The Solvolysis of 21-Hydrocortisone Esters and Hemiesters

EDWARD R. GARRETT*

Research Laboratories of The Upjohn Company, Kalamazoo, Mich.

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The 21-hemiester of hydrocortisone and succinic acid is hydrolyzed at faster rates in the neutral pH region than would be predicted from studies on specific hydrogen and hydroxyl ion catalysis. This can be attributed to either intramolecular catalyzed hydrolysis by the terminal carboxylate anion or by an enhanced rate of specific hydroxyl ion attack on the undissociated hemiester.

No highly significant difference in hydrolysis of the hemiester anion by specific hydroxyl ion catalysis under comparable conditions was observed for increased length of the dicarboxylic acid, from the hemisuccinate to the hemisuberate. Steric blocking by substituents on the α or β carbons of the carboxyalkyl group is more effective in modification of the alkaline hydrolysis rate than anion distance from the ester group for the hemiesters studied, $ROOC(CH_2)_nCOO^-$, $n \geq 2$. Simple monocarboxylic esters of hydrocortisone are more susceptible to alkaline hydrolysis than the hemiester anion. The structure of the 21-hydroxy-21-keto side chain in hydrocortisone is the significant factor in the facile hydrolysis of 21-hydrocortisone esters.

Modification of 21-hydroxysteroids by esterification with dicarboxylic acids greatly enhanced their clinical utility.[1,2,3] The formulation of the water-soluble lyophilized sodium salt of hydrocortisone 21-hemisuccinate[4] permitted facile parenteral administration of hydrocortisone in a reconstituted homogeneous solution where immediately high levels of circulating hydrocortisone are required. It is apparent that the choice of an appropriately soluble, stable, and biologically active hemiester of hydrocortisone and other steroids is of pharmaceutical and clinical importance.

The hydrolysis of the sodium salt of hydrocortisone 21-hemisuccinate (I) ($n = 2$), at pH values above 8 is specific hydroxyl ion cata-

* College of Pharmacy, University of Florida, Gainesville, Florida

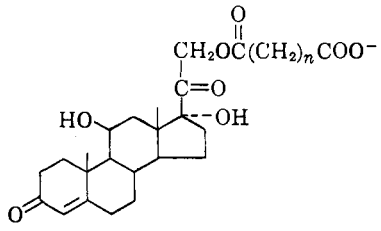
(1) R. H. Orr, V. C. DiRaimondo, M. D. Flanagan, and P. H. Forsham, *J. Clin. Endocrinol.*, **15**, 765 (1955).

(2) M. H. Kuizenga and G. F. Cartland, *Endocrinology*, **27**, 647 (1940).

(3) J. C. Melby and M. St. Cyr, *Metabolism*, **10**, 75 (1961).

(4) Hydrocortisone hemisuccinate (Na salt): Solu-Cortef®.

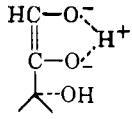
lyzed and unusually fast.[5] For example, the estimated bimolecular



I

rate constant for the hydroxyl ion catalyzed hydrolysis of hydrocortisone hemisuccinate is 1.0 l./*M*/sec. in water[5] and much greater than the rate constant for the hydrolysis of ethyl hemisuccinate, 0.0277.[6] However, the bimolecular rate constant for the hydrolysis of glycol monoacetate is 0.272 l./*M*/sec.,[6] which is of an order of magnitude similar to the rate of steroid hemiester hydrolysis.

These facts imply that the structure of the steroid 21-hydroxyl side-chain is more responsible for promoting hydroxyl ion catalyzed hydrolysis of the hydrocortisone 21-hemiester than the nature of the esterified acid. The nature of the steroid side chain could promote hydroxyl ion attack or facilitate decomposition of the activated intermediate by stabilization of the leaving group, a group such as an enediol anion.[5]



Certainly, the negatively charged hemiester would be expected to inhibit hydroxyl ion catalyzed hydrolysis. Yet if the structure of the steroid side chain is the principal rate determining factor, it follows that the comparison of the rates of hydrolysis of an homologous series of hydrocortisone hemiesters of dicarboxylic acids among themselves and with the simple monocarboxylic esters as reported in this paper may not show great differences. However, introduction of alkyl groups in the dicarboxylic acids to sterically hinder hydroxyl

(5) E. R. Garrett, *J. Pharm. Sci.*, in press.

(6) "Table of Chemical Kinetics: Homogeneous Reactions," National Bureau of Standards Circular 510, V.S. Department of Commerce, Washington, D. C., (1951) and Supplement 1 (1956).

ion catalyzed hydrolysis should produce more stable salts of hydrocortisone hemiesters.

Recent evidence of intramolecular catalyzed hydrolysis of esters by a neighboring carboxylate anion[7-11] suggests that "spontaneous" hydrolysis may significantly contribute to the hydrolysis of the sodium salts of hydrocortisone hemiesters at and around neutrality.

Study of the rate-pH profile is thus necessary to the understanding of the hydrolysis of hydrocortisone hemiesters in the physiological pH range.

Experimental

Procedure for Ester Assay and Kinetic Studies of Hydrocortisone Hemisuccinate in 30% Ethanol by the Hydroxamic Acid Procedure.—The procedure used was the hydroxamic acid procedure for the spectrophotometric determination of esters[12] as modified for steroid esters by Forist and Theal.[13] The preparation of reagents was as reported in this reference except that tenfold amounts of materials were prepared.

A stock solution of hydrocortisone hemisuccinate in absolute ethanol was prepared at 12 mg./ml. The stock solution was diluted 3:10 with varying concentrations of standard aqueous HCl, acetic acid–acetate buffers, and phosphate buffers so that the resultant solutions were 30% ethanol and of specific molarities in HCl and buffers as given in Table I. The hydrocortisone hemisuccinate was 0.008 *M* in the final solutions. The solutions were immersed in a 70° constant temperature bath and aliquots were assayed at recorded intervals. Aliquots of 2.5 ml. were pipetted into 25-ml. volumetric flasks and followed by 1.5 ml. of alkaline hydroxylamine reagent.[13] This resultant solution reacted at room temperature for 30 min. It was diluted to 25 ml. with ferric perchlorate reagent[13] and removed from light for 10 min. The absorbance at 530 mμ against a similarly prepared reagent blank was read on the Beckman Model B spectrophotometer.

In the case of phosphate buffers, the resultant solutions after reacting for 30 min. at room temperature were filtered through a medium glass filter to remove the precipitated phosphate salts. Subsequently, 3 ml. of filtrate were transferred to a 25-ml. volumetric flask and diluted to volume with ferric perchlorate solution. The resultant solution was removed from light for 10 min. and then centrifuged for 2 min. The clear supernatant was transferred to a 1-cm. cell and the absorbance at 530 mμ against a similarly prepared reagent blank was read on the Beckman Model B spectrophotometer. These modifications of the Forist and Theal[13] procedure were necessary to remove precipitated phosphate salts and increase

(7) H. Morawetz and E. W. Westhead, *J. Polymer Sci.*, **16**, 273 (1955).
(8) E. R. Garrett, *J. Am. Chem. Soc.*, **79**, 3401 (1957).
(9) M. L. Bender, F. Chloupek and M. C. Neveu, *ibid.*, **80**, 5386 (1958).
(10) E. Gaetjens and H. Morawetz, *ibid.*, **83**, 5327 (1960).
(11) T. C. Bruice and U. K. Pandit, *ibid.*, **82**, 5858 (1960).
(12) R. F. Goddu, N. F. LeBlanc, and C. M. Wright, *Anal. Chem.*, **27**, 1251 (1955).
(13) A. A. Forist and S. Theal, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 320 (1957).

the ferric salt concentration to overcome consumption by phosphate. This ferric ion-hydroxamate procedure was not satisfactory for the assay of hydrocortisone hemi-β,β'-dimethylglutarate so it was not possible to follow ester hydrolysis of hindered esters of this type in acid and buffered neutral regions by this procedure. Attempts to use aqueous organic solvents where esterification of the acid products would not interfere with the ester assay were unsuccessful. Acetonitrile, Diglyme, dimethylsulfoxide, isopropyl alcohol, *t*-butyl alcohol, dioxane, and dimethylformamide in aqueous solution either did not dissolve sufficient hemiester or reacted adversely in the ferric ion-hydroxamate ester assay.

Procedure for Kinetic Studies on Hydrolysis of Hydrocortisone Hemisuccinate and Other Hemiesters of Hydrocortisone by Constant pH Titration.—The Cannon di-functional titrator[14] was standardized before each run with buffer solution which had been equilibrated a minimum of 5 min. in the temperature-controlled beaker of the titrator so it was at the same temperature as the hydrocortisone hemiester solution to be studied. The temperature compensator of the Cannon titrator was set to read the temperature of the buffer in the temperature-controlled beaker. The pH meter was then adjusted to read the pH of the buffer for the corresponding temperature. The pH meter was always standardized with a buffer closest in pH to the pH of the run and then checked with a second buffer which was also equilibrated to the temperature of the run.

The hemiester of the dibasic acid and hydrocortisone to be studied was dried at 50° in high vacuum for 24 hr. A weighed sample, *ca.* 56 mg., was dissolved in absolute ethanol in the temperature-controlled beaker and thermally equilibrated. Standard NaOH was added in stoichiometric amount to form the sodium salt of the steroid hemiester so that a total volume of 15 ml. resulted. The beaker was sealed as tightly as possible with orifices for glass-saturated calomel electrodes, an air-driven agitator, and nitrogen tubing to blow nitrogen on the surface of the solution in the beaker. The control pH was set and the plot of milliliters of 1.929 *M* NaOH *vs.* time was recorded. At the end of each run, the temperature and volume of the solution in the beaker were recorded. The detailed procedure has been given previously.[5]

The hydrocortisone hemiesters studied were the hemiadipate, hemipimelate, hemi-α,α'-diethylsuccinate, hemi-α,α'-diethylglutarate, hemisuberate, hemisuccinate, and hemi-β,β'-dimethylglutarate. The acetate and acrylate esters of hydrocortisone were also studied.

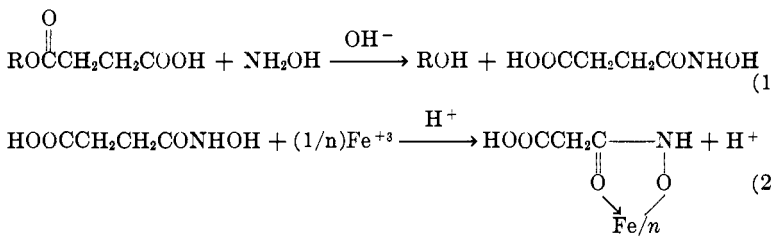
The pK_a' values of hydrocortisone hemisuccinate as determined by the pH at half-neutralization at various percentages of ethanol by volume were: 4.50, 20%; 4.75, 30%; and 5.36, 50%.

Calculations and Results

Kinetics of Hydrocortisone Hemisuccinate Hydrolysis and Succinic Acid Esterification in 30% Ethanol at Varying HCl Concentrations.—The spectrophotometric assay of the highly colored ferric complexes of the derived hydroxamic acids[12,13] which can be related to the non-

(14) J. B. Neilands and M. D. Cannon, *Anal. Chem.*, **27**, 29 (1955).

hydrolyzed hydrocortisone hemisuccinate, $ROCOCH_2CH_2COOH$, was used in the studies on the kinetics of hydrolysis of hydrocortisone hemisuccinate in 30% ethanol by volume at various HCl molarities.



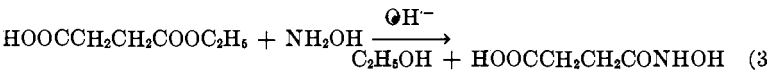
(1)

(2)

The absorbance of the highly colored ferric complexes of the derived hydroxamic acids, presumably formed from the non-acid-hydrolyzed hydrocortisone hemisuccinate ester, did not decrease to zero with time. An asymptotic absorbance was reached, as is shown in a plot of a typical example, Fig. 1, Curve A, where 0.008 *M* hydrocortisone hemisuccinate was hydrolyzed in 0.2 *M* HCl, 30% ethanol, at 70°.

Realization that the HCl also catalyzed esterification of the derived succinic acid with the ethanolic portion of the solvent was confirmed by following the formation of hydroxamic acid-ferric ion complex on similar ethanol-water systems that contained equimolar succinic acid and the same HCl molarities. A typical curve for the development of absorbance with time is also given in Fig. 1, Curve B, for 0.008 *M* succinic acid in 0.2 *M* HCl, 30% ethanol, at 70°. The absorbance increased to an asymptotic value that was the same as that to which the absorbance of the complex derived from the hydrocortisone hemisuccinate decreased.

These asymptotic values were the same in all HCl molarities studied, *i.e.*, from 0.025 to 0.20 *M*, and showed that all derived succinic acid was esterified by ethanol to the same extent, independent of the HCl concentration but at rates which can be presumed to be functions of the HCl molarity.



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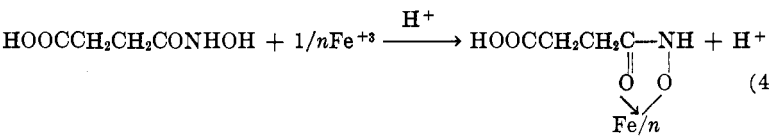

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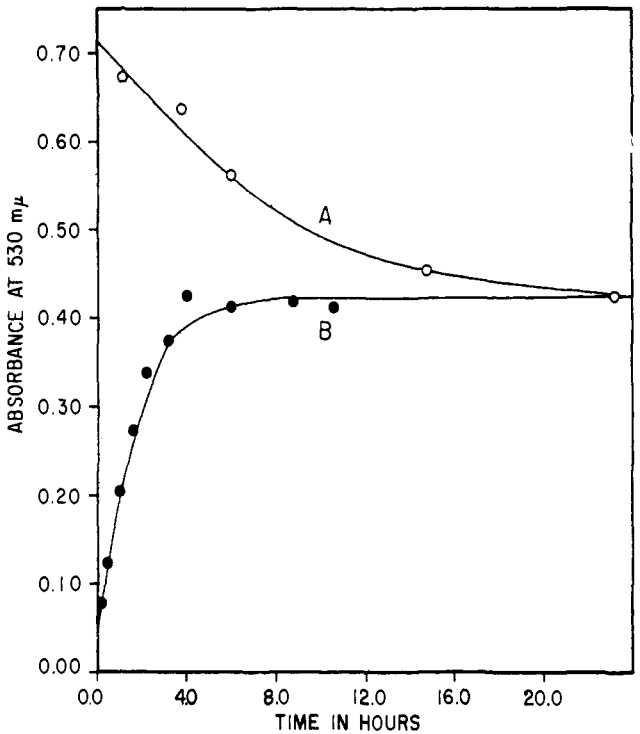


Fig. 1.—Typical curves for the ester assay of 0.008 *M* hydrocortisone hemisuccinate and 0.008 *M* succinic acid in 30% ethanol, 0.2 *M* HCl, 70°. Absorbance at 530 mμ of ferric-hydroxamate complexes against time in hours: ⊙, hydrocortisone hemisuccinate, Curve A; ●, succinic acid, Curve B.

The apparent first order rate constants for the hydrolysis of hydrocortisone hemisuccinate by strong acid catalysis in 30% ethanol at 70° can be determined from the slopes of plots of the logarithm of the difference of absorbance, A, at 530 mμ, and the asymptotic absorbance, $A_\infty = 0.400$, *vs.* time as by the equation

$$\log[A - A_\infty] = -kt/2.303 + \text{constant} \qquad (5)$$

These derived values of k in sec.$^{-1}$ are given in Table I.

Similarly, the apparent first order rate constants for the esterification of succinic acid in 30% ethanol at 70° can be determined from the slopes of similar plots. These derived values of k in sec.$^{-1}$ are given

TABLE I

CONDITIONS AND OBSERVED FIRST ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF 0.008 *M* HYDROCORTISONE HEMISUCCINATE IN 30% ETHANOL AT 70° (DETERMINED BY COLORIMETRIC ASSAY OF HYDROXAMIC ACID-FERRIC COMPLEXES DERIVED FROM INTACT ESTER)

Run	Composition of buffer [HCl]			pH	$10^6 k$ (sec.$^{-1}$)
1		0.20		0.85[a]	27.7[c]
2		0.10		1.14[a]	13.9[c]
3		0.05		1.40[a]	6.11[c]
4		0.025		1.69[a]	3.33[c]
	[CH_3COOH]	[CH_3COO^-]	[NaCl]		
5	0.1334	0.0333		4.00[b]	1.54[c]
6	0.0326	0.0837		4.78[b]	2.75[c]
7	0.004	0.098		5.30[b]	4.04[c,d]
8	0.142	0.158	0	4.99	2.22[c,d]
9	0.092	0.108	0.05	4.95	3.06[c,d]
10	0.072	0.088	0.07	4.96	2.90[c,d]
11	0.057	0.073	0.085	5.0	2.69[c,d]
12	0.042	0.058	0.100	5.01	2.72[c,d]
	[KH_2PO_4]	[NaOH]			
13	0.1263	0.0758		6.76	6.26[c]
14	0.1035	0.0994		7.46	18.1[c]

[a] Calculated pH = $-\log f$(HCl) where f is the mean activity coefficient for HCl in ethanol-water mixture at 70°.[15] [b] pH decreased during run. The pH recorded was average pH. [c] Calculated from 2.303 × slope of log ($A_{530\ m\mu} - A_\infty$) *vs.* time where A_∞ is asymptotic value of ferric hydroxamate complex absorbance at 530 mμ. The various runs and their asymptotic values, A_∞, were runs 1–4, 0.400; run 5, 0.307; runs 6 and 7, 0.240; runs 8 and 9, 0.151; run 10, 0.212; run 11, 0.220; run 12, 0.204. Runs 13 and 14 apparently decreased to a zero value of absorbance with time. [d] Calculated from application of the Guggenheim procedure.[16]

in Table II. Typical apparent first order plots as per equation (5) for the hydrolysis of 0.008 *M* hydrocortisone hemisuccinate and the esterification of 0.008 *M* succinic acid in 30% ethanol at 70° at 0.05 *M* HCl are given in Fig. 2.

In both cases the pH is calculated from the literature values for the mean activity coefficient, f, in ethanol–water mixtures at 70°[15] where

$$\text{pH} = -\log f[\text{HCl}] \qquad (6)$$

The apparent bimolecular rate constant for the acid catalyzed

(15) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publishing Co., New York, N. Y., 3rd ed., 1958, p. 719.

TABLE II

APPARENT FIRST ORDER RATE CONSTANTS[a] FOR THE ESTERIFICATION OF 0.008 *M* SUCCINIC ACID IN 30% ETHANOL AT 70°

[HCl]	pH[b]	$10^4 k$ (sec.$^{-1}$)
0.20	0.85	1.84
0.10	1.14	1.00
0.05	1.40	0.531
0.025	1.69	0.281

[a] Determined by colorimetric assay of hydroxamic acid–ferric complex derived from intact ester. [b] pH = − log [f(HCl)] where f is the mean activity coefficient for HCl in ethanol-water mixture at 70°.[15]

esterification of succinic acid in 30% ethanol at 70° may be given by the expression

$$k = k_{H^+}[H^+] \tag{7}$$

or

$$\log k = \log k_{H^+} - \text{pH} \tag{8}$$

where pH is defined by equation (6) and k is the apparent first order rate constant in sec.$^{-1}$. A plot of log k against this calculated pH is given in Fig. 3 and shows the expected slope to equal −1 with an intercept of log k_{H^+} = −2.875 so that $k_{H^+} = 1.33 \times 10^{-3}$ l./M/sec. for the bimolecular rate constant for the acid catalyzed esterification of succinic acid in 30% ethanol at 70°.

Similarly, a plot of log k against the calculated pH for the hydrolysis of hydrocortisone hemisuccinate at 70° in 30% ethanol is given as the left branch of the curve drawn in Fig. 4. It shows the expected slope to equal −1 with an intercept of log k_{H^+} = −3.70 in conformity with equations (6), (7), and (8) so that $k_{H^+} = 2.00 \times 10^{-4}$ l./M/sec. for the bimolecular rate constant for the specific acid catalyzed hydrolysis of hydrocortisone hemisuccinate in 30% ethanol at 70°.

Kinetics of Hydrocortisone Hemisuccinate Hydrolysis in 30% Ethanol in Various Acetate and Phosphate Buffers.—The derived hydroxamic acid–ferric ion complex absorbance was used to follow the kinetics of hydrocortisone hemisuccinate hydrolysis in 30% ethanol and in various acetate and phosphate buffers. The observed rate constants, k in sec.$^{-1}$, and the conditions of the reactions are given in Table I. The logarithms of these rate constants are plotted at the base of the curve in Fig. 4 as a function of the average of the pH values observed during the course of the reaction.

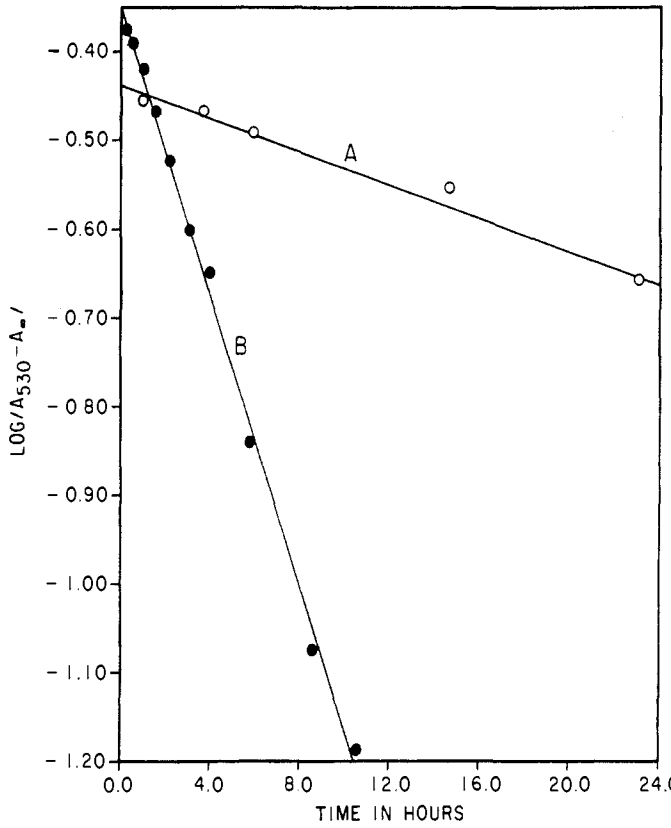


Fig. 2.—Typical first order plots for the hydrolysis of 0.008 *M* hydrocortisone hemisuccinate (Curve A) and esterification of 0.008 *M* succinic acid (Curve B) in 30% ethanol, 0.05 *M* HCl at 70° as derived from absorbance of derived ferric–hydroxamate complex at 530 mμ; ⊙, hydrocortisone hemisuccinate, log (*A* at 530 mμ − 0.4); ●, succinic acid, log (0.445 − *A* at 530 mμ).

The absorbance of the derived hydroxamic–ferric ion complex approached zero as a function of the time of hydrolysis in phosphate buffers. No significant esterification of succinic acid was indicated at pH values greater than 5.9. Asymptotic absorbances were observed in acetate buffers, however, and are given in footnotes to Table I. These asymptotic absorbances were less than those encountered in strong acid catalyzed hydrolysis in 30% ethanol.

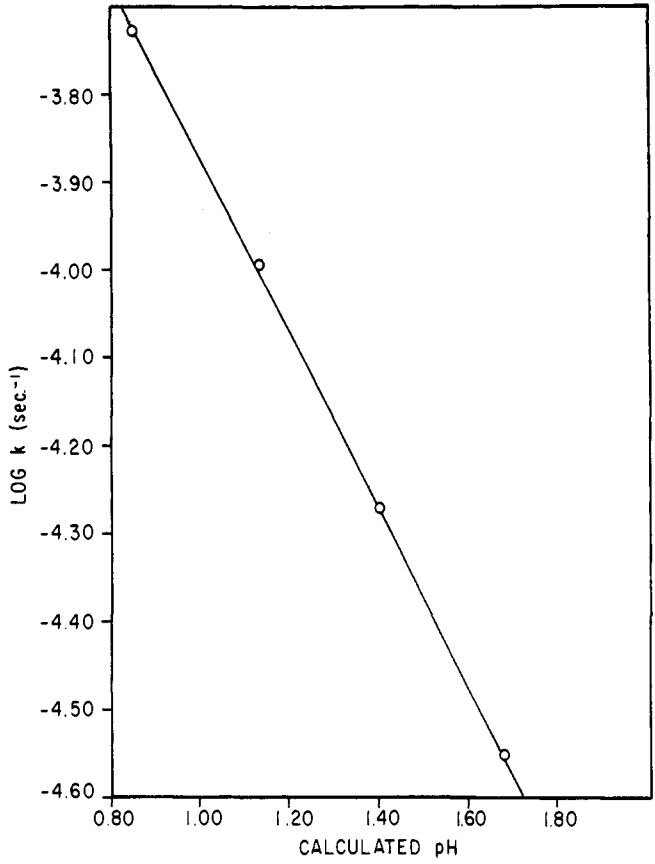


Fig. 3.—Apparent first order rate constant for the esterification of 0.008 *M* succinic acid in varying molarities of HCl at 70°, 30% ethanol.

The effect of acetic acid–acetate buffer concentration at constant ionic strength and at constant pH was evaluated by varying the buffer concentrations at pH *ca.* 5.0 and adjusting the ionic strength with sodium chloride (see runs 8–12, Table I). No significant variation of the apparent first order rate constant with buffer concentration was observed at constant pH and constant ionic strength where the rate constants were calculated by the Guggenheim method.[16]

(16) F. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1936).

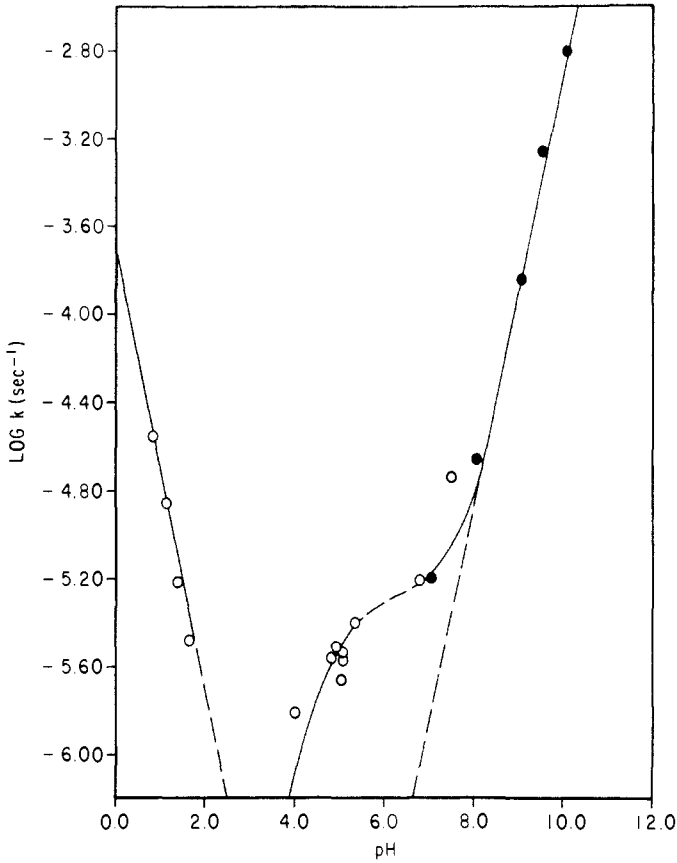


Fig. 4.—Rate constants for the hydrolysis of 0.008 *M* hydrocortisone hemisuccinate in 30% ethanol at 70°: ⊙, rate constants determined by colorimetric assay of unhydrolyzed ester; ●, rate constants determined by constant pH titrations.

Kinetics of Hydrocortisone Hemisuccinate Hydrolysis in 30% Ethanol at Various Alkaline pH Values.—The Cannon titrator plots the consumption of standard alkali against time while the solution of hydrocortisone hemisuccinate is maintained at a given pH. Typical curves for the standard alkali consumption for pH 9 and 10 at 66° in 30% ethanol are given in Fig. 5. The plotted curves are expected to become asymptotic with time. However, this was observed not to be

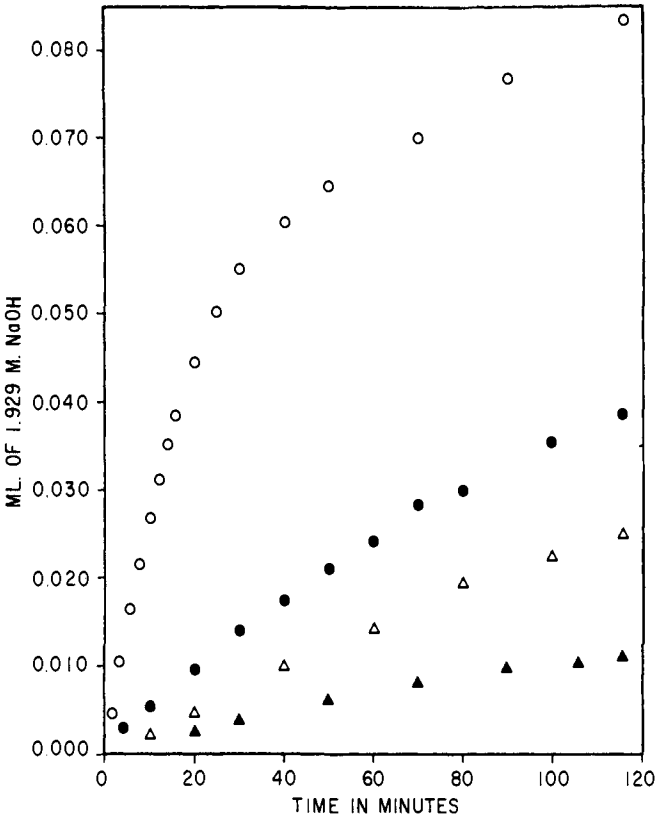


Fig. 5.—Typical curves for the hydrolysis of 0.008 *M* hydrocortisone hemisuccinate and 0.008 *M* hydrocortisone in 30% ethanol at 67°, pH's 9 and 10: ⊙, hydrocortisone hemisuccinate at pH 10; ●, hydrocortisone hemisuccinate at pH 9; △ = hydrocortisone at pH 10; ▲, hydrocortisone at pH 9.

the case since the first order cumulative consumption of alkali tailed off into an apparent zero order consumption.

Although some error is introduced into the estimates of the apparent first order rates by some loss of 30% ethanol-water solvent at 70°,[5] the major source of deviation is apparently due to the consumption of alkali by the product of hemiester hydrolysis, the steroid hydrocortisone.

Included in Fig. 5 are plots of the consumption of standard alkali

by hydrocortisone of the same molarity as the hydrocortisone hemisuccinate studied for hydrolysis rates. These curves clearly show that alkali consumption by the steroid is a function of the hydroxyl ion concentration. They also show that, superimposed on the consumption of alkali by the hydrolyzing ester in 30% alcohol–70% water is the consumption by the non-esterified steroid. A method of calculation of the apparent first order rate constant was used so that the interference of these above factors would be minimized. The slope of the line, m, tangent to the curve of alkaline consumption *vs.* time was determined and the apparent first order rate constant was calculated by

$$k = (N \times m)/(ml \times M) \tag{9}$$

where N is the normality of the standard NaOH consumed, ml = ml. of solution used for constant pH titration, and M = molarity of the solution in the steroid hemiester at zero time.

The apparent first order rate constants, k in sec.$^{-1}$, calculated by equation (9) are listed in Table III for several constant pH titrations

TABLE III

APPARENT FIRST ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF 0.008 *M* HYDROCORTISONE HEMISUCCINATE IN 30% ETHANOL (DETERMINED BY CONSTANT pH TITRATIONS)

pH	Temp., °C.	10^5k (sec.$^{-1}$)	log k^a (sec.$^{-1}$) for 70°	$10^5k_{70}{}^a$ (sec.$^{-1}$)
10	66.1	110[b]	−2.810	155
9.5	66.2	38.6[b]	−3.270	56.9
9.0	65.9	9.95[b]	−3.846	14.3
8.0	66.3	1.56[c]	−4.670	2.41
7.0	66.7	0.475[c]	−5.197	0.635

[a] Calculated from $\log k_{70} = -4.327 \times 10^3 [1/T_{70} - 1/T] + \log k$ were T is 273 + °C. of rate study of constant k. [b] 10–25% loss of volume by end of run. [c] 25–50% loss of volume by end of run.

from 7 to 10. To correlate these rate constants at *ca.* 66° with the complete log k *vs.* pH profile of Fig. 4, they are corrected to 70° by use of the derived Arrhenius expression obtained from the alkaline hydrolysis of hydrocortisone hemisuccinate in water[5] where

$$\log k_{70} = -4.327 \times 10^3 \left[\frac{1}{T_{70}} - \frac{1}{T_{66}}\right] + \log k_{66} \tag{10}$$

where T_{66} is the appropriate absolute temperature at which k_{66}

is observed. The calculated k_{70} values also are given in Table III.

The apparent bimolecular rate constant for the hydroxyl ion catalyzed hydrolysis of hydrocortisone hemisuccinate in 30% ethanol at 70° is given by the expression

$$k = k_{OH^-}[OH^-] \tag{11}$$

or

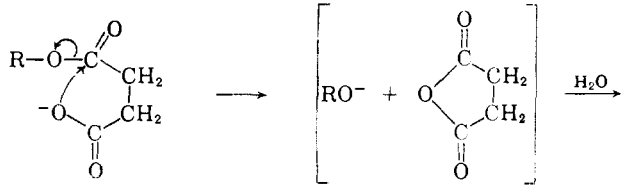
$$\log k = \log k_{OH^-} - pOH$$
$$= \log k_{OH^-} + pH - pK_w \tag{12}$$

Such a plot of log k against the pH is given in the right branch of the curve of Fig. 4. It shows the expected slope to equal +1 with an intercept of log k_{OH^-}-pK_w = 12.80 in conformity with equations (11) and (12) so that k_{OH^-}-K_w = 1.58×10^{-3}. It follows that the apparent first order rate constant k for alkaline hydrolysis of the hemiester at 70° in 30% alcohol-70% water can be calculated from

$$k = k_{OH^-}K_w/[H^+] = 1.58 \times 10^{-13}10^{pH} \tag{13a}$$
$$= 1.58 \times 10^{(pH-13)} \tag{13b}$$

The Deviations from Summation of Specific Acid and Specific Hydroxyl Ion Catalyzed Hemiester Hydrolysis in the Intermediate pH Regions.—It is apparent that the rate constants for the hydrolysis of hydrocortisone hemisuccinate in the intermediate pH ranges greatly exceed the sum of the values predictable for these pH values from the extrapolations of the right and left arms of the curve of Fig. 4. Between the pH values 3 and 6.5 the apparent first order rate constants of hydrolysis in 30% ethanol at 70° are predicted on the premise of specific acid-base catalysis to not exceed 10^{-6} sec.$^{-1}$ (log k = −6.00). These values are exceeded, however, by the experimentally determined rate constants (Table I). In light of possible expectation of intramolecular catalysis by the anion of the hemiester[8-11] and the lack of significant general acid-base catalysis as evidenced by the nonvariation of rate at constant pH with varying acetic acid–acetate buffer concentrations (Table I), the deviations from specific hydrogen and hydroxyl ion catalyzed hydrolysis could be assigned to the monomolecular hydrolysis of the anionic form of the hemiester.[10,11]

The concentration of the anionic form will be a function of the dissociation constant of the hemiester in the same solvent, *viz.*, K_a



$$ROH + HOOCCH_2CH_2COO^- \qquad (14)$$

$= 1.78 \times 10^{-5}$, and the data in the intermediate pH values can be fitted by the expression[17]

$$k = k_{OH^-}[OH^-] + k_{H^+}[H^+] + k_0[ROCOCH_2CH_2COO^-] \qquad (15a)$$

$$= k_{OH^-}K_w/[H^+] + k_{H^+}[H^+] + k_0/(1 + [H^+]//K_a) \qquad (15b)$$

$$= 1.58 \times 10^{(pH-13)} + 1.33 \times 10^{-3}\cdot 10^{-pH} + 5.2 \times 10^{-6}/(1 + [H^+]/1.78 \times 10^{-5}) \qquad (15c)$$

where the constants in (15c) have been estimated and the k is in sec.$^{-1}$. The k_0 for the intramolecular catalyzed hydrolysis of the hemiester is 5.2×10^{-6} sec.$^{-1}$.

The log k values were calculated from equation (15c) and are drawn as a function of pH in Fig. 4, and demonstrate the consistency of the model with the plotted experimental data. The data do not deny completely the possibility of a rate contribution from the hydroxyl ion catalyzed hydrolysis of the undissociated hemiester. The analogy to aspirin hydrolysis is apparent.[8,17]

Hydrolytic Rates of Various Esters of Hydrocortisone.—The hydrolyses of various hemiesters of hydrocortisone and dicarboxylic acids were studied by constant pH titration at 57° and 66° in 20% and 30% ethanol in water. Since most of the hemiesters were supplied in the free acid form, the alcoholic solutions were necessary to provide sufficient quantities instantaneously in homogeneous solution and to permit comparison with the alkali consumption by the hydrocortisone alcohol and the hydrolysis of esters of monocarboxylic acids.

Typical plots of the ml. of 2 N NaOH titer consumed as a function of time are given for various hydrocortisone hemiesters and hydrocortisone, 15 ml. of 0.008 M, at 57° at pH 10.0 in 20% ethanol, in Fig. 6.

The apparent first order rate constants k in sec.$^{-1}$ and the half-lives

(17) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).

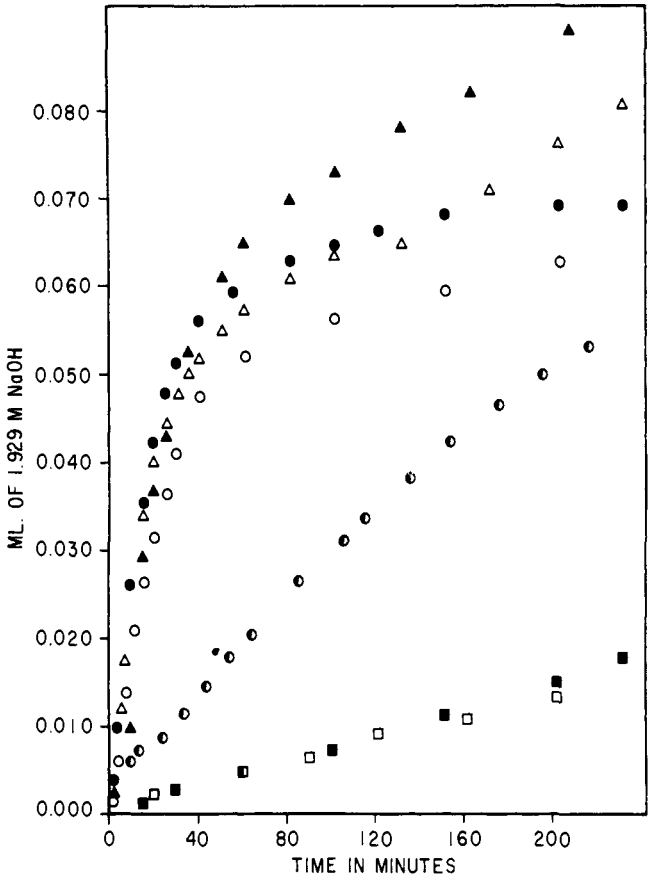


Fig. 6.—Typical curves for the hydrolysis of 0.008 *M* hemiesters and hydrocortisone in 20% ethanol, pH 10.0, at 57°: ⊙, hydrocortisone hemisuberate; ●, hydrocortisone hemipimelate; △, hydrocortisone hemisuccinate; ⊡, hydrocortisone hemi-α,α'-diethylsuccinate; ▲, hydrocortisone hemiadipate; ■, hydrocortisone; ◐, hydrocortisone β,β'-dimethylglutarate.

are listed in Table IV. When the rate was slow and when alkaline consumption by the free steroid significantly contributed to the total sodium hydroxide to maintain pH, especially at the higher ethanolic concentrations, the apparent first order rate was calculated from the initial zero order slope using equation (9). Alternative methods of

TABLE IV

PSEUDO-FIRST ORDER RATE CONSTANTS GIVEN AS 10^4k(sec. $^{-1}$) AS BASED ON ALKALI CONSUMPTION IN VARYING PER CENT. ETHANOL-WATER AT CONSTANT pH

Hydrocortisone ester	Temp. →	66°				57°			
	% Ethanol →	20		30		20		3	
	pH →	10	9	10	9	10	9	10	9
Hemisuccinate		25[a]	2.1[a]	7.3[a]	1.0[a]	7.0[a]	0.84[a]		
Hemiadipate		18[a]	0.98[a]			5.2[a]	0.88[a] 1.0,[b] 0.82[b,d]		
Hemipimelate		13[a]	1.7[a]			8.6[a]	0.59[a] 0.59[b]		
Hemisuberate		14[a] 16[c]	0.60[a]			5.3[a]	0.85[a] 0.78[b,d]		
Acetate					5.6[a] 6.1[c]				2.2[a] 2.5[c] 3.1[a,d] 3.6[c,d]
Acrylate				25[a] 22[c]	2.9[a] 3.1[c]			9.0[a] 9.1[c]	1.7[a] 1.9[c]
Hydrocortisone[e]				0.71[a]	0.098[a]	0.21[a]	0.018[a] 0.023[a,d] 0.0771[a,f]	0.67[a]	0.096[a]
Hemi-α,α'-diethylglutarate									
Hemi-β,β'-dimethylglutarate[e]				1.5[a] 1.9[a,d]	0.19[a]	1.3[a]	0.077[a]	0.39[a]	0.077[a,f]
Hemi-α,α'-diethylsuccinate[e]		0.25[a,f]	0.12[a,f]			0.17[a,f]	0.042[a,f]		

[a] Calculated from initial zero order slope, m, of ml. of N normal titer consumed per second on reaction of m' ml. of M molar hydrocortisone ester, *i.e.*, $k = (N \times m)/(m' \times M)$. [b] Calculated from a Guggenheim plot.[16] [c] Calculated from first order expression $\log (\lambda_\infty - \lambda) = kt/2.303 + \log \lambda_\infty$ where λ_∞ is the theoretical volume of standard titer consumed on complete saponification and λ is the amount consumed at time, t. [d] A separate study from one listed above. [e] Although calculated as first order, these studies were closer to zero order for the duration of the study. [f] Apparent zero order for as long as 10 hours.

calculation were also used including the Guggenheim method[16] and classical first order plots

$$\log (m_\infty - m) = -kt/2.303 + \log m_\infty \qquad (16)$$

where m_∞ is the ml. of titer theoretically necessary to completely saponify the ester and m is the ml. of titer consumed at time, t.

Equation (16) or the Guggenheim equations were applicable to estimate rate constants for the relatively fast hydrolyzing hydrocortisone esters such as the hemisuberate, hemipimelate, hemisuccinate, hemiadipate, acrylate, and acetate since the subsequent consumption of alkali by the free hydrocortisone alcohol was relatively small.

However, the hydrocortisone esters of β,β'-dimethylglutarate and α,α'-diethylsuccinate at the ethanolic concentrations studied hydrolyze at rates of similar magnitude to the rate of alkali consumption by the free hydrocortisone alcohol. The probable sequence is

$$\text{hydrocortisone ester} + OH^- \longrightarrow \text{hydrocortisone alcohol} \xrightarrow{OH^-} \text{degradation product (etio acid and/or formic acid)} \qquad (17)$$

The fact that the estimated rate constants for the hydrolysis of hydrocortisone β,β'-dimethylglutarate at 57°, pH 9 and 10 in 30% ethanol, and of hydrocortisone α,α'-diethylsuccinate at 60°, pH 9 and 10 in 20% ethanol, are equivalent to or less than those estimated for alkali consumption by hydrocortisone under the same conditions is consistent with equation (17) (see Table IV).

The rate of alkali consumption for hydrocortisone, hemi-α,α'-diethylglutarate, hemi-β,β'-dimethylglutarate, and hemi-α,α-diethylsuccinate is not clearly first order as it was for the other esters and for most of the alkali consumption is closer to zero order, thus demonstrating the complex sequential nature of the reactions for the difficultly hydrolyzable esters in aqueous alcohol (see Figs. 5 and 6).

Discussion

Hydrolysis Studies on Hydrocortisone Hemisuccinate in Aqueous Ethanol and the Ferric Hydroxamate Ester Assay.—The fact that the absorbances from hydroxamic acid–ferric ion complexes reached the same asymptote on catalyzed hydrolysis of 0.008 M hydrocortisone hemisuccinate with various HCl molarities showed that the succinic acids derived from the hydrolyses were esterified by the ethanol of the 30% ethanol–water solvent to the same extent. This is confirmed

by the fact that the 0.008 *M* succinic acid, dissolved in the same media and reacting under the same conditions, gave the same asymptotic absorbance independent of HCl molarity.

A point of interest, however, is that the absorbance of the hydroxamic acid–ferric ion complex when derived from the hydrocortisone hemisuccinate was much greater (see Fig. 1, Curve A, at times less than 8 hours) than the absorbance when derived from the ethyl hemisuccinate (see Fig. 1, Curve B, asymptotic absorbance).

By equations (1) through (4), the absorbance is logically attributed to the ferric succinohydroxamate in both cases. These facts imply that the alkaline catalyzed hydroxamic acid formation is competitive with the alkaline catalyzed hydrolysis, that hydrocortisone hemisuccinate is the more readily transformed to the succinohydroxamic acid than is the ethyl hemisuccinate. An alternate explanation is that the hydrocortisone hemisuccinate is di-esterified to a greater extent in the acid ethanol-water solvent than is the ethyl hemisuccinate, that a greater amount of succino-dihydroxamic acid results from the assay procedure in the case of the hydrocortisone hemisuccinate.

The decrease in asymptotic absorbance of the derived hydroxamic-ferric ion complex in acetate and phosphate buffers from hydrocortisone hemisuccinate is consistent with the slowing down of the rate of esterification of the 30% ethanol in water solvent with the succinic or acetic acids. It can be calculated from equation (8) where log $k_{H^+} = -2.875$ that at pH 4 the apparent first order rate constant of succinic acid esterification is 1.33×10^{-7} sec.$^{-1}$ and at pH 5 is 1.33×10^{-8} sec^{-1}. This is of the order of 10% or less of the rate constants of hydrolysis of hydrocortisone hemisuccinate in the acetate and phosphate buffer systems studied and thus should have negligible effect on the approach of the measured absorbance to a zero value with time, at least in the phosphate buffers. The presence of acetic acid in the acetate buffers would predict a higher asymptotic absorbance as was observed (footnotes to Table I).

The lack of significant variation of the rate constant for hydrocortisone hemisuccinate at constant pH and constant ionic strength with varied acetate-acetic acid buffer concentrations implies no significant general acid-base catalysis, that hydrogen ion and hydroxyl ions are the significant catalytic species.

Yet a deviation from specific hydrogen ion and hydroxyl ion catalyzed hydrolysis is apparent in Fig. 4 and could be assigned to the

monomolecular hydrolysis of the anionic form of the hemiester.[10,11] Because the rate of hemisuccinate hydrolysis by hydroxyl ion is of such a high order of magnitude, this possible intramolecular catalyzed hydrolysis would contribute little above pH 7.5.

Comparison of Hydrolytic Rates of Various Esters of Hydrocortisone.—Comparison of the apparent first order rates of alkali consumption by esters of hydrocortisone (Table IV) at the same pH values (9 and 10), solvent conditions (20% ethanol), and reaction temperatures (57° and 66°) showed no highly significant difference in hydrolysis rate for the hemiester of hydrocortisone

$$ROOC(CH_2)_n\,COO^- + OH^- \longrightarrow ROH + {}^-OOC(CH_2)_n\,COO^- \quad (18)$$

where $n \geq 2$.

Thus it must be concluded that the distance of the negative charge from the ester grouping has little effect on the hydroxyl ion catalyzed hydrolysis of the hydrocortisone hemiester above pH 9 for unbranched chain dicarboxylic acids of four or more carbons.

A field effect, in that the catalytic hydroxyl ion is repelled in part by the hemiester anion, is indicated by the comparison of the rates of hydrolysis of hydrocortisone acetate and acrylate with hydrocortisone hemisuccinate in 30% ethanol at 66° (Table IV).

The hindered hemiesters of hydrocortisone, the hemi-α,α'-diethylglutarate, hemi-β,β'-dimethylglutarate, and the hemi-α,α'-diethylsuccinate show a dramatic decrease in hydroxyl ion catalyzed hydrolysis (Table IV). There appears to be very little difference between the hemi-α,α'-diethylglutarate and the hemi-β,β'-dimethylglutarate on the basis of the data available in Table IV. This is to be expected on the basis of Newman's "rule of six."[18,19]

The data of Table IV favor the hemi-α,α'-diethylsuccinate ester as the most stable to hydroxyl ion catalysis of the hydrocortisone esters studied.

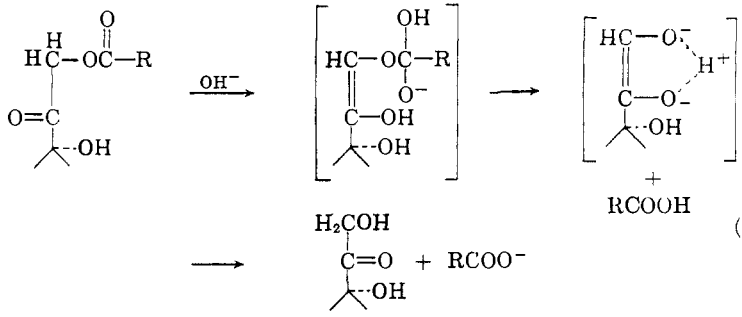
Possible Mechanisms for the Hydrolysis of Esters of Hydrocortisone.—The relatively fast hydrolysis of hydrocortisone esters with respect to similar esters of the other alcohols has been discussed previously with specific reference to the hemisuccinate ester.[5] This fact is also valid with respect to the acetate ester.

The accelerated rate and lower heat of activation,[5] *i.e.*, 7 kcal./mole,

(18) K. L. Loening, A. B. Garrett, and M. S. Newman, *J. Am. Chem. Soc.*, **74**, 3929 (1952).
(19) M. S. Newman, *ibid.*, **72**, 4783 (1950).

over ordinary aliphatic esters of similar dicarboxylic or monocarboxylic acids, *e.g.*, acetates and acrylates, 9–14 kcal./mole, must be attributed to the configuration of the steroid side chain.

A possible mechanism is the stabilization of the steroid side chain as a leaving group to enhance the hydrolysis rate.



(19)

An argument against (19) is that the steroid alcohol is regenerated on hydrolysis of the ester whereas some aldehyde sidechain may be expected. However, the ratio of keto to aldehyde form for dihydroxyacetone is 20:1[20] at equilibrium so that equation (19) cannot be rejected on these grounds. Also, in triose phosphate the equilibrium favors the keto form by a 20:1 ratio.[21,22]

Ease of deprotonation can be predicted for the steroid side chain. Work now in progress shows that 20-keto, 21-hydroxysteroids are base titratable in pyridine.[23]

An explanation for the enhancement of hydrolysis rate of hydrocortisone hemisuccinate in the neutral pH range was given previously. Intramolecular nucleophilic attack by carboxylate anion could significantly contribute to the hydrolysis. This is kinetically described by the contribution

$$d[\text{Ester}]/dt = -k[\text{ROC}(\text{CH}_2)_2\text{CO}^-] \qquad (20)$$

However

$$[\text{ROC}(\text{CH}_2)_2\text{CO}^-] = [\text{ROC}(\text{CH}_2)_2\text{COH}]Ka/[\text{H}^+] \qquad (21)$$

(20) A. A. Forist, Doctoral Thesis, Michigan State University, 1952.
(21) M. F. Utter and C. H. Wekman, *J. Bact.*, **42**, 665 (1941).
(22) O. Meyerhof and R. Junowicz-Kocholaty, *J. Biol. Chem.*, **149**, 71 (1943).
(23) E. R. Garrett and D. J. Weber, to be published.

and

$$1/[H^+] = [OH^-]/K_w \qquad (22)$$

so that in substitution of (21) and (22) into (20)

$$d[\text{Ester}]/dt = \frac{kK_s}{K_w}[OH^-]\ [RO\overset{O}{\overset{\|}{C}}(CH_2)_2\overset{O}{\overset{\|}{C}}—OH] \qquad (23)$$

Thus, intramolecular catalysis (equation 20) need not necessarily be favored over its kinetic equivalent (equation 23).

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Certain Steroid Ketals and Their Biological Activity

WILLIAM S. ALLEN, HENRY M. KISSMAN, SIDNEY MAUER, IRA RINGLER, AND MARTIN J. WEISS[1]

Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

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By straightforward procedures the 20-ketals of 6α-methylhydrocortisone, 6α-methylprednisolone, 6α-methylprednisone, 9α-fluoro-6α-methylprednisone, 21-deoxy-9α-fluoro-6α-methylprednisone, prednisone, 9α-fluorocortisone, 9α-fluoroprednisone, 9α-fluoro-16α-hydroxycortisone, triamcinolone, 2α-methylhydrocortisone, 2α,6α-dimethylhydrocortisone, and 2,6α-dimethylprednisolone have been prepared. These ketal derivatives, although less active than the parent 20-ones, showed substantial glucocorticoid activity.

The observation made in the course of routine screening of steroid intermediates, that 6α-methylhydrocortisone 20-ethylene ketal (II), prepared as a substrate for ethoxalylation studies at C-2 of the steroid nucleus, possesses substantial glucocorticoid activity, prompted the study which is the subject of this paper. To our knowledge biolog-

(1) To whom inquiries concerning this paper should be addressed.