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Association of Deoxycholic Acid in Organic Solvents

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Abstract □ The distribution of deoxycholic acid (I) between aqueous buffer and an organic phase consisting of isooctane–1-octanol (70:30, v/v) (System A) or isooctane–chloroform (80:20, v/v) (System B) was studied. The distribution isotherms suggested that I associates strongly in the organic Systems A and B unlike in pure 1-octanol. Therefore, a previous model, describing distribution of bile salts between 1-octanol and aqueous buffer, was modified to include association of I in the organic phases to describe distribution behavior. The treatment suggested that I exists as monomer and dimer in System A with a dimerization constant of 820 M^{-1}. A model consisting of monomer–tetramer–hexamer in the organic phase best describes the data for System B. The data support the view that association in the organic phase is due to hydrogen bonding between bile acid molecules.

Keyphrases □ Deoxycholic acid—association in organic solvents □ Bile salts—association of deoxycholic acid in organic solvents □ Partition coefficient—association of deoxycholic acid in organic solvents

In the preceding paper (1), the distribution behavior of bile salts between aqueous buffer and 1-octanol was reported. By vapor pressure osmometry data, it was shown that bile acids exist primarily as monomers in 1-octanol[1]. A recent paper (2) reported strong association of bile acid esters in nonaqueous solvents such as carbon tetrachloride and chloroform using vapor pressure osmometry. The association in carbon tetrachloride is much stronger than in chloroform. The relatively low association in chloroform was attributed to the hydrogen bonding ability of chloroform. This probably also explains the monomeric state of bile acids in 1-octanol. If this were true, then modifying the hydrogen bonding solvent (*e.g.*, 1-octanol or chloroform) by addition of a nonhydrogen bonding nonpolar solvent like isooctane should result in an increased association of bile acid in such organic systems. In the present report the state of association of deoxycholic acid (I) is examined in two such modified solvent systems: isooctane–1-octanol (70:30) (System A) and isooctane–chloroform (80:20) (System B) using the distribution behavior of I between the organic phase and aqueous buffer. Partition equilibria were used to study the association of solutes in the organic phase on the basis of the previously determined association pattern in the aqueous phase.

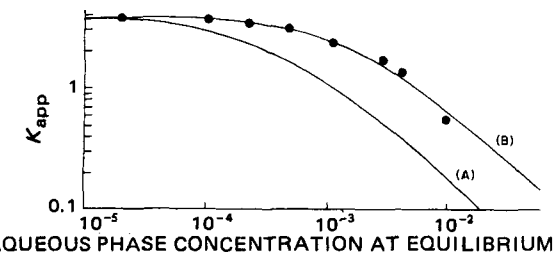

Figure 1—*Distribution isotherm for sodium deoxycholate at 25°. Curve A is based on the model in Scheme IA. Curve B is calculated on the basis of the model in Scheme IB. Key: (●) experimental data.*

[1] It was found that bile salts exist primarily in the acidic form in 1-octanol and in the anionic form in the aqueous phase under the experimental conditions of this investigation.

EXPERIMENTAL

Materials—The purity of sodium deoxycholate[2] (>99%) was confirmed by TLC and titration with perchloric acid in glacial acetic acid [^{3}H,(G)]deoxycholic acid in ethanol, specific activity 4 Ci/mmole[3], radiochemical purity 98%; 1-octanol[4]; 2,2,4-trimethylpentane (isooctane)[5] were used as obtained. The same scintillation cocktail[6] was used for both aqueous and organic samples.

Experimental details of partition coefficient measurements are given in the preceding paper (1).

RESULTS AND DISCUSSION

The experimental results obtained for the partition of I between 0.02 *M* tromethamine buffer (pH 8) and two mixed organic phases are summarized in Figs. 1 and 2. Here, the partition coefficient defined as the ratio of the bile salt (and acid) concentration in the organic phase to the concentration of bile salt (and acid) in the aqueous phase, is plotted as a function of the equilibrium total bile salt and acid concentration (moles/liter) in the aqueous phase.

The nature of the distribution isotherm obtained for System A is similar to one obtained for 1-octanol in the previous study (1). The distribution of I between 1-octanol and aqueous buffer was explained on the basis of reversible association of I in the aqueous phase and no association in the organic phase as shown in Scheme IA. In the present case, since

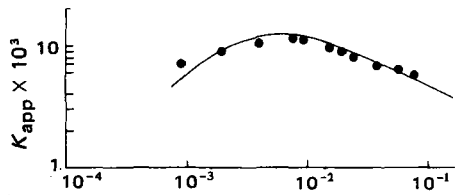

Figure 2—*Distribution isotherm for sodium deoxycholate at 25°. Organic phase isooctane–octanol (80:20, v/v). Aqueous phase 0.02 M tromethamine buffer (pH 8.0). Solid line represents distribution isotherm calculated on the basis of the 1-4-6 association model in organic phase. Key: (●) experimental data.*

[2] Calbiochem, LaJolla, Calif.
[3] New England Nuclear, Boston, Mass.
[4] Fisher Scientific Co., Pittsburgh, Pa.
[5] Eastman Kodak Co., Rochester, N.Y.
[6] Aquasol, New England Nuclear, Boston, Mass.

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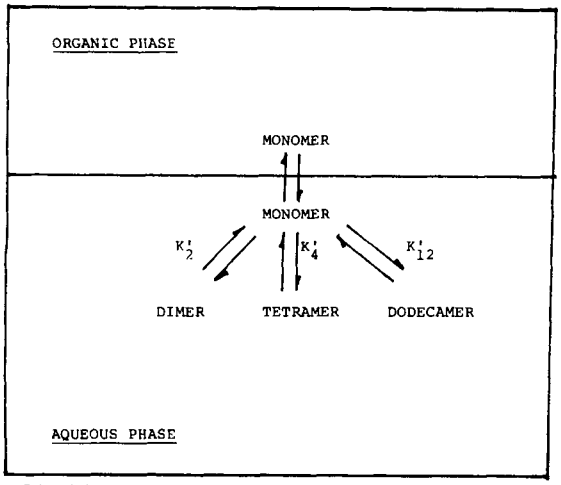


Scheme IA—The possible equilibria involved in the partitioning of bile salts between 1-octanol and aqueous solution.

the aqueous phase is not changed[7], association constants obtained previously are expected to remain constant and should describe the total concentration of I in the aqueous phase according to:

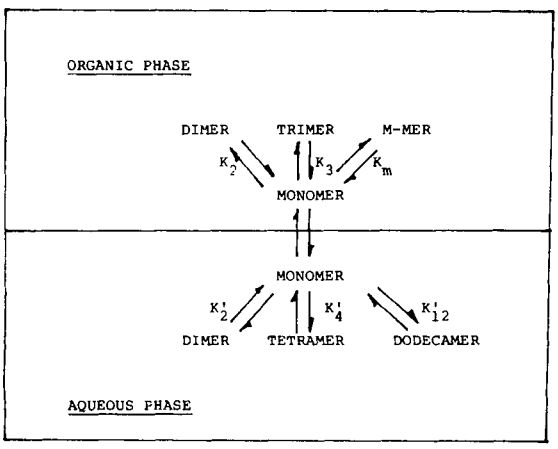
$$[C]_{aq} = [M]_{aq} + 2K'_2[M]^2_{aq} + 4K'_4[M]^4_{aq} + 12K'_{12}[M]^{12}_{aq} \quad \text{(Eq. 1)}$$

where, $[C]_{aq}$ is the total concentration of bile salt in the aqueous phase in moles per liter, $[M]_{aq}$ is the monomer concentration of bile salt in the aqueous phase[8] in moles per liter, and K'_2, K'_4, and K'_{12} represent association constants in the aqueous phase where subscripts refer to association numbers.

If the assumption that I does not associate in the organic solvent is true for System A, then the model shown in Scheme IA with the above association constants should describe the distribution isotherm. Calculations based on this model generated Curve A in Fig. 1. It is apparent from Fig. 1 that experimental points have significant positive deviation from Curve A. This positive deviation of partition coefficients from expected values based on the previous model suggests that there is much more solute in the organic phase than expected by assuming only monomers. This may be explained by considering reversible association of solute in the organic phase as shown in Scheme IB. This model assumes that only the monomer is transported from one phase to another, and higher aggregates are formed by the association of monomers in both phases. Thus, it can be shown that the concentration of bile salt and acid in the organic phase may be written as:

$$[C]_{org} = [M]_{org} + 2K_2[M]^2_{org} + 3K_3[M]^3_{org} + \ldots \quad \text{(Eq. 2)}$$

where, $[C]_{org}$ is the total concentration of I in the organic phase in moles per liter, $[M]_{org}$ is the monomer concentration of I in the organic phase



Scheme IB—The possible equilibria involved in the partitioning of bile salts between aqueous solution and Solvent Systems A or B.

[7] This assumes that the two organic phases either do not modify the aqueous phase or modify it to the same extent.

[8] This may be acid, anion, or a mixture of both.

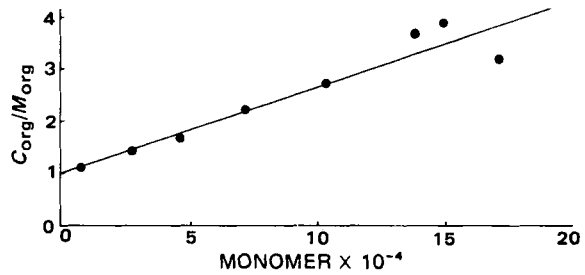


Figure 3—*A plot testing the possible occurrence of dimers in organic phase System A according to Eq. 6.*

in moles per liter, and K_2, K_3 . . . are association constants for I in the organic phase. Also:

$$K_{app} = [C]_{org}/[C]_{aq} \quad \text{(Eq. 3)}$$

$$K^0_{app} = [M]_{org}/[M]_{aq} = \lim_{c \to 0} K_{app} \quad \text{(Eq. 4)}$$

where, K_{app} is the apparent partition coefficient at any given concentration, and K^0_{app} is the apparent partition coefficient at infinite dilution.

By using carrier-free radiolabeled samples, K^0_{app} was obtained at concentration $<10^{-10}$ *M*. From Eq. 1 $[M]_{aq}$ was computed where $[C]_{aq}$ was known experimentally and aqueous association constants were used from previous studies (1). Using this computed value of $[M]_{aq}$ in Eq. 4, $[M]_{org}$ was calculated.

If the monomer and dimer are the primary species in the organic system, then Eq. 2 reduces to:

$$[C]_{org} = [M]_{org} + 2K_2[M]^2_{org} \quad \text{(Eq. 5)}$$

Rearranging gives:

$$([C]_{org}/[M]_{org}) = 1 + 2K_2[M]_{org} \quad \text{(Eq. 6)}$$

Thus, a plot of $([C]_{org}/[M]_{org})$ *versus* $[M]_{org}$ should give a straight line with unit intercept and slope = $2K_2$. This relationship can be seen in Fig. 3 for System A. It gives the estimate of $K_2 = 820\ M^{-1}$. Using this value of K_2 and Eqs. 1, 3, and 5, the distribution isotherm was generated as shown by Curve B in Fig. 1. It is seen that the curve calculated on the basis of the proposed model is in close agreement with the experimental data.

The distribution isotherm for System B exhibits a maximum (Fig. 2). This may be anticipated if the association in the organic phase is stronger than that in the aqueous phase until the CMC (critical micelle concentration) in the aqueous phase (CMC_{aq}) is reached. Under this circumstance the partition coefficient will increase until the CMC_{aq} is reached, the association in the aqueous phase being greater, the partition coefficient would be expected to decrease. Thus, a maximum in the isotherm may occur approximately in the concentration region corresponding to the CMC_{aq}. The value of the CMC thus estimated from this study is $\sim 7 \times 10^{-3}$ *M*, which compares reasonably well with literature values of 5–6 $\times 10^{-3}$ *M* (3–6).

A monomer–dimer treatment similar to the one used for System A did not yield a straight line relationship for System B. Instead, a positive

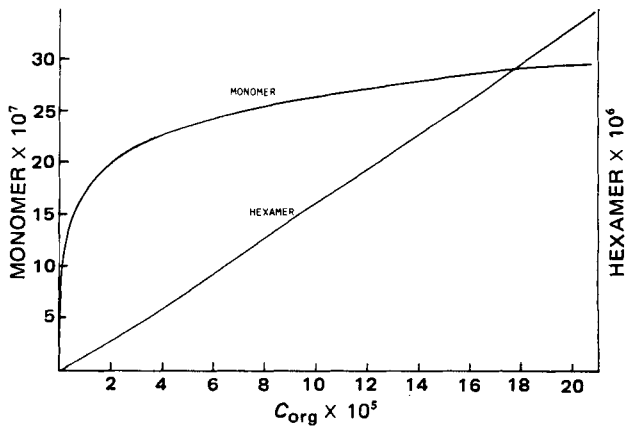


Figure 4—*A plot showing the distribution of deoxycholic acid among its various forms in Solvent System B.*

deviation was observed suggesting that higher aggregates exist in System B. The data for the system, therefore, were treated by a nonlinear least-squares fit to Eq. 2. The best fit was obtained for the monomer–tetramer–hexamer model in the organic phase. The estimated association constants were $K'_4 = 9.96 \times 10^2\ M^{-3}$, $K'_6 = 4.79 \times 10^{28}\ M^{-5}$. The distribution isotherm based on these constants is in good agreement with the experimental data (Fig. 2). Figure 4 shows the distribution of deoxycholic acid among its various forms in Solvent System B.

In studies with the methylester of deoxycholic acid (2), it was found that the methylester exists as monomers and dimers in chloroform ($K_2 \approx 14.0\ M^{-1}$) and monomers and tetramers in carbon tetrachloride ($K_4 \approx 2.8 \times 10^5\ M^{-3}$). Although no report was found in the literature for comparison with the present observations, a qualitative comparison can be made with the data derived previously (2). In pure octanol, hydrogen bonding interactions between solvent molecules and the hydroxyl and carboxyl groups of the bile acid are considerable. Consequently, this interaction precludes any appreciable self association of steroidal monomers. Solvent System A is a solvent of low polarity. Octanol is, therefore, expected to self associate to a significant extent in this solvent system (7). Thus, there is likely to be only weak interaction between 1-octanol and bile acid molecules. This leads to self association of solute molecules either between hydroxyl groups, carboxylic acid groups, or both. It was found in the preceding study (1) that under the condition of the experiment it is the free acid form that is partitioned in the organic phase. An acid form, because of its relatively more polar nature compared to the ester form, is expected to associate more strongly.

Even higher aggregation is expected, therefore, in Solvent System B, since this solvent is even less polar than Solvent System I. The organic phase has been rendered less polar, by increasing the isooctane concentration and by substituting 1-octanol (dielectric constant 10.34) with chloroform (dielectric constant 4.81).

These results have important significance in terms of mixed micelles of bile salts with lecithin (8). In a low dielectric inert medium such as the interior of a lecithin bilayer or liposome, pairwise association of bile salt molecules hydrogen-bonded to each other through their hydroxyl and/or carboxylic acid groups is plausible. Such a mixed disk model for bile salt lecithin micelles in which hydrogen bonded bile salt anion pairs are found within the interior of the micelle has been proposed previously (9). It cannot be decided on the basis of the present or previous (9) work whether the associated species are bile salt anions or free acid molecules. Molecular models suggest that the hydroxyl groups on the trihydroxy bile acids and dihydroxy bile acids can align to form hydrogen bonded pairs. It was suggested (10) that this hydrogen-bonded pairing occurs in aqueous solvents. However, it has been shown (1, 11) that in aqueous solution, bile salts are associated by hydrophobic forces. The previous hydrogen-bonded pairing model (10) appears to be the most likely structure in a low dielectric, nonhydrogen-bonded medium. The partition of bile salts from an aqueous to a lipid membrane phase would thus involve an inversion from hydrophobic back-to-back association in the aqueous phase, to hydrogen-bonded association in the lipid phase.

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Serum Prolactin Level Increase in Normal Subjects Following Administration of Perphenazine Oral Dosage Forms: Possible Application to Bioavailability Testing

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Abstract □ Two pilot studies were performed to determine if oral phenothiazine products could generate a significant increase in serum levels of the hormone prolactin. The two studies employed three and four healthy normal male subjects, respectively. In the first study the subjects received a screening dose, a placebo, one 8-mg perphenazine tablet, and two 8-mg perphenazine tablets. In the second study, the subjects were dosed with two 10-mg amitriptyline tablets, one 10-mg amitriptyline tablet with one combination tablet containing 10 mg of amitriptyline and 4 mg of perphenazine, and two combination tablets, each containing 10 mg of amitriptyline and 4 mg of perphenazine. In both cases the drug treatments produced a significant rise in the serum prolactin levels *versus* a placebo or control. This increase was defined as a prolactin response. The possible utility of this response in bioavailability testing is discussed.

Keyphrases □ Prolactin—serum prolactin level increase following administration of perphenazine oral dosage forms, application to bioavailability testing □ Bioavailability—application to testing, serum prolactin level increase following administration of perphenazine □ Perphenazine—oral dosage forms, serum prolactin level increase following administration, application to bioavailability testing

An adequate methodology for determining the bioavailability and bioequivalence of phenothiazine dosage forms has been sought for some time. The phenothiazines are a vital psychopharmaceutic tool in combating mental and emotional illnesses. In addition, these drugs are produced and marketed in a very large number of dosage

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