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Influence of Premicellar and Micellar Association on the Reactivity of Methylprednisolone 21-Hemiesters in Aqueous Solution

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Abstract □ Self-association of drug molecules at formulation concentrations can have a major impact on formulation properties. In this study a homologous series of methylprednisolone 21-hemiesters were found to undergo self-association in aqueous solution. The effect of aggregate formation on the solution degradation of these compounds was examined. To determine the nature and extent of association of these steroidal esters, partition coefficients between butyronitrile and aqueous buffer (pH 8.5) were measured as a function of ester concentration. The partitioning data were found to be consistent with dimer formation at low concentration followed by true micelle formation at higher concentration. Chain length increases favored micelle formation, but appeared to have little effect on dimerization. The first-order rate constants for ester hydrolysis and 21 → 17 acyl migration in aqueous buffer (pH 8.5) were also found to be dependent on ester concentration. The kinetic data are consistent with a model which assumes stabilization by both dimer and micelle formation, the limiting factor at high concentration being the reactivity of the ester in the micelles. The degree of stabilization due to self-association was found to increase with chain length.

Keyphrases □ Methylprednisolone—synthesis of 21-hemiester homologues, influence of premicellar and micellar association on reactivity □ Steroids—methylprednisolone, synthesis of 21-hemiester homologues, influence of premicellar and micellar association on reactivity □ Association, micellar—influence on the reactivity of methylprednisolone 21-hemiesters □ Association, premicellar—influence on the reactivity of methylprednisolone 21-hemiesters

Self-association of hydrophobic drug molecules in aqueous solution can have a profound effect on formulation properties due to the reduced effective concentration of drug at high total concentration. Specifically, molecular aggregation may result in higher drug solubility, increased or decreased solution stability, or transient masking of local biological effects.

While there is voluminous literature on the effect of micelle-forming additives on the chemical reactivity of various substrates, very few cases have been reported in which a labile substrate itself forms molecular aggregates resulting in self-stabilization. The few studies which do exist suggest that reactivity can be significantly altered either favorably or unfavorably by substrate self-aggregation into micelles (1, 2). Premicellar aggregation has also been found to dramatically alter reactivity (3–5).

Steroidal molecules, particularly bile salts, are known to undergo self-association in aqueous solution to form aggregates varying in size from dimers to much larger oligomers (6–8). Self-association has also been observed for the corticosteroid methylprednisolone 21-phosphate (9). In this case a marked acceleration in reactivity in more concentrated solutions was attributed to micelle formation.

A recent study showed that methylprednisolone 21-succinate[1] decomposes initially in aqueous solution *via* two parallel pathways (10). In addition to the well-known ester hydrolysis reaction, acyl migration from the 21- to the 17-OH occurs at a rate comparable to hydrolysis (Scheme I).

Since it was suspected that methylprednisolone 21-succinate may self-associate at formulation concentrations, a study was initiated to determine: (*a*) the nature and extent of self-association, (*b*) the effect of aggregation on the solution kinetics, and (*c*) the effect of molecular modification (increasing the hydrophobicity through increases in hemiester chain length) on both the aggregation and kinetics. To determine unambiguously the nature and extent of self-association, a partitioning method was developed enabling calculation of the monomer concentration as a function of total concentration. The initial rates of ester hydrolysis and acyl migration as a function of concentration were then combined with the partitioning data to elucidate the relative reactivities of monomeric and aggregated species.

EXPERIMENTAL

Materials—All reagents and chemicals were either analytical reagent grade or known to be of high purity. Methylprednisolone 21-hemisucci-

[1] SOLU-MEDROL (Upjohn brand of methylprednisolone sodium succinate).

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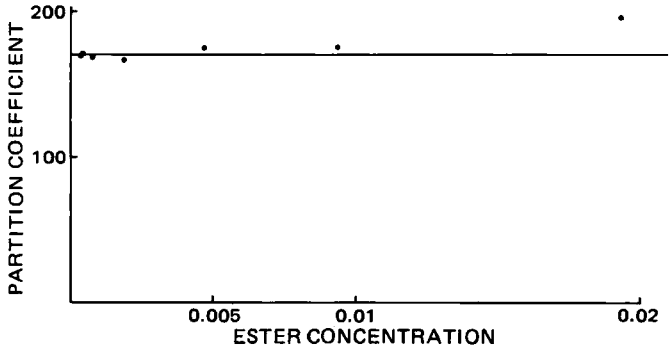


Figure 1—*Butyronitrile–0.01* N *HCl partition coefficient* versus *concentration of methylprednisolone 21-succinate in the butyronitrile phase.*

nate[2] was used as supplied without further purification. Methylprednisolone[2] for use as a high-performance liquid chromatography (HPLC) reference standard was recrystallized from tetrahydrofuran. The remaining methylprednisolone esters used in this study were synthesized as described.

Synthesis—*6α-Methylprednisolone 21-Hemiadipate*—A mixture of 5 g of 11β,17α-dihydroxy-21-iodo-6α-methylpregna-1,4-diene-3,20-dione[2], 14.6 g of adipic acid, and 34.8 ml of *N,N*-diisopropylethylamine in 45 ml of dimethylformamide and 20 ml of acetone was allowed to stand at 25° for 1 hr. The mixture was extracted with ethyl acetate (250 ml) and washed with 0.08 *M* citric acid. The organic phase was rapidly extracted (pH 10), and the resulting aqueous phase was readjusted to pH 5 and then extracted with ethyl acetate. The solvent was evaporated under reduced pressure, and the resulting solid was recrystallized twice from ethyl acetate–hexane to give an analytical specimen, mp 164.3–165.6; NMR[3a,b]: δ 7.2–7.4 (d, 1, C_1—H), 6.0–6.2 (d, 1, C_2—H), 5.9 (s, 1, C_4—H), 4.6–5.3 (m, 2, C_{21}—H_2), 4.4 (broad, 1, C_{11}—H).

Anal.—Calc. for $C_{28}H_{38}O_8$: C, 66.92; H, 7.62. Found: C, 67.01; H, 7.91.

6α-Methylprednisolone 21-Hemisuberate—The ester was prepared in the aforementioned manner using 5 g of 11β,17α-dihydroxy-21-iodo-6α-methylpregna-1,4-diene-3,20-dione, 17.4 g of octanedioic acid, 34.8 ml of *N,N*-diisopropylethylamine, 67 ml of dimethylformamide, and 20 ml of acetone to give, after recrystallization from ethyl acetate–hexane, an analytical specimen, mp 192.1–195.4; NMR[3a,b]: δ 7.2–7.4 (d, 1, C_1—H), 6.0–6.2 (d, 1, C_2—H), 5.9 (s, 1, C_4—H), 4.6–5.3 (m, 2, C_{21}—H_2), 4.4 (broad, 1, C_{11}—H).

Anal.—Calc. for $C_{30}H_{24}O_8$: C, 67.91; H, 7.98. Found: C, 67.58; H, 8.33.

6α-Methylprednisolone 17-Hemisuccinate—The synthesis and structure elucidation were reported previously (10).

6α-Methylprednisolone 17-Hemisuberate—Methylprednisolone 21-hemisuberate (4 g) was dissolved in 400 ml of water by the slow addition of 1 *N* NaOH. The pH of the final solution was maintained ≤9.5 over several hours and the 17-ester formation was monitored by HPLC. The concentration of 17-ester plateaued at ~9–10% of the total solution concentration. The reaction was stopped by acidification and products were isolated by extraction with ethyl acetate. The solvent was removed under reduced pressure and the remaining solid purified by HPLC: (*a*) reverse-phase[4] using acetonitrile–water (9:11) containing 0.05 *M* acetic acid at a flow rate of 18 ml/min[5] and (*b*) silica gel[6] using butylchloride–ethyl acetate (1:1) containing 1% acetic acid at 18 ml/min[5]. The solvent was removed to give a white amorphous solid which was reprecipitated from ethyl acetate–hexane. No methylprednisolone nor 21-suberate were detected by HPLC. UV[7]: λ_{max} = 244 nm, $\epsilon = 1.48 \times 10^4$ (methylprednisolone 17-succinate λ_{max} = 244, $\epsilon = 1.50 \times 10^4$). NMR[3] (acetone-$d_6$): δ 7.3–7.5 (d, 1, C_1—H), 6.1–6.3 (d, 1, C_2—H), 5.90–5.95 (s, 1, C_4—H), 4.55 (broad, 1, C_{11}—H), 4.2–4.25 (s, 2, C_{21}—H_2).

Sample Preparation—All sample solutions for both kinetic and partitioning studies were prepared in 0.1 *M* boric acid buffer adjusted to pH 8.47 ± 0.01 with 50% sodium hydroxide. The ionic strength of the buffer was 0.5 *M* (potassium chloride).

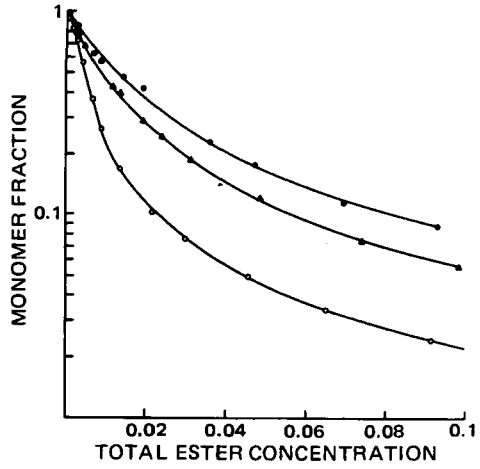


Figure 2—*Semilog plots of monomer fraction [PC/PC(O)]* versus *total concentration in ester in aqueous buffer at pH 8.47 and 25°. Key: (●) 21-succinate; (▲) 21-adipate; and (○) 21-suberate.*

Stock solutions containing ~0.1 *M* 21-ester were prepared by weighing an appropriate amount in a volumetric container and adding buffer containing the amount of excess sodium hydroxide required to neutralize the carboxylic acid. Sonication and vigorous mixing were required to rapidly dissolve the sample. The stock solutions were frozen (−20°) for later use. Aliquots of these stock solutions were diluted and pH was adjusted to 8.47 ± 0.01 if necessary. Temperature of the diluted samples was maintained at 25.0° during all studies.

Partition Coefficient Determinations—After the appropriate dilution of one of the aforementioned stock solutions, a 1–5 ml aliquot was transferred to a test tube containing 1–5 ml of butyronitrile. The biphasic mixture was brought to 25°, vortexed vigorously, centrifuged, and allowed to stand in the 25° water bath for 15–30 min. Aliquots from each layer were removed. The butyronitrile was evaporated under a nitrogen stream, and the residues from both the aqueous and butyronitrile phases were diluted with an acidified acetonitrile–water mixture. HPLC analysis was carried out as described below using standard solutions of the corresponding 21-ester in acetonitrile–water (1:3) at pH 3–3.2.

Kinetic Studies—The initial rates of product formation (up to 3%) were monitored by HPLC as a function of initial 21-ester concentration. Standards containing methylprednisolone and the corresponding 17-ester (when available) at concentrations of $\sim 1 \times 10^{-6}$–1×10^{-4} *M* were prepared in acetonitrile–water (1:3) adjusted to pH 3.2.

At suitable time intervals precise aliquots of samples prepared by

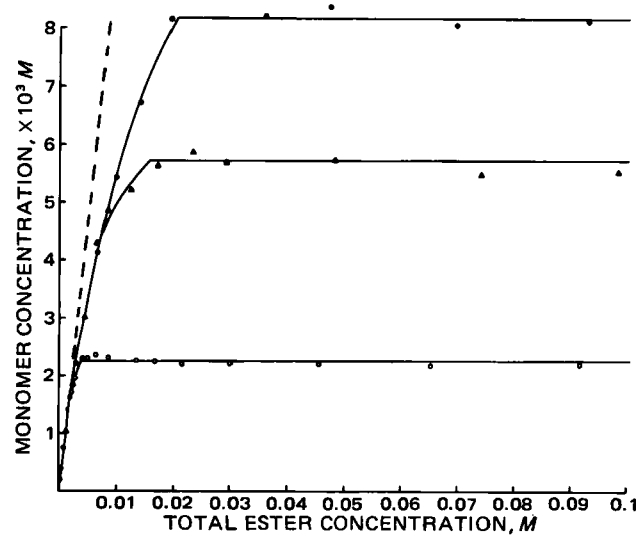


Figure 3—*Monomer concentration* versus *total ester concentration in aqueous solutions at pH 8.47 and 25°. Key: (●) 21-succinate; (▲) 21-adipate; and (○) 21-suberate. Dashed line represents the monomer concentration expected in the absence of self-association.*

[2] The Upjohn Co., Kalamazoo, Mich.

[3] (a) Model T-60 NMR Spectrometer, Varian Assoc. (b) UNISOL-d is a 4:1 mixture of $CDCl_3$–DMSO-d_6, Norell, Inc., Landisville, N.J..

[4] LOBAR—Size B RP-8 columns (2) from E. Merck, Darmstadt, Germany.

[5] Milton Roy Mini-Pump, Laboratory Data Control, Riviera Beach, Fla.

[6] LOBAR—Size B silica gel columns (3) from E. Merck, Darmstadt, Germany.

[7] Zeiss DMR-21 spectrophotometer.

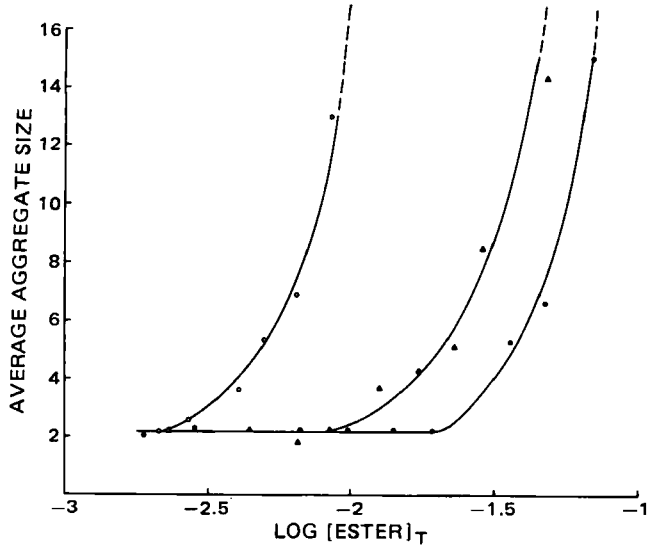


Figure 4—*Average aggregate size determined from Eq. 13* versus *log of ester concentration in aqueous buffer (pH 8.47 and 25°). Key: (●) 21-succinate; (▲) 21-adipate; and (○) 21-suberate.*

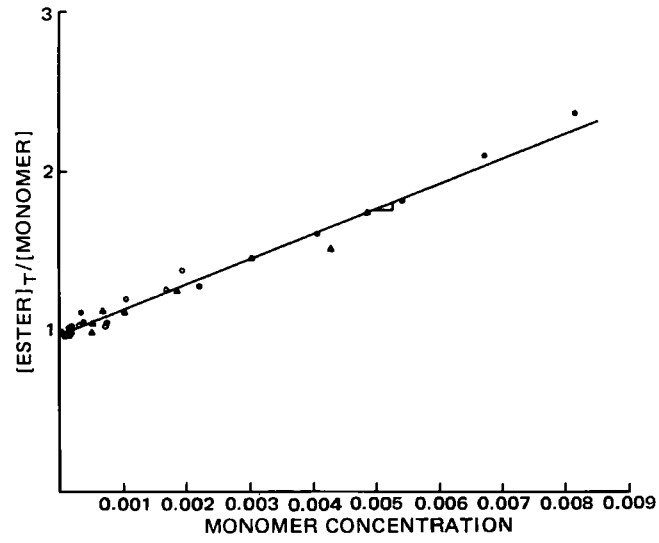


Figure 5—*Plots of* $C_T/[Mon]$ versus *monomer concentration (see Eq. 15) for estimation of dimerization equilibrium constants. Key: (●) 21-succinate; (▲) 21-adipate; and (○) 21-suberate.*

appropriately diluting the stock solutions described previously were transferred to vials containing an acetonitrile–water mixture acidified such that the final solution pH was 3–5 to immediately quench the reaction. Samples (≤100 μl) were analyzed by HPLC employing a modular chromatographic system consisting of an automated sample injector[8], a constant-flow pump[9] operated at 1.5–2.0 ml/min, a reverse-phase column[10], a variable-wavelength UV detector[11] operated at 244 nm, and a digital integrator[12].

Mobile phase compositions for the 21-succinate, 21-adipate, and 21-suberate systems were, respectively, 0.05 *M* acetic acid in acetonitrile–water (1:2) adjusted to pH 5.3 with NaOH, 0.05 *M* acetic acid in acetonitrile–water (2:3) adjusted to pH 5.0, and 0.05 *M* acetic acid in acetonitrile–water (9:11) adjusted to pH 5.2.

RESULTS AND DISCUSSION

Self-Associated Systems and Methods for Their Investigation—Although the nature of the forces in water which give rise to a hydrophobic effect are still not well understood (11–13), the tendency of hydrophobic solutes (molecules containing large regions of exposed organic groups) to self-associate in water is widely recognized (14). Typical micelle-forming surfactants, such as flexible-chain compounds with polar head groups, can form large aggregates containing ~30–120 monomers per multimer (15).

If the aggregates formed are sufficiently large, containing >20 monomers, a simple monomer–micelle model adequately represents the equilibria involved. Such systems are characterized by a distinct break point in plots of any number of properties *versus* concentration. The point at which this discontinuity occurs is termed the critical micelle concentration (CMC). Below the CMC the concentration of micelles is negligible (monomer concentration equals total concentration), while above the CMC the activity of monomer is virtually constant.

Careful studies, however, generally show curvature rather than a distinct discontinuity at the apparent CMC (14), suggesting that small aggregate formation occurs prior to true micelle formation. Even flexible long-chain fatty acid anions have been shown to dimerize in dilute aqueous solutions (16). Steroids consisting of inflexible alicylic fused-ring systems, such as the bile acid salts and the corticosteroid derivatives of interest in this study, are also quite likely to form smaller aggregates. Light scattering and solubilization studies of sodium cholate, for example, suggest that it self-associates to form dimers (6) and other small aggregates (17).

The existence of premicellar aggregates complicates the interpretation of experimental data obtained by methods that do not allow direct calculation of the monomer concentration. Most experimental methods are unsuitable, because each multimer must be described by both a formation constant and a parameter reflecting its contribution to the property being measured. Conductance methods, for example, require the estimate of an equivalent conductance for each species (18). Spectral methods require an estimate of absorptivity for each species present (19).

Partitioning Method for Monitoring the Self-Association of Methylprednisolone Hemiesters—The determination of partition equilibria has been shown to be a useful technique in monitoring self-association (16). The partition coefficient, PC(C), of an amphiphile between an organic and an aqueous solvent reflects the relative thermodynamic activity coefficients $\gamma_{H_2O}(C)$ and $\gamma_{org}(C)$, of solute in the two solvents where C is the concentration of solute in the aqueous phase.

At equilibrium, the thermodynamic activities of solute in the two phases are equal:

$$a_{H_2O} = a_{org} \qquad \text{(Eq. 1)}$$

where

$$a_{H_2O} = \gamma_{H_2O} C_{H_2O} \text{ and } a_{org} = \gamma_{org} C_{org}$$

At infinite dilution, $\gamma_{H_2O}(O)$ is defined such that:

$$\lim \gamma_{H_2O}(C) = \gamma_{H_2O}(O) = 1$$
$$C_{H_2O} \to 0 \qquad \text{(Eq. 2)}$$

Also at infinite dilution, from Eq. 1 and the definition of the partition coefficient:

$$PC(O) = \lim (C_{org}/C_{H_2O}) = \gamma_{H_2O}(O)/\gamma_{org}(O)$$
$$C_{H_2O} \to 0 \qquad \text{(Eq. 3)}$$

and, substituting the value of $\gamma_{H_2O}(O)$ from Eq. 2:

$$\gamma_{org}(O) = 1/PC(O) \qquad \text{(Eq. 4)}$$

Assuming that the activity coefficient of solute in the organic phase, γ_{org}, remains constant with changes in solute concentration:

$$\gamma_{org}(C) = \gamma_{org}(O) = 1/PC(O) \qquad \text{(Eq. 5)}$$

A further assumption is that deviations in the activity coefficient of solute in aqueous solution are due solely to self-association, so at any concentration the value of γ_{H_2O} is equal to the fraction of monomer ([Mon]) present:

$$\gamma_{H_2O}(C) = \frac{[Mon]_{H_2O}}{C_{H_2O}} \qquad \text{(Eq. 6)}$$

Two useful relationships are then obtained from Eqs. 1–6, enabling the direct calculation of monomer concentration and monomer fraction from measurements of partitioning equilibria:

$$[Mon]_{H_2O} = C_{org}/PC(O) \qquad \text{(Eq. 7)}$$

[8] Wisp Model 710A, Waters Associates, Milford, Mass.
[9] Altex Model 110A, Altex Scientific Inc, Berkeley, Calif.
[10] 10-μm LICHROSORB RP-18 column, Brownlee Labs, Berkeley, Calif.
[11] Altex/Hitachi Model 153-00, Altex Scientific.
[12] Model 3380A, Hewlett-Packard, Avondale, Mass.

and

$$[\text{Mon}] = \gamma_{H_2O}(C) = PC(C)/PC(O) \quad \text{(Eq. 8)}$$

A pH of 8.5 was selected for both the partitioning and kinetic studies, because the solutes were highly soluble at this pH and the reaction rates were in a range which could be conveniently measured in a reasonable length of time. At this pH, nonpolar solvents (isooctane, toluene, *etc.*) were unacceptable for use in partitioning studies, because partition coefficients were too low, while most polar hydrogen-donating or hydrogen-accepting solvents extracted too much solute. Butyronitrile—a polar, aprotic solvent which is immiscible with water—was found to be suitable.

The pH dependence of the partition coefficient of methylprednisolone 21-succinate suggested that only the free acid partitions into the organic phase. At pH 2 the intrinsic partition coefficient of solute, $PC_I(O)$ was found to be ~170, while the value of $PC(O)$ at pH 8.47 was 0.031. Assuming that only the free acid partitions and that the pK_a for the succinate is 4.7 (10), the predicted value of $PC(O)$ at pH 8.47 can be calculated from:

$$PC(O)_{pH\ 8.5} = \frac{PC_I(O)[H^+]}{[H^+] + K_a} = 0.029 \quad \text{(Eq. 9)}$$

which is in good agreement with the observed value.

A critical assumption on which Eqs. 7 and 8 are based is that the activity coefficient of solute in the organic phase is constant with concentration (Eq. 5). Changes in the activity coefficient of the solute due to association in the organic phase, therefore, either must be negligible or must be considered significant. Carboxylic acids are known to dimerize in nonpolar solvents (19, 20), but the equilibrium constant for dimerization in the more polar solvent butyronitrile should be substantially smaller.

To determine whether dimerization in butyronitrile is significant over the solute concentration range of interest in the pH 8.5 partitioning studies (0 to ~3.3×10^{-3} *M*), the partition coefficient of methylprednisolone 21-hemisuccinate between butyronitrile and 0.01 *N* HCl was determined as a function of concentration (Fig. 1). Since the partition coefficient is large at low pH, the aqueous concentration of solute approaches infinite dilution over the entire butyronitrile concentration range of interest. Self-association in the aqueous phase at pH 2 is, therefore, unimportant. Although there does appear to be an upward trend consistent with a very small deviation in γ_{org}, this trend is negligible over the concentration range of 0–3.3×10^{-3} *M*, indicating that self-association of solute in the organic phase can be neglected.

Partition coefficients at pH 8.47 (μ = 0.5) were determined over a concentration range of 0–0.1 *M*. Values of $PC(O)$ were estimated by linear extrapolation of the partitioning data at concentrations below 3×10^{-3} *M* to infinite dilution. The estimated values of $PC(O)$ for the succinate, adipate, and suberate are 0.031, 0.167, and 1.48, respectively. As expected, partition coefficients increase by a factor of 2.5–3 per $-CH_2-$ group increment consistent with literature values for the methylene group contribution to partitioning into polar organic solvents (21).

The monomer fraction was calculated by applying Eq. 8 to the partitioning data. Semilog plots of the monomer fraction *versus* concentration in aqueous buffer are shown in Fig. 2. Two conclusions can be drawn from the data in Fig. 2: changes in monomer fraction are observed at very low concentration (~1×10^{-3} *M*) and increased chain length results in greater association. Surface tension measurements on the succinate did not show any aggregation below ~0.02–0.03 *M*[13], while the partition coefficients clearly change at much lower concentrations. This suggests that partition coefficients are a more sensitive measure of the aggregation phenomena.

Applying Eq. 7 to the partitioning data one obtains the monomer concentration at any total ester concentration (Fig. 3). A striking observation from these plots is the existence of at least two distinct types of association—premicellar association exemplified by curvature at low concentrations and micelle formation indicated by the constancy in monomer concentration after an apparent discontinuity in each curve.

Premicellar Association—In very dilute solutions, monomer concentration very nearly equals total concentration as indicated by the dashed line in Fig. 3. The marked deviations from the extrapolated line prior to the apparent CMC suggest that premicellar association occurs. Premicellar aggregation is perhaps more noticeable in Fig. 2, since monomer fractions change markedly prior to the apparent critical micelle concentrations observed in Fig. 3.

A calculation of the average aggregate size as previously described (22) is derived in the following manner.

[13] S. L. Nail, The Upjohn Company, private communication.

Table I—Apparent Critical Micellar Concentrations, Equilibrium Constants for Dimerization, and Contribution of Each Species to Total Concentration at 0.1 *M* for Corticosteroid 21-Hemiesters Varying in Side-Chain Length

Ester	Apparent[a] CMC, *M*	$K_{1:2}$ liter mole^{-1}	Species Contribution at 0.1 *M* Monomer, *M*	Dimer, *M*	Micelle, *M*
21-Succinate	0.02	80 ± 4[b]	8.2×10^{-3}	1.1×10^{-2}	8.1×10^{-2}
21-Adipate	0.01–0.015	70 ± 7	5.7×10^{-3}	4.6×10^{-3}	9.0×10^{-2}
21-Suberate	0.003	85 ± 22	2.3×10^{-3}	8.6×10^{-4}	9.7×10^{-2}

[a] Apparent critical micelle concentration. [b] 95% Confidence limits from linear regression.

The total concentration of ester (C_T) can be expressed as,

$$C_T = [\text{Mon}] + 2K_{1:2}[\text{Mon}]^2 + 3K_{1:3}[\text{Mon}]^3 + \ldots \quad \text{(Eq. 10)}$$

where aggregated species of all sizes are allowed without any assumptions as to their tendency to form. The total species concentration (S_T) is:

$$S_T = [\text{Mon}] + K_{1:2}[\text{Mon}]^2 + K_{1:3}[\text{Mon}]^3 + \ldots \quad \text{(Eq. 11)}$$

By inspection it is clear that:

$$S_T = \int_0^{\text{Mon}} C_T/\text{Mon}\, d\text{Mon} \quad \text{(Eq. 12)}$$

The average polymer size at any concentration is defined as:

$$\text{average polymer size} = \frac{C_T - \text{Mon}}{S_T - \text{Mon}} \quad \text{(Eq. 13)}$$

The integral in Eq. 12 was evaluated by trapezoidal integration.

The results of average polymer size calculations *versus* log C_T are shown in Fig. 4. The premicellar region is indicated by the flat portion of the diagrams at an average polymer size of ~2 which is very broad for the succinate, less so for the adipate, and barely distinguishable for the suberate due to its low apparent CMC. The broad region with average polymer size of 2 is consistent with dimer formation. Since the average polymer size does not gradually increase in the premicellar region, trimers or other small oligomers do not appear to be present.

If dimerization is the major mode of association in the premicellar region, systems in this region should be adequately described by:

$$C_T = \text{Mon} + 2K_{1:2}\text{Mon}^2 \quad \text{(Eq. 14)}$$

where

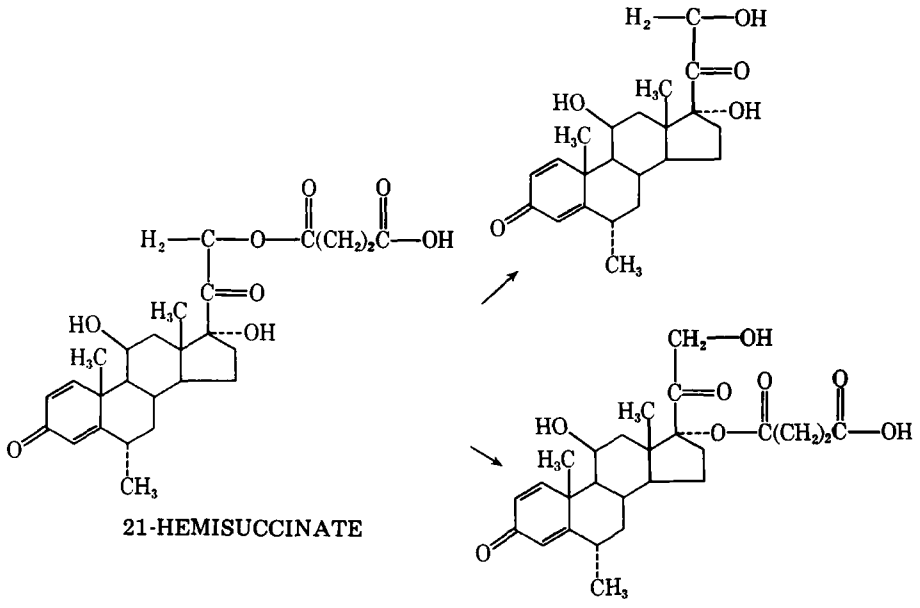
$$K_{1:2} = \frac{[\text{Dimer}]}{[\text{Mon}]^2} \quad \text{(Eq. 15)}$$

Therefore, plots of C_T/Mon *versus* [Mon] should be linear with slopes of $2K_{1:2}$. These plots are shown to be approximately linear in Fig. 5. The values of $K_{1:2}$ estimated from a linear regression on the data in Fig. 5 are 80 ± 4, 70 ± 7, and 85 ± 22 liter/mole for the succinate, adipate, and suberate, respectively. $K_{1:2}$ is not affected noticeably by chain length suggesting that the C_{21} side chains are not participating in the dimerization interaction.

It is possible that dimers arise from the stacking of the steroidal portion of the molecules with the C_{21} side chains directed away from each other. Another possibility is that the side chains may be folded back in a hydrophobic interaction with the side of the steroidal nucleus not involved in dimer formation. If the latter were true, folding back of the side chain would be expected in the monomer as well. Since the infinite dilution partition coefficients increase with chain length by the magnitude predicted, this explanation does not seem likely.

Micelle Formation—The break points in Fig. 3 after which monomer concentration remains constant suggest the formation of much larger aggregates or micelles. The critical micelle concentrations for the three homologues (the concentrations at which micelles are first detectable) are listed in Table I along with the dimerization constants discussed previously. Above the CMC, the concentrations of monomer and dimer are invariant. Estimates of these concentrations are also shown in Table I. An interesting observation is that the actual monomer concentration is much lower than the apparent CMC, particularly for the succinate and adipate. In a simple monomer–micellar model, the monomer activity is generally assumed to be given by the CMC. As shown in Table I, estimates of monomer concentration from apparent critical micelle concentrations may be very misleading if premicellar association occurs.

Numerous reports in the literature cite a relationship between CMC



Scheme I

and the number of methylene groups in the surfactants. The relationship generally takes the form:

$$\log \text{CMC} = A - bn \qquad \text{(Eq. 16)}$$

where b is usually ~0.28–0.3 (23). A plot of log CMC *versus* chain length in the series of corticosteroid esters gave a slope of 0.24, which is close to values generally reported in the literature. The effect of chain length on monomer concentration, however, was much less (Table I) than predicted from Eq. 16 due to the importance of dimer formation.

It is difficult to speculate on the structure of these corticosteroid micelles other than to point out that they are large (average polymer size in Fig. 4 increases sharply above the CMC), and their formation is enhanced by increases in side-chain length.

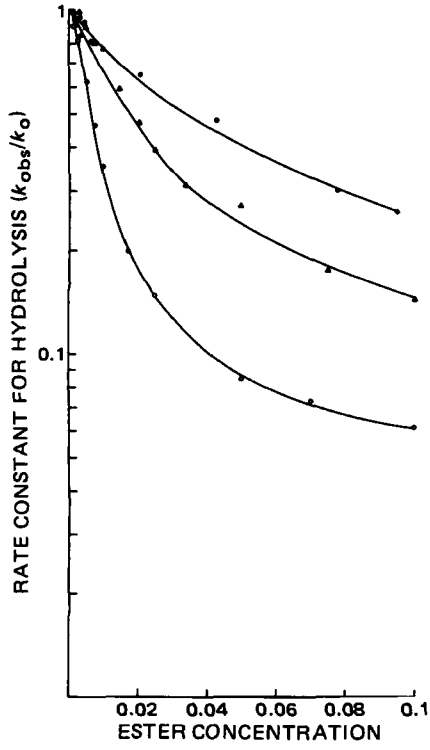


Figure 6—*Semilog plots of the ratio of the rate constant for hydrolysis at the given concentration to the infinite dilution rate constant* (k_{obs}/k_0) versus *ester concentration. Key: (●) 21-succinate; (▲) 21-adipate; and (○) 21-suberate.*

One hint as to the nature of the environment in the micelles may be obtained from UV measurements[14]. The $\pi \rightarrow \pi^*$ transition of the steroidal A-ring in the 21-esters of methylprednisolone undergoes a bathochromic shift as the polarity of the solvent is increased, due presumably to a reduction in the energy level of the excited state accompanying dipole–dipole interaction and hydrogen bonding (24). For example, λ_{max} of methylprednisolone succinate in tetrahydrofuran is 236 nm while in water it is 244 nm. A thin film of a 0.1 *M* aqueous solution of methylprednisolone succinate spread between two cuvettes exhibited a λ_{max} of ~245 nm, very similar to that observed in dilute solutions, indicating that there is no significant change in absorbance accompanying the partitioning of molecules into micelles. This observation tentatively supports the hypothesis that the environment surrounding the A-ring in the micelle is very polar or the A-ring is involved in hydrogen bonding within the micelle.

Kinetics of Hydrolysis and Acyl Migration in Aggregated Corticosteroid Systems—Catalysis and inhibition of reactions in associated systems have been the subjects of extensive research (25, 26). Of greatest interest from a pharmaceutical standpoint are those studies in which reaction rates have been markedly retarded by micelle-forming additives (27–31). Base-catalyzed hydrolysis of esters is generally inhibited by anionic surfactants (26), and since previous studies have shown (10) that both hydrolysis and 21 → 17 ester migration in methylprednisolone succinate are hydroxide ion catalyzed, it is reasonable to expect that both reactions might be slowed by self-association. The concentration of monomer in 0.1 *M* solutions of the corticosteroid homologues show a range of $2.3–8.2 \times 10^{-3}$ (Table I). If degradation were completely suppressed in the aggregates as has been shown in at least one case (27), significant stabilization should result from self-association in 0.1 *M* solutions.

As predicted, hydrolysis and 21 → 17 acyl migration (Scheme I) of the methylprednisolone 21-ester linkage is suppressed at higher concentrations. Plots of k_{obs}/k_0 *versus* concentration for hydrolysis and acyl migration, are shown in Figs. 6 and 7 respectively, where k_0 is the pseudo first-order rate constant at high dilution. From Figs. 6 and 7 the following conclusions are drawn: (*a*) significant suppression of reactivity occurs prior to the critical micelle concentrations (obtained from partitioning data) suggesting that dimerization as well as micelle formation stabilizes these esters toward hydroxide ion attack; (*b*) the overall magnitude of stabilization is much less than would be observed if monomers were the only reactive species; (*c*) increases in chain length which were earlier shown to promote micelle formation also result in larger rate suppression with increasing concentration; and (*d*) the magnitude of stabilization is about the same for both the hydrolysis reaction and 21 → 17 acyl migration.

Whereas the equilibrium constants for dimerization, critical micelle

[14] J. R. Cardinal, College of Pharmacy, University of Utah, private communication.

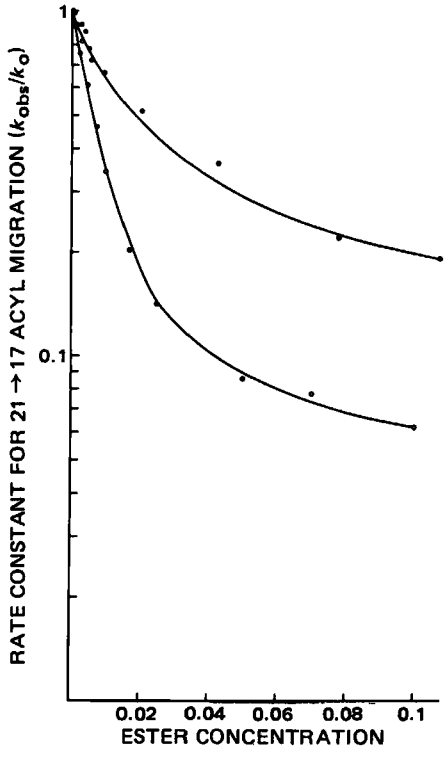


Figure 7—*Semilog plots of the ratio of the observed 21 → 17 acyl migration rate constant at the given ester concentration to the infinite dilution acyl migration rate constant* (k_{obs}/k_0) versus *ester concentration (pH 8.47 and 25°). Key: (●) 21-succinate and (○) 21-suberate.*

concentrations, *etc.* were determined in butyronitrile-saturated aqueous buffer, the kinetic data were obtained in aqueous buffer containing no butyronitrile. The use of the dimerization constants in Table I to calculate rate constants for reaction of dimers from the kinetic data is, therefore, probably not advisable. A few kinetic studies in butyronitrile-saturated buffer were done, however, and similar rate suppression *versus* concentration curves were observed even though absolute reaction rates differed. Qualitatively at least, it is apparent from Figs. 6 and 7 that reaction rates in the dimers are substantially reduced since reactivities decline with concentration even at very low concentrations.

While the percentages of monomer reported in Table I are 2–8% of the total ester at 0.1 *M*, reaction rates at 0.1 *M* are lower by a lesser amount (6–27%). Clearly, reactivity is not totally suppressed in the aggregated species. This is evident in the plots of the absolute hydrolysis rate *versus* the total ester concentration (Fig. 8). Although break points are not as sharp in the kinetic data as in the partitioning data, apparent critical micelle concentrations estimated from extrapolation of the two linear portions of each curve agree fairly well with the apparent critical micelle concentrations reported in Table I.

Reaction rates continue to increase above the critical micelle concentrations indicating that reactivity in the micelles is not totally suppressed. From the slopes above the critical micelle concentrations in Fig. 8, rate constants for hydrolysis in the micelles can be obtained. Values of pseudo first-order rate constants for monomeric and micellar species, k_{mon} and k_{mic}, are listed in Table II for both hydrolysis and acyl migration. At high concentration (0.1 *M*), the reactions occurring in the micelles are actually the major contribution to the overall degradation rate. This accounts for the fact that stabilization is less than predicted based on the monomer concentrations.

Table II—Reaction Rate Constants for Monomeric and Micellar Species of Corticosteroid 21-Esters

Ester	Hydrolysis, min^{-1}		21 → 17 Acyl Migration, min^{-1}	
	k_{mon}	k_{mic}	k_{mon}	k_{mic}
21-Succinate	4.2×10^{-4}	7.4×10^{-5}	3.2×10^{-4}	3.9×10^{-5}
21-Adipate	3.1×10^{-4}	2.1×10^{-5}	—	—
21-Suberate	2.6×10^{-4}	8.1×10^{-6}	2.8×10^{-4}	9.5×10^{-6}

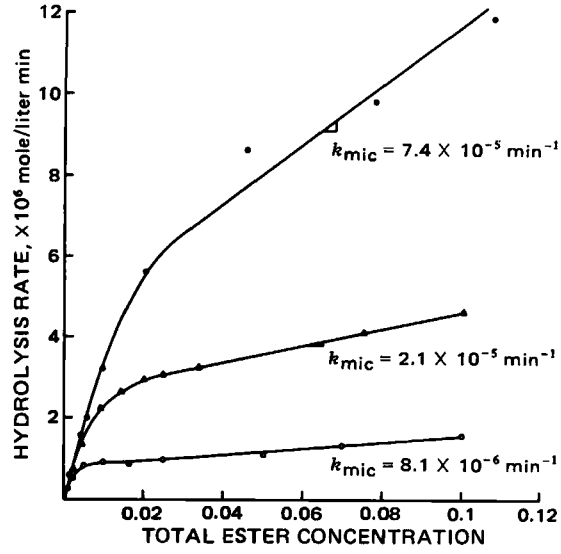


Figure 8—*Absolute hydrolysis rates* versus *total ester concentration (pH 8.5 and 25°). Key: (●) 21-succinate; (▲) 21-adipate; and (○) 21-suberate.*

The effect of chain length on stability was investigated for a similar series of hydrocortisone hemiesters by Garrett in 1962 (32). These studies were conducted in diluted alcoholic solutions, however, and it was concluded that chain length has no significant effect on corticosteroid ester stability. The results of the present study suggest that chain length does have a significant effect on stability, especially at higher concentrations. The 21-suberate of methylprednisolone is nearly an order of magnitude more stable than the 21-succinate at a 0.1 *M* concentration. This greater stability is due to a combination of factors: (*a*) a slight steric effect which increases with chain length is apparent in the reactivity of the monomeric ester; (*b*) self-association increases with chain length providing added stability to long-chain hemiesters by lowering monomer concentration; and, most important, (*c*) reactivity in the micelle apparently decreases with increasing chain length. As a result of these factors, methylprednisolone 21-suberate is >16-fold more stable at high concentration (>0.1 *M*) than in dilute solution.

By comparing Figs. 6 and 7 it is apparent that 21 → 17 acyl migration is suppressed by self-association to an extent similar to the suppression of hydrolysis. Since the approach of hydroxide ion is required for both reactions at pH 8.5, the similarity in behavior with self-association can be rationalized from simple electrostatic considerations (33). The decrease in the rate constants of both reactions with increasing chain length is not explained as readily. Since the effect is not specific for one mechanism, this must also reflect differences in the hydroxide ion activity in the immediate environment of the 21-ester linkage.

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Effect of Ethyl Cellulose in a Medium-Chain Triglyceride on the Bioavailability of Ceftizoxime

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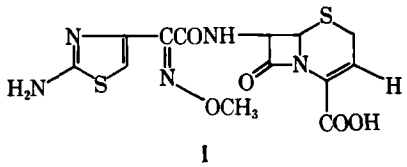
Received February 22, 1982 from the *Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka 532, Japan.* Accepted for publication July 16, 1982.

Abstract □ The oral bioavailability of new formulations of ceftizoxime sodium was investigated in animals and humans. In rats, one of the formulations tested showed significant improvement, with a urinary excretion of 47.7% (0–24 hr). Good results were obtained also in dogs. In humans, the mean peak serum level was 3.6 μg/ml at 3.3 hr postadministration for formulation 10. The average ceftizoxime AUC at 0–8 hr was 17.3 μg hr/ml and urinary excretion of ceftizoxime was 9.6% (0–24 hr). The concentrations in the serum exceeded the minimum inhibitory concentrations for most of the commonly encountered bacterial pathogens.

Keyphrases □ Bioavailability—oral ceftizoxime in rats, dogs, and humans, effect of ethyl cellulose in medium-chain triglyceride □ Ceftizoxime—bioavailability of oral formulations in rats, dogs, and humans, effect of ethyl cellulose in medium-chain triglyceride □ Ethyl cellulose—effect with medium-chain triglyceride on the oral bioavailability of ceftizoxime in rats, dogs, and humans □ Triglyceride, medium-chain—effect with ethyl cellulose on the oral bioavailability of ceftizoxime in rats, dogs, and humans

Ceftizoxime (I) is a new cephalosporin antibiotic which is active against both Gram-positive and Gram-negative bacteria.



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The activity of ceftizoxime *in vitro* has been confirmed (1), and the reports on its clinical efficiency are numerous (2). The metabolism and pharmacokinetics of this drug have also been described (3). Ceftizoxime is administered parenterally for effective systemic action since it is poorly absorbed from the GI tract[1]. The purpose of this investigation was to determine the oral bioavailability of ceftizoxime after the administration of its sodium salt to animals and humans in new formulations produced with a combination of medium-chain triglyceride and ethyl cellulose as additives.

Attempts to improve the oral bioavailability of poorly absorbed drugs by devising different pharmaceutical formulations have been reported (4–6). Similarly, our efforts have been directed to the development of new oral formulations of ceftizoxime. We have systematically studied a number of hydrophilic and hydrophobic vehicles, surfactants, and nonsurfactants as additives and found that a combination of ethyl cellulose and a medium-chain triglyceride enhanced the oral absorption of ceftizoxime in rats, dogs, and humans.

EXPERIMENTAL

Materials—Ceftizoxime sodium[2] was prepared as described in the patent (7). Commercially available ethyl cellulose[3], polyethylene glycol 400[4], a medium-chain triglyceride[5], olive oil[4], and ethyl alcohol[4] were used in the suspensions.

Formulations—Formulations containing various amounts of ethyl cellulose were tested. A medium-chain triglyceride (50 ml) and ethyl cellulose (500 mg) dissolved in 1 ml of ethyl alcohol were mixed with stirring. The ethyl alcohol was removed under reduced pressure. Ceftizoxime sodium (5 g-potency) was then dispersed in the resulting vehicle to give the formulation of ceftizoxime used. Similar formulations containing olive oil or polyethylene glycol 400 instead of the medium-chain triglyceride were also investigated.

Absorption Studies—*Rats*—Six-week-old male Sprague-Dawley rats, weighing 160–230 g, were fasted for 18 hr. The rats were given the ceftizoxime sodium formulations (Table I) using a gastric tube at a dose equivalent to 20 mg/kg. Urine was collected for 24 hr, stored at −20°, and

[1] Unpublished data.

[2] Epocelin; Fujisawa Pharmaceutical Co., Ltd. Osaka, Japan.
[3] Ethocel, 10, 45, and 100 cps; Dow Chemical Co.
[4] Hayashi Pure Chemical Industries, Ltd., Japan.
[5] Miglyol 812; Dynamit Nobel Co.

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