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STUDIES ON THE STABILITY OF CORTICOSTEROIDS V. THE DEGRADATION PATTERN OF HYDROCORTISONE IN AQUEOUS SOLUTION *

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SUMMARY

The degradation pattern of hydrocortisone in aqueous solution was investigated utilizing an HPLC procedure capable of separating and quantitating hydrocortisone and its major degradation products. The product distribution was characterized qualitatively and quantitatively as a function of pH in the range 0–11, nature of buffers and trace metal impurities. Two major decomposition pathways were observed, an oxidative degradation leading to the formation of 21-dehydrohydrocortisone which subsequently degraded to a 17-carboxylic acid and a 17,20-dihydroxy-21-carboxylic acid derivative, and a non-oxidative reaction giving a 17-oxo, 17-deoxy-21-aldehyde and 17-deoxy-20-hydroxy-21-carboxylic acid derivative.

The analytical consequences of the formation of steroid-glyoxals (21-aldehyde derivatives) in the evaluation of the stability-indicating properties of some spectrophotometric assays for corticosteroids are discussed as are possible means to prevent the conversion of corticosteroids to these products recently characterized as potentially immunogenic substances possibly involved in corticosteroid-mediated allergic reactions.

INTRODUCTION

21-Hydroxy corticosteroids are capable of undergoing degradation in various pharmaceutical preparations and/or in aqueous solutions. The degradation results almost exclusively from reactions of the C_{17}-dihydroxyacetone side chain and a variety of both oxidative and non-oxidative reactions have been postulated to occur (for refs., see below).

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However, only restricted information is available on the degradation pattern under various conditions including those of pharmaceutical relevance.

As a continuation of studies aiming at providing more detailed knowledge on the decomposition kinetics and mechanisms of corticosteroids (Hansen and Bundgaard, 1979, 1980a; Bundgaard and Hansen, 1980), an investigation of the degradation pattern of hydrocortisone in aqueous solution was undertaken. By means of a recently developed HPLC procedure (Hansen and Bundgaard, 1980b) kinetic data for hydrocortisone degradation were obtained and the product distribution was characterized both qualitatively and quantitatively as a function of pH, nature of buffers and trace metal impurities.

MATERIALS AND METHODS

Chemicals and apparatus

Hydrocortisone, corticosterone and 11β-hydroxy-4-androsten-3,17-dione were purchased from Sigma Chemicals, St. Louis. The preparation of the various degradation products of hydrocortisone has been described in a previous paper (Hansen and Bundgaard, 1980b). 3-Methylbenzothiazol-2-one-hydrazone hydrochloride (MBTH) and tetrabutylammonium bromide were obtained from Fluka AG, Switzerland and were used as received. Buffer substances and all other chemicals used were of reagent grade.

A Perkin-Elmer 124 spectrophotometer and 1 cm quartz cells were used for the spectral measurements. The pH measurements were made at the temperature of study using a Radiometer Type PHM 26 instrument. High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500 B instrument equipped with a variable-wavelength UV detector (8-μl 1 cm flow cells) and a 10-μl loop injection valve. The detector was connected to a Servogor RE 541 potentiometric recorder. The column used, 10 cm long and 4.5 mm i.d., was packed with LiChrosorb RP-8 (5 μm particles).

Determination of hydrocortisone and its degradation products

Quantitative determination of hydrocortisone and its major degradation products was performed by means of the HPLC procedure recently developed (Hansen and Bundgaard, 1980b). The reversed-phase column was eluted isocratically with a mixture composed of acetonitrile (20% v/v) in 0.01 M phosphate buffer, pH 6.5, containing 0.01 M tetrabutylammonium bromide. The flow rate was 1.6 ml · min^{-1} and the column effluent was monitored at 242 nm. The procedure allows separation and simultaneous quantitation of hydrocortisone and the degradation products shown in Fig. 1. The content of hydrocortisone and degradation products in the sample (10 μl) injected was determined by comparing the peak heights with those of standards chromatographed under similar conditions.

In some cases the concentration of undegraded hydrocortisone was determined by the spectrophotometric assay previously described (Bundgaard and Hansen, 1979). Besides by HPLC the steroid glyoxal products III and IV were identified and quantitated by reaction with a MBTH reagent as previously reported (Bundgaard, 1978; Bundgaard and Hansen, 1980; Hansen and Bundgaard. 1980a).

Kinetic measurements

All kinetic experiments were carried out in aqueous buffer solutions (acetate, phos-

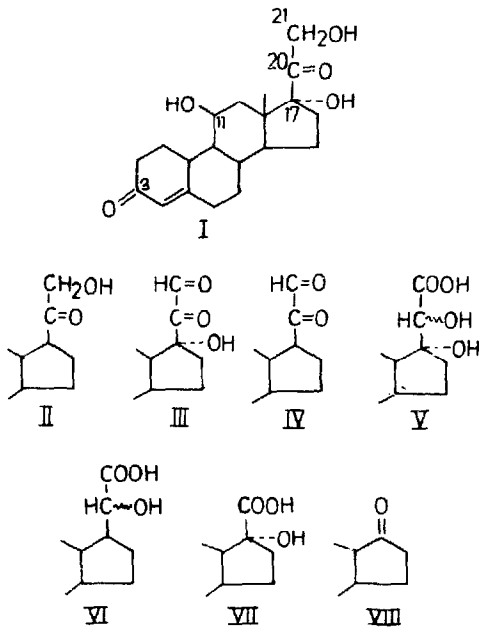


Fig. 1. Chemical structures of hydrocortisone (I), corticosterone (II), 11β,17α-dihydroxy-3,20-dioxo-4-pregnene-21-al (III), 11β-hydroxy-3,20-dioxo-4-pregnene-21-al (IV), 11β,17α,20-trihydroxy-3-oxo-4-pregnene-21-oic acid (V), 11β,20-dihydroxy-3-oxo-4-pregnene-21-oic acid (VI), 11β,17α-dihydroxy-3-oxo-4-androsten-17β-carboxylic acid (VII) and 11β-hydroxy-4-androsten-3,17-dione (VIII).

phate, borate and carbonate) at a constant temperature (50 or 70°C). The ionic strength (μ) of the solutions was maintained at 0.5 by addition of a calculated amount of potassium chloride. The reaction solutions were kept in a water bath in screw-capped test tubes and no attempt was made to exclude oxygen from the solutions. After addition of 1000 µl of ethanolic solutions of hydrocortisone to 25.00 ml of pre-heated buffer solution to give an initial concentration of $3-16 \times 10^{-4}$ M, samples were taken at appropriate intervals and analyzed for hydrocortisone and degradation products as described above. First-order rate constants for the overall degradation were determined from the slopes of the straight lines obtained by plotting the logarithm of the residual steroid against time.

RESULTS AND DISCUSSION

Kinetics of overall degradation

At constant pH and temperature the disappearance of hydrocortisone displayed strict first-order kinetic behaviour in agreement with previous findings over the pH range 0–12 at 70°C (Hansen and Bundgaard, 1979, 1980a). In alkaline solutions, however, and at initial hydrocortisone concentrations exceeding about 10^{-3} M deviations from first-order kinetics were observed in the initial stage of degradation (Fig. 2). The observed pseudo-first-order rate constants in the pH range 5–10 are listed in Table 1. Identical (within ±3%) rate constants were obtained in the runs followed both by HPLC and the spectrophotometric assay.

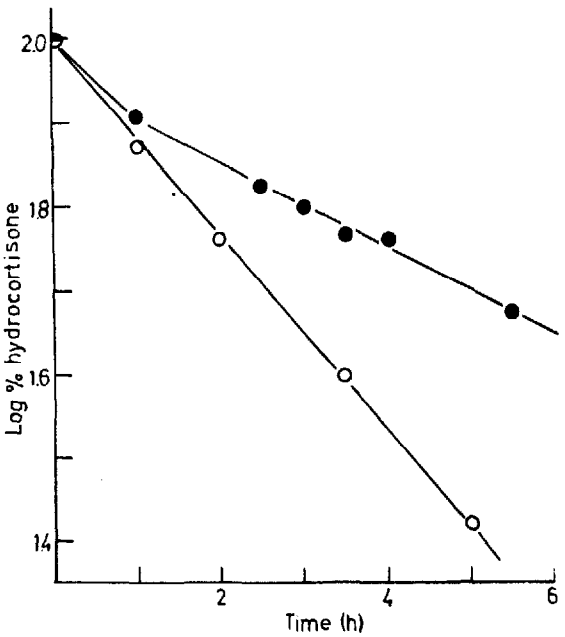


Fig. 2. Semilogarithmic plots showing the rate of degradation of hydrocortisone at different initial concentrations (3.1×10^{-4} M (○) and 15.2×10^{-4} M (●)) in 0.1 M carbonate buffer solution of pH 10.16 ($\mu = 0.5$) at 50°C.

As seen from Table 1 inclusion of disodium edetate in the solutions markedly decreased the degradation rate. It has previously been shown that phosphate, borate and carbonate buffers accelerate the degradation of hydrocortisone and that the effect is due

TABLE 1

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR DEGRADATION OF HYDROCORTISONE AT 70°C IN VARIOUS BUFFER SOLUTIONS ($\mu = 0.5$) WITH AND WITHOUT ADDITION OF DISODIUM EDETATE

pH	Buffer	Disodium edetate conc. (M)	k_{obs} (h^{-1})
5.07	0.1 M acetate	0	5.8×10^{-3}
		4×10^{-5}	4.7×10^{-3}
6.19	0.1 M phosphate	0	5.8×10^{-2}
		4×10^{-5}	6.5×10^{-3}
7.09	0.1 M phosphate	0	6.5×10^{-2}
		4×10^{-5}	5.3×10^{-3}
9.29 [a]	0.1 M borate	0	4.2×10^{-2}
		4×10^{-5}	1.1×10^{-2}
10.16 [a]	0.1 M carbonate	0	2.7×10^{-1}
		4×10^{-5}	4.6×10^{-2}

[a] At 50°C

to trace metal contaminants (Hansen and Bundgaard, 1979). Similar findings have been reported for prednisolone and some of these buffer substances (Oesterling and Guttman, 1964).

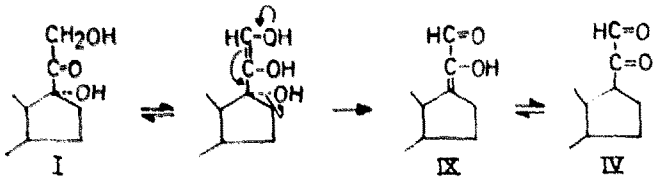
Degradation pattern

The degradation pattern of hydrocortisone was examined in aqueous buffer solutions of various pH and with and without addition of a metal complexing agent. The degradation of hydrocortisone was followed until most of the drug was destroyed and the major products formed were identified and quantified by means of HPLC.

Acidic solution. At pH <2 the rate of degradation of hydrocortisone shows a first-order dependency on the hydrogen ion concentration and is independent of trace-metal impurities (Hansen and Bundgaard, 1980a). The almost exclusive recognizable product formed by the specific acid-catalyzed degradation of hydrocortisone as well as of various other corticosteroids was the 17-deoxy glyoxal derivative (IV) (Hansen and Bundgaard, 1980a). The formation of this product occurred to an extent of 80–90% in 0.01–2.0 M hydrochloric acid solutions and it may most likely take place by elimination of water through an initial enolization of the C_{20}-keto group as proposed by Lewbart and Mattox (1964) (Scheme 1). A minor (≤5%) degradation product was postulated to be the enol (IX) (Hansen and Bundgaard, 1980a). Such a product was recently identified for beta-methasone by degradation in 0.1 M hydrochloric acid–dioxane solutions (Hidaka et al., 1980). In agreement with the findings of Hansen and Bundgaard (1980a), Dekker (1980a) has recently reported the isolation and identification of 17-deoxy-21-dehydroprednisolone as a decomposition product of prednisolone in solutions of pH <6.

Weakly acidic and neutral solutions. The product distributions observed for reaction solutions of pH 5.07 and 6.19 are shown in Tables 2 and 3. It is apparent that hydrocortisone under these conditions degrades by two parallel processes, a non-oxidative reaction leading to 17-deoxy-21-dehydrohydrocortisone (IV) as in strongly acidic solutions and a reaction involving oxidation of the 21-alcohol group to an aldehyde group giving 21-dehydrohydrocortisone (III). These two degradation pathways leading to steroid-glyoxals account almost exclusively for the total decomposition, their relative importance being dependent on trace-metal impurities in the reaction solutions. In solutions without addition of disodium edetate, minor amounts (≤5%) of the 17-ketosteroid (VIII) were detected at the end of the hydrocortisone degradation.

The decomposition of corticosteroids to the corresponding 21-dehydro derivatives in aqueous or alcoholic solutions containing trace metal (especially copper) impurities has been observed by several investigators (Lewbart and Mattox, 1963; Sunaga and Koide, 1968; Monder, 1968; Connor, 1974; Jackson et al., 1975; Bundgaard, 1978; Bundgaard and Hansen, 1979, 1980). A detailed study (Bundgaard and Hansen, 1980) of the kinetics



I IX IV

Scheme 1

TABLE 2

DEGRADATION OF HYDROCORTISONE (4×10^{-4} M) IN 0.1 M ACETATE BUFFER SOLUTION pH 5.07 (μ = 0.5) WITH OR WITHOUT ADDITION OF DISODIUM EDETATE (70°C)

Hours	Produce distribution (mole%)				
	I	III	IV	I [a]	IV [a]
21	90	3	5	92	10
41	79	5	10	84	15
71	69	10	15	78	25
113	56	10	25	60	35
142	41	5	35	53	45
310				26	75

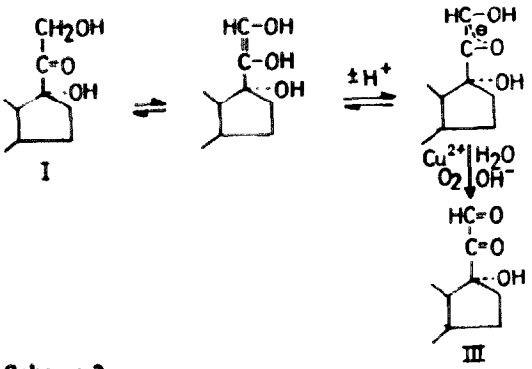
[a] For solutions containing 4×10^{-5} M disodium edetate.

of formation as well as of the degradation of 21-dehydrohydrocortisone has shown that the formation rate for the product is markedly enhanced by iron (III), nickel (II) and, especially, by copper (II) ions in neutral and alkaline aqueous solutions. Copper (II) ions showed a significant catalytic effect in a concentration as low as 3×10^{-3} ppm, which is well below the level of contamination of analytical grade buffer substances. It was further shown that the rate of the metal ion-catalyzed oxidation of hydrocortisone to the 21-dehydro derivative increased in a sigmoid fashion with pH over the range pH 6.5–10 and that this pH–rate relationship could be explained by assuming the occurrence of spontaneous as well as hydroxide ion-catalyzed reaction of the enolate form of the corticosteroid (Scheme 2). The metal ion-catalyzed degradation appears solely to be a catalysis of 21-dehydro steroid formation (Bundgaard and Hansen, 1980) which is supported by the results in Tables 2 and 3. The increase in the degradation rate for hydrocortisone in the buffer solutions without disodium edetate as compared with the rate in solutions contain-

TABLE 3

DEGRADATION OF HYDROCORTISONE (4×10^{-4} M) IN 0.1 M PHOSPHATE BUFFER SOLUTION pH 6.19 (μ = 0.5) WITH OR WITHOUT ADDITION OF DISODIUM EDETATE (70°C)

Without addition of disodium edetate				With addition of 4×10^{-5} disodium edetate			
Hours	Product distribution (mole %)			Hours	Product distribution (mole %)		
	I	III	IV		I	III	IV
1.25	91	10	–	21	90	–	10
4.5	79	20	–	41	78	–	20
6.75	71	25	5	71	64	5	30
25	23	55	10	113	52	5	35
29	19	55	10	142	42	5	45

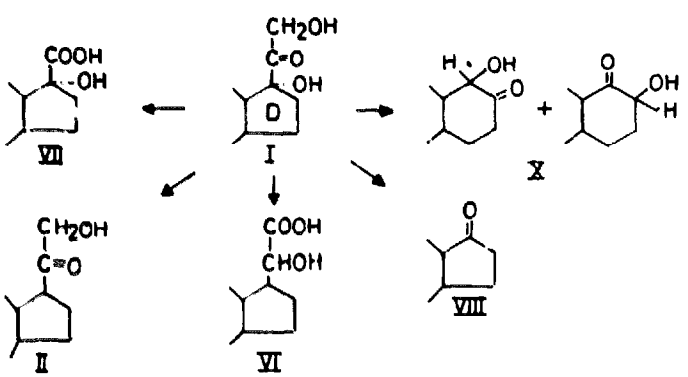


Scheme 2

ing this agent (cf. Table 1) is accompanied by a closely related predominance of the 21-dehydro steroid decomposition pathway. In a 0.1 M phosphate buffer solution of pH 7.54 and containing 0.2 ppm copper (II) ions this pathway was previously found to make up 45% of the overall degradation (Bundgaard and Hansen, 1980).

The steroid-glyoxals III and IV are highly unstable in alkaline solutions, being subject to specific base-catalyzed degradation (Bundgaard and Hansen, 1980). Compound III is about 4 times more labile than compound IV and it is apparent from the data in Tables 2 and 3 that 21-dehydrohydrocortisone degrades slowly following its formation.

Basic solutions. Previous studies have indicated that a variety of both oxidative and non-oxidative reactions of the C_{17}-dihydroxyacetone side chain in corticosteroids may occur in basic solutions (Scheme 3). In the presence of oxygen, the predominant reaction appears to involve an oxidative cleavage of the side chain to yield the corresponding etio-acid (VII) (Velluz et al., 1947; Herzig and Ehrenstein, 1951; Chulski and Forist, 1958; Hidaka et al., 1980). In the absence of air, two hydroxide ion-catalyzed reactions have been reported yielding the 17-ketosteroid (VIII) and the glycolic acid (VI) (Mason, 1938; Wendler and Graber, 1956; Chulski and Forist, 1958; Guttman and Meister, 1958; Dekker and Buijs, 1980a; Hidaka et al., 1980). In addition to these 3 products a 17-deoxy steroid (II) has been identified as a product formed during the anaerobic decomposition (at pH 8) of prednisolone (Dekker, 1979) and dexamethasone (Dekker and Buijs, 1980b). Under similar degradation conditions Dekker (1980b) has further reported the transformation of



Scheme 3

TABLE 4

THE PERCENTAGE YIELDS (MOLE%) OF THE PRODUCTS V, VI, VII AND VIII AFTER COMPLETE DEGRADATION OF HYDROCORTISONE IN 0.1 M CARBONATE BUFFER SOLUTION pH 10.21 (μ = 0.5) AT 50°C

Degradation product	Initial conc. of hydrocortisone (M × 10^4)		
	3.0	6.6	3.0 [a]
V	25	25	0
VI	5	5	20
VII	55	55	0
VIII	5	5	30

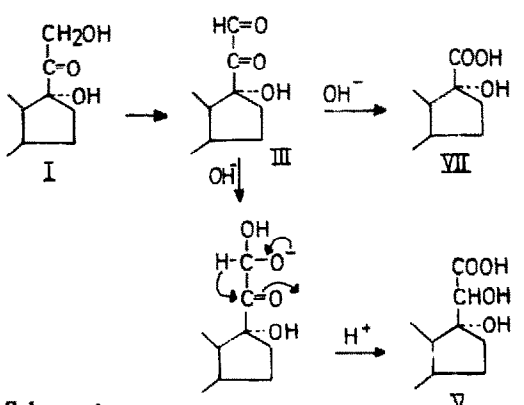
[a] The solution was added 4×10^{-5} M disodium edetate.

prednisolone to D-homosteroids (X). Such a derivative has also been reported from degradation of triamcinolone in alkaline solution (Smith et al., 1960).

In accordance with these previous findings the degradation pattern of hydrocortisone was found to be more complex in basic aqueous solution and several products were observed at pH 9–11. In a 0.1 M carbonate buffer solution of pH 10.21 with no content of disodium edetate the major decomposition products were found to be the etienic acid (VII) and the glycolic acid (V) (Table 4). Only minor amounts of the 17-ketosteroid (VIII) and the glycolic acid (VI) were formed; these 4 products accounted for about 90% of the total degradation (Table 4).

The glycolic acid (V) has apparently not previously been reported as a decomposition product of corticosteroids. As it appears from Table 4 its formation as well as the formation of the etienic acid (VII) are completely blocked when disodium edetate is included in the solutions. Thus, both V and VII must arise from metal-catalyzed reactions.

The compounds V and VII are suggested to be formed from the intermediate 21-dehydrohydrocortisone (III). This steroid glyoxal was previously shown to decompose to V and VII in alkaline solutions (Bundgaard and Hansen, 1980), compound V most likely being formed by an intramolecular Cannizzaro reaction and compound VII by a simple oxidation (Scheme 4). Further support for the postulated reaction sequence involving 21-



Scheme 4

dehydrohydrocortisone as an intermediate is obtained from experiments using borate buffer solutions as reaction media. Borate ions exert a pronounced stabilizing effect on the steroid glyoxal by complexing with the glyoxal moiety (Bundgaard and Hansen, 1980) and utilizing this effect it has been demonstrated that the decomposition of hydrocortisone to the 21-dehydro product amounts to about 70% in alkaline aqueous solution containing catalytic trace-metal impurities (Bundgaard and Hansen, 1980). In Fig. 3 are shown the time-courses for hydrocortisone, 21-dehydrohydrocortisone and the etienic acid (VII) in a 0.1 M borate buffer solution of pH 9.47 and at 50°C. It is readily seen that the formation of compound VII goes through an induction period. Kinetic treatment of the data for 21-dehydrohydrocortisone in a manner previously described (Bundgaard and Hansen, 1980) showed that the decomposition pathway leading to this product accounts for approximately 75% of the overall degradation. It is remarkable that no significant amounts of the glycolic acid (V) were formed on degradation in borate buffers in contrast

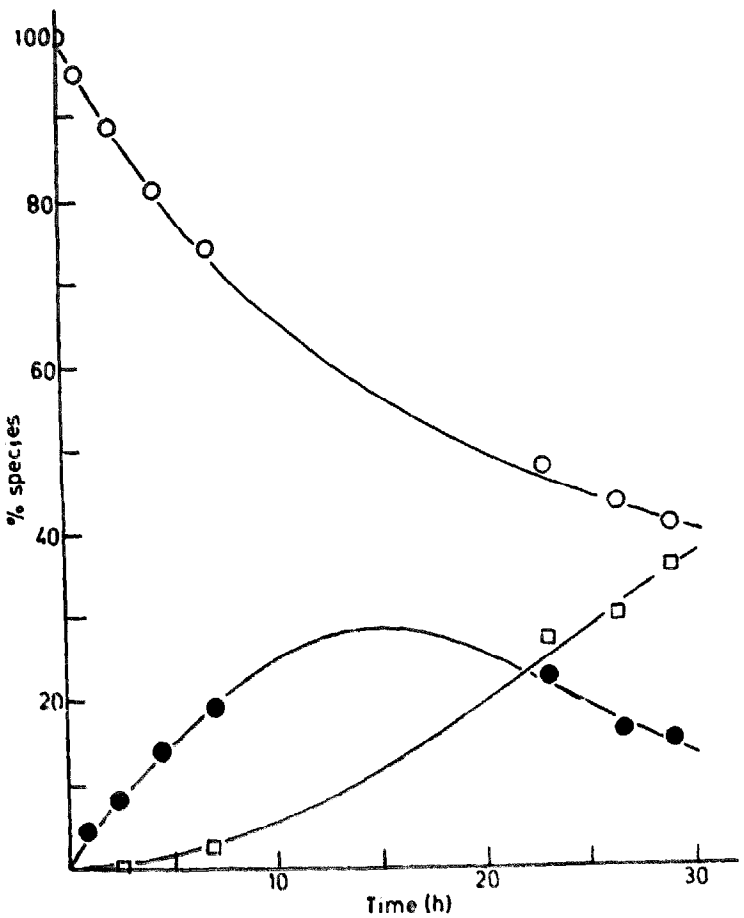


Fig. 3. Time-courses for hydrocortisone (○), 21-dehydrohydrocortisone (III) (●) and the etioacid (VII) (□) in the degradation of hydrocortisone (4×10^{-4} M) in 0.1 M borate buffer solution of pH 9.21 (μ = 0.5) at 50°C.

to carbonate or sodium hydroxide solutions. This specific effect of borate on the product distribution may possibly be connected with its ability to complex with the intermediary steroid-glyoxal.

It can be shown kinetically that the formation of the products V and VII (and the intermediate III) accounts quantitatively for the metal ion-catalyzed oxidative degradation of hydrocortisone in alkaline solutions. Letting k_1 and k_2 represent pseudo-first-order rate constants for the oxidative and non-oxidative reactions, respectively, the following equations can be written:

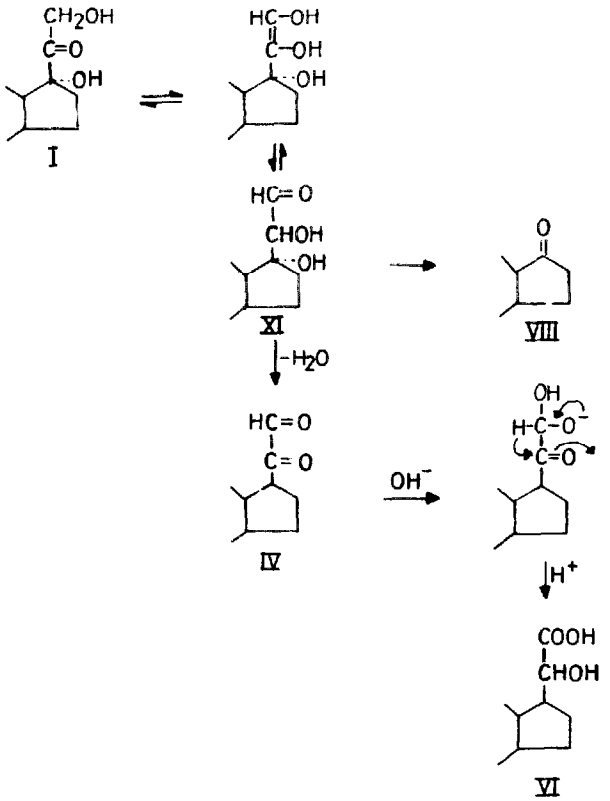
$$k_1 = k_{obs} \frac{[V]_\infty + [VII]_\infty}{[HC]_0} \quad (1)$$

$$k_2 = k_{obs} - k_1 \quad (2)$$

where k_{obs} is the pseudo-first-order rate constant for the overall degradation of hydrocortisone, $[V]_\infty$ and $[VII]_\infty$ are the concentrations of the respective compounds at complete disappearance of hydrocortisone and $[HC]_0$ is the initial concentration of hydrocortisone. Degradation experiments were performed at 50°C in 0.1 M borate buffer pH 9.29 and 0.1 M carbonate buffer pH 10.16 with and without the addition of disodium edetate. The k_{obs} values obtained are listed in Table 1 and on basis of Eqn. 1 the following values of k_1 were found: $k_1 = 0.029\ h^{-1}$ (pH 9.29) and $k_1 = 0.22\ h^{-1}$ (pH 10.16). From Eqn. 2 k_2 was calculated to be: $k_2 = 0.013\ h^{-1}$ (pH 9.29) and $k_2 = 0.05\ h^{-1}$ (pH 10.16). These values of k_2 as obtained from measurements of k_1 are seen to be in favourable agreement with the rate constants directly determined in solutions containing disodium edetate (cf. Table 1). The k_1 values derived show a direct proportionality to the hydroxide ion concentration and a second-order rate constant for the specific base catalysis of the oxidative decomposition of $275\ M^{-1}\ h^{-1}$ (50°C) was calculated.

In alkaline solutions containing disodium edetate the 17-ketosteroid (VIII) and the 17-deoxy-20-hydroxy-21-acid (VI) were formed in a total yield of about 50% (Table 4). Possible mechanisms for the formation of VIII and VI may involve the equilibrium formation of the dihydroxy aldehyde tautomer (XI), providing a system capable of breakdown by two pathways: by β-elimination of water followed by an intramolecular Cannizzaro reaction of the intermediary 17-deoxy steroid glyoxal (IV) to give the glycolic acid (VI) and by a retroaldolization to form the 17-ketone (VIII) (Scheme 5) (Wendler, 1967). An alternative mechanism for the formation of compound VIII may involve a direct attack of hydroxide ions at C_{17} (Dekker and Buijs, 1980a).

The products VI and VIII only account for approximately 50% of the total degradation under the non-oxidative conditions. The identity of other products formed remains to be elucidated. Since 17-deoxy derivatives have been reported as anaerobic decomposition products of prednisolone and dexamethasone as mentioned above a search was made for the formation of the corresponding derivative (i.e. corticosterone (II)) from hydrocortisone. However, in no cases could even traces of corticosterone be detected by the HPLC procedure used.



Scheme 5

Analytical consequences of steroid-glyoxal formation

The finding of the steroid-glyoxals III and IV as being major and accumulating degradation products of hydrocortisone under conditions similar to those often encountered in pharmaceutical preparations (pH 5–7) may have consequences in the evaluation of the stability-indicating properties of some commonly used spectrophotometric assay procedures for corticosteroids such as the method of Porter and Silber (1950) and its various modifications (Lewbart and Mattox, 1961; Görög and Szepesi, 1972; Chafetz et al., 1974; Chafetz and Tsilifonis, 1972). These procedures involve conversion of corticosteroid to the glyoxal (IV) or (III) and subsequent condensation of these products with a chromogenic reagent. The procedures have been proposed to be stability-indicating on the assumption that the glyoxals due to their instability may be expected to occur in only insignificant amounts in a decomposed corticosteroid preparation (Chafetz, 1971; Kingsford, 1975). The present results show clearly that such assumption is not generally valid. In order to eliminate any interference from the steroid-glyoxals in the assay procedures a blank measuring the amount of steroid-glyoxals already present in the sample to be analyzed should be included. Procedures involving such a differential measurement has recently been described and shown to be selective for corticosteroids in the presence of glyoxal products (Bundgaard, 1978; Bundgaard and Hansen, 1979).

Potential immunological aspects of steroid-glyoxal formation

The steroid-glyoxals (III) and (IV) have recently been characterized as potentially immunogenic substances and suggested to be implicated as pro-antigens in allergic reactions caused by various corticosteroid preparations (Bundgaard, 1980). For this reason their presence in steroid preparations should be avoided. An efficient means to prevent the formation of the 21-dehydro derivatives (III) would be inclusion of a metal-sequestering agent like disodium edetate in the preparations. If alkaline conditions occur borate should be avoided because of its stabilizing effect on the glyoxals at pH >8.5 (Bundgaard and Hansen, 1980). To prevent the formation of the 17-deoxy steroid-glyoxals (IV) strongly acidic conditions should be avoided. The stability of hydrocortisone in aqueous solutions shows an optimum at pH around 4 (Hansen and Bundgaard, 1979).

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