nonmetal-catalyzed reaction exhibited a strong hydroxide-ion dependency at high pH values (Fig. 4), whereas the metal-catalyzed reaction was pH independent in this range (Fig. 5). Thus, at a very high concentration of hydroxide ion, the contribution of the metal-catalyzed reaction to the ob-

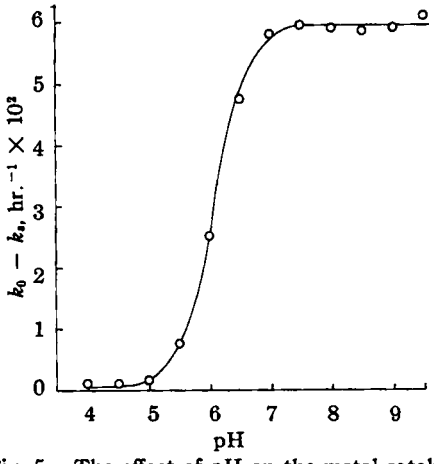

Fig. 5.—The effect of pH on the metal-catalyzed degradation of prednisolone at 70° in 0.1 *M* phosphate buffers.

served rate constant would be small. It should be pointed out, however, that trace metals markedly accelerate the degradation of corticosteroids in the pharmaceutically important pH range. The presence of these impurities should be a prime factor in steroid stability considerations.

SUMMARY

1. The decomposition of prednisolone in aqueous solution was markedly accelerated by contaminants present in buffer reagents. Evidence has been presented which strongly indicates that the contaminants were trace metals. However, no attempt was made to identify the metals or to determine the nature of their influence on the degradative reactions.

2. Addition of a sequestering agent provided a method to isolate and quantitate the rate of the apparent metal-catalyzed reaction.

3. The rate of the apparent metal-catalyzed reaction was pH independent above pH 7 and below pH 5 and exhibited a first-order dependency on hydroxide-ion in the intermediate range.

4. In the presence of sequestering agent, the reaction rate was strongly dependent on hydroxide-ion concentration above pH 8 and only slightly dependent at lower pH values.

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Ultraviolet Spectrophotometric Estimation of Arylglycolate Drugs

By LESTER CHAFETZ*

Oxidation of arylglycolic acids to carbonyl compounds affords ultraviolet chromophores which make possible the spectrophotometric estimation of the acids with greatly increased sensitivity and selectivity. Oxidative spectrophotometric methods for representative arylglycolic acids were studied; the procedures were applied to the estimation of some arylglycolate drugs in dosage forms.

SALTS OF mandelic acid have been employed as urinary antiseptics, and several arylglycolate esters are used in medicine for antispasmodic, mydriatic, psychotropic, and other pharmacological activities. A study of spectrophotometric procedures was carried out using mandelic acid, benzilic acid, α-cyclopentylphenylglycolic acid, and α-cyclopentyl-2-thiopheneglycolic acid. The application of the procedures to the assay of representative arylglycolate drugs is reported.

Phenylglycolic acids and their esters have benzenoid ultraviolet spectra which are neither sufficiently intense to afford sensitivity in determination nor sufficiently characteristic to serve for identification (1). The majority of the analytical methods reported for arylglycolate drugs are based on the presence of a tertiary amine or quaternary ammonium function in the alcohol moiety, and few of the methods are selective for unhydrolyzed drug (2–8).

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A number of workers have described procedures for oxidation of arylglycolic acids with decarboxylation to carbonyl compounds. The reagents used included "zinc manganite" (9–11), sodium bismuthate (12), *N*-bromosuccinimide (13), lead tetraacetate (14), periodate (14–16), and ceric sulfate (17–19). Some of these oxidative procedures were made the basis of titrimetic assay methods.

Spectrophotometry of the carbonyl compound products of the oxidation of arylglycolic acids appeared to offer a promising means for analysis of arylglycolate drugs. The increase in ultraviolet absorption effected by thus obtaining a carbonyl group in conjugation with an aromatic ring is evident in a comparison of the molar absorptivities, ϵ, of the acids with values reported in the literature for the carbonyl compounds. Mandelic acid has $\epsilon_{max.}$ 212 and benzaldehyde $\epsilon_{max.}$ 14,000 (20); benzilic acid has $\epsilon_{max.}$ 538 and benzophenone $\epsilon_{max.}$ 19,500 (21); and α-cyclopentylphenylglycolic acid has $\epsilon_{max.}$ 276 compared with $\epsilon_{max.}$ of 12,900 (22) for cyclopentyl phenyl ketone.