Solubilization of Some Steroids in Aqueous Solutions of a Steroidal Nonionic Surfactant

ARVIND L. THAKKAR and PAUL B. KUEHN*

Abstract □ The solubility of testosterone, methyltestosterone, and testosterone propionate in aqueous solutions of unpurified and partially purified ethoxylated cholesterol was determined. The solubilizing capacities of both the surfactant samples were of the order: testosterone propionate > methyltestosterone > testosterone. The UV spectral characteristics of the three steroids in aqueous solutions of both the surfactant samples were similar to those in various polyethylene glycols. The spectral evidence suggests a mechanism of solubilization involving association of the steroid with the polyoxyethylene portion of the surfactant.

Keyphrases □ Steroid solubility—ethoxylated cholesterol □ Testosterone, methyltestosterone, testosterone propionate—solubility □ UV spectrophotometry—analysis

The solubilization of steroid hormones by aqueous solutions of nonionic surfactants has not been extensively investigated. Most previous reports (1–4) in this area have dealt with polysorbates or polyoxyethylene sorbitan esters of fatty acids. A study by Guttman *et al.* (5) dealt with the solubilization of three anti-inflammatory steroids by an ethoxylated tertiary octylphenol formaldehyde polymer. Sjöblom (4) has recently reviewed the applications of micellar solubilization to pharmaceutical systems.

The present report is concerned with the solubilization of three structurally related androgenic steroids, testosterone, testosterone propionate, and methyltestosterone, by aqueous solutions of a steroidal nonionic surfactant. This surfactant, described as ethoxylated cholesterol,[1] is prepared by ethoxylating a fraction of lanolin alcohols consisting principally of cholesterol. The structure of this surfactant may be summarized as R—O—R′, where R represents 24 polyoxyethylene units and R′ the cholesterol and associated lanolin alcohols. Steroid solubilization by this nonionic surfactant appeared interesting in view of its principally steroidal nonpolar portion. The surfactant was used both in the form in which it is commercially available and in a partially purified form. The partially purified sample did not contain any polyethylene glycols, the usual contaminants of commercial polyoxyethylene type surfactants. It was hoped that quantitative data on the solubilizing capacities of the two samples of the surfactant for the three steroids would provide some information about the effect of the steroid structure, and about the influence of small amounts of polyethylene glycols in the surfactant upon solubilization.

The alterations in the electronic spectra of solubilized molecules have assisted in the interpretation of the mechanism of solubilization (3, 5–7). In the case of steroids, it has been previously proposed that solubilization by ethoxylated surfactants probably takes place by association of the polar groups of the steroid with the hydrated polyoxyethylene chain of the surfactant (3). The present paper reports UV spectral studies which support the earlier proposal.

EXPERIMENTAL

Materials—Testosterone NF, methyltestosterone NF, testosterone propionate USP, commercial grade ethylene glycol, and polyethylene glycols 200, 300, and 400 were used without further purification. Ethoxylated cholesterol was used as received (hereafter referred to as the unpurified sample) and, also after partial purification by Weibull's method (8). The absence of polyethylene glycols in the partially purified sample was ascertained by the thin layer chromatographic procedure of Thakkar *et al.* (9). Distilled water and reagent grade solvents were used in the study.

Solubility Determinations—To a series of vials each containing 10 ml. of a surfactant solution of known concentration, sufficient steroid was added to ensure an excess at equilibrium. The vials were closed with aluminum foil-lined rubber stoppers, sealed, and were rotated in a water-bath shaker[2] at 30 ± 0.25° for 5–7 days. Preliminary experiments showed that this time was more than sufficient to establish equilibrium. The contents of the vials were filtered through 0.45-μ filters (Millipore) and analyzed spectrophotometrically[3] following appropriate dilution with reagent grade methanol and water such that the final methanol concentration of all the solutions was 50% v/v. Matched rectangular silica cells of 10-mm. pathlength were used. The amount of surfactant remaining in solution following multifold dilution with methanol-water was so small that it did not affect either the wavelength of maximum absorbance ($\lambda_{max.}$) or the absorbance of the steroid solutions. The $\lambda_{max.}$ and the molar absorptivity (ϵ), respectively, of the steroids in 50% (v/v) aqueous methanol were as follows: testosterone, $\lambda_{max.}$ = 245 mμ, $\epsilon = 16.2 \times 10^3$; methyltestosterone, $\lambda_{max.}$ = 244 mμ, $\epsilon = 16.6 \times 10^3$; testosterone propionate, $\lambda_{max.}$ = 244 mμ, $\epsilon = 16.8 \times 10^3$. The applicability of Beer's law was confirmed in all cases.

Spectral Studies—Spectral recordings of the steroids in various concentrations of ethoxylated cholesterol were made with undiluted, saturated aqueous solutions. The blanks used were the appropriate ethoxylated cholesterol solutions. Absorbance cells of various light path lengths, ranging from 0.1 to 50 mm., were used for this purpose. When recording the spectra in pure solvents, the steroid concentration was adjusted to give suitable spectra. All the spectra were recorded in duplicate on a spectrophotometer[3] (Cary model 15) at room temperature.

RESULTS AND DISCUSSION

Solubility Determinations—Figures 1 and 2 show the solubilization of the three steroids in aqueous solutions of the unpurified and the partially purified surfactant, respectively. All the plots are linear, indicating a direct relationship between the quantity of steroid solubilized and the concentration of the surfactant. No attempt was made to determine the critical micelle concentrations of the two samples. It may be seen from Figs. 1 and 2 that the solubilizing capacities of both the samples are of the order: testosterone propionate > methyltestosterone > testosterone. The

[1] Marketed as Solulan C-24 by American Cholesterol Products, Inc., Edison, N. J.

[2] Model G77, New Brunswick Scientific Corp., New Brunswick, N. J.

[3] A Cary model 15 spectrophotometer.

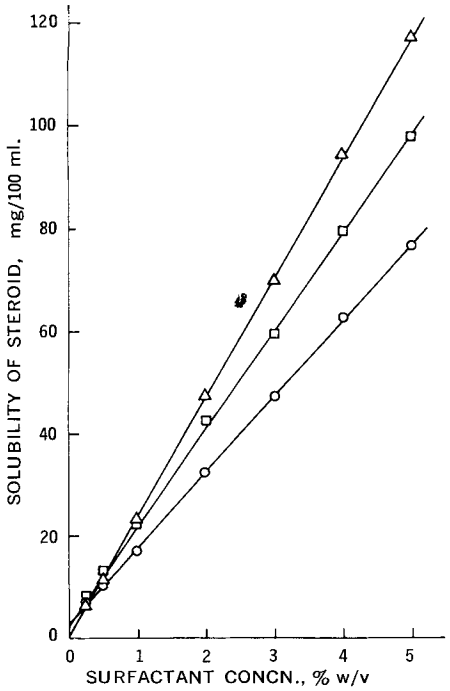

Figure 1—*Solubility of steroids in aqueous solutions of unpurified ethoxylated cholesterol; testosterone* (○), *methyltestosterone* (□), *testosterone propionate* (△). *The intercept values are the result of extrapolation of data points when the systems contain the surfactant. These values do not correspond to the water solubility values.*

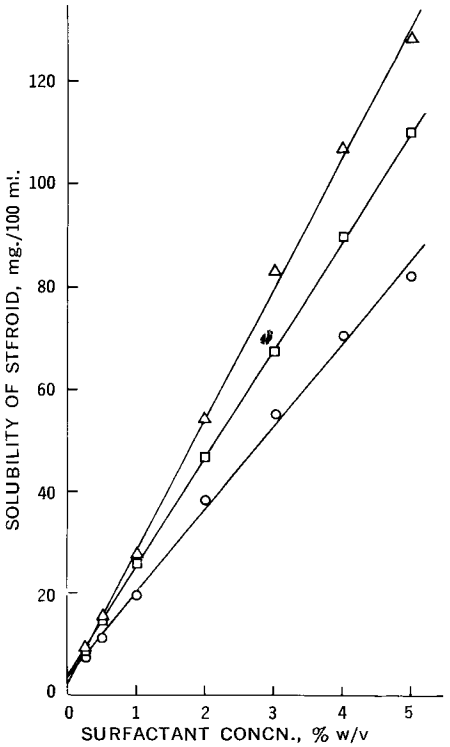

Figure 2—*Solubility of steroids in aqueous solutions of partially purified ethoxylated cholesterol: testosterone* (○), *methyltestosterone* (□), *testosterone propionate* (△). *The intercept values are the result of extrapolation of data points when the systems contain the surfactant. These values do not correspond to the water solubility values.*

numerical values of the solubilizing capacities, calculated from the linear plots, are summarized in Table I.

The values of solubilizing capacities are not presented in molar terms in view of the heterogeneity of the surfactant. Although the molecular weights of the steroids increase in the order testosterone < methyltestosterone < testosterone propionate, the values of solubilizing capacities in terms of moles steroid per gram surfactant remain in the same order as the values in terms of milligrams steroid per gram surfactant. Guttman *et al.* (5), in their work on the solubilization of three anti-inflammatory steroids, observed that the greater the aqueous solubility of a steroid the greater its ability to become solubilized. In the present work, the aqueous solubilities of the steroids are in the order: methyltestosterone > testosterone > testosterone propionate, but their ability to become solubilized in both the samples of the surfactants are in the order: testosterone propionate > methyltestosterone > testosterone. Thus it appears that the observation of Guttman *et al.* may not be a general one. The results of the present work bear out, however, the generalization by Sjöblom (4) that, for androgenic steroids, the introduction of a methyl group at C-17 and esterification of the hydroxyl group at this position result in increased solubilization.

Another observation that may be made from the numerical values of the solubilizing capacities is that the partially purified sample of the surfactant is a more efficient solubilizer than the unpurified sample which contains small amounts of polyethylene glycols. This observation may be explained as being due to the fact that removal of the polyethylene glycols results in a sample richer in the more efficient ethoxylated cholesterol, the solubilizing surfactant.

It is interesting to note also that the order of magnitude of the solubilizing capacities of this surfactant is comparable to those of other polyoxyethylene type surfactants (3, 4). Although the hydrocarbon portion of the surfactant under consideration consists

Table I—Solubilizing Capacity of Ethoxylated Cholesterol for Steroids at 30°.

Steroid (mol. wt.)	Solubility in Water, mg./100 ml.	Solubilizing Capacity of Ethoxylated Cholesterol, mg. steroid/g. surfactant	
		Unpurified	Partially Purified
Testosterone (288.41)	2.56	14.80	16.19
Methyltestosterone (302.44)	3.08	18.89	21.21
Testosterone propionate (344.48)	0.21	23.45	25.62

Table II—Wavelength of Maximum Absorbance of Steroids in Selected Solvents and in Aqueous Solutions of Ethoxylated Cholesterol.

Solvent	Wavelength of Maximum Absorbance, mμ		
	Testosterone	Methyl-testosterone	Testosterone Propionate
Methanol	240	240	240
50% methanol (v/v)	245	244	244
Ethylene glycol	243	243	243
Polyethylene glycol 200	239	238	238
Polyethylene glycol 300	238	238	237
Polyethylene glycol 400	238	238	238
Ethoxylated cholesterol (unpurified), % w/v			
0.25	242	242	240
0.50	241	241	240
1.00	241	240	240
2.00	239	239	239
3.00	239	239	239
4.00	239	239	239
5.00	239	239	239
Ethoxylated cholesterol (partially purified), % w/v			
0.25	243	242	239
0.50	242	241	240
1.00	240	241	240
2.00	239	239	239
3.00	239	239	239
4.00	239	239	239
5.00	239	239	239

principally of the steroid cholesterol, the solubility of the steroids examined is not of a magnitude that would lead one to propose a "like dissolves like" situation. From the solubilizing capacities which are comparable to those of other polyoxyethylene-type surfactants and from the spectral evidence, discussed in the following section, it seems likely that solubilization of the steroids takes place by association with the polyoxyethylene exterior of the surfactant micelles.

Spectral Studies—The wavelengths of maximum absorbance ($\lambda_{max.}$) of the three steroids in a few selected solvents and in various aqueous solutions of both unpurified and partially purified ethoxylated cholesterol are presented in Table II.

It may be seen that with an increase in the surfactant concentration up to 2% (w/v) there is a progressive shift toward lower wavelengths. This is in good agreement with the results of a previous study dealing with testosterone and polysorbates (3). At surfactant concentrations above 2% (w/v) the $\lambda_{max.}$ becomes essentially constant. These constant values of $\lambda_{max.}$ compare well with the $\lambda_{max.}$'s of the three steroids in polyethylene glycols 200, 300, and 400. The fact that the surfactant solutions were aqueous might account for the difference of 1 mμ. From these results it would thus appear that the polarity of the environment offered by aqueous ethoxylated cholesterol solutions to the Δ^4-3-keto chromophore of the three steroids is similar to that offered by polyethylene glycols. This observation further substantiates the previous proposal that solubilization of steroids by nonionic ethoxylated surfactants involves association of the steroid with the polyoxyethylene portion of the surfactant.

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Chemotherapy of Tuberculosis, Part IX: Synthesis and Screening of New Thiazolyl Thiocarbanilides

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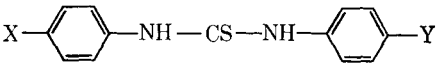
Abstract □ Nearly eighty substituted thiocarbanilides—*viz.*, *p*-(2-thiazolyl)-, *p*-(4-thiazolyl)- and *p*-(5-thiazolyl)-*p*-alkoxy-thiocarbanilides, and *p*-*p'*-bis(4-thiazolyl)-thiocarbanilides, along with a few thiazolyl thiocarbanilides having halogens on the phenyl ring containing the *p'*-alkoxy group, have been synthesized and studied for *in vitro* antitubercular activity. Also described are over 40 new substituted thiazoles prepared as intermediates. *p*-(4-Thiazolyl)-*p'*-alkoxy-thiocarbanilides in general showed the maximum *in vitro* tuberculostatic activity in the present study. *p*-(2,5-Dimethyl-4-thiazolyl)-*p'*-*n*-propoxythiocarbanilide had the same *in vitro* tuberculostatic activity as isonicotinic acid hydrazide (INH) (0.04 mcg./ml.) but did not produce tuberculostatic serum concentration at the same oral dose level as INH.

Keyphrases □ Tuberculosis chromotherapeutic agents □ Thiazolyl thiocarbanilides—synthesis □ Antitubercular activity—thiazolyl thiocarbanilides

Since the discovery of antimycobacterial activity of thioureas (1) numerous publications have appeared showing the pronounced activity of thiocarbanilides both *in vitro* as well as in experimental animals coupled with only a low rate of development of resistance (2–5). Among the most potent compounds of this class are the thiocarbanilides bearing alkoxy groups in *para* positions (6–8). Some of these—*viz.*, 4,4'-diisoamyloxythiocarbanilide (9–11) (I), 4,4'-diethoxythiocarbanilide (12–15) (II) and 4-butoxy-4'-dimethylaminothiocarbanilide (16, 17) (III), have been used clinically for the treatment of tuberculosis and leprosy.



I, X = Y = isoamyloxy
II, X = Y = ethoxy
III, X = butoxy and Y = $N(CH_3)_2$
IV, X = 2-pyridyl and Y = isobutoxy

Doub *et al.* (18) have extended the series of alkoxythiocarbanilides by incorporating a heterocyclic ring—*viz.*, pyridyl as the substituent, and observed potentiation of antimycobacterial activity. One of the compounds from their series called thiocarbanidine (IV) showed high degree of antitubercular action in mice and guinea pigs (19). However, in clinical trials it was not effective, possibly due to poor absorption (20).

METHODS

Since thiazole and pyridine are isosteric and several thiazole derivatives are potent antibacterial agents, it seemed worthwhile to synthesize and study thiocarbanilides having thiazoles as substituents. Accordingly, the synthesis of *p*-(2-thiazolyl)-, *p*-(4-thiazolyl)-, and *p*-(5-thiazolyl)-, *p'*-alkoxythiocarbanilides was undertaken. A few thiazolyl thiocarbanilides having halogens on the phenyl ring