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Solubilization of Some Steroid Hormones in Aqueous Solutions of Bile Salts

ARVIND L. THAKKAR

Abstract □ Solubilities of testosterone propionate, methyltestosterone, and 19-nortestosterone in aqueous sodium cholate and deoxycholate were determined. Solubilizing capacity values show that deoxycholate is a better solubilizer than cholate and that both bile salts solubilize more 19-nortestosterone than other testosterone derivatives. A possible mode of solubilization is discussed.

Keyphrases □ Steroid hormones—solubilization, aqueous solutions, bile salts □ Testosterone derivatives—solubilizing effect of sodium cholate, deoxycholate □ UV spectrophotometry—analysis

The ability of bile salts to enhance the water solubility of steroid hormones was noted as early as 1944 by Cantarow *et al.* (1). Since that time, micellar solubilization of steroids has been studied extensively by Ekwall (2) and Sjöblom (3). However, bile salt solubilization of hormonal steroids appears not to have been examined in detail. This study was undertaken to examine the solubilization of testosterone propionate, methyltestosterone, and 19-nortestosterone by the anionic surfactants, sodium cholate and sodium deoxycholate, and is part of a larger study of the solubilization of steroidal hormones by steroidal surfactants. A recent report from this laboratory (4) dealt with the solubilization of some androgenic steroids by ethoxylated cholesterol, a nonionic surfactant.

EXPERIMENTAL

Materials—Sodium cholate,[1] sodium deoxycholate,[1] methyltestosterone NF, testosterone propionate USP, and 19-nortestosterone[2] were used as received. Moisture contents of the bile salts, determined by drying overnight *in vacuo* at 110°, were taken into consideration when recording their weights. Bile salt solutions, prepared with distilled water, were not buffered; their pH ranged from 7.0 to 7.8 for cholate and from 7.2 to 8.6 for deoxycholate.

Solubility Determinations—Solubilities were determined by equilibration of several concentrations of bile salt solutions with the steroids, followed by spectrophotometric analyses of suitably diluted aliquots, as described previously (4). For solutions in which enhancement of steroid solubility was minimal, dilution with 50% (v/v) methanol was still necessary to lower the bile salt concentration to a point where it would not interfere with the UV spectrophotometric analytical procedure. In such cases, cells of 5-cm. pathlength were used. 19-Nortestosterone, which was not included in the previous study, has maximum absorbance in 50% (v/v) aqueous methanol at 244 mμ, with a molar absorption coefficient of 17.3×10^3.

[1] Special enzyme grade, Mann Research Laboratories, Inc., New York, N. Y.
[2] Purchased from Organon, Inc., West Orange, N. J.

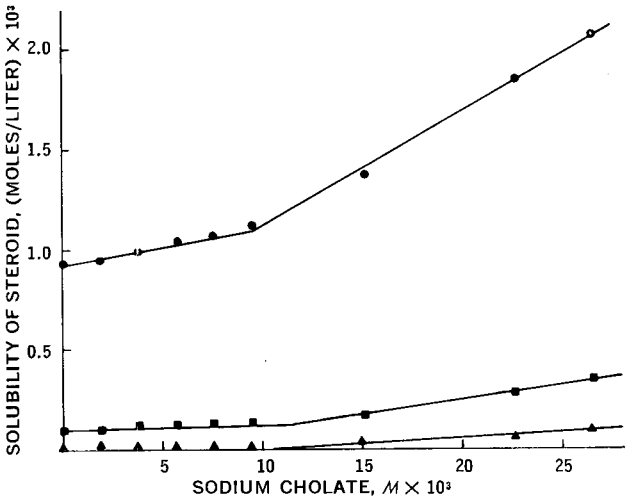

Figure 1—*Solubility of steroids in aqueous solutions of sodium cholate at 30°. Key:* ●, *19-nortestosterone;* ■, *methyltestosterone; and* ▲, *testosterone propionate.*

RESULTS AND DISCUSSION

Figures 1 and 2 show the relationship between solubility of the steroid solubilizates and the concentration of sodium cholate and sodium deoxycholate, respectively. At low concentrations of bile salts, only a marginal change in steroid solubility is observed. After these initial stages, up to $\sim 1.0 \times 10^{-2}$ *M* for sodium cholate and $\sim 6.0 \times 10^{-3}$ *M* for sodium deoxycholate, steroid solubility increases linearly with bile salt concentration. This behavior conforms well to the general features of micellar solubilization, but it is at variance with the report of Lach and Pauli (5) who found that the solubility of testosterone increased at a higher rate below the apparent critical micelle concentration (CMC) of deoxycholate than above it. In a comprehensive paper dealing with the solubiliza-

Table I—Solubilizing Capacity of Bile Salts for Steroids at 30°

Steroids	Solubilizing Capacity,[a] —(Mole Steroid/Mole Bile Salt) × 10^3— Sodium Cholate	Sodium Deoxycholate	Reciprocal of Solubilizing Capacity, —Mole Bile Salt/Mole Steroid— Sodium Cholate	Sodium Deoxycholate
Testosterone propionate	6.61	34.19	151	29
Methyltestosterone	16.20	30.49	62	32
19-Nortestosterone	62.11	163.2	16	6

[a] These values are calculated by the method of least squares from the linear plots, above the apparent CMC, in Figs. 1 and 2. Correlation coefficients of the linear relationships were higher than 0.90 in all the cases.

tion of bile acids, cholesterol, and several other lipoidal substances by bile salts, Ekwall (2) has shown that micelle formation in aqueous bile salt solutions takes place in a number of discrete stages. In the present work, the bile salt concentration ranges in which the rather sharp increases in steroid solubility commence are in reasonably good agreement with the "first concentration limits," 1.3–1.8 × 10^{-2} *M* for cholate and 5–6 × 10^{-3} *M* for deoxycholate, reported by Ekwall (2), and also with the CMC values reported by Bates *et al.* (6), using other solubilizates.

The solubilizing capacities of the bile salts for the steroids examined are listed in Table I. It is evident from Table I that deoxycholate is a better solubilizer than cholate. This is in agreement with previous findings (2, 6) with other solubilizates. Table I also shows that both bile salts solubilize 19-nortestosterone to the greatest extent. Water solubilities (in moles/liter at 30°) of the steroids examined are in the following order: 19-nortestosterone (9.59 × 10^{-4}) > methyltestosterone (1.02 × 10^{-4}) > testosterone propionate (0.06 × 10^{-4}). The ability of 19-nortestosterone to become solubilized to the greatest extent may be due to its inherently high affinity for water. Sjöblom (3) has also reported similar data for solubilization of 19-nortestosterone in aqueous solutions of polysorbate 20. In the concentration range examined, both of the bile salts solubilized more methyltestosterone than testosterone propionate. However, as Table I shows, the solubilizing capacity of deoxycholate for testosterone propionate is greater than that for methyltestosterone. These results and those of a previous study (4) thus show that there is no apparent relationship between the water solubility of a steroid and its property of becoming micellarly solubilized.

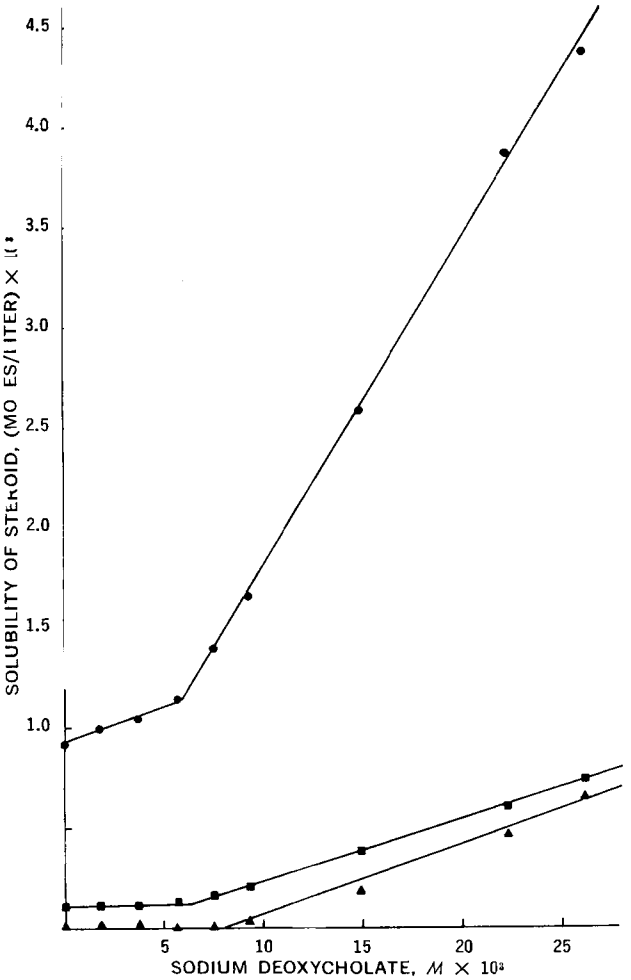

Figure 2—*Solubility of steroids in aqueous solutions of sodium deoxycholate at 30°. Key:* ●, *19-nortestosterone;* ■, *methyltestosterone; and* ▲, *testosterone propionate.*

A closer examination of Figs. 1 and 2 shows that significant increases in the solubility of 19-nortestosterone take place before the apparent CMC's of the bile salts are exceeded. This may be due to formation of mixed micelles of the bile salt and 19-nortestosterone, or it may be that the bile salts increase the solubility of 19-nortestosterone by cosolvation effects. It is interesting that 19-nortestosterone, a steroid molecule without one angular methyl group, is considerably more hydrophilic than other testosterone derivatives.

Recently, Small *et al.* (7, 8) proposed that cholate and deoxycholate micelles are formed by hydrophobic association of the hydrocarbon backs of the rigid steroid nuclei in such a way that the hydrophilic sides, containing the hydroxyl groups and the negatively charged ionic groups, are exposed to water. In the absence of excess counterion concentration, as in this study, these micelles remain small in comparison with classical detergent micelles, their aggregation number ranging from 2 to 9 (7). Upon examining space-filling (Stuart–Breiglib) molecular models, Small has suggested that up to 9 or 10 cholate molecules may associate hydrophobically.

Small and Admirand (9) have shown that at 30° about 6–9 moles of bile salt will solubilize 1 mole of lithocholate. The reciprocal solubilizing capacity values for 19-nortestosterone determined in this study are similar to those for lithocholate. There is a structural similarity between lithocholate and 19-nortestosterone; both have polar groups at each end of their molecules. These observations suggest that the mechanism responsible for the solubilization of these two steroids may be similar. Working with space-filling molecular models (Corey–Pauling–Koltun), it is possible to surround one molecule of either 19-nortestosterone or lithocholate by bile salt molecules in such a way that the hydrophobic parts of the molecules are in contact and the polar functions are exposed to the surroundings. The number of bile salt molecules required for this purpose is consistent with the reciprocal solubilizing capacity values.

For methyltestosterone and testosterone propionate, the solubility data do not permit postulation of the solubilization mechanism. It seems reasonable, however, to expect that the effective average micelle size and volume would change when these solubilizates are incorporated, regardless of the mechanism, in micellar solutions of bile salts. Light scattering, viscosity, and ultracentrifugation studies would reflect such changes and might, therefore, provide useful clues for understanding these solubilized systems.

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Alkaloids of Tylophora I: Isolation of Six New Alkaloids

KOPPAKA V. RAO*, RICHARD WILSON, and BERNICE CUMMINGS

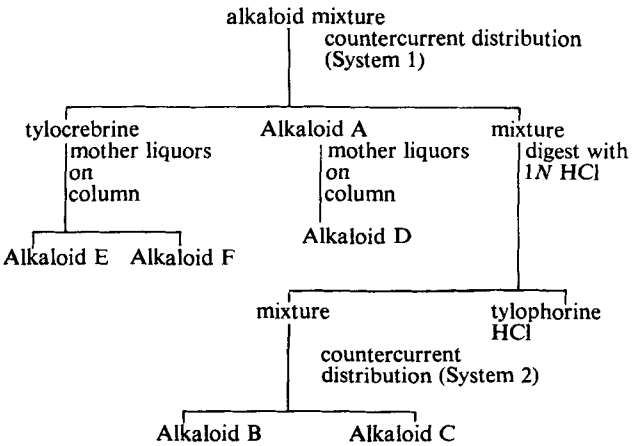
Abstract □ *Tylophora crebriflora* (N. O. Asclepiadaceae) is a slender vine found chiefly in northeastern Australia. In a detailed examination of the plant, six alkaloids have been isolated which have not previously been shown to be present in this genus. The methods for their isolation and their physical characteristics are described.

Keyphrases □ *Tylophora crebriflora*—separation, isolation, physical properties, six alkaloids □ IR spectrophotometry—structure, identification □ UV spectrophotometry—structure, identification

In a series of papers during the 1950's, Govindachari *et al.* (1–5) described the isolation, structure, and synthesis of two alkaloids, tylophorine and tylophorinine, present in the Indian plant *Tylophora indica*. These alkaloids are built up of a dibenzo[*f,h*]-pyrrolo[1,2*b*]-isoquinoline skeleton. From a related Australian plant, *Tylophora crebriflora*, Gellert *et al.* (6) described the isolation of a third member named tylocrebrine, together with a minor amount of tylophorine. The two were shown to be isomeric, differing in the arrangement of the methoxyl groups. During routine screening by the Cancer Chemotherapy National Service Center (CCNSC), it was observed that tylocrebrine showed significant antileukemic activity. At the request of CCNSC to provide tylocrebrine for possible clinical trials, these studies were initiated.

EXPERIMENTAL

The dried plant, *Tylophora crebriflora*, was obtained from Australia.[1] The total alkaloid fraction could be readily isolated by the following steps: (*a*) extraction with 1% methanolic acetic acid; (*b*) concentration; (*c*) partition between ethyl acetate and 0.2 *N* HCl (aq.); and (*d*) extraction of the aqueous layer at pH 9–10 with chloroform. The crude mixture of alkaloids represented a yield of approximately 0.15%.



Scheme I—Separation of the alkaloids of Tylophora crebriflora. *System 1: 3% aqueous acetic acid–chloroform–ethyl acetate (10:7:3) System 2: 3% aqueous acetic acid–chloroform–*n*-butanol (5:4:1)*

The mixture was separated into its components by the use of countercurrent distribution and chromatography on a commercial adsorbent,[2] as indicated in Scheme I. In addition to the two known members, tylocrebrine and tylophorine, the extracts yielded six new alkaloids.

Tylocrebrine and Alkaloid A are the major components, each being present to the extent of about 40% of the total. Next in abundance are tylophorine and Alkaloids B and C, which account for approximately 4–5% each. The rest is made up of the other three members, Alkaloids D, E, and F.

The analytical data and physical properties of the new members are shown in Tables I and II. In general, Alkaloids A–E show

[1] The plant material used in this study was collected, identified, and supplied by the Department of Forestry of Queensland, Brisbane, Queensland, Australia, in 1964. (A voucher specimen was preserved at Chas. Pfizer & Co., Inc., Maywood, N. J.)

[2] Florisil, Floridin Co., Pittsburgh, Pa.