[Chem. Pharm. Bull. 26(9)2832—2837(1978)]

UDC 615.357.011.4.014.2.074 : 547.295.72.09

Solubilization of Steroid Hormones by Polyoxyethylene Lauryl Ether

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(Received March 27, 1978)

As a further approach to an understanding of a relationship between the magnitudes of micellar solubilization and the hydrophobicities of solubilizates, steroid hormones (19 species) were selected to explore the meaning of the intercept b value. Two linear free energy relationships were established between the aqueous-polyoxyethylene lauryl ether micellar partition coefficient (K) and the partition coefficient ($P_{octanol}$) between aqueous solution and n-octanol. One consisted of the steroids carrying a fluorine atom at a carbon 9 and the other of those carrying no fluorine atom. The b value predicted that the fluorine steroids are solubilized in the outer layer of the mantle while the others are incorporated into rather inner position, which was consistent with the experimental results.

Keywords——solubilization by surfactant; steroid hormones; polyoxyethylene lauryl ether; solubility; partition coefficient between aqueous and micellar phases; partition coefficient between water and n-octanol; linear free energy relationship

Many of the steroid hormones are of low aqueous solubility, which has brought the necessity of the solubilization by macromolecular adjuvants such as surfactants.

The solubilization of steroids by surfactants has been reported by several investigators,[2–7] especially Sjöblom and co-workers.[5–7] They have concerned mainly with finding solubilizing capacities of various surfactants, discussing the slopes of solubility curves in terms of the steroid molecular structure and the effect of surfactants on a particular steroid. Also, some works attempted to elucidate the solubilization mechanism involved. However, because of complexities of steroid structure it seems difficult to describe quantitatively the effect of surfactants on the steroid solubilization.

Previously, on the solubilization of a set of benzoic acid derivatives by polyoxyethylene lauryl ether, the authors showed the linear free energy relationship between the aqueous-micellar partition coefficient and the aqueous-n-octanol partition coefficient.[8] The relationship was classified into four groups in terms of the intercept value, b, which might be related to the configurational difference of solubilizates incorporated in micelles.

The purpose of this study is to extend the above consideration to the micellar solubilization of steroids by polyoxyethylene lauryl ether (PLE) and to investigate further what the intercept b value implies.

Experimental

Materials——Polyoxyethylene (23) lauryl ether (PLE) was purified from commercially available Brij 35 as previously mentioned.[9] The steroid hormones used are listed in Table I, which were used without further purification. All other materials and solvents were of analytical grade.

1) Location: *Tanabe-dori 3, Mizuho-ku, Nagoya, 467, Japan.*
2) T. Nakagawa, *Yakugaku Zasshi*, **73**, 469 (1953).
3) D.E. Guttman, W.E. Hamlin, J.W. Shell, and J.G. Wagner, *J. Pharm. Sci.*, **50**, 305 (1961).
4) A.L. Thakker and N.A. Hall, *J. Pharm. Sci.*, **56**, 1121 (1967).
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7) L. Sjöblom, "Solvent Properties of Surfactant Solutions," edited by K. Shinoda, Marcel Dekker, New York, 1967, p. 189.
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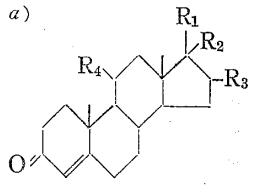
TABLE I. Steroids used in This Study

No.	Compound	Structure			
		R_1	R_2	R_3	R_4
1	Hydrocortisone[a,d]	$COCH_2OH$	α-OH	H	β-OH
2	Corticosterone[a,g]	$COCH_2OH$	H	H	β-OH
3	Deoxycorticosterone[a,h]	$COCH_2OH$	H	H	H
4	Cortisone[a,h]	$COCH_2OH$	α-OH	H	=O
5	Hydrocortisone acetate[a,g]	$COCH_2OCOCH_3$	α-OH	H	β-OH
6	Cortisone acetate[a,g]	$COCH_2OCOCH_3$	α-OH	H	=O
7	Deoxycorticosterone acetate[a,g]	$COCH_2OCOCH_3$	H	H	H
8	11α-Hydroxy progesterone[a,g]	$COCH_3$	H	H	α-OH
9	Progesterone[a,h]	$COCH_3$	H	H	H
10	Testosterone[a,g]	OH	H	H	H
11	Prednisolone[b,d]	$COCH_2OH$	α-OH	H	β-OH
12	Prednisolone acetate[b,e]	$COCH_2OCOCH_3$	α-OH	H	β-OH
13	Triamcinolone[c,f]	$COCH_2OH$	α-OH	α-OH	β-OH
14	Triamcinolone acetonide[c,f]	$COCH_2OH$	$-O>C<^{CH_3}_{CH_3}$		β-OH
15	Triamcinolone diacetate[c,f]	$COCH_2OCOCH_3$	α-OH	α-$OCOCH_3$	β-OH
16	Dexamethasone[c,d]	$COCH_2OH$	α-OH	α-CH_3	β-OH
17	Betamethasone[c,e]	$COCH_2OH$	α-OH	β-CH_3	β-OH
18	Dexamethasone acetate[c,h]	$COCH_2OCOCH_3$	α-OH	α-CH_3	β-OH
19	Betamethasone 17-valerate[c,e]	$COCH_2OH$	α-$OCOC_4H_9$	β-CH_3	β-OH

a)



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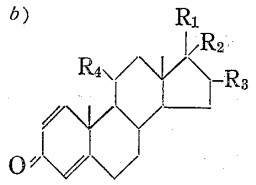
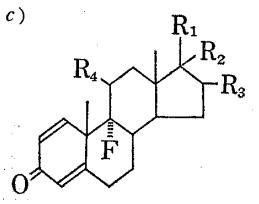


c)



d) Donated from Sankyo Co., Ltd.
e) Donated from Shionogi Pharmaceutical Co., Ltd.
f) Donated from Japan Lederle Co., Ltd.
g) Obtained from Tokyo Kasei Co., Ltd.
h) Obtained from Sigma Chemical Co., Ltd.

Solubility Measurements——The buffer solution used in the solubility and partition studies was 0.05 M phosphate buffer, pH 7.0. The ionic strength was adjusted to 0.1 by adding NaCl. An excess of steroid was added to aqueous solutions (10 ml) containing various concentrations of PLE. The solutions were shaken for 3 days at a constant temperature, 25±1°. After equilibration sample solutions were filtered through a Whatman No. 44 filter paper. Then, the samples were suitably diluted with 95% methanol and assayed spectrophotometrically.

Measurements of Partition Coefficients——Partition coefficients between aqueous solution and *n*-octanol were determined by the method of Fujita, *et al.*[10] 50—900 ml of aqueous solution containing steroids were added to 5—20 ml of *n*-octanol and the mixture was agitated vigorously for 30 minutes at 25±1°. The initial concentration of most of the steroids in aqueous phase was 1.0×10^{-4} M. For some sparingly soluble steroids the saturated solutions were used. After equilibration the aqueous phases were centrifuged for 30 minutes to obtain clear solutions and then assayed.

Determination of Partial Molar Volume of PLE——The density of PLE solutions was measured by a Lipkin-Davison type pycnometer. The calculated partial molar volume of PLE was 1.18 l/mol at 25±0.1°.

Results and Discussion

Solubilities in PLE solutions and partition coefficients between aqueous solution and *n*-octanol, $P_{octanol}$ have been determined for the 19 steroid hormones. Fig. 1 shows a typical solubility curve on hydrocortisone, in which the solubility curve is linear with respect to PLE concentration from 0.004 to 0.02 M and the intercept on the ordinate of the solubility curve

10) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

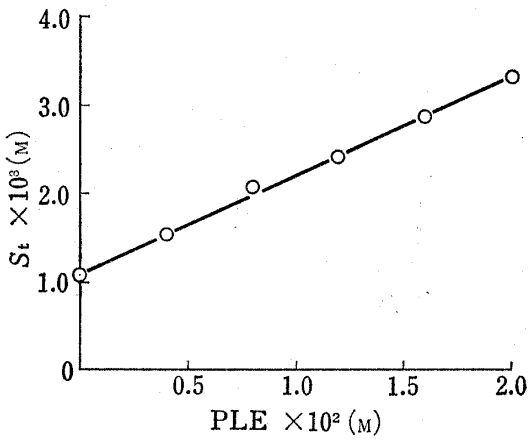


Fig. 1. Solubility of Hydrocortisone in Different Concentrations of Polyoxyethylene Lauryl Ether at pH 7.0 and 25±1°

coincides with the aqueous solubility. Similar results were obtained for the other steroid-PLE systems. In PLE solution, the total solubility, S_t, can be expressed as equation (1).

$$S_t = S_w (K-1) \cdot PMV \cdot [PLE] + S_w \qquad (1)$$

where S_w is the aqueous solubility of steroid and PMV is the partial molar volume of PLE. K is the partition coefficient between aqueous and micellar phases, which was defined by

$$K = \frac{S_m}{S_w} \qquad (2)$$

where S_m is the solubility in micellar phase.

Aqueous solubilities, S_w, the slopes of the solubility curves, R, aqueous-micellar partition coefficients, K, and partition coefficients between aqueous solution and *n*-octanol, $P_{octanol}$, are summarized in Table II. Partition coefficients between water and ether, P_{ether}, which were reported by Flynn[11] are also shown.

Table II. Solubilization Parameters for the Steroids and Partition Coefficients between Water and *n*-Octanol, $P_{octanol}$, and Water and Ether, P_{ether} at 25±1°

No.	Compound	S_w (M)	$R^{a)}$	K	$P_{octanol}$	$P_{ether}^{b)}$
1	Hydrocortisone	1.08×10^{-3}	0.115	97.9	35.7	1.60
2	Corticosterone	5.79×10^{-4}	0.131	187	86.5	4.52
3	Deoxycorticosterone	3.55×10^{-4}	0.157	399	798	52.0
4	Cortisone	5.32×10^{-4}	0.0388	66.7	26.2	1.40
5	Hydrocortisone acetate	4.58×10^{-5}	0.0116	229	154	26.0
6	Cortisone acetate	6.18×10^{-5}	0.0140	205	126	25.1
7	Deoxycorticosterone acetate	2.35×10^{-5}	0.0187	718	1190	95.5[c)]
8	11α-Hydroxy progesterone	1.53×10^{-4}	0.0459	271	227	35.5[c)]
9	Progesterone	3.79×10^{-5}	0.0559	1330	7410	604
10	Testosterone	8.26×10^{-5}	0.0579	633	1960	87.3
11	Prednisolone	6.54×10^{-4}	0.0794	110	41.4	1.13
12	Prednisolone acetate	4.22×10^{-5}	0.0131	281	250	21.1
13	Triamcinolone	2.07×10^{-4}	0.0219	96.3	10.8	0.757
14	Triamcinolone acetonide	4.95×10^{-5}	0.0312	569	205	14.6
15	Triamcinolone diacetate	7.41×10^{-5}	0.0245	299	83.7	—
16	Dexamethasone	2.58×10^{-4}	0.0721	253	67.8	3.87
17	Betamethasone	1.71×10^{-4}	0.0535	283	87.7	4.76
18	Dexamethasone acetate	1.25×10^{-5}	0.0134	967	806	70.8[c)]
19	Betamethasone 17-valerate	1.95×10^{-5}	0.0387	1790	3070	509

a) The slope of the solubility curve.
b) The data taken from ref. 11. The experimental was carried out at 23±1°.
c) The value estimated from the data in ref. 11.

Linear free energy relationships were observed between $P_{octanol}$ and K values as shown in Fig. 2. This indicates that the solubilization of the steroids by PLE micelles is directly dependent upon the lipophilicity of steroids. Similar observations were reported for a series of the benzoic acids[8,12] and the phenol derivatives.[13]

11) G.L. Flynn, *J. Pharm. Sci.*, **60**, 345 (1971).
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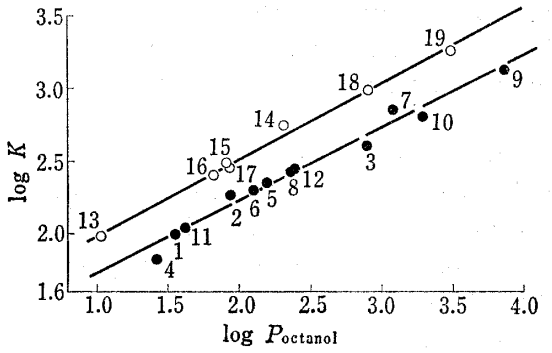

Fig. 2. Relationship between K and $P_{octanol}$

Numbers refer to the steroids listed in Table I.
Open circle represents the steroids carrying a fluorine atom at carbon 9.
Closed circle represents the steroids carrying no fluorine atom.

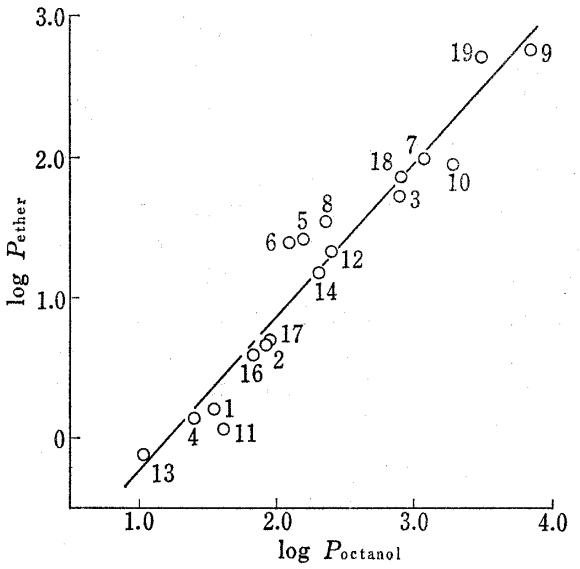

Fig. 3. Relationship between $P_{octanol}$ and P_{ether} of Steroids

Numbers refer to the steroids listed in Table I.

Furthermore, two linear relationships were obviously seen to run parallel with each other, which can be expressed by the following equations derived by the least-squares method.

	n	r	s	
$\log K = 0.494 \log P_{octanol} + 1.24$	12	0.986	0.066	(3)
	n	r	s	
$\log K = 0.523 \log P_{octanol} + 1.46$	7	0.995	0.044	(4)

where n is the number of points used in the regression, r is the correlation coefficient, and s is the standard deviation. A main structural difference among the steroids which constitute these two groups is whether or not they carry a fluorine atom at carbon 9. It should be noted that considering the magnitude of micellar solubilization as a function of $P_{octanol}$, all of the steroids carrying a fluorine atom are more solubilized than those carrying no fluorine atom. A similar observation was reported by Barry and El Eini[14] who studied the solubilization of hydrocortisone, dexamethasone, testosterone and progesterone by polyoxyethylene surfactants and showed the linear relationship between R_m values and logarithms of the partition coefficients (K_m) between aqueous and micellar phases. Dexamethane, which was only one fluorine compound in their study, is solubilized to higher degree than the expectation from the R_m value. With regard to the steroid hormones examined in the present study, all of the fluorine compounds clearly form a group, expressed by equation (4), with more positive b value than that of the other steroids.

In the previous report,[8] the authors applied the linear free energy relationship generally expressed by equation (5) to relate K to $P_{octanol}$ with regard to a set of benzoic acid derivatives.

$$\log K = a \log P_{octanol} + b \quad (5)$$

where a was used as a measure of micelle's sensitivity to changes in the lipophilicity of a selected set of solubilizates and b was a measure of the distribution of lipophilicity in the PLE micelles. The original meanings of a and b terms were given by Leo and Hansch,[15] but the b value might predict a measure of relative positions of the solute incorporated in the PLE micelles in addition when they are characterized by different b values with reference to

14) B.W. Barry and D.I.D. El Eini, *J. Pharm. Pharmacol.*, **28**, 210 (1976).
15) A. Leo and C. Hansch, *J. Org. Chem.*, **36**, 1539 (1971).

octanol water content. Benzoic acid derivatives carrying more electronegative group such as nitro and cyano compounds and dicarboxylic acids gave a positive b value, which corresponds to more hydrophilic environment for the solutes solubilized in the PLE micelles with reference to octanol. Similarly, it is assumed that these steroids favor more hydrophilic environment in the PLE micelles with reference to octanol and within two groups the fluorine steroids do more hydrophilic region than the others.

Meanwhile, it would be of interest to survey the relationship between P_{octanol} and P_{ether} for these steroids. Flynn[11] has measured the partition coefficients of a variety of steroid hormones between water and ether, and showed the establishment of the additive constitutive rule of the partition coefficient. As shown in Fig. 3, the relationship between P_{octanol} and P_{ether} is substantially linear, which can be expressed by

$$\log P_{\text{ether}} = 1.10 \log P_{\text{octanol}} - 1.34 \qquad n = 18 \quad r = 0.968 \quad s = 0.227 \qquad (6)$$

No deviation owing to the fluorine atom at carbon 9 could be observed. This indicates that the steroids can be considered as a set of compounds in respect to the partitioning in these two organic solvent systems, but again the analogous problem arises from the steroid partitioning to that in the partitioning of benzoic acid derivatives[8]: namely, a single linear correlation is sufficient to express the relationship between log P_{ether} and log P_{octanol} while not sufficient between log K and log P_{octanol}. As previously pointed out,[8] the hydration of PLE micelles varies from the surface toward the interior core. Such distribution of water content may offer to some extent alternative environments along with polyoxyethylene unit for the interaction behavior of steroids which have different nature in polarity. Unlike PLE micelles, the octanol phase offers a homogeneous one in water content.

Changes of ultraviolet absorption maximum have been frequently used to elucidate the site of incorporation of solubilizates.[3,4] The absorption maxima of six representative steroids were measured in the PLE solutions of different concentrations and in methanol, as summarized in Table III. It is evident that in cases of hydrocortisone, prednisolone acetate and progesterone which have no fluorine atom at carbon 9, their absorption maxima shift

Table III. Ultraviolet Absorption Maxima of Steroids in Various Concentrations of PLE and Methanol

Solvent	$\lambda_{\max}$ (nm)[a]		
	Hydrocortisone	Prednisolone acetate	Progesterone
PH 7.0 Buffer	247.5	247.5	247.5
PLE (1×10^{-3} M)	246.5	246.0	243.0
PLE (5×10^{-3} M)	245.0	245.0	242.0
PLE (1×10^{-2} M)	244.5	244.5	242.0
Methanol	242.0	242.5	241.5

Solvent	$\lambda_{\max}$ (nm)[a]		
	Triamcinolone	Dexamethasone	Betamethasone 17-valerate
PH 7.0 Buffer	241.0	241.0	241.0
PLE (1×10^{-3} M)	241.0	241.0	240.0
PLE (5×10^{-3} M)	241.0	241.0	240.0
PLE (1×10^{-2} M)	241.0	240.5	240.0
Methanol	238.0	238.5	238.5

a) The $\lambda_{\max}$ was measured by a Hitachi model 124 spectrophotometer at slit width 0.5 nm.

to shorter wavelengths as the concentration of PLE increases. On the other hand, the maxima of fluorine steroids, triamcinolone, dexamethasone and betamethasone 17-valerate were little affected by PLE. These results indicate that the latter steroids are solubilized at the outer layer of the polyoxyethylene portion of the micelles compared with the former ones. From the analysis of thermodynamic parameters, Barry and El Eini[14] reported that polar steroids are most probable to be incorporated in the outer layer of the polyoxyethylene mantle and less polar ones are located in the less hydrated region of the mantle. Thus, it is very likely that steroids are generally located in the palisade layer when solubilized and the site of solubilization depends upon their polarity.

These facts are consistent with what predicted by the difference of b values: namely the fluorine steroid group is solubilized at the outer layer of the polyoxyethylene mantle while other less polar one is located in the closer region to the interior core. Although the b value is the summation of free energy terms of unknown interactions but partitioning process for each group, further investigations of the b value should take account of a configurational factor of solutes solubilized by polyoxyethylene type surfactants. Such consideration may provide a single linear relationship for the cases mentioned above.

Acknowledgement The authors are most grateful for the generous supports of the materials to Japan Lederle Co., Ltd., Sankyo Co., Ltd., and Shionogi Pharmaceutical Co., Ltd.