Micellar properties of sodium fusidate, a steroid antibiotic structurally resembling the bile salts

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ABSTRACT The properties of sodium fusidate micelles were determined by a spectral shift technique, surface tension measurements, and ultracentrifugal analysis. The critical micellar concentrations, mean molecular areas, and apparent aggregation numbers were estimated as a function of the concentration of counterion (0.001–1.0 M Na^+) at 20°C. The critical micellar concentrations were studied over a temperature range of 10°C to 40°C at one counterion concentration (0.001 M Na^+), and from these data the standard thermodynamic functions of micellization were calculated. The ability of sodium fusidate solutions to solubilize the insoluble swelling amphiphiles, lecithin and monoolein, was investigated, and the results were compared with the solubilizing properties of sodium taurocholate.

The critical micellar concentrations of sodium fusidate approximated those of sodium taurocholate. The values fell in the range of 1.44–4.56 mM, varying with the technique used, counterion concentration, and temperature. The percentage of counterions bound to fusidate micelles in water, calculated from the log critical micellar concentration–log Na^+ curve, was estimated to be negligible, which compares with sodium taurocholate micelles. The critical micellar concentration of sodium fusidate exhibited a minimum at 20°C, a phenomenon observed with other ionic detergents and with bile salts. Micelle formation in sodium fusidate solutions was shown to be primarily entropy-driven at 10° and 20°C, whereas at 30° and 40°C the enthalpy factor predominated. From the surface tension measurements the molecular areas of sodium fusidate and sodium taurocholate were calculated. The mean molecular area of fusidate was 101 A^2, whereas sodium taurocholate possessed a molecular area of 88 A^2. It was demonstrated that the sodium fusidate molecule, like a bile salt molecule, lies with its longitudinal axis horizontal at an air–water interface. The apparent aggregation number of sodium fusidate micelles increased from 5 to 16 as the concentration of counterion increased from 0.01 to 0.60 M Na^+. These values are slightly larger than the corresponding aggregation numbers of sodium taurocholate micelles.

Sodium fusidate is thus similar to the bile salt sodium taurocholate in its micellar properties. Furthermore, saturation ratios similar to those found with bile salt solutions were obtained when liquid crystalline suspensions of lecithin or monoglyceride were solubilized by sodium fusidate solutions. As excretion of orally administered fusidate occurs mainly via the biliary tract, this finding may prove to be of considerable biological importance. A model of the fusidate micelle similar to that previously proposed for the bile salt micelle is suggested.

SUPPLEMENTARY KEY WORDS Fucidin · critical micellar concentration · sodium taurocholate · bile · aggregation number · spectral shift · surface tension · ultracentrifugal analysis · molecular areas · micellar size · micellar charge · mixed micelles with lecithin and monoolein · gallstones

SODIUM FUSIDATE,[1] a fungal metabolite isolated from the fermentation products of *Fusidium coccineum* (K. Tubaki), was originally developed in 1962 by Leo Research (Denmark) as an antibiotic active against the

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Abbreviations: TLC, thin-layer chromatography; CMC, critical micellar concentration; NaTC, sodium taurocholate (sodium salt of 3α,7α,12α-trihydroxy-5β-cholanoyl taurine); Ag#, aggregation number (number of monomers per micelle).

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[1] Proprietary name, Fucidin; the sodium salt of 3α,11α-dihydroxy-16β-acetoxyl-fusida[17,20(16-21 *cis*)24]-diene-21-oic acid.

penicillinase-producing strains of *Staphylococcus aureus* (1, 2). After extensive trials in man had shown that it possessed no toxic side effects, fusidate enjoyed considerable therapeutic vogue prior to the advent of the semisynthetic penicillins. Chemically, it belongs to the group of tetracyclic triterpenes and has been shown to possess the structure depicted in Fig. 1, *a* (3, 4). Studies in man showed that sodium fusidate is rapidly absorbed from the gut to give high and well-maintained blood levels (5, 6). It is excreted in the urine only in very small amounts, whereas high concentrations are found in bile (7, 8). Godtfredsen and Vangedal (8) detected seven different metabolites of sodium fusidate by TLC in the bile of a patient who received the antibiotic. Only a small amount of the steroid was excreted unchanged, but a major metabolite was shown to be the monoglucuronide of fusidic acid.

Our interest was initially stimulated by the obvious structural similarity of this steroid to the bile acids, e.g., cholic acid, shown for comparison in Fig. 1, *b*. Though the conventional chemical representations (Fig. 1, *a* and *b*) of both steroids bear a superficial resemblance to one another, their stereochemical configurations are fundamentally different. Essentially, fusidic acid possesses a saturated cyclopentenophenanthrene ring system with an A/B *trans* ring juncture (5α-hydrogen). The B ring of fusidic acid takes up the unusual "boat" conformation as depicted in the perspective stereochemical formula in Fig. 1, *c*. The A/B ring juncture of cholic acid (Fig. 1, *d*) is *cis* (5β-hydrogen) and all three cyclohexane

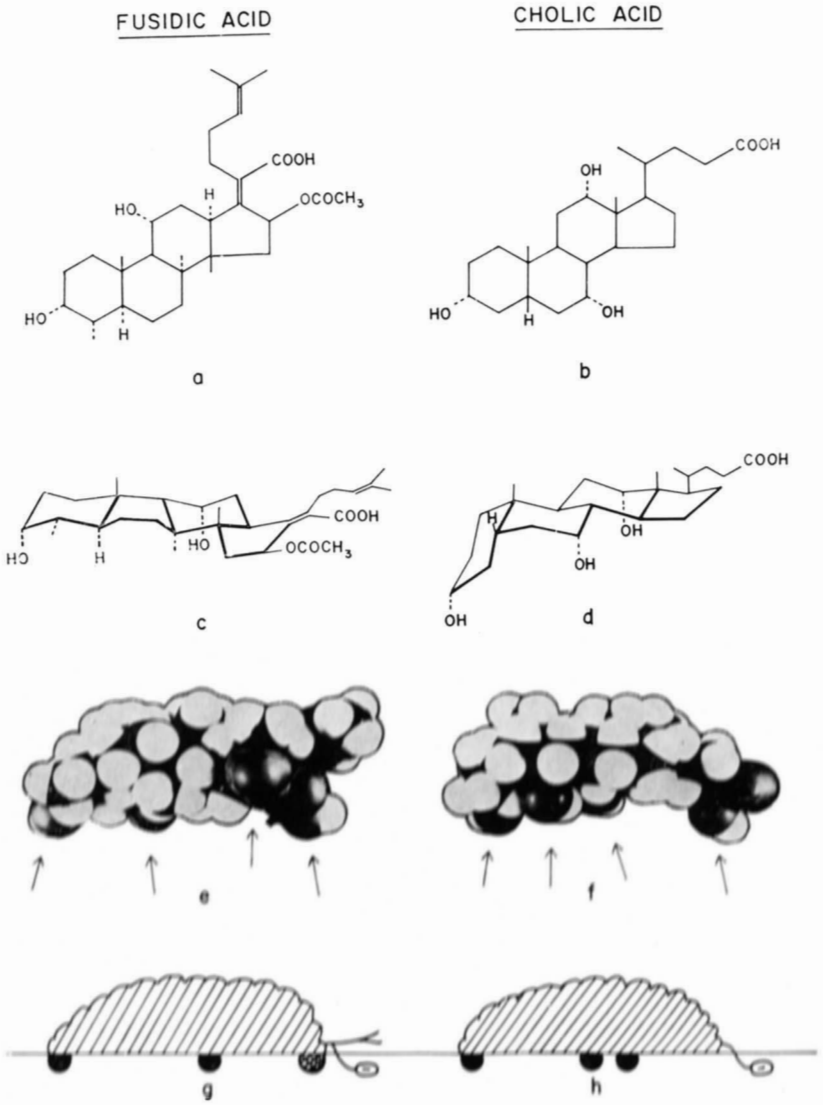

FIG. 1. Fusidic and cholic acids: *a*, *b*, conventional chemical configuration; *c*, *d*, perspective structural formulas; *e*, *f*, space-filling (Stuart-Briegleb) molecular models (the positions of the polar groups are indicated by arrows on the undersurface of each molecule); *g*, *h*, the authors' shorthand representations of the molecules as they would lie at an air–water or oil–water interface.

rings exist as "chair" conformational isomers. Nevertheless, when the space-filling (Stuart-Briegleb) molecular models of both steroids are studied (Fig. 1, *e* and *f*), the polar groups of each molecule lie on one side of their bulky hydrocarbon parts. The space-filling models are represented diagrammatically in Fig. 1 (*g* and *h*) at an air–water interface. The closed circles represent hydroxyl groups, and the crosshatched circle of the fusidic acid molecule represents the acetoxyl group. The open ovals with negative signs represent the ionizable carboxyl groups. These polar groups are at the head of short, mobile aliphatic chains positioned at one end of each molecule. In the case of fusidic acid there exists a further unsaturated aliphatic chain which branches upwards from the ionic side chain. This is shown as the forked line in Fig. 1, *g*.

In preliminary experiments, we verified by three physical techniques (spectral shift, solubilization, and surface tension) that sodium fusidate formed micelles in aqueous solution. These observations, together with the fact that orally administered fusidate is excreted in the bile, prompted us to investigate systematically the colloid–chemical characteristics of this material.

The present paper deals with the properties of pure sodium fusidate micelles, and a comparison is made between these properties and the micellar properties of a trihydroxy bile salt, sodium taurocholate (NaTC). Further, the remarkable ability of sodium fusidate solutions to solubilize lecithin and monoolein as mixed micelles is compared with the solubilizing capacity of bile salts (NaTC) for these lipids.

EXPERIMENTAL

Materials

Sodium fusidate[2] was of the highest purity (>99%), as assessed by TLC, calculation of the equivalent weight by potentiometric titration, and the absence of minima in surface tension vs. log concentration curves. On irradiation with UV light of sulfuric acid-sprayed TLC plates, sodium fusidate produced a purple fluorescence quite distinct from the fluorescence of cholesterol (pink-violet) or bile salts (yellow) under similar conditions. Rhodamine 6G was purchased from the Allied Chemical Company (National Biological Stains Department) and was spectrophotometrically pure as described previously (9). The method of preparing, purifying, and storing egg lecithin was reported earlier (10). Utilizing the method of Wuthier (11), two-dimensional paper chromatography of the lecithin just before use showed that only trace amounts (<1%) of lysolecithin and sphingomyelin were present. The monoglyceride was a commercial product purchased from the Eastman Kodak Co., Rochester, N.Y. This was used without further purification. The other chemicals employed (NaCl, NaOH, $NaHCO_3$, and Na_2CO_3) were of analytical quality. The water was doubly distilled from an all-Pyrex still. The pH of all fusidate solutions was 10.0 ± 0.2. This was attained either by direct adjustment of the pH of each solution with the addition of stock solutions of NaOH (dye titration and solubilization studies) or by preparing the fusidate solutions in carbonate–bicarbonate buffer, pH 10.0 (12), to which weighed amounts of NaCl had been added to achieve the desired ionic strength (surface tension and ultracentrifugal studies).

Methods

2-ml portions of concentrated sodium fusidate solutions were prepared gravimetrically in cuvettes with 2.5 × 10^{-6} M rhodamine 6G solution, each containing a fixed molarity of NaCl (0.001, 0.01, 0.05, 0.15, 0.30, 0.60, and 1.0 M). Each of these solutions, which were well above the CMC of fusidate, was scanned through its absorption maximum in a Beckman spectrophotometer. Serial scans were carried out after repeated dilutions of each solution with the corresponding rhodamine 6G–NaCl buffer solutions. This afforded scans covering a wide concentration range of fusidate above and below the CMC. The temperature of the cuvettes in the spectrophotometer was kept at 20 ± 0.5°C by means of a double-walled jacket through which water was constantly circulated from a thermostatically controlled bath. The CMC's were taken as the concentration of sodium fusidate corresponding to the first break points in the curves obtained when the spectral maxima were plotted against the logarithm of the sodium fusidate concentration. The rationale for taking this value as an estimate of the CMC has been described in detail previously (9). The CMC's were also estimated as a function of temperature (10°C–40°C) at a counterion concentration of 0.001 M Na^+. At 50°C precipitation of the salt occurred due to hydrolysis of the acetoxyl group of the fusidate molecule, resulting in the formation of the sparingly soluble sodium salt of 16-epidesacetyl-fusidic acid (3).

Surface tension measurements were carried out with a torsion wire surface tension apparatus employing a platinum Wilhelmey blade of 1 × 2 cm dimensions. Calibration of the instrument was carried out with the use of milligram weights from a Cahn Electrobalance. The solutions were contained in thick-walled glass containers, 4.5 cm I.D., which were kept covered with tightly fitting ground-glass caps until the surface tension measurements were begun. All readings were carried out

[2] A generous gift of Dr. W. O. Godtfredsen, Leo Pharmaceutical Products, Ballerup, Denmark.

at 20°C in a temperature-controlled room (tolerance, ± 0.2°C). In each study, 15 ml of 0.125, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10, 15, and 20 mM sodium fusidate solutions were made up in 0.01 M sodium carbonate–bicarbonate buffer. Each set was then brought to a final Na^+ concentration of 0.01, 0.05, 0.15, 0.30, and 0.60 M by the addition of NaCl. The Wilhelmey blade was carefully rinsed with fresh distilled water and heated to incandescence in a Bunsen flame after each measurement. Preliminary surface tension readings of water and several organic liquids (methanol, ethanol, benzene, and hexane) agreed well with published values. Before all measurements the surface of each sodium fusidate solution was cleaned by sweeping with an air jet and aspirating the surface, which was marked with a few grains of purified talc. Surface tensions were measured as a function of time to obtain constant surface tension readings. In some cases, especially with low sodium fusidate concentrations, surface tension readings did not attain constant values until about 120 min after surface sweeping. All glassware used in these experiments had been chromic–sulfuric acid-washed for 24 hr prior to use.

The method of apparent anhydrous micellar weight (MW_{app}) determination was the technique of Svedberg and Pedersen (13) for estimating the molecular weight of macromolecular compounds. The formula used is

$$MW_{app} = \frac{RTs}{D(1 - \bar{V}\rho)}$$

where R is the gas constant (8.314×10^7 ergs per degree per mole), T is the absolute temperature (in this case, 293.2°K), s is the sedimentation velocity and D is the diffusion constant, both measured in a Beckman Model E analytical ultracentrifuge, $\bar{V}$ is the partial specific volume of sodium fusidate, and ρ is the density of the solution under study. In the estimation of the diffusion constants of macromolecules such as proteins, the usual practice is to diffuse the buffer or solvent against a solution of macromolecules using a synthetic boundary cell in the ultracentrifuge. Utilizing standard schlieren optics, the diffusion constant is then calculated from the variation in the ratio of [area]^2 to [height]^2 of the schlieren tracings as a function of time. However, the "solvent" used in the experiments described herein was a solution of sodium fusidate slightly above its CMC (4 mM). The "solution" was a more concentrated solution (20 mM). Therefore, since both "solvent" and "solution" possessed the same monomeric concentration (equivalent to the CMC), the diffusion constant calculated in this way gave the value for the fusidate micelles only. The partial specific volume ($\bar{V}$) of sodium fusidate was measured by pycnometry in 0.15 M Na^+ (pH 10.0, 27.3°C) as a 20 mM solution. The value found was 0.774 ml/g. Partial specific volumes of bile salts do not change appreciably when the ionic strength of the medium is varied (14). Thus, it was assumed for the purpose of these calculations that the partial specific volume of sodium fusidate does not vary significantly with added counterion. The densities of the solutions were calculated from density data for NaCl, $NaHCO_3$, Na_2CO_3, and water published in the International Critical Tables (15). The micellar weights of sodium fusidate were measured in four concentrations of counterion (0.01, 0.15, 0.30, and 0.60 M Na^+).

In the solubilization experiments, sodium fusidate plus lecithin and sodium fusidate plus monoolein were intimately mixed together in all proportions in a mutual solvent (ethanol or chloroform) in glass tubes of 20-ml capacity. A series of matched control mixtures employing NaTC instead of sodium fusidate were also prepared. All mixtures were evaporated to dryness under a stream of N_2 and then rigorously desiccated in vacuo over phosphorus pentoxide for 48 hr. Water adjusted to pH 10.0 was then added to give the desired final concentrations of 5% (monoolein study) and 2.5% (lecithin study). After the contents were thoroughly mixed (Vortex mixer), all tubes were allowed to stand at room temperature for 48–72 hr, a period of time that is probably sufficient to allow equilibration to occur (10). The gross appearances of the solutions were then recorded. The absorbance at 750 nm of each solution was measured in a spectrophotometer. A solution was judged isotropic (i.e., micellar) if its extinction coefficient, measured against a water reference, was less than 0.025. The presence of micelles in these solutions was then confirmed by the shift in the absorption maximum of rhodamine 6G.

RESULTS

Critical Micellar Concentrations

The results of surface tension measurements are represented in Fig. 2 as plots of the surface tension vs. the logarithm of the sodium fusidate concentration for solutions containing various amounts of Na^+ (NaCl plus carbonate–bicarbonate buffer). The CMC's which correspond to the concentration of sodium fusidate at the break points in these curves are listed in Table 1. In the same table the CMC values obtained for sodium fusidate by the spectral shift technique are also tabulated. For comparison, the CMC values of NaTC obtained by the latter method (9) are included. In Fig. 3 all of these values are plotted logarithmically as a function of the logarithm of the counterion concentration. The temperature variation of the CMC of sodium fusidate is plotted in Fig. 4.

TABLE 1 MICELLAR DATA AND MOLECULAR AREAS OF SODIUM FUSIDATE AND NaTC AT 20°C

Sodium Fusidate							Bile Salt, NaTC			
Na^+ Concentration	CMC, Spectral Shift Method	CMC, Surface Tension Method	Area per Molecule	Diffusion Constant of Micelles	Apparent Anhydrous Micellar Weight	Rounded Off Aggregation Number	CMC, Spectral Shift Method*	Area per Molecule†	Apparent Anhydrous Micellar Weight‡	Rounded Off Aggregation Number
M	mM	mM	A^2	$cm^2/sec \times 10^6$			mM	A^2		
0.001	3.10						3.10			
0.01	2.74	4.20	93	2.67	2870	5	3.10		1230§	3
0.05	2.65	3.65	98				3.15		2240	4
0.15	2.46	3.70	107	1.71	4610	8	3.12	88	2450	5
0.30	2.37	3.70	104	1.44	5740	10	3.22		3220	6
0.60	2.35	3.30	104	1.00	8730	16				
1.0	1.44						2.20		4840	9

* From Refs. 9 and 16.
† Carey, M. C., and D. M. Small, unpublished experiments.
‡ From Refs. 14 and 16.
§ Value for sodium cholate (Ref. 14).

The CMC values for sodium fusidate may be summarized as follows: In 0.001 M Na^+ the CMC of fusidate by the spectral shift technique is 3.1 mM. The CMC shows a gradual fall with added counterion to a value of 2.35 mM at 0.60 M Na^+. A steep break in the curve then ensues as the counterion concentration is increased further (Fig. 3), so that at 1.0 M Na^+ the CMC is 1.44 mM. Above this concentration of Na^+, fusidate precipitates from solution. The CMC's by the surface tension method (Fig. 3 and Table 1) are, on the average, approximately 1.2 mM higher than the values obtained by the spectral shift method. Different physical methods are well known to give different CMC values under the same experimental conditions (17). However, the changes in CMC with added counterion, i.e., the slopes of the curves, are practically identical and equal to −0.04. The slope of the log CMC–log counterion plot has been shown (9, 18) to give the percentage of counterions bound to an ionic micelle in water. Thus, the value for fusidate micelles is negligible (4%). In other words, a micelle of fusidate binds practically no counterions in water. For comparison, the CMC's of NaTC derived by the spectral shift method (9, 16) at the same temperature (20°C) are shown in increasing counterion concentrations (Fig. 3). In 0.001 M Na^+ the CMC of NaTC is identical with that of sodium fusidate (3.1 mM). However, in contrast to the behavior of sodium fusidate with added counterion, the CMC of NaTC shows no appreciable variation whatsoever as the counterion concentration is increased. The CMC decreases sharply, however, to a value of 2.2 mM in 1 M Na^+ (Fig. 3). As the CMC of NaTC possesses a zero slope in response to added counterion between a NaCl concentration of 0 and 0.7 M, its micelles bind no counterions in water (9).

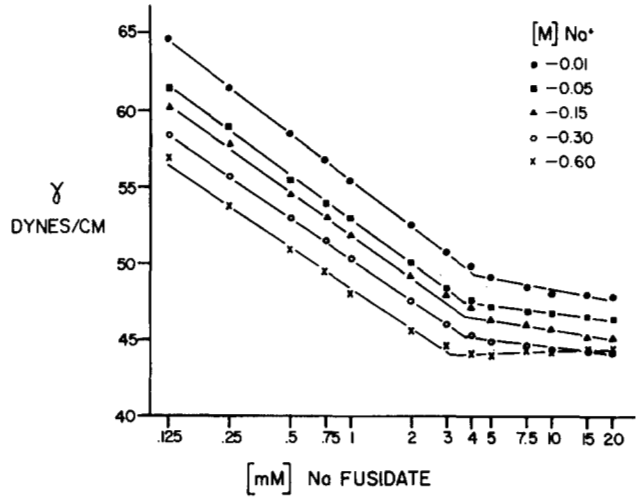

FIG. 2. Concentration dependence of surface tension of solutions of sodium fusidate at 20°C (pH 10.0).

The CMC's of sodium fusidate at 10, 20, 30, and 40°C in 0.001 M Na^+ are 3.80, 3.10, 3.37, and 4.56 mM, respectively (Fig. 4). At 50°C (pH 10) hydrolysis of the acetoxyl group occurs and the salt precipitates from solution as indicated earlier. The temperature variation of the CMC of sodium fusidate shows that a minimum occurs at about 20°C. Thereafter, the CMC rises sharply to the value at 40°C. The CMC of NaTC exhibits a similar pattern as a function of temperature (9).

Molecular Areas

The area occupied by a fusidate molecule at the air–water interface was calculated from the slopes of the surface tension vs. log sodium fusidate concentration plots (Fig. 2) using the Gibbs adsorption isotherm:

$$\Gamma = -\frac{1}{RT}\left(\frac{d\gamma}{d\ln c}\right)$$

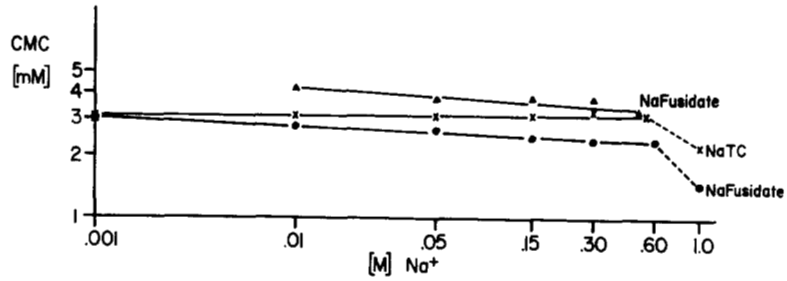

FIG. 3. Double logarithmic plot of CMC's as a function of counterion concentration. CMC's of sodium fusidate by surface tension (▲—▲) and spectral shift (●—●), and CMC's of NaTC by spectral shift (from Refs. 9 and 16) (X—X).

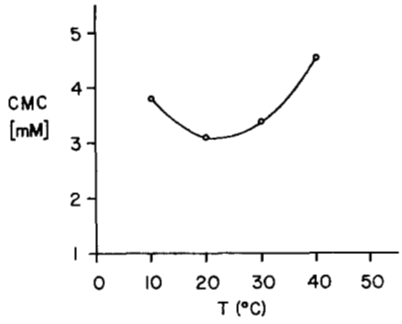

FIG. 4. Temperature dependence of CMC's of sodium fusidate solutions by spectral shift in 0.001 M Na^+ (pH 10.0).

where Γ represents the surface excess concentration, γ is the surface tension in dynes/cm, c is the concentration of sodium fusidate in g/ml, and R and T are the gas constant and absolute temperature, respectively. This simplified form of the Gibbs equation assumes that a swamping excess of neutral electrolyte is present and that activity coefficients approach unity. The molecular areas (A) were then obtained from

$$A = \frac{1}{\Gamma N_A}$$

where N_A is Avogadro's number. These areas and the area obtained for the bile salt NaTC by the same technique at 20°C in 0.15 M NaCl[3] are given in Table 1. The area per fusidate molecule was between 93 and 107 A^2 with a mean of 101 A^2. The molecular area of NaTC was 88 A^2 in 0.15 M NaCl. The values for the molecular areas of the dihydroxy bile salt sodium taurodeoxycholate from the work of Kratohvil and DelliColli (19) were from 73 to 95 A^2 per molecule, with a mean of 87 A^2. Thus, both bile salts possess similar molecular areas which are significantly less than the molecular area of sodium fusidate. Confirmatory studies of the area per molecule of fusidic acid were carried out with an automatic recording surface balance.[3] A monolayer of fusidic acid spread on 3 M NaCl (pH 1.0) at 24°C had a collapse pressure of 23 dynes/cm and a mean molecular area of 107 A^2. The areas occupied by a molecule of sodium fusidate or NaTC were estimated using Stuart-Briegleb molecular models. The outlines of the models positioned vertically or lying flat were traced on high quality drafting paper. The outlined areas were cut out, weighed accurately, and compared with the weight of a reference cutting of the same paper 15 cm square (equivalent to an actual molecular area of 100 A^2, as 1.5 cm on these models corresponds with reasonable accuracy to 1 A on a molecule). The traced area of sodium fusidate positioned vertically was 62 A^2, and lying flat it was 98 A^2. The corresponding areas for NaTC were 52 and 93 A^2, respectively. The average experimental values for the interfacial area of sodium fusidate (101 and 107 A^2) and NaTC (88 A^2) by surface tension and surface balance methods agree well with those of the molecular models lying flat. Thus, molecules of sodium fusidate, like bile salt molecules, apparently lie with their longitudinal axes parallel to the surface at the air–water interface.

Micellar Weights and Aggregation Numbers

The apparent anhydrous micellar weights of sodium fusidate micelles are listed in Table 1. The Ag#'s were obtained by dividing the micellar weights by the anhydrous weight of the monomer. For comparison, the apparent anhydrous micellar weights and Ag#'s of NaTC micelles (14) are also listed. These values were measured by equilibrium ultracentrifugation, a technique which gives values for micellar weights which agree with values obtained by the sedimentation–diffusion method (16).

The apparent micellar weights of sodium fusidate and NaTC micelles and, therefore, their respective Ag#'s increase with increases of counterion concentration. The Ag#'s of fusidate micelles are slightly greater at any particular counterion concentration than those of NaTC. These Ag#'s are plotted vs. counterion concentration in Fig. 5. As the counterion concentration is increased beyond 0.05 M Na^+, this disparity is widened.

[3] Carey, M. C., and D. M. Small. Unpublished experiments.

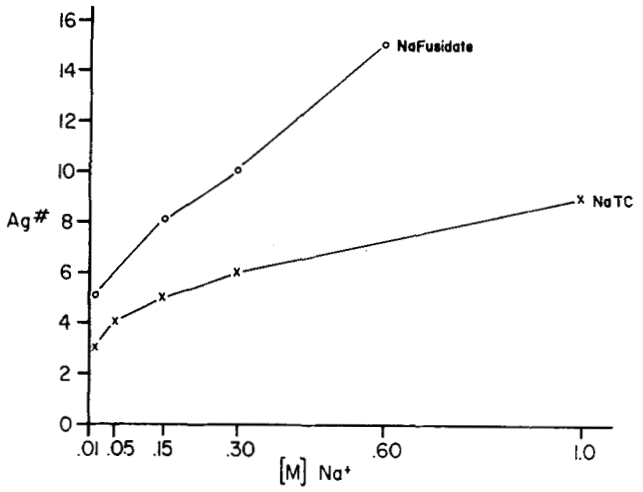

FIG. 5. Variation of Ag#'s as a function of counterion concentration at 20°C. Sodium fusidate, O—O; NaTC (from Ref. 14), X—X.

Thus, both the CMC's and Ag#'s of sodium fusidate micelles are less resistant to the effects of counterion than the trihydroxy bile salt NaTC.

Solubilization of Lecithin and Monoolein

Sodium fusidate solubilized egg lecithin as a clear micellar solution in increasing proportions of lecithin up to a lecithin–fusidate weight ratio of 7:3, which is equivalent to a molar ratio of 2 moles of lecithin to 1 mole of sodium fusidate. Monoolein was solubilized as a micellar solution to a weight ratio of 5:5, which is equivalent to a molar ratio of 1.5 moles of monoolein to 1 mole of sodium fusidate. The results of these experiments indicated that sodium fusidate solubilized both lecithin and monoolein in ratios identical to the solubilizing capacities of NaTC for these lipids (10, 16).

DISCUSSION

Some physical properties of fusidic, cholic and taurocholic acids and their sodium salts are listed in Table 2. The basic physical properties, such as melting point and pK'_a, of fusidic acid and the water solubility, Krafft point, and partial specific volume of its sodium salt are remarkably similar to those of cholic acid and its sodium salt. Taurocholic acid, however, differs from the former acids in possessing a taurine side chain which confers acid resistance (pK'_a, 1.85) and increased water solubility on the molecule. All three acids are soluble amphiphiles above their precipitation pHs. They all aggregate into micelles above their respective CMC's and none exhibits mesomorphic behavior in water; thus they can be classified as Class III B polar lipids (21–23).

An investigation of the structure of the sodium fusidate molecule (Fig. 1) may explain many features of its interesting colloid behavior. Sodium fusidate, owing to its *trans* A/B ring juncture, is a slightly more "extended" molecule than a bile salt molecule, which is "kinked" due to its *cis* A/B ring juncture (Fig. 1). The hydrophobic unsaturated aliphatic side chain of fusidate which could lie on the water surface may be the major contributing factor to the larger surface area (107 A^2/molecule) of this molecule compared with bile salts (88 A^2/molecule of NaTC). The apparent constancy in the molecular area at higher concentrations of counterion (0.15–0.60 M Na^+) is best explained by the fact that only in a swamping excess of neutral electrolyte is the simplified form of the Gibbs adsorption isotherm valid. Furthermore, the areas at high counterion concentrations (104–107 A^2/molecule) agree better with the molecular area of fusidic acid (107 A^2/molecule) obtained by the surface balance technique.

Sodium fusidate contains 31 carbon atoms, and NaTC

TABLE 2 PHYSICAL CONSTANTS OF FUSIDIC, CHOLIC, AND TAUROCHOLIC ACIDS

	Fusidic Acid*	Cholic Acid†	Taurocholic Acid†
Appearance	White crystals	White crystals	White crystals
Empirical formula	$C_{31}H_{48}O_6$	$C_{24}H_{40}O_5$	$C_{26}H_{45}NO_7S$
Molecular weight	516.7	408.6	515.7
Melting point	192–193°C	198–199°C	125–126°C
pK'_a (1% solution; by potentiometry)‡	5.35	5.35	1.85
Water solubility (Na salt; dry weight of a saturated solution; 23°C; w/w)	63.5 g%§	56.0 g%§	All proportions soluble
Lipid class (Na salt; by polarizing microscopy)	Soluble amphiphile (class III B polar lipid, i.e., no liquid crystalline phase)	Soluble amphiphile (class III B polar lipid)	Soluble amphiphile (class III B polar lipid)
Krafft point (Na salt)	<0°C	<0°C	<0°C
Partial specific volume (Na salt; ml/g; by pycnometry)	0.774	0.750	0.750

* From this work and Refs. 20 and 22.
† From Ref. 16.
‡ pK'_a, apparent pK.
§ Grams per 100 grams of solution.

contains 26 carbon atoms. Each molecule contains four hydrophilic groups, three of which are nonionic and the other ionic. Sodium fusidate can thus be considered to be more hydrophobic than NaTC. The 16β-acetoxyl group is essential for aqueous solubility and thus for micelle formation in the case of sodium fusidate. The negligible water solubility of the sodium salt of 16-epidesacetyl-fusidic acid, which possesses three hydroxyl groups (3), attests to the more hydrophobic nature of the fusidate molecule. It is not readily apparent why this should be the case. It is possible that the 3α-, 11α-, and 16β-hydroxyl groups of the desacetyl derivative are so far separated from one another, due mainly to the *trans* nature of the A/B ring juncture, that they cannot act together to solubilize the molecule. It is also possible that the β-epimer position of the 16-hydroxyl group may prevent its solubilizing action. Furthermore, it is known that acetoxyl groups have a greater aqueous solubility than hydroxyl groups. The removal of one hydroxyl group from either the 7 or the 12 carbon of NaTC to form the corresponding dihydroxy bile salt decreases solubility slightly but does not cause the salt to precipitate from solution. Nevertheless, the removal of both the 7- and 12-hydroxyl groups results in lithocholates which are quite insoluble (24). On the other hand, the removal of the hydroxyl group from the 3-position of any bile salt results in a marked decrease in solubility (25). In spite of possessing five additional carbon atoms, the CMC of sodium fusidate in 0.001 M Na^+ is identical with the CMC of NaTC in 0.001 M Na^+ (3.1 mM). In other words, one would expect the bulkier hydrocarbon part of the fusidate molecule to keep the CMC considerably lower than that of NaTC; however, the powerful aqueous solubility of the fusidate polar groups, particularly the acetoxyl group, counterbalances this tendency, and the CMC's are thus the same in water.

Added counterion lowers the CMC of ionic detergents mainly by progressively neutralizing the ionic charges. NaTC is unique in exhibiting resistance to the CMC-lowering effects of added counterion up to about 0.6 M Na^+ (9). Conversely, sodium fusidate shows a slight but progressive fall in CMC as the counterion concentration is increased (Fig. 3). On the other hand, the CMC's of dihydroxy bile salts fall progressively with added counterion (9, 19), the rate of change in the CMC being greater than with sodium fusidate. At about 0.60 M Na^+, steep breaks are observed in the curves of the CMC's of both NaTC and fusidate (Fig. 3). It is likely that this is the concentration of counterion at which salting out of the nonionic polar groups begins.

Similar explanations can be proposed to explain the increases in Ag# of sodium fusidate micelles as the counterion concentration is increased, as shown in Fig. 5. In 0.01 M Na^+, the fusidate micelle consists of five monomers and the NaTC micelle consists of three monomers. The larger fusidate micelle seems to be a function of the larger hydrophobic area of the fusidate molecule and perhaps of the ease of packing due to its flatter shape compared with NaTC. The Ag# of NaTC increases only gradually as the counterion concentration is increased to 1.0 M Na^+. Sodium fusidate micelles enlarge with minor increments of counterion and the slope of the curve diverges widely from that of NaTC (Fig. 5). This behavior seems to inversely mimic the counterion variation of the CMC's (Fig. 3). The increase in the Ag# of fusidate in response to added counterion is better compared with the dihydroxy series of bile salts, which can form very large micelles at high counterion concentrations (14).

The variation of the CMC of sodium fusidate with temperature (Fig. 4) can be partly explained by considering the balance of "hydrophobic" and electrostatic forces which influence the CMC at any particular temperature. Increases of temperature stabilize "hydrophobic interactions" (26), thus, neglecting other factors in micelle stability, the CMC should fall. As the temperature is increased further, monomer solubility increases (27) and the dielectric of the solvent medium is reduced (28). Thus, increased electrostatic repulsion of the ionized groups should cause the CMC to rise. The CMC of an ionic detergent at any particular temperature is, accordingly, a resultant of these opposing forces. In the case of fusidate the hydrophobic interactions predominate between 10 and 20°C, whereas increased solubility and ionic repulsion forces tend to predominate at higher temperatures.

An estimate of the standard thermodynamic functions of micellization was calculated on the assumption that micelles and monomers of fusidate can be treated mathematically as a mass action equilibrium (9, 18). The free energy change (ΔF_m) in kcal per mole was obtained from

$$\Delta F_m = 2.303RT \log CMC$$

where R, T, and CMC have the usual meanings (ΔF_m = −3.14, −3.37, −3.43, and −3.35 at 10, 20, 30, and 40°C, respectively). The change in enthalpy (ΔH_m) in kcal per mole was derived from the Clausius-Clapeyron equation using the same model:

$$\Delta H_m = \frac{-RT^2 d\ln(CMC)}{dT}$$

where $\frac{d\ln(CMC)}{dT}$ represents the change in logarithm of the CMC as a result of a small increment in temperature (1°C) (ΔH_m = +4.20, −1.10, −5.43, and −6.43 at 10, 20, 30, and 40°C, respectively). Therefore, the en-

tropy in kcal per mole ($T\Delta S_m$) follows from the Gibbs relationship,

$$T\Delta S_m = \Delta H_m - \Delta F_m$$

($T\Delta S_m$ = +7.34, +2.27, −2.00, and −3.08 at 10, 20, 30, and 40°C, respectively). Thus, micelle formation in sodium fusidate solutions is an entropy-directed process at 10°C and 20°C. At 30°C and 40°C there is a decrease in entropy so that the large enthalpy component now appears to be the principal driving force in micellization. The transition in the sign of $T\Delta S_m$ (entropy-directed to enthalpy-directed) occurs at a much higher temperature (60°C) in the case of NaTC (9).

On the basis of the assumption that the fusidate micelle is spherical, as has been shown by viscometry (29) for the bile salt micelle at low counterion concentrations, the most likely model for the fusidate micelle is similar to that proposed for the bile salt micelle (14). The micelles of sodium fusidate in 0.01 M Na^+ would consist of five monomers "bonded" back to back in a ring fashion, exposing their hydrophilic groups to the surrounding water. From examination of models, this number of molecules can be fitted together without leaving an empty center. Sodium fusidate micelles of higher Ag# (i.e., in Na^+ concentration > 0.15 M) would probably consist of aggregates of the small "primary" micelles hydrogen-bonded through their hydroxyl groups to one another to form "secondary" micelles. This phenomenon was previously proposed for dihydroxy bile salt micelles in high counterion concentration (14).

The most reasonable way to explain the marked solubility of lecithin and monoolein in sodium fusidate solutions is to assume that mixed micelle formation occurs and that the structure of this micelle is similar to that proposed for the bile salt–lecithin mixed micelle (16, 23, 30). As far as we can determine, this is the only detergent known that mimics bile salts in their remarkable ability to solubilize large amounts of these biologically important, insoluble swelling amphiphiles. Since fusidic acid and its metabolic products are excreted almost exclusively in the bile of man after oral administration (7, 8, 20), the unique physicochemical properties of fusidate may have important biological applications.

What pathophysiological importance fusidate or a similar molecule may have therapeutically in the future is impossible to predict. However, there is a great need for some type of an artificial bile salt for possible use in bile salt deficiency states, for the prevention of cholesterol precipitation from supersaturated micellar solutions of hepatic bile in population groups predisposed to gallstones (31–33), and for the dissolution of gallstones in vivo, particularly in those patients with the so-called "silent" stone (34).

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