Carboxyl Group Catalysis of Acyl Transfer Reactions in Corticosteroid 17- and 21-Monoesters

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Abstract □ Succinate esters, although frequently employed as water-soluble prodrugs of poorly soluble parent drugs, are not sufficiently stable to allow long-term storage in solution. Intramolecular catalysis of ester hydrolysis by the terminal succinate carboxyl group is a contributing factor to this instability. Methylprednisolone 21-succinate has recently been reported to undergo both hydrolysis and 21 ⇄ 17 acyl migration in aqueous solutions. Intramolecular catalysis by the terminal carboxyl group is seen in both reactions, but the catalytic mechanisms are not well understood. While acyl migration can only be catalyzed *via* the carboxyl group acting as a general acid or general base, hydrolysis may undergo either nucleophilic or general acid–base catalysis. To gain further insight into the catalytic mechanism, hydrolysis of methylprednisolone 21-succinate was carried out in aniline buffers to trap any succinic anhydride (as the anilide) that would form if the catalysis were nucleophilic. The nucleophilic mechanism was shown to account for only 15–20% of the overall catalysis. Comparisons of the rates of the intramolecularly catalyzed reactions of methylprednisolone 21- and 17-succinate were made with the same reactions of methylprednisolone 21- and 17-acetate catalyzed intermolecularly by acetate ion. Interestingly, intramolecular catalysis appears to favor acyl migration over hydrolysis. Hence, the hydrolysis of methylprednisolone 21-succinate is faster in basic solutions (pH > 7.4), while acyl migration becomes the dominant reaction in the catalyzed region of the pH profile between pH 3.6 and 7.4. Arguments are presented to account for these differences in catalytic efficiency in terms of the transition-state structures for the two reactions.

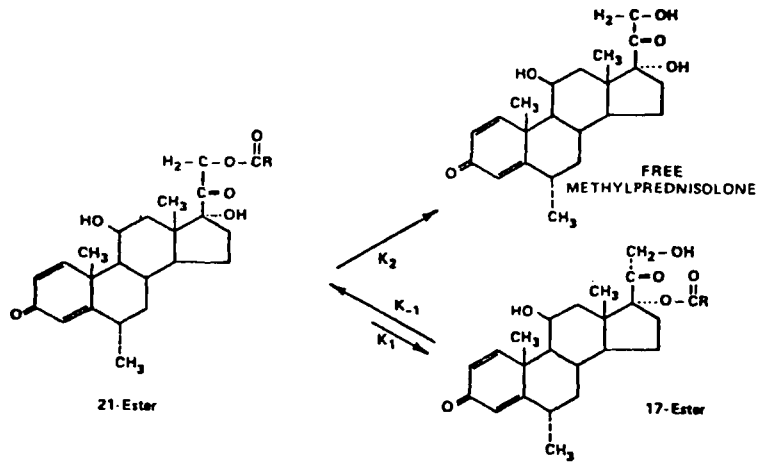
Keyphrases □ Acyl transfer reactions—methylprednisolone 17- and 21-monoesters, carboxyl group catalysis □ Methylprednisolone 17- and 21-monoesters—acyl transfer reactions, carboxyl group catalysis □ Carboxyl group catalysis—methylprednisolone 17- and 21-monoesters, acyl transfer reactions

Carboxyl group catalysis of acyl transfer reactions is an important phenomenon in a host of chemical and biological processes involving esters or amides. Many examples of carboxyl groups acting as nucleophilic, general acid, or general base catalysts either inter- or intramolecularly have been cited (for general reviews on the subject: 1–3). Yet, more work is needed since it is still not possible to predict with certainty the extent to which carboxyl groups in the immediate environment will accelerate a given acyl transfer reaction or *via* what mechanism this will occur.

The role of carboxyl catalysis in the degradation of hemiesters of dicarboxylic acids has been of particular interest from a pharmaceutical perspective because hydroxyl-containing drugs having low water solubility are frequently solubilized for parenteral administration by forming their succinate esters. A classic example of the clinical utility of this approach was the development of soluble 21-succinate esters of corticosteroids for parenteral use. A serious drawback of such derivatives is the limited shelf life of their solutions, which is due in part to intramolecular catalysis by the terminal succinate carboxyl group.

In aqueous solution, 21-esters of corticosteroids undergo ester hydrolysis and 21 ⇆ acyl migration as depicted in Scheme I for esters of methylprednisolone (4–6). Garrett recognized in the early 1960's that the solvolysis of the 21-succinate ester of hydrocortisone is catalyzed by the terminal carboxyl group and suggested that intramolecular nucleophilic catalysis is involved (4, 5). Recently, studies of the degradation of methylprednisolone 21-succinate confirmed that hydrolysis is catalyzed intramolecularly and also revealed that 21 ⇄ 17 acyl migration which was found to occur at an initial rate comparable to the hydrolysis rate is also subject to intramolecular catalysis (6).

Shown in Fig. 1 are the pH–rate profiles for the hydrolysis and 21 → 17 acyl migration of methylprednisolone 21-succinate, reported in an earlier study (6). Intramolecular catalysis of hydrolysis is clearly seen as a deviation of the hydrolysis curve from the simple V-shaped profile (dashed line) typically observed for the hydrolysis of esters. Between pH 4.1 and 6.5, the dominant hydrolytic reaction is hydroxide-ion attack on



Scheme I—Degradation pathways of 21-esters of methylprednisolone in aqueous solution.

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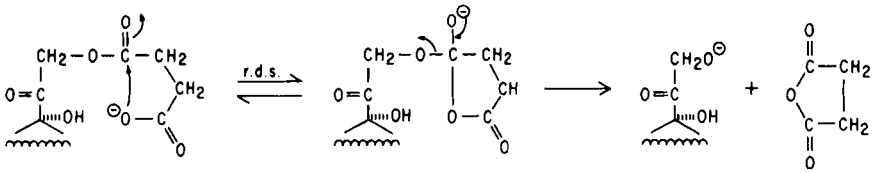
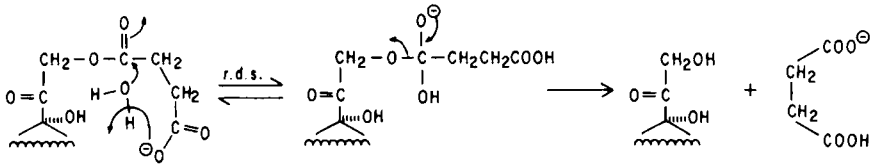
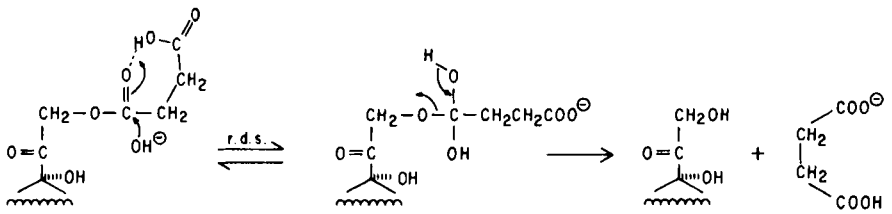


Scheme II—Intramolecular nucleophilic catalysis of 21-ester hydrolysis.



Scheme III—Intramolecular general base catalysis of 21-ester hydrolysis by water.



Scheme IV—Intramolecular general acid catalysis of attack by hydroxide ion on the 21-ester.

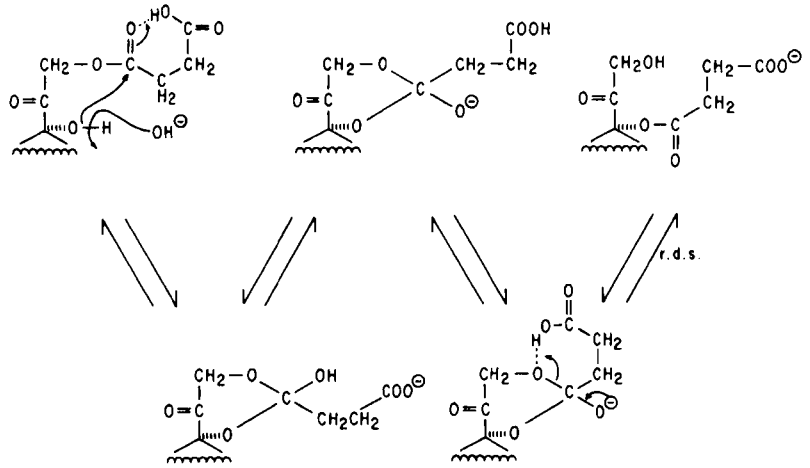
the ionized ester or the kinetically equivalent water attack on the anion. Mechanistically, the increase in rate over that expected from hydrolysis in more basic solutions can be interpreted as being due to one or more of the following kinetically equivalent reactions: (*a*) intramolecular nucleophilic attack by the terminal carboxylate anion; (*b*) intramolecular general base catalysis of attack by water; (*c*) intramolecular general acid catalysis of attack by hydroxide ion, or (*d*) repulsion of hydroxide ion by the negatively charged carboxylate. Mechanisms *a*–*c* are depicted in Schemes II–IV, respectively.

Intramolecular nucleophilic attack by carboxylate anion (Scheme II) results in the formation of succinic anhydride, which in a subsequent step then hydrolyzes in water to give succinic acid. The other possible mechanisms yield succinic acid directly.

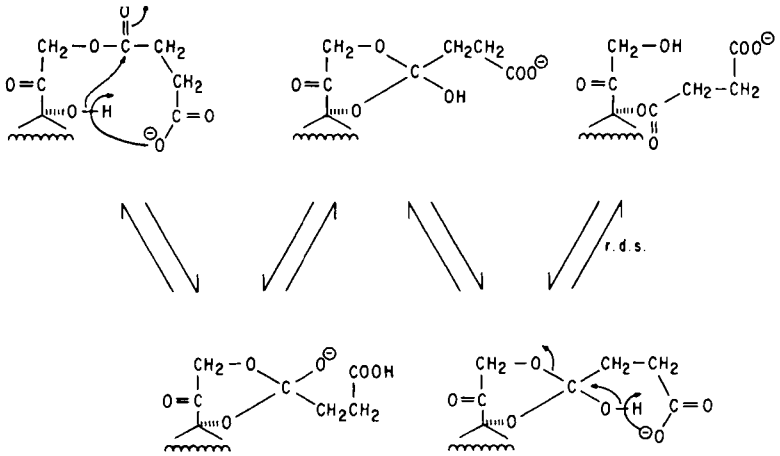
Acyl migration is also catalyzed intramolecularly by the terminal succinate carboxyl group of methylprednisolone 21-succinate (or 17-succinate), as evident in Fig. 1, from the acceleration of the migration rate below pH ~ 7.4 compared with that expected based on the rate in more basic solutions. Two possible mechanisms may account for this acceleration: intramolecular general acid-specific base catalysis, or general base catalysis. Two plausible representations of these catalytic mechanisms are shown in Schemes V and VI, respectively, for the 21 → 17 migration. (These diagrams assume that breakdown of the tetrahedral intermediate is rate determining in going from 21 → 17 and, therefore, formation of the tetrahedral intermediate would be rate determining in going from 17 → 21.)

Since catalysis of acyl migration must occur *via* a general acid–base mechanism, it appeared likely that a general catalytic mechanism may also be operative in the hydrolysis. To ascertain the importance of the intramolecular nucleophilic mechanism in hydrolysis, an attempt was made to trap the succinic anhydride (as succinanilic acid) that would form from the intramolecular nucleophilic attack of carboxylate anion on the ester, by carrying out the hydrolysis in aniline buffers.

Of further interest was the observation that intramolecular catalysis brings about a reversal in the relative rates of the 21 → 17 migration and 21-ester hydrolysis. In solutions of pH > 7.4, hydrolysis is slightly faster than 21 → 17 acyl migration, while acyl migration is dominant in the intramolecularly catalyzed region between pH 3.6–7.4. To understand better the



Scheme V—General acid-specific base catalysis of 21 → 17 acyl migration. (Reverse reaction reflects general base catalysis of 17 → 21 acyl migration.)



Scheme VI-- General base catalysis of 21 → 17 acyl migration. (Reverse reaction reflects general acid-specific base catalysis of 17 → 21 acyl migration.)

differences in catalysis of these two similar reactions and to generate additional background information on O → O acyl migration in corticosteroid esters, studies of the degradation of methylprednisolone 21- and 17-acetate in water and in acetate buffers were also conducted.

EXPERIMENTAL

Reagents—Methylprednisolone[1], methylprednisolone 21-hemisuccinate[2], and methylprednisolone 21-acetate[3] were commercially available and used without further purification. Methylprednisolone 17-hemisuccinate was reported previously (6).

Methylprednisolone 17-acetate was prepared as described previously (7); UV: λ_{max} 242 nm (ϵ = 14,900); ^{1}H-NMR ($CDCl_3$-DMSO-d_6, 4:1)[4] δ 7.2-7.35 (d, 1, C_1— H), 6.35-6.5 (d, 1, C_2—H), 5.9 (s, 1, C_4—H), 4.4 (br, 1, C_{11}—H), and 4.15 ppm (s, 2, C_{21}—H_2).

Anal.- Calc. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.75. Found: C, 68.84; H, 7.66.

Succinanilic acid was prepared from succinic anhydride and aniline, mp 145.8-146.8°C [lit. (8) mp 150°C].

Anal.—Calc. for $C_{10}H_{11}NO_3$: C, 62.16; H, 5.74; N, 7.25. Found: C, 62.18; H, 5.82; N, 7.27.

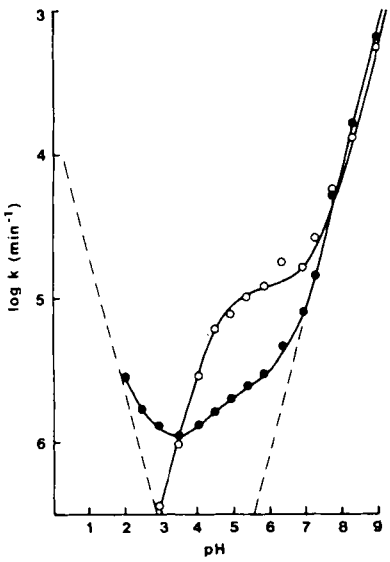


Figure 1—*pH-Log rate profiles for hydrolysis (●) and 21 → 17 acyl migration (○) of the 21-succinate ester of methylprednisolone in aqueous solutions at 25°C.*

All other reagents were of analytical or reagent grade and were used without further purification, except for aniline which was redistilled before use.

Procedure for the Anilide Trapping Experiment—Solutions of methylprednisolone 21-succinate were prepared at a concentration of 5×10^{-4} M in aqueous aniline buffers varying from 4×10^{-4} to 0.04 M aniline with pH values of 5.0, 5.5, and 6.0. At these pH values the intramolecularly catalyzed reaction dominates in the hydrolysis.

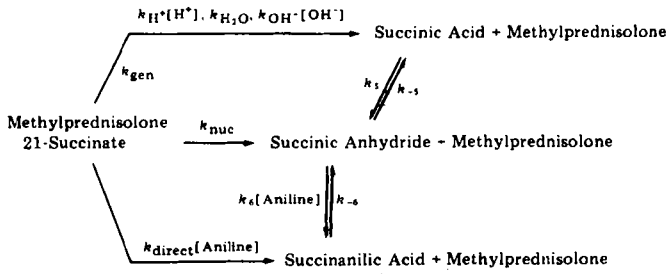
The initial rates of formation of methylprednisolone and succinanilic acid were determined at 25°C by measuring the solution concentrations of these reaction products at various times by HPLC. The solutions were monitored until ~1% of the ester had hydrolyzed to methylprednisolone. For the HPLC analysis a 25 cm × 4.6 mm i.d. reverse-phase column[5] packed with 10-μm Lichrosorb RP-8[6] was utilized with a mobile phase consisting of methanol-water-dimethyloctylamine (50:50:0.3) and 0.02 M MES buffer adjusted to pH 5.8. The solvent was pumped[7] at a flow rate of 1.3 mL/min, with sample detection at 254 nm[8].

Methylprednisolone 21- and 17-Acetate Hydrolysis, Acyl Migration Kinetics, and Acetate Buffer Catalysis Studies—Stock solutions of methylprednisolone 17- and 21-acetate in dimethylformamide were diluted 100-fold into aqueous buffers to a concentration of 2.5×10^{-5} M. These solutions were reacted at 25°C during the study. The initial rates of formation of decomposition products were determined by HPLC analysis using one of the following systems.

1. For the degradation of the 21-acetate, a reverse-phase column packed with 5-μm Spheri-5 RP-18[9] was used with a methanol-water (57:43) mobile phase. The flow rate was 1.6 mL/min[10], detection was at 243 nm[11], and the injection size was 500 μL[12].

2. The 17-acetate decomposition kinetics were monitored using conditions similar to those above, but with a mobile phase consisting of methanol-acetonitrile-water (10:32:58).

For comparison with data reported previously for the 21-succinate, the hydrolysis of methylprednisolone 21-acetate was carried out in 0.01 M ionic strength buffers. The studies of catalysis by acetate buffer were carried out



Scheme VII—Possible reactions of methylprednisolone 21-succinate in aniline buffers.

[1] Medrol; The Upjohn Co., Kalamazoo, Mich.
[2] Solu-Medrol (methylprednisolone sodium succinate); The Upjohn Co.
[3] Depo-Medrol, The Upjohn Co.
[4] Unisol-*d*; Norell, Inc., Landisville, N.J.
[5] Altex Scientific, Berkeley, Calif.
[6] E. Merck, Dermstadt, West Germany.
[7] Milton Roy Mini-Pump; Laboratory Data Control, Riviera Beach, Fla.
[8] Model 840000-901; DuPont.
[9] MPLC 10-cm column + 3-cm guard column; Brownlee Laboratories, Berkeley, Calif.
[10] Model 110A pump; Altex Scientific, Berkeley, Calif.
[11] Altex/Hitachi model 153-00; Altex Scientific, Berkeley, Calif.
[12] Wisp model 710A; Waters Associates, Milford, Mass.

Table I—Values of Rate Constants for Methylprednisolone 21-Succinate Hydrolysis and Succinanilic Acid Formation in Aniline Buffers at 25°C

Parameter	Value	95% Confidence Limit
k_{H^+}[a]	0.012 L/mol	±60%
k_{H_2O}[a]	6×10^{-5} h^{-1}	±34%
k_{cat}[a]	1.5×10^{-4} h^{-1}	±24%
k_{OH^-}[a]	4.4×10^3 L/mol-h	±19%
k_{direct}	Indeterminate[c]	—
k_{nuc}	2.28×10^{-5} h^{-1}	±13%
k_{gen}	1.3×10^{-4} h^{-1}	±30%
k_6[b]	3.6×10^4 L/mol-h	—
k_{-5}[b]	9 h^{-1}	—

[a] From Ref. 6. [b] From Ref. 9. [c] Not significantly different from zero.

in buffers containing 0.05–0.5 M total acetate adjusted to 0.5 ionic strength with NaCl.

RESULTS

Hydrolysis of Methylprednisolone 21-Succinate in Aniline Buffers— In water, methylprednisolone 21-succinate hydrolyzes *via* several kinetically distinguishable routes (6) as expressed by the following equation for the overall hydrolysis rate constant, k_{hyd}:

$$k_{hyd} = k_{H^+}[H^+]f_u + k_{H_2O} \cdot f_u + k_{cat} \cdot f_i + k_{OH^-}[OH^-]f_i \quad \text{(Eq. 1)}$$

where k_{H^+} and k_{H_2O} represent hydrogen ion and water catalysis of the degradation of the un-ionized fraction, f_u, and k_{cat} and k_{OH^-} represent water- and hydroxide-ion catalyzed breakdown of the ionized fraction, f_i.

The catalyzed region clearly evident in the plot of the logarithm of the hydrolysis rate constant for methylprednisolone 21-succinate *versus* pH in Fig. 1 between pH 4.1–6.5 is the region in which the k_{cat} term in Eq. 1 dominates. This term may represent (*a*) intramolecular nucleophilic attack by the terminal carboxylate anion on the ester linkage, as proposed by Garrett (4) (Scheme II); (*b*) intramolecular general base catalysis of attack by water (Scheme III) or its kinetic equivalent, intramolecular general acid catalysis of hydroxide ion attack (Scheme IV); or (*c*) a composite of all possibilities as expressed by:

$$k_{cat} = k_{nuc} + k_{gen} \quad \text{(Eq. 2)}$$

While they are kinetically indistinguishable, k_{nuc} (representing nucleophilic catalysis) can be differentiated from k_{gen} (representing general acid–base catalysis) since succinic anhydride would be formed as an intermediate in the nucleophilic mechanism and should be detectable by trapping with aniline as demonstrated by Higuchi *et al.* (9). In aniline buffer systems, the reactions shown in Scheme VII are envisioned.

In addition to hydrolysis by water as expressed by k_{hyd} (see Eq. 1), methylprednisolone 21-succinate may also undergo nucleophilic attack by aniline in aniline buffers, so the rate of methylprednisolone (MP) formation is expressed by:

$$\frac{d(MP)}{dt} = k_{hyd}[Ester] + k_{direct}[Free\ Aniline][Ester] \quad \text{(Eq. 3)}$$

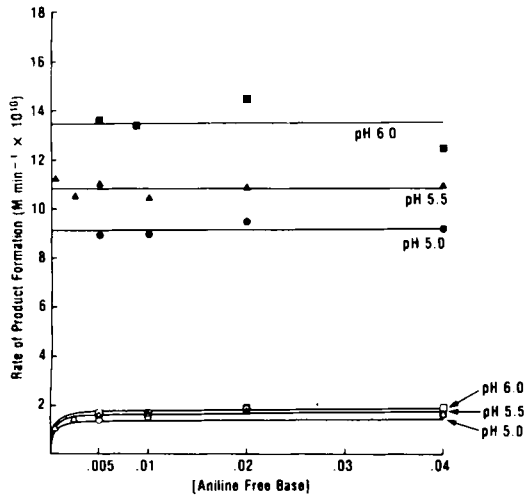


Figure 2—*Plots of the initial rates of methylprednisolone (closed symbols) and succinanilic acid (open symbols) formation* versus *aniline concentration at pH 5.0, 5.5, and 6.0: ester concentration = 5×10^{-4} M; temperature = 25°C.*

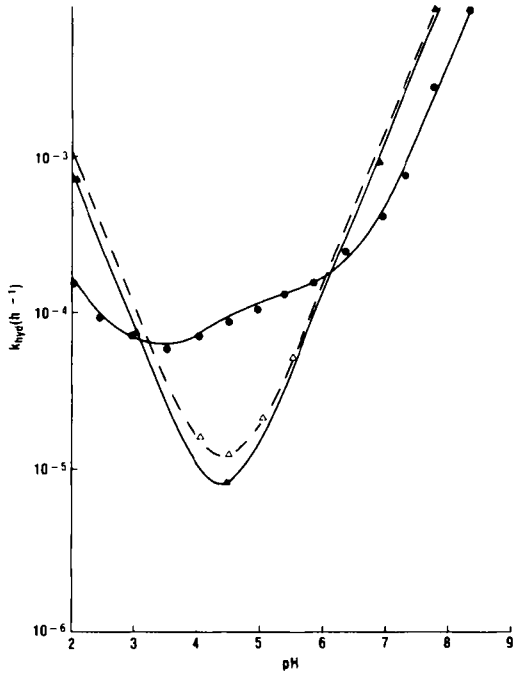


Figure 3—*pH- Hydrolysis rate profiles for methyprednisolone 21-succinate and 21-acetate at 25°C. Key: (●) 21-succinate, μ = 0.01; (▲) 21-acetate, μ = 0.01; (△) 21-acetate, μ = 0.5.*

Succinanilic acid, the product of the reaction of aniline with any succinic anhydride formed as a result of the intramolecular attack by the succinate carboxyl group on the ester linkage, could also be formed from direct attack of aniline on the ester. The rate of formation of succinanilic acid is therefore given by:

$$\frac{d[Anilide]}{dt} = k_{direct}[Free\ Aniline][Ester] + k_6[Free\ Aniline][Anhydride] - k_{-6}[Anhydride] \quad \text{(Eq. 4)}$$

The formation of succinic anhydride, the intermediate of interest, can be expressed by:

$$\frac{d[Anhydride]}{dt} = k_{nuc} \cdot f_i[Ester] - k_6[Free\ Aniline][Anhydride] - k_{-5}[Anhydride] + k_{-6}[Anilide] \quad \text{(Eq. 5)}$$

The rate of direct formation of succinic anhydride from succinic acid was calculated from literature data (9) to be very small relative to the other terms in Eq. 5 and was therefore disregarded. This was also verified experimentally by monitoring anilide formation in a solution containing 5×10^{-6} M succinic acid (equivalent to the amount formed from 1% hydrolysis of a 5×10^{-4} M solution of methylprednisolone 21-succinate) in 0.01 M aniline buffer at pH 5.5. No succinanilic acid could be detected over a 47-h period.

Assuming that the concentration of succinic anhydride would rapidly reach a low steady-state level, i.e., $d[Anhydride]/dt = 0$, this concentration can be expressed as follows:

$$[Anhydride] = \frac{k_{nuc} \cdot f_i \cdot [Ester] + k_{-6}[Anilide]}{k_6[Free\ Aniline] + k_{-5}} \quad \text{(Eq. 6)}$$

Table II—Methylprednisolone 21-Acetate Hydrolysis Rate Constants[a]

Rate Constant	Ionic Strength	Value	95% Confidence Limit
k_{H^+}	0.5 M	0.114 L/mol·h	±38%
k_{H_2O}	0.5 M	4.8×10^{-6} h^{-1}	±66%
k_{OH^-}	0.5 M	1.65×10^4 L/mol·h	±16%
k_{ga} (Acetate)	0.5 M	2.7×10^{-5} L/mol·h	±13%
k_{gb} (Acetate)	0.5 M	1.17×10^{-4} L/mol·h	±8%
k_{H^+}	0.01 M	8.5×10^{-2} L/mol·h	±82%
k_{H_2O}	0.01 M	1.9×10^{-6} h^{-1}	± >100%
k_{OH^-}	0.01 M	1.45×10^4 L/mol·h	±80%

[a] See Eq. 8.

Table III—Migration Rate Constants for Methylprednisolone Esters at 25°C [a]

Ester	Reaction	Rate Constant	Value	95% Confidence Limit
Succinate	21 → 17	k_{OH^-}	3.5×10^3 L/mol·h	±27%
		k_{cat}	7.3×10^{-4} h^{-1}	±26%
	17 → 21	k_{OH^-}	1.74×10^4 L/mol·h	±34%
		k_{cat}	3.0×10^{-3} h^{-1}	±17%
Acetate	21 → 17	k_{H^+}	–	–
		k_{H_2O}	3.9×10^{-6} h^{-1}	±22%
		k_{OH^-}	2.5×10^4 L/mol·h	±10%
		k_{ga}	1.1×10^{-5} L/mol·h	±33%
		k_{gb}	1.0×10^{-4} L/mol·h	±10%
	17 → 21	k_{H^+}	5.8×10^{-3} L/mol·h	±19%
		k_{H_2O}	3.1×10^{-5} L/h	±12%
		k_{OH^-}	3.1×10^5 L/mol·h	±13%
		k_{ga}	9.7×10^{-5} L/mol·h	±78%
		k_{gb}	1.4×10^{-3} L/mol·h	±15%

[a] See Eqs. 10 and 11.

Substituting the anhydride concentration from Eq. 6 into Eq. 4 gives, for the rate of anilide formation:

$$\frac{d[\text{Anilide}]}{dt} = k_{\text{direct}}[\text{Free Aniline}][\text{Ester}] + \frac{k_6[\text{Free Aniline}](k_{\text{nuc}}f_i[\text{Ester}] + k_{-6}[\text{Anilide}])}{k_6[\text{Free Aniline}] + k_{-5}} - k_{-6}[\text{Anilide}] \quad \text{(Eq. 7)}$$

Note in Eq. 7 that when k_6 [Free Aniline] is large relative to k_{-5}, the succinic anhydride hydrolysis rate constant, a plot of the initial rate of anilide formation *versus* free aniline concentration at constant ester concentration should be linear, with a slope equal to k_{direct} [Ester] and intercept of $k_{\text{nuc}}f_i$ [Ester]. Higuchi *et al.* (9) give rate constants for k_6 and k_{-5} of 10 L/mol·s and 2.5×10^{-3} s^{-1}, respectively. Therefore, k_6 [Free Aniline] $\gg k_{-5}$ (by 20-fold) when the free aniline concentration is ≥0.005 M. Similarly, plots of the initial rate of methylprednisolone formation *versus* free aniline (Eq. 3) should be linear with the same slope (in the absence of general catalysis by aniline), but with an intercept equal to the total hydrolysis rate in pure water, k_{hyd} [Ester].

Plots of the initial rates of methylprednisolone and succinanilic acid formation *versus* aniline concentration are shown in Fig. 2 at three pH values. The solid lines in Fig. 2 are the theoretical plots representing the rate constants in Eqs. 3 and 7 as reported in Table I.

The data clearly show that nucleophilic catalysis of the hydrolysis does occur as indicated by the level of significance of k_{nuc} in Table I and the positive intercept for the rate of anilide formation in Fig. 2. From the ratio of intercepts (anilide formation/methylprednisolone formation rates) one can obtain the percentage of the overall reaction which is catalyzed *via* the nucleophilic mechanism. These ratios are 0.12, 0.16, and 0.14 at pH 5.0, 5.5, and 6.0, respectively. Since k_{cat} accounts for 85, 88, and 76% of the total hydrolysis at these pH values, based on Eq. 1 and the rate constants in Table I, general catalysis must account for 62–73% of the total. Therefore, both general catalysis and nucleophilic catalysis occur, but the nucleophilic mechanism is a minor component accounting for only 15–20% of the overall catalysis.

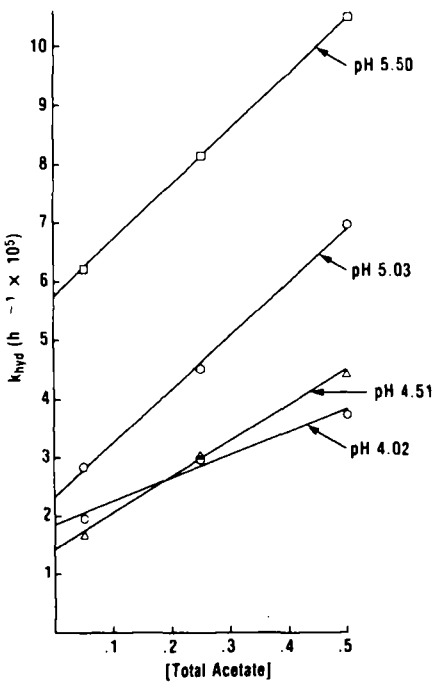


Figure 4—*Hydrolysis rate constant for methylprednisolone 21-acetate* versus *acetate buffer concentration at varying pH.*

Hydrolysis and Acyl Migration of Methylprednisolone 21-Acetate in Water and in Acetate Buffers—*Hydrolysis*—The changeover from nucleophilic to general acid-base catalysis of ester hydrolysis by a catalyst acting intermolecularly occurs when the pK_a of the leaving group is greater than that of the catalyst by ~2.5 units (10). Since the pK_a of the steroid side chain leaving group is ~11 due to possible enolization (11), while that of acetic acid is 4.8, it is assumed that acetate buffer is a general acid-base catalyst in the degradation of methylprednisolone 21-acetate.

The hydrolysis of methylprednisolone 21-acetate can be described by the following rate equation:

$$k_{hyd} = k_{H^+}[H^+] + k_{H_2O} + k_{OH^-}[OH^-] + (f_{A^-}k_{gb} + f_{HA}k_{ga})[Ac_T] \quad \text{(Eq. 8)}$$

where the final term represents the expected influence of the total acetate buffer [Ac_T] acting as either a general base catalyst ($k_{gb} \cdot f_{A^-}$) or general acid catalyst ($k_{ga} \cdot f_{HA}$). The k_{hyd} is plotted *versus* pH in Fig. 3 for systems at ionic strengths of 0.01 and 0.5 and buffer concentrations approaching infinite dilution. Superimposed on these plots is the logarithm of the hydrolysis rate constant *versus* pH for methylprednisolone 21-succinate for comparative purposes.

Figure 4 shows the acetate ester hydrolysis rate constant, k_{hyd}, at various pH values and total acetate buffer concentrations. The rate constants k_{ga} and k_{gb} were determined by rearranging Eq. 8 and plotting the left-hand side of Eq. 9 *versus* the fraction of acetate ion, f_{A^-}:

$$\frac{k_{hyd} - (k_{H^+}[H^+] + k_{H_2O} + k_{OH^-}[OH^-])}{Ac_T} = k_{ga} + (k_{gb} - k_{ga})f_{A^-} \quad \text{(Eq. 9)}$$

This plot is shown in Fig. 5. Values of the rate constants for the hydrolysis of methylprednisolone 21-acetate are shown in Table II. Kinetically, acetate buffer acts primarily as a general base catalyst in this reaction, with $k_{gb} > k_{ga}$ by about fivefold. Whether or not this is true general base catalysis or its kinetic equivalent, general acid-specific base catalysis, cannot be ascertained from the kinetic data.

21 ⇄ 17 Acyl Migration—A previous study of acyl migration in methylprednisolone succinate fit the kinetic data to an equation of the following form (6):

$$k_{mig}^{su} = k_{OH^-}^{mig}[OH^-]f_i + k_{cat}^{mig} \cdot f_i \quad \text{(Eq. 10)}$$

The 21 ⇄ 17 migration of acetate in methylprednisolone acetate esters subjected to acetate buffer can be described by the following rate equation:

$$k_{mig}^{ace} = k_{H^+}^{mig}[H^+] + k_{H_2O}^{mig} + k_{OH^-}^{mig}[OH^-] + (k_{ga}^{mig} \cdot f_u + k_{gb}^{mig} \cdot f_i)[Ac_T] \quad \text{(Eq. 11)}$$

The last term in Eq. 11 again expresses the possible role of acetate [Ac_T] acting as an intermolecular general acid, $k_{ga}^{mig} \cdot f_u$ or general base catalyst, $k_{gb}^{mig} \cdot f_i$. Plots of the logarithm of the forward and reverse migration rate constants *versus*

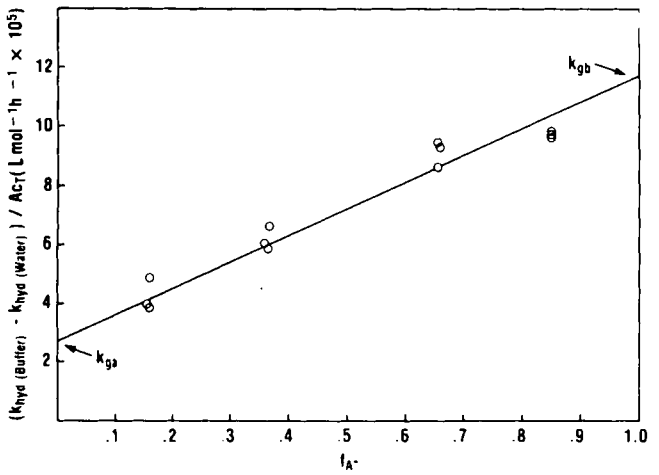


Figure 5—*Plot of* $(k_{hyd\ (buffer)} - k_{hyd\ (water)})/Ac_T$ versus *the fraction of acetate ionized for methylprednisolone 21-acetate (see Eq. 9).*

pH are shown in Fig. 6 for both methylprednisolone succinate and acetate esters in buffers approaching infinite dilution.

The influence of acetate buffer acting intermolecularly as a general acid-base catalyst in the migration (21 ⇄ 17) of acetate in methylprednisolone acetate was assessed by plotting the quantity: $[k_{mig}^{acc}$ (in buffer) − k_{acc} (no buffer)]/Ac_T *versus* the fraction of acetate buffer, Ac_T, in ionized form (f_{A^-}) as done previously for hydrolysis according to Eq. 9. These plots were linear for both the 21 → 17 and 17 → 21 reactions. Values for k_{ga}^{mig} and k_{gb}^{mig} obtained from these plots and all other migration rate constants for both the acetate and succinate esters are listed in Table III.

As observed in the hydrolysis, intermolecular catalysis of migration by acetate buffer appears to involve the acetate acting primarily as a general base in both directions. Mechanistically, however, this is most unlikely.

Calculation of the "Effective Molarity" of the Succinate Carboxyl Group Acting as a General Acid-Base Catalyst— It is a common practice to assess the effect of intramolecularity by comparing the catalytic rate constant in an intramolecular reaction with that of an analogous intermolecular one (12). While the hydrolysis and acyl migration reactions in methylprednisolone acetate are similar to those in the succinate, a correction should be made for the differences in intrinsic reactivity of the two esters. This can be done by dividing the catalytic constants k_{gen} and k_{gb} by k_{OH^-}. (Refer to Tables I and II for the values of these constants.) Thus, for the 21-succinate hydrolysis k_{gen}/k_{OH^-} is 3.0×10^{-8} M and for the 21-acetate hydrolysis k_{gb}/k_{OH^-} is 7.1×10^{-9}M. The ratio of these quantities is the effective concentration of the carboxylate anion in the hydrolysis of the hemisuccinate ester, 4.2 M.

The k_{H_2O} of 6×10^{-5} h^{-1} for the un-ionized succinate ester hydrolysis (Table I) is an order of magnitude greater than that of the acetate ester hydrolysis of 4.8×10^{-6} h^{-1} (Table II). This is evidence for intramolecular catalysis in k_{H_2O} by the terminal carboxyl group, but an effective molarity of the terminal carboxylic acid was not calculated for this reaction.

Following a procedure identical to that described above, the effective molarity of the terminal carboxylate anion of the hemisuccinate ester in catalyzing acyl migration can also be calculated. For the 21 → 17 ester migration (see Table III) k_{gb}/k_{OH^-} for the acetate ester is 4×10^{-9} M and k_{cat}/k_{OH^-} for the succinate ester is 2.1×10^{-7} M, for an effective molarity of ~50 M. In the 17 → 21 ester migration k_{gb}/k_{OH^-} for the acetate ester is 4.7×10^{-9} M and k_{cat}/k_{OH^-} for the succinate ester is 1.7×10^{-7} M, for an effective molarity of ~40 M. These numbers are the same without experimental error, but are significantly greater than the effective concentration of the terminal carboxylate anion of the succinate ester in catalyzing hydrolysis.

DISCUSSION

Comparison of the pH-Rate Profiles of Methylprednisolone Acetate and Succinate Esters—The primary evidence that hydrolysis and acyl migration in methylprednisolone succinate esters are catalyzed intramolecularly by the terminal succinate carboxyl group comes from the observation that the pH rate profiles for these reactions deviate from classical behavior. This is best illustrated by comparisons of the pH-hydrolysis rate and pH-acyl migration profiles of methylprednisolone succinate esters with those of methylprednisolone acetate esters, which exhibit the expected classical behavior (Figs. 3 and 6).

The pH-rate profile obtained for hydrolysis of the acetate ester in Fig. 3 has the typical U-shape generally observed for aliphatic ester hydrolysis in the absence of catalysis (2) and can be described by Eq. 8 (neglecting the acetate buffer term). In basic solution, the hydrolysis rate of methylprednisolone 21-succinate *versus* pH is parallel to that of methylprednisolone 21-acetate. This region represents hydroxide ion attack on the ionized succinate ester or on the neutral acetate ester. In acidic solutions (pH <2) it appears that the two curves again become parallel. This region represents H^+-catalyzed attack of water on the neutral esters. From a comparison of k_{H^+} and k_{OH^-} values in Tables I and II, it is estimated that methylprednisolone 21-succinate is more stable than the 21-acetate in acid by nearly an order of magnitude and in base by roughly fourfold. The lower reactivity of the 21-succinate is assumed to be due largely to a steric effect. Electrostatic repulsion of OH^- attack by the negatively charged succinate carboxylate anion does not appear to be a significant factor in the basic hydrolysis of methylprednisolone 21-succinate, since steric effects alone can account for its decreased reactivity. Steric effects are assumed to be equal in acidic and basic ester hydrolysis, as originally proposed by Ingold (13).

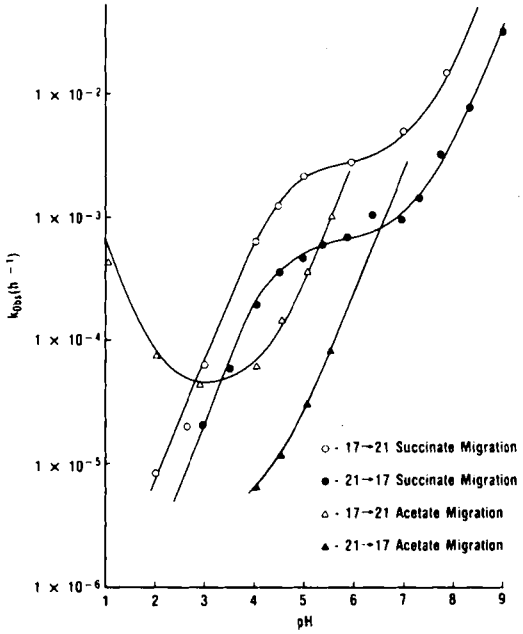


Figure 6—*pH-17 → 21 and pH-21 → 17 migration rate profiles for methyprednisolone acetate and succinate esters at 25°C.*

Between pH ~3-7 the pH-methylprednisolone 21-succinate hydrolysis rate profile deviates from the classical U-shape exhibited by the acetate ester profile. This behavior suggests intramolecular catalysis by the terminal succinate carboxyl group, as discussed previously.

From an examination of the pH-21 ⇄ 17 acyl migration rate profiles in Fig. 6 or from a comparison of k_{OH^-} values in Table III, it is evident that ester migration occurs faster in basic solution in methylprednisolone acetate esters than in the succinate esters. This decreased reactivity at high pH is again attributed to the greater steric bulk of the succinate esters. The pH-rate profiles for acetate ester migration are linear down to a pH of ~4-5, while the succinate ester pH-migration rate profiles deviate from linearity at ~pH 7. Again this behavior is rationalized as intramolecular catalysis of migration by the terminal succinate carboxyl group.

Mechanism of Intramolecular Catalysis of the Hydrolysis of the 21-Succinate— Studies on the solvolysis of 21-hemiesters of hydrocortisone (4, 5) led Garrett to conclude that intramolecular catalysis by the terminal succinate carboxyl group was nucleophilic. This mechanism has been observed in other systems and was further supported by the fact that general acid-base catalysis by acetate buffer was not observed in the reaction. However, in light of the recent observation that 21 ⇄ 17 acyl migration in methylprednisolone 21-succinate is also catalyzed intramolecularly, coupled with the realization that nucleophilic catalysis is not a plausible mechanism for catalysis of acyl migration, it seemed appropriate to reexamine the evidence for the nucleophilic mechanism in the hydrolysis and attempt to establish or refute its existence by trapping any succinic anhydride formed as a result of nucleophilic attack of the terminal carboxylate anion on the ester.

The anilide trapping experiment clearly demonstrates that, while nucleophilic catalysis does occur, the predominant mechanism is general acid-base catalysis. It is reasonable that the mechanism of intramolecular catalysis of methylprednisolone succinate should lie on the borderline between general acid base and nucleophilic but favoring general acid-base catalysis from a

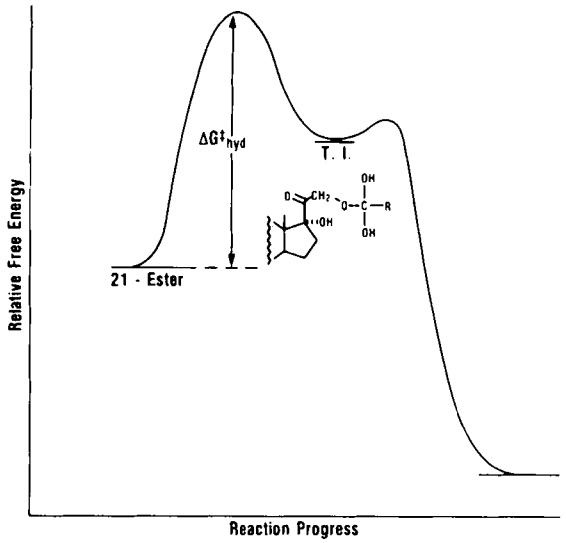


Figure 7—*Relative free energy* versus *reaction progress diagram for methylprednisolone 21-ester hydrolysis.*

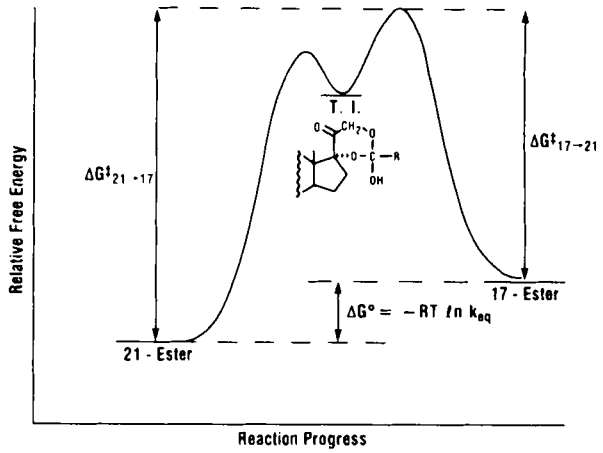


Figure 8—*Relative free energy* versus *reaction progress diagram for 21 → 17 acyl migration in methylprednisolone esters.*

consideration of the catalyst and leaving group pK_a values. Fersht and Kirby (10) have shown that in an exocyclic displacement (that is, when the leaving group does not stay attached to the intermediate anhydride formed on nucleophilic attack by a catalyst), the borderline between intramolecular general base and nucleophilic catalysis lies in the region where the pK_a of the leaving group is 5.5–6.5 units more basic than the attacking nucleophile. Due to possible enolization, the pK_a of the C-17 side chain of methylprednisolone is near 11 (11), while the terminal succinate carboxylic acid is ~4.5, for a pK_a difference of 6.5.

If the *intramolecular* catalytic mechanism is largely general acid-base, then one would expect the reaction to also be catalyzed *intermolecularly* by general acids or bases. Yet, general acid base catalysis by acetate was not seen by Garrett in his studies of the hydrolysis of hydrocortisone succinate (4,5). This is of general importance, since such information is often the primary support for or against the intramolecular nucleophilic mechanism.

General catalysis was not observed in Garrett's studies, since the reaction is already catalyzed intramolecularly. If the magnitude of intramolecular catalysis (as determined by the effective molarity of the carboxylate anion) in hydrocortisone succinate is equivalent to that which would result intermolecularly from a 4 M acetate buffer, then the increase in rate brought about by a 0.3 M solution of acetate (the highest concentration used in Garrett's study) would be only ~7% and may well be overlooked. Thus, the apparent absence of intermolecular general acid-base catalysis in a reaction that is already intramolecularly catalyzed may not be sufficient evidence to rule out a general acid-base mechanism in the intramolecular reaction.

It is more appropriate to test the presence or absence of intermolecular general acid base catalysis in a reaction that is similar to the intramolecularly catalyzed reaction but is not already catalyzed intramolecularly. The results of this study showing that acetate buffer catalyzes the hydrolysis of methylprednisolone 21-acetate make more plausible the conclusion that intramolecular general acid-base catalysis occurs in the hydrolysis of methylprednisolone 21-succinate.

Intramolecular Catalysis Favors Acyl Migration Over Hydrolysis—As shown in Fig. 1 and pointed out earlier, hydrolysis of methylprednisolone 21-succinate is slightly faster than 21 → 17 acyl migration above pH 7.4, while acyl migration dominates between pH 3.6 and 7.4. This dramatic reversal in relative reaction rates is due to the fact that intramolecular catalysis of acyl migration is 4–6 times greater than that of hydrolysis. These differences in catalytic efficiency can be rationalized from a consideration of differences in the transition states of the two reactions.

Referring to the hypothetical diagrams in Figs. 7 and 8 of relative free energy *versus* reaction progress, it is assumed that the transition state for 21-ester hydrolysis (Fig. 7) is a very early transition state, occurring prior to the formation of the tetrahedral intermediate, since OH^- is a much poorer leaving group than the 21-alcoholate. However, the transition state for the 21 → 17 migration (Fig. 8) is quite likely the breakdown of the tetrahedral intermediate. The 17-ester is thermodynamically less favored and, applying Hammond's postulate (14), the transition state should more closely resemble the less stable 17-ester than the 21-ester. In further support of the conclusion that the transition state in the migration occurs between the tetrahedral intermediate and the 17-ester are data showing that the 17,21-hemiorthoester of betamethasone benzoate, which has been isolated, breaks down in acid to the 21-ester (15). Differences in the magnitude of intramolecular catalysis of hydrolysis and 21 → 17 acyl migration are not surprising in view of the fact that the transition states are probably quite different.

Whereas *intramolecular* catalysis by the terminal succinate carboxyl group of 21 → 17 acyl migration is 4–6 times greater than intramolecular catalysis of hydrolysis, this does not appear to be the case in the analogous *intermolecularly* acetate buffer catalyzed reactions occurring in methylprednisolone 21-acetate. Comparing the ratios k_{gb}/k_{OH^-} (see Tables II and III), the magnitude of intermolecular catalysis by acetate buffer of the hydrolysis of methylprednisolone 21-acetate is nearly equal to that of 21 → 17 migration.

It is suggested that intermolecular catalysis of migration by acetate is rendered less effective due to steric (entropic) factors that come into play in the intermolecular reaction but not in the intramolecular mechanism. Using molecular models, it appears that the conformational freedom of the succinate moiety in the transition state is about the same for both hydrolysis and migration. An intermolecular general acid or base appears, however, to be conformationally more restricted in the transition state during migration as compared to hydrolysis.

Ambiguity in the Assignment of the Site of Catalysis—The acetate buffer-catalyzed 21 ⇄ 17 acyl migration reaction in methylprednisolone 21-acetate provides an excellent demonstration of what Jencks refers to as the ambiguity of the assignment of the site of catalysis (16). Applying the principle of microscopic reversibility (17), it is clear that the same transition state is involved in both the 21 → 17 and 17 → 21 migrations. This means mechanistically that if the reaction is general base catalyzed in one direction, it must be general acid-specific base catalyzed in the other. Yet, *kinetically*, both directions appear to be primarily general base catalyzed (Table III). By convention, both reactions are referred to as general base catalyzed even though mechanistically this cannot be. None of the data generated by this study specifically support one general catalytic mechanism over its kinetic equivalent. Indeed, the same ambiguity exists in much of the published literature wherein general base or general acid catalytic mechanisms have been postulated.

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Degradation Kinetics in Aqueous Solution of Cefotaxime Sodium, a Third-Generation Cephalosporin

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Abstract □ The degradation kinetics of a 3-acetoxymethylcephalosporin, cefotaxime sodium salt, in aqueous solution investigated by HPLC under different conditions (pH, ionic strength, temperature) and using different buffers. The scheme of degradation involves a cleavage of the β-lactam nucleus and the deacetylation of the side chain. In highly acidic medium, the deacetylated derivative is easily converted to the lactone. The degradation rate constants were calculated at three pH values (1.9, 4.0, and 9.0) by measuring the residual cephalosporin and the main decomposition products. The degradation pathway is both supported by the results of a primary salt effect and by the agreement between the theoretical pH-rate profile and the experimental values. In the pH range from 3.0 to 7.0, the main process is a slow water-catalyzed or spontaneous cleavage of the β-lactam nucleus with intramolecular participation of the side chain amido fraction in the 7-position. In alkaline or strongly acidic medium, the hydrolysis is a base- or acid-catalyzed reaction. Of the buffer systems investigated, carbonate buffer (pH 8.5) and borate buffers (pH 9.5 and 10.0) are found to increase the degradation rates, while acetate buffer decreases the degradation rates. The apparent activation energies determined at different pH values are compatible with a solvolysis mechanism and similar to those previously given in the literature for other cephalosporins. Cefotaxime in aqueous solution is slightly less stable than the main cephalosporin derivatives, despite its high resistance to the β-lactamases and its remarkable biological activity.

Keyphrases □ 3-Acetoxymethylcephalosporins—stability in aqueous solutions, HPLC, pH effect, primary salt effect, buffer effect, temperature effect □ Cefotaxime—kinetics and mechanism of degradation, pH-rate profile □ Antibiotics—cefotaxime, stability, degradation profile, kinetics

Systematic studies on the degradation of cephalosporin derivatives are of interest for several reasons: (*a*) a correlation between degradation and antibiotic activity has been shown in first- and second-generation cephalosporins (1), (*b*) some degradation products may be involved in allergic reaction (2), and (*c*) the stability of the compounds has to be known for the synthesis of derivatives (3) and the formulation of drugs. The kinetics of first- and second-generation cephalosporins have been reported in a few instances (1, 3–6). These studies concern a quantitative analysis of the antibiotic itself and sometimes the kinetics of the major degradation product (1, 3). In this report, a systematic kinetic study of a recently commercially available third-generation cephalosporin, cefotaxime sodium salt[1], has been carried out. The quantitation of this cephalosporin and its major decomposition products allowed the proposal of a degradation pathway.

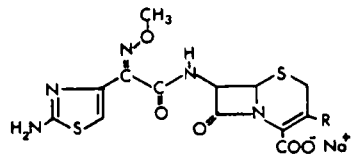
BACKGROUND

Cefotaxime sodium (I) is sodium [6(*R*)-[6α,7β(*Z*)]]-3-[(acetyloxy)-methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate]. The possible *in vitro* degradation products are deacetylcefotaxime (II), deacetoxycefotaxime (III), deacetylcefotaxime lactone (IV), thiazoximic acid (V), and 7-aminocephalosporanic acid (VI). The *anti* isomer which could be expected as a potential degradation product of I has been shown to be formed only in nonaqueous medium (7) and has not been taken into consideration in this study.

Cefotaxime sodium (I) is an original cephalosporin derivative with a 2-amino-4-thiazolyl side chain and an α-methoximino group in the *syn* position. The former is probably responsible for the very great affinity for transpeptidase, involved in the construction of the bacterial wall, and of the great activity against Gram-negative bacilli. The latter is probably responsible for the stability of the drug against most β-lactamases (8, 9). An exhaustive review of the bacteriological and clinical properties of I has been presented (10).

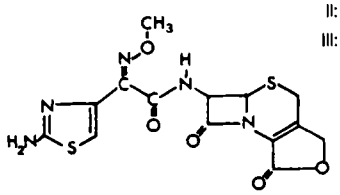
EXPERIMENTAL

Materials—Compounds I–VI were used as received[2]. UV, IR, and NMR spectra were used to confirm the structure of these compounds. All other chemicals were analytical reagent grade.

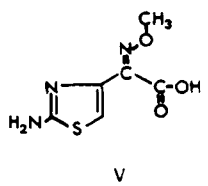


R

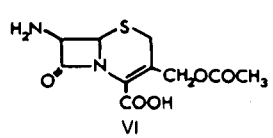
I: R=CH_2-O-CO-CH_3
II: R=CH_2-OH
III: R=CH_3



IV



V



VI

[1] Trade names: Claforan, Tarivid, Zariviz, and Primafen.

[2] Gifts from Roussel UCLAF Laboratories.

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