Prediction of Stability in Pharmaceutical Preparations XI

Increased Stability of Soluble Steroid Hemiesters by Steric Hindrance

By EDWARD R. GARRETT† and MAX E. ROYER

Since alkyl substitution on the mono-esterified dicarboxylic acid of 21-steroid hemiesters decreases the rate of specific hydroxyl ion catalyzed hydrolysis, the kinetics of aqueous hydrolysis of prednisolone and 6α-methylprednisolone hemi-β,β′-dimethylglutarates have been studied by constant pH titration. The effect of steroid 6α-substitution on rate of 21-ester hydrolysis has been indicated. The stabilities of sodium salts of such esters at various pH values between 8 and 10 and at various temperatures have been predicted. It has been shown that steric hindrance due to alkyl substitution on the monoesterified dicarboxylic acid increases stability by more than tenfold over the previously reported stability of the nonhindered hydrocortisone hemisuccinate. A more precise procedure for constant pH titration is described.

THE PHARMACEUTICAL utility of a steroid has been enhanced by formation of a soluble derivative that still possessed biological potency and stability as the derivative. The criteria of solubility and biological potency have been well fulfilled by the sodium salt of the hemisuccinate ester of hydrocortisone (1–3). It has been shown that the stability of 21-steroid hemiesters is not significantly affected by increasing the chain length of the monoesterified dicarboxylic acid (4). Resistance to specific hydroxyl ion catalysis is promoted, however, by alkyl substitution on the dicarboxylic acid in accordance with Newman's rule of "six" (5, 6).

This paper reports on the detailed kinetic studies of the specific hydroxyl ion catalyzed hydrolysis of such sterically hindered esters, the hemi-β,β′-dimethylglutarate esters of prednisolone and 6α-methylprednisolone in aqueous media.

EXPERIMENTAL

The procedure for the constant pH titrations of the sodium salt of the hemi-β,β′-dimethylglutarate ester of prednisolone (Δ¹-hydrocortisone) was similar to that reported previously (4, 7). However, the experimental techniques for the constant pH titrations of the sodium salt of the hemi-β,β′-dimethylglutarate ester of 6α-methylprednisolone were considerably improved. The material was weighed and placed in a beaker fitted with a close-fitting rubber insert through which the single Beckman compound electrode extended to the bottom of the beaker. A metal clamp was also used to seal this rubber cap around the beaker. A needle was placed through the rubber insert and tubing led to another needle sealed in the electrode side orifice so that the pressure atop the electrode salt solution was the same as that within the beaker. The beaker containing the weighed material and a magnetic bar was submerged in the constant temperature bath atop a magnetic stirrer in a watertight can with a conduit periscoped above the water to permit egress for the insulated electric cord.

Fifteen milliliters of nitrogen-purged water, previously equilibrated in the temperature bath, was added by means of a syringe and needle thrust through the rubber cap. An additional needle had been thrust through the insert to act as a vent.

The resultant solution was *ca.* 0.004 *M* in hemiester. Both of these needles were removed and a 25-gauge 2-in. needle, bent at a right angle, was inserted. This was for the titrant delivery from a gas-tight 1-ml. "microliter" syringe.[1] The rubber cap bulged slightly from the internal vapor pressure but was definitely vaportight.

The Cannon difunctional titrator had been standardized previously as reported (4, 7).

CALCULATIONS AND RESULTS

Typical plots of the consumption of standard sodium hydroxide by hemi-β,β′-dimethylglutarate esters of a steroid alcohol are given in Fig. 1. These plots are for the hydrolysis of the 6α-methylprednisolone ester at 70.0° at several pH values and are given as chart divisions against time. Each chart division, *CD*, represents 0.008052 meq. of sodium hydroxide added as 1.0175 *M*. In some instances, as at pH 10.0, the possible rate of addition of alkali was slower than the consumption so that the only valid part of the curve is when the desired constant pH was attained and maintained.

The dashed lines in Fig. 1 represent the tangents to the curves at the origin and the apparent first-order rate constants, *k*, at the constant pH values can be calculated from

$$k(\text{sec.}^{-1}) = (N \times m)/(m' \times M) = (CD \times 0.008052)/(m' \times M) \quad \text{(Eq. 1)}$$

where *m* is the number of ml. of *N* normal sodium hydroxide consumed per second at the constant pH

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† Present address: College of Pharmacy, University of Florida, Gainesville.

[1] Syringe No. 1001, Hamilton Co., Whittier, Calif.

TABLE I.—RATE CONSTANTS (10^5 k in sec.$^{-1}$) FOR THE APPARENT FIRST-ORDER HYDROLYSIS OF 0.004 M STEROID HEMIESTERS OF β,β'-DIMETHYLGLUTARATE AT VARIOUS TEMPERATURES AND pH VALUES BY CONSTANT pH TITRATION OF 15 ML. WITH 1.0 M SODIUM HYDROXIDE

	6α-Methylprednisolone			Prednisolone		
pH	70.0°	60.0°	50.0°	67.5°	57.5°	50.5°
10.50	...	...	...	...	...	97.3,[a] 98.3[b]
10.00	126,[a] 116[b]	27.8,[a] 25.3[b] 38.8,[a,c] 28.5[b,c]	9.93,[a] 9.43[b]	91.6,[a] 94.4	37.8,[a] 38.7[b]	24.4,[a] 25.5[b]
9.50	37.5,[a] 34.5[b]	10.5,[a] 10.1[b] 8.86,[a,c] 8.69[b,c]	3.68,[a] 2.88[b]	30.7,[a] 24.2[b]	14.9,[a] 13.5[b]	6.49,[a] 4.96[b] 6.72,[a,c] 6.20[b,c]
9.00	10.1,[a] 8.58[b]	2.81,[c] 2.78[b]	0.878,[a] 0.863[b]	17.0,[a] 13.9[b]	...	1.96,[a] 1.79[b]
8.75	4.59,[a] 3.40[b]	...	...	...	...	...
8.54	...	...	...	...	2.10[a]	...
8.50	3.28,[a] 3.40[b]	...	...	3.15,[a] 3.33[b]	...	...

[a] Calculated from slope of plot of 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. [b] Calculated from initial zero-order slope, m, of ml. of N normal titer consumed per second by m' ml. of M molar steroid hemiester, $k = (N \times m)/(m' \times M)$. [c] A different study than the one above it.

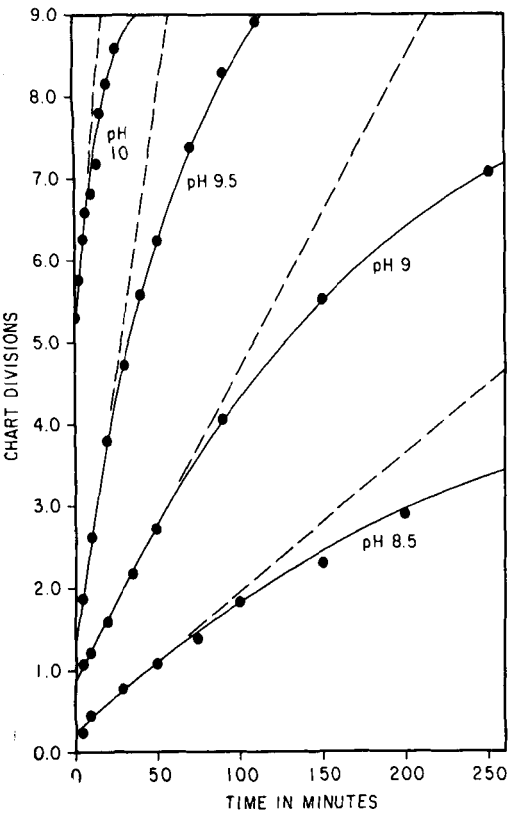


Fig. 1.—Typical plots of consumption of 1.000 M sodium hydroxide by 15 ml. of 0.004 M 6α-methylprednisolone hemi-β,β'-dimethylglutarate at constant pH and at 70.0° as a function of time. Each chart division represents 0.008052 meq. sodium hydroxide. The dashed lines are estimates of the initial rate of sodium hydroxide addition. At pH 10, the titer addition did not initially keep up with speed of reaction.

by m' ml. of M molar hemiester, or where CD is the number of chart divisions where each chart division represents 0.008052 meq. sodium hydroxide added.

The first-order rate constants, k in sec.$^{-1}$, derived from these tangents at the origin to the plots of alkali consumption with time are given in Table I for both 6α-methylprednisolone and prednisolone hemi-β,β'-dimethylglutarate.

Typical first-order plots are given in Fig. 2 as based on the expression

$$\log (CD_\infty - CD) = -kt/2.303 + \log CD_\infty \quad \text{(Eq. 2)}$$

where CD_∞ is the number of chart divisions which should be traveled at theoretically complete saponification of the *ca.* 0.004 M ester in the 15 ml. of water.

The apparent first-order rate constants, k in sec.$^{-1}$, estimated from the slopes of such plots are also given in Table I. The fine consistency of the rates determined by this method and calculated from the tangent to the origin is gratifying.

Plots of the logarithm of the rate constants against pH for the 6α-methylprednisolone and prednisolone hemiesters are given in Figs. 3 and 4, respectively.

These plots are characterized by the equation

$$k = k_{OH^-}[OH^-] \quad \text{(Eq. 3)}$$

or in logarithmic form

$$\log k = \log k_{OH^-} - \text{pOH} = \log k_{OH^-} + \text{pH} - \text{pKw} \quad \text{(Eq. 4)}$$

The intercepts of the plots of Figs. 3 and 4 are $\log k_{OH^-} - \text{pKw}$ and are given in Table II. The $\log k_{OH^-}$ can thus be calculated from the knowledge of the pKw at the given temperature as listed in the literature (8).

The Arrhenius plots of $\log k_{OH^-}$ against the reciprocal of the absolute temperature, $1/T$, are given in Fig. 5 as per the expression

$$\log k_{OH^-} = -S/T + \log P = \Delta H_a/2.303\ RT + \log P \quad \text{(Eq. 5)}$$

where for 6α-methylprednisolone hemi-β,β'-dimethylglutarate S = 2,995, $\log P$ = 8.506, and ΔH_a = 13.7 Kcal./mole and for prednisolone hemi-β,β'-dimethylglutarate S = 2,422, $\log P$ = 7.050, and ΔH_a = 11.1 Kcal./mole.

Values of $\log k_{OH^-}$ at 25, 30, and 40° can be calculated for both hemiesters from Eq. 5 with proper substitution of the derived parameters and they are given in Table II. The apparent first-order rate constants can be calculated from the derived values of Table II by insertion into Eq. 4 and are given for various pH values in Table III.

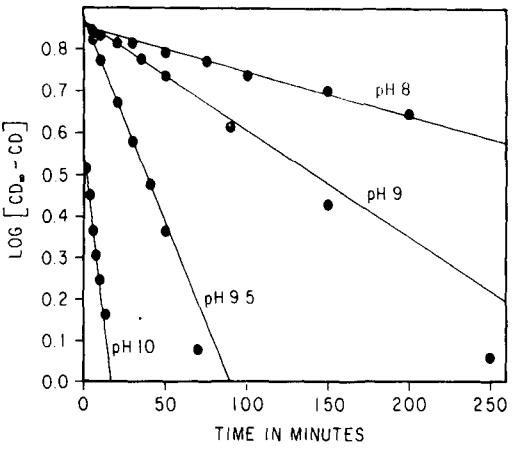


Fig. 2.—Typical first-order plots of hydrolysis of 6α-methylprednisolone hemi-β,β′-dimethylglutarate at constant pH and at 70°. Each chart division, *CD*, represents 0.008052 meq. sodium hydroxide added as 1 *M* to 15 ml. of *ca.* 0.004 *M* steroid hemiester. CD_∞ is the theoretical value at complete saponification.

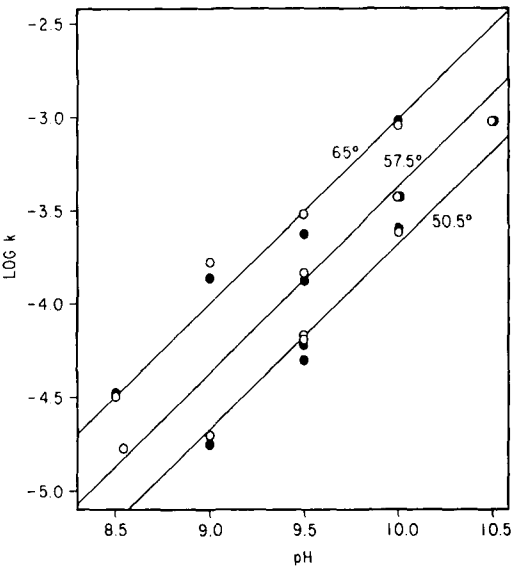


Fig. 3.—Logarithm of the apparent first-order rate constants, *k* in sec.$^{-1}$, for the hydrolysis of 6α-methylprednisolone hemi-β,β′-dimethylglutarate as a function of pH. The open circles represent *k* values derived from first-order plots; the solid circles represent *k* values derived from the initial zero-order rate of alkali consumption; the half-open circles are coincident values.

The half-life, $t_{1/2}$ in days, is calculated from

$$t_{1/2}(da) = 8.00 \times 10^{-6}/k(\text{sec.}^{-1}) \quad \text{(Eq. 6)}$$

The fraction, *f*, of nonhydrolyzed ester remaining at one year at the given temperature and pH and given in Table III is calculated from

$$\log f = -1.367 \times 10^{7} k(\text{sec.}^{-1}) \quad \text{(Eq. 7)}$$

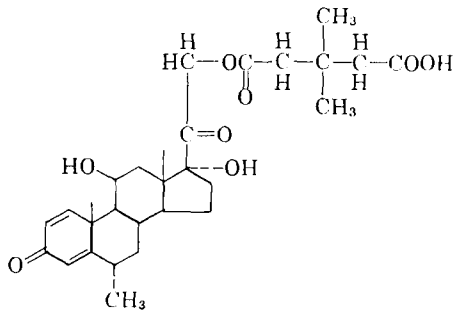
TABLE II.—ESTIMATES OF THE BIMOLECULAR RATE CONSTANT k_{OH^-} (1/*M*/sec.) FOR THE HYDROXYL ION CATALYZED HYDROLYSIS OF HEMIESTERS OF β,β′-DIMETHYLGLUTARATE

	°C.	log k_{OH^-} − pKw[a]	pKw[b]	log k_{OH^-}	(1/*M*/sec.) k_{OH^-}
6α-Methyl-prednisolone	70.0	−13.00	12.76	−0.24	0.58
	60.0	−13.50	13.02	−0.48	0.32
	50.0	−14.03	13.26	−0.77	0.17
	40.0[c]	−14.59	13.53	−1.06	0.087
	30.0[c]	−15.22	13.84	−1.38	0.042
	25.0[c]	−15.55	14.00	−1.55	0.028
Prednisolone	65.0	−13.01	12.89	−0.12	0.76
	57.5	−13.37	13.07	−0.30	0.50
	50.5	−13.68	13.25	−0.43	0.37
	40.0[c]	−14.22	13.53	−0.69	0.20
	30.0[c]	−14.78	13.84	−0.94	0.12
	25.0[c]	−15.08	14.00	−1.08	0.083

[a] Intercept of plot of logarithm of apparent first-order rate constant, *k* in sec.$^{-1}$, against pH by the expression log k = log k_{OH^-} − pKw + pH. [b] As determined from ref. 8. [c] The listed values for these temperatures were calculated from the derived Arrhenius' expression log $k_{OH^-} = -S/T + \log P$ where *S* and *P* for the 6α-methylprednisolone hemiester are $2.995 \cdot 10^3$ and 8.506, respectively, and for the prednisolone hemiester are $2.442 \cdot 10^3$ and 7.050, respectively. From these data and the pKw, the other values can be derived.

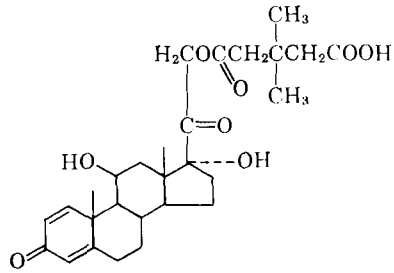
DISCUSSION

The specific hydroxyl ion catalyzed hydrolysis of 6α-methylprednisolone hemi-β,β′-dimethylglutarate (I) would be expected to have rates similar to the



I

specific hydroxyl ion catalyzed hydrolysis of prednisolone hemi-β,β′-dimethylglutarate (II) since the



II

former compound differs from the latter by only an α-methyl at the 6 position. Surprisingly, the latter compound hydrolyzes faster by an order of magnitude greater than two. Although the experimental conditions were not as elegant for the constant pH hydrolyses of the prednisolone compound as for the 6α-methylprednisolone since the improvements were subsequently introduced, the difference in magnitude of rates is considered significant.

TABLE III.—PREDICTED HYDROLYSIS RATES[a] OF STEROID ESTERS OF HEMI β,β'-DIMETHYLGLUTARATE[b]

pH	25° 10^8k (sec.$^{-1}$)[a]	25° $t_{1/2(da)}$[c]	25° f[d]	30° 10^8k (sec.$^{-1}$)[a]	30° $t_{1/2(da)}$[c]	30° f	40° 10^7k (sec.$^{-1}$)[a]	40° $t_{1/2(da)}$[c]	40° f[d]
				6α-Methylprednisolone					
10	282	2.84	...	602	1.50	...	257	0.311	...
9	28.2	28.4	0.00	60.2	15.0	0.00	25.7	3.11	...
8	2.82	284	0.41	6.02	150	0.15	2.57	31.1	...
7	0.282	2,840	0.92	0.602	1,500	0.83	0.257	311	0.45
				Prednisolone					
10	831	0.961	...	1,660	0.482	...	602	0.133	...
9	83.1	9.61	...	166	4.82	...	60.2	1.33	...
8	8.31	96.1	0.07	16.6	48.2	0.005	6.02	13.3	...
7	0.831	961	0.77	1.66	482	0.59	0.602	133	0.15

[a] Log k_{OH^-} was calculated from log $k_{OH^-} = -2.995 \cdot 10^3(1/T) + 8.506$ for 6α-methylprednisolone hemiester, log $k_{OH^-} = -2.422 \cdot 10^3(1/T) + 7.050$ for prednisolone hemiester where T = °C. + 273°. Subsequently, k for a given pH and temperature was estimated by log k = log k_{OH^-} − pKw + pH, where pKw for a given temperature is given in Table II from the literature (8). [b] The ester of 6α-methylprednisolone hydrolyzes 0.029× as fast as hydrocortisone hemisuccinate at 25°, 0.036× as fast at 30°, and 0.051× as fast at 40°. The ester of prednisolone hydrolyzes 0.085× as fast as hydrocortisone hemisuccinate at 25°, 0.10× as fast at 30°, and 0.12× as fast at 40°. [c] Half-life in days at the given pH, $t_{1/2(da)} = 8 \times 10^{-6}/k$(in sec.$^{-1}$). [d] The fraction of nonhydrolyzed ester remaining at one year at the given temperature and pH.

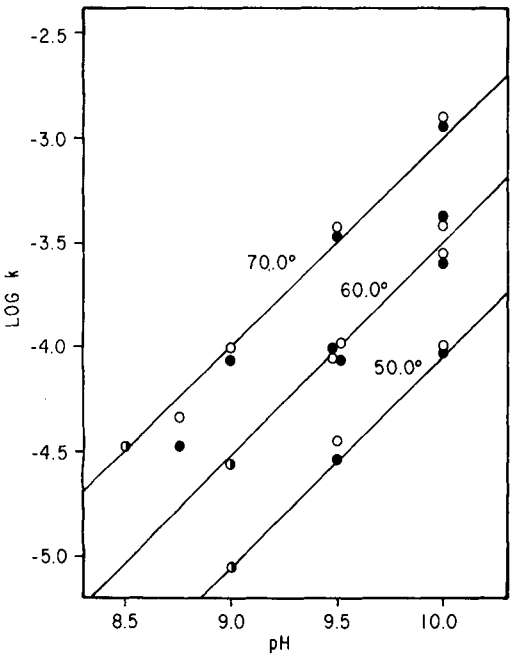


Fig. 4.—Logarithm of the apparent first-order rate constants, k in sec.$^{-1}$, for the hydrolysis of prednisolone hemi-β,β'-dimethylglutarate as a function of pH. The open circles represent k values derived from first-order plots; the solid circles represent k values derived from the initial zero-order rate of alkali consumption.

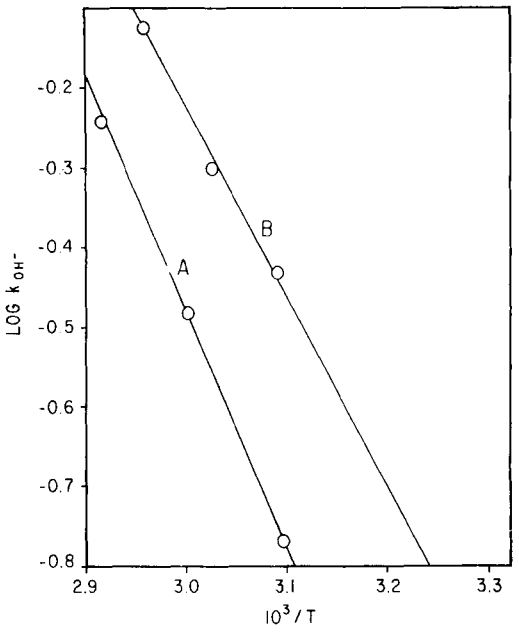
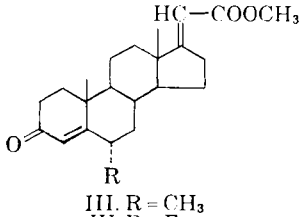


Fig. 5.—Arrhenius plot of the bimolecular rate constant, k_{OH^-}(1/M/sec.) for the hydroxyl ion catalyzed hydrolysis of steroid hemiesters of β,β'-dimethylglutarate. Curve A is for 6α-methylprednisolone; curve B is for prednisolone.

It is also significant that the coulometric bromination of methyl-6α-methyl-4,17(20)-[*cis*]pregnadien-21-oate (III) proceeds exceedingly fast whereas the coulometric bromination at fifty times the bromine concentrations as used for III is very much slower for methyl-6α-fluoro-4,17(20)-[*cis*]-pregnadien-21-oate (IV) (9).

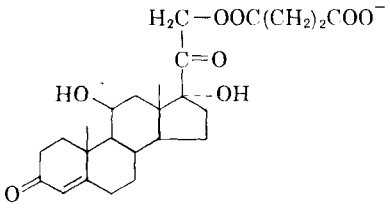


III. R = CH_3
IV. R = F

In addition, it has been shown by derivative ultraviolet spectroscopy that a definitive interaction exists between the 3-keto-Δ^4 system of the A ring modified by a 6α-methyl substitution and an α-hydroxyl substituent at the 17 position (10).

The hydrolysis studies were conducted down to a pH of 8.5 and only specific hydroxyl ion catalysis was indicated. This does not permit rejection of intramolecularly abetted "spontaneous" hydrolysis or, its kinetic equivalent, specific hydroxyl ion catalyzed hydrolysis of the undissociated hemiester

which would make a significant contribution to the total hydrolysis rate at lower pH values, specifically in the neutral pH region 4–7 as a function of pKa. This phenomenon had been indicated previously for hydrocortisone hemisuccinate(V) (4).



V

Thus the predicted stabilities (Table III) of the β,β′-dimethylglutarate hemiesters of these steroids at pH values closer to neutrality are the most optimistic estimates. Intramolecular catalysis, if present, would tend to increase the hydrolysis rate at neutral pH values over that predicted from specific hydroxyl ion catalysis alone.

As was expected, stability to specific hydroxyl ion catalyzed hydrolysis is greatly enhanced by alkyl substitution over that for the hemisuccinate or other straight chain aliphatic hemiesters (4, 7) and is in accordance with Newman's rule (5, 6). It is interesting to note, however, that if intramolecular catalyzed hydrolysis is present, and significantly contributes in the neutral pH region 4–7, it would be predicted to be greater in the case of the alkyl substituted esters than for the nonalkylated hemiesters of dicarboxylic acids such as the straight chain glutaric or succinic acids (11).

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Solubilization in Nonpolar Solvents

Influence of the Chain Length of Solvent on the Solubilization of Water by Dioctyl Sodium Sulfosuccinate

By W. I. HIGUCHI and JAGDISH MISRA

The water solubilizing capacities of the solutions of dioctyl sodium sulfosuccinate (Aerosol OT, AOT) in normal aliphatic hydrocarbon solvents from *n*-heptane to *n*-octadecane were determined as functions of the AOT concentration and temperature. The method involved adding water to the AOT solutions up to the turbidity point. The solubilizing capacity was found to be strongly dependent on the solvent chain length, increasing with decreasing chain length. For example, in octane solutions at 30° about 50 moles of water per mole AOT can be solubilized while only 5 moles can be solubilized in hexadecane solutions. The thermodynamic activity of the solubilized water was determined by a modified isopiestic method. These data indicated that the binding of water in all systems studied was essentially the same. A tentative theory based on the solvation of the micelle exterior is proposed to explain the two sets of data.

THE PHENOMENON of micellar solubilization in aqueous systems has recieved considerable attention, this subject having recently been covered extensively in a book by McBain and Hutchinson (1). The applications of the principles and concepts of solubilization in aqueous media to pharmacy have been demonstrated (2). The drug activity, release characteristics, and stability (3, 4) may be improved by a suitable choice and amount of these solubilizing agents.

There appears to have been little or no study of micellar solubilization of drugs in nonaqueous systems. It is not implied by this statement that the practice of pharmacy has not involved the use of this type of solubilization. However, the mechanisms of incorporation of water and highly polar drugs in hydrophobic vehicles by agents have not received much consideration. One should know whether the formulation is

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