Fluid and Chiral Ephedrinium Myristate Micellar Fibers

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Abstract: Myristic acid crystallizes as 1:1 mixture with its sodium salt (acid soap) at pH 9.8, as pure sodium salt (soap) upon addition of sodium chloride and gives long fibers (curds) of a carboxylate monohydrate at pH 11.5. Transmission electron microscopy shows micellar fibers as structural units of the curds. Curvature of the myristate bilayer is enforced by hydration, not by repulsive forces between anions. Ephedrinium and pseudoephedrinium myristate produce stable isolated fibers of bimolecular thickness in water. They dissolve magnesium octaethylporphyrin in a chiral arrangement and show strong CD effects. The aqueous solution is viscoelastic. Twisted ribbons were obtained from the cationic analogue cetyltrimethylammonium with the chiral adenosine monophosphate counterion.

Introduction

Sodium palmitate and myristate fibers of micrometer width in soap gels ("curds") presumably constitute the oldest supramolecular assemblies known.[1,2] These fibers are stable in dilute aqueous solutions and have been isolated and spun in the dry state.[3] Titrations with sodium hydroxide showed that they were only produced at pH ≥ 13 and that they rearranged to form planar crystals[2,4] at pH 9. Thiele proposed that an increase in Coulombic repulsion was responsible for the crystal to fiber conversion,[4] although there should not be much difference in carboxyl group deprotonation at pH values of 9 and 13.

More recently, micellar fibers made of cetyltrimethylammonium bromide in presence of large salt concentrations[5] or salicylate counterions[6−8] have found considerable interest. These fibers are fluid,[7] dissolve massive amounts of small, hydrophobic molecules, e.g. benzene,[8] and show the effect of viscoelasticity in aqueous suspensions.[9]

We have studied both the anionic curds and the cationic viscoelastic fibers with respect to their functionalization with amphiphilic[10,11] or hydrophobic[12] metalloporphyrins. Such fluid fibers of practically infinite lifetime would be ideal counterparts of the solid porphyrin fibers characterized recently.[10,11] It was then found, that fluid fibers made of ephedrinium and pseudoephedrinium myristate not only dissolved porphyrins but also induced strong circular dichroism. Such an effect has so far not been observed in fluid micellar systems. Cationic amphiphiles with chiral counterions were not as effective.

Experimental Section

Methods. ^{1}H-NMR spectra were measured on Bruker AM 270 Sy and AMK 500 spectrometers, and CD spectra with a Jasco J-500 A spectropolarimeter with data processor. Light microscopy was carried out with an Olympus BH-2 microscope, and transmission electron microscopy with a Philips CM-12 apparatus. The probes for cryo electron microscopy were obtained with a cryo environment vitrification system. The sample solution was placed on a carbon sheet covering a grid and dried with filter paper, and the 100−200 nm film was plunged into liquid ethane. A cryo specimen holder (Gatan, U.S., Model 626) was used maintaining the specimen temperature at −175 °C.

Sodium Myristate−Myristic Acid Cocrystals (Acid Soap). Myristic acid (11.45 mg, 50 μmol) was dissolved in 10 mL of 5 mM sodium hydroxide and sonicated (320 W) at 50 °C for 45 min. After the mixture was cooled to room temperature, a fine precipitate of sodium myristate−myristic acid (1:1) crystals was formed. Light microscopy showed square platelets only. Anal. Calcd for $C_{28}H_{55}NaO_4$ (478.74): C, 70.25; H, 11.58. Found: C, 70.22; H, 11.31. The infrared spectrum showed strong, sharp bands at 1725 cm^{-1} (COOH) and 1450 cm^{-1} (hydrogen-bonded carboxylate).

Sodium Myristate Crystals and Fibers. The same procedure as above was followed, but 20 mM of NaCl or NaF was added. A milky white gel was formed instead of a clear solution. Light microscopy revealed a mixture of hexagonal platelets and thin fibers. The elemental analysis now corresponded to pure sodium myristate. Anal. Calcd for $C_{14}H_{27}NaO_2$ (250.36): C, 67.16; H, 10.87. Found: C, 66.94; H, 10.46. The infrared spectrum showed a carboxylate bond at 1568 cm^{-1}.

Sodium Myristate Hydrate Fibers. Pure fibers without admixture of any crystals were obtained by raising the pH from 9.5 to values above 11 with sodium hydroxide (10 mM). The fibers were removed from the viscous aqueous solution with tweezers and dried in air. The analysis corresponded to a slightly dehydrated monohydrate. Anal. Calcd for $C_{14}H_{27}NaO_2xH_2O$ (268.37): C, 63.66; H, 10.14. Found: C, 63.76; H, 9.91.

Viscoelastic Ephedrinium Myristate Solutions. Myristic acid (11.45 mg, 50 μmol) and 8.3 mg (50 μmol) of ephedrine or pseudoephedrine were dispersed in 5 mL of water and sonicated (300 W) for 45 min at 50 °C. Clear, viscoelastic solutions were obtained after cooling to room temperature. Rotating air bubbles changed their sense of rotation upon a sudden halt of the vessel's rotation.

Magnesium octaethylporphyrin was dissolved in methanol, and 50 μL containing 278 μg (0.1 mM) was added to 5 mL of the viscoelastic, 50 μmol ephedrinium myristate solutions. The mixture was again sonicated, and clear red solutions were obtained, which were stable in the dark for several months.

Results

Sodium Myristate: Crystallites and Solid Fibers. Solutions (5×10^{-3} M) of myristic acid and sodium hydroxide (pH

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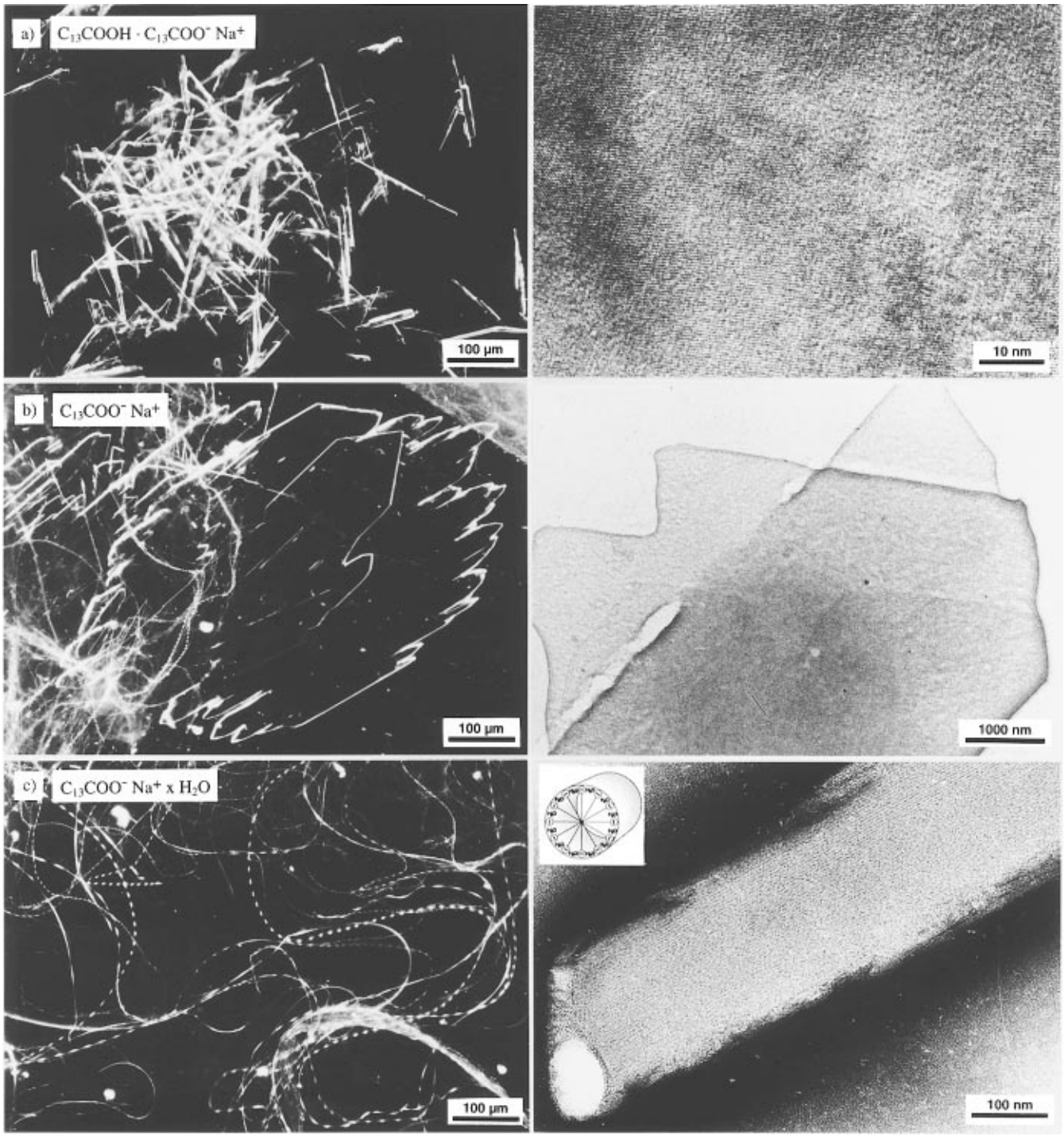

Figure 1. Light and electron micrographs of sodium myristates crystals and fibers: (a) acid soap, producing a hexagonal pattern of head groups, (b) soap, showing a smooth surface, and (c) soap monohydrate crystals made of 3.8 nm micellar fibers.

9.8) gave a clear solution upon stirring or ultrasonication at 50 °C. Cooling to room temperature yielded small crystallites under the light microscope (Figure 1a). High-resolution TEM and laseroptical diffraction revealed a head group distance of 0.44 nm in a hexagonal arrangement (Figure 1a, inset). Combustion analysis of the isolated crystals showed one sodium ion per two myristic acid molecules. The infrared spectrum of the 3D crystals also indicated the formation of a 1:1 compound between myristic acid and its sodium salt ("acid soap"). The carboxyl band occurred at 1725 cm^{-1}. There was no carboxylate band at 1568 cm^{-1}. This was replaced by a strong band at 1450 cm^{-1} presumably shifted by hydrogen bonding.

If, however, sodium ions were applied in excess of myristate (pH 9.8 and 5×10^{-3} M NaCl) mixtures of very thin crystals and fibers were seen under the light microscope (Figure 1b). Combustion analysis of an isolated probe gave one sodium ion for each myristic acid. The crystal platelets were thus made of pure sodium myristate and produced the expected carboxylate infrared band at 1568 cm^{-1} only.

A 2-fold excess of sodium in the form of hydroxide (pH 11.5) finally produced exclusively bundles of 3.8 nm micellar fibers (Figure 1c). Negatively stained, unstained, cryomicroscopic, and Pt/C shadowed preparations all showed the same assemblies. The combustion analysis now corresponded to sodium myristate monohydrate. The fibers showed the 1568 cm^{-1} carboxylate band together with a strong water absorption at 3433 cm^{-1} which was absent in the other two preparations.

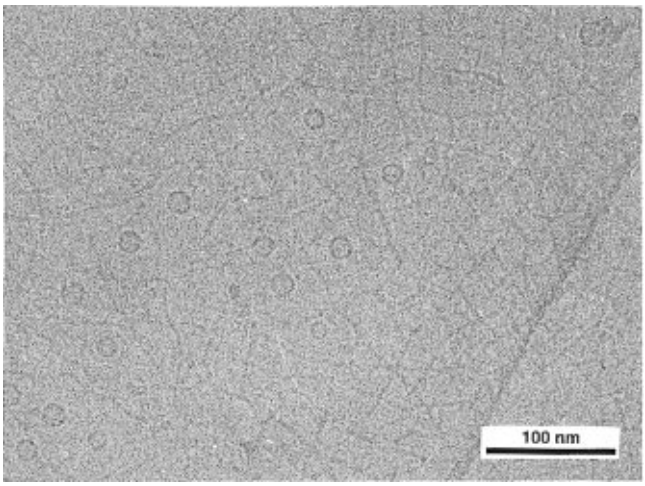

Figure 2. Cryo TEM micrograph of a viscoelastic solution of 20 mM myristic acid/20 mM ephedrine showing a few vesicles and an entanglement of thin micellar fibers.

A comparison of eight fatty acid sodium salts $CH_3(CH_2)_n$-COONa with n = 8, 10, 11, 12, 13, 14, 16, and 18 under identical conditions under the light microscope showed that the shorter fatty acid salts remained dissolved and produced 3D crystals upon concentration while the longer fatty acids gave very short fibers only. Exchange of sodium ions by lithium, potassium, ammonium, or ethylenediammonium ions destroyed the fibers. Potassium always leads to stable clear solutions, the other ions yielded always platelike precipitates. Curvature is, under our conditions, limited to sodium myristates. The pH is not important, provided it is above 9.8.

The sodium myristate curds dissolved hydrophobic methylene blue very well, but the larger porphyrin dyes were ejected from hot micellar solutions upon cooling and subsequent fiber formation.

Ephedrinium Myristate: Fluid Fibers. We then studied myristates with various large, organic ammonium counterions, e.g. brucine, strychnine, and cinchonidine to provide space for porphyrins. Cooling of hot solutions usually produced crystallites. Only quinine, ephedrine, and pseudoephedrine gave fibers which were detectable by transmission electron microscopy (TEM). The quinine fibers tended to precipitation and were not further investigated. The ephedrine fibers, on the other hand, were indefinitely stable and produced viscoelastic solutions as evidenced by the back bounce of air bubbles in rotating vessels after a halt. A net of extended 3−4.5 nm micellar fibers was found in cryo electron micrographs (Figure 2). There was no difference between pure enantiomer and racemic ephedrinium myristate fibers. All fibers disintegrated upon lyophilization.

^{1}H-NMR spectra of ephedrinium and pseudoephedrinium myristate fibers in deuterium oxide showed narrow signals and upfield shifts for all phenyl protons with respect to the pure ephedrine bases (Figure 3).

Both, the ephedrinium and pseudoephedrinium myristate fibers in water dissolved magnesium octaethylporphyrin (MgOEP) up to a concentration of 10^{-4} M, which corresponds to a ratio of ephedrinium myristate:MgOEP of about 100. The visible spectrum showed the Soret band at 411 nm with a half-bandwidth of 15 nm corresponding to monomolecular porphyrin solutions. Nevertheless, depending on the chirality of the ephedrines, we found mirror image CD spectra for enantiomers of ephedrines ($[\theta] = 30\,000$ deg cm^2 dmol^{-1}) and pseudoephedrines ($[\theta] = 60\,000$ deg cm^2 dmol^{-1}). The CD effects were temperature-dependent in both fibers and disappeared at 60 °C. Addition of a hundred-fold excess of pyridine, which is known to form 5-coordinate complexes with MgOEP in apolar solvents, quenched the CD effect in the case of the ephedrine fibers (Figure 4). Covalent ephedrine−myristylamides also did not produce stable molecular assemblies in water.[13]

Ethyltrimethylammonium Adenosine Monophosphate Fibers. It was also tried to produce chiral cationic viscoelastic fibers from cetyltrimethylammonium salts. The best results were obtained with D- and L-configured adenosine monophosphates. These CTA-AMP salts produced helical ribbons similar to those made of double-chain nucleolipids.[14] The racemates only gave planar sheets, which provided another example for the chiral bilayer effect.[15] They showed, however, a strong tendency to precipitate and did not dissolve MgOEP.[16]

Discussion

Sodium Myristates. Myristic acid and sodium myristate show three different forms of molecular assemblies at pH values between 9.8 and 11.5: acid soap crystals with bilayer striations in TEM, soap crystals with a smooth surface in TEM, and soap hydrate fibers again with striations. The striations in transmission electron micrographs (TEM) presumably indicate high molecular ordering by hydrogen bonds, which is lost in the dry soap crystallites. This behavior is not reproduced as cleanly by shorter or longer fatty acids. Shorter homologues tend much more toward crystallization; longer homologues give shorter fibers. The clean transformation of acid soap needles to soap platelets at pH 9.8 could in our hands only be achieved with myristic acid. "Acid soaps" have been described earlier,[17,18] but they were obtained at pH values below 7. The persistence of the free acid at a pH which is 5 orders of magnitude higher than the pK_a can be explained with an reversible displacement of sodium ions by protons upon formation of stacks of monolayers. This displacement allows both the formation of a nonhydrated sodium ion layer between the carboxylate end groups and the formation of intralayer hydrogen bonds. At pH 9.8 acid soaps thus remain stable in crystallites.

Addition of excess sodium ions without changing the pH displaced the equilibrium toward the carboxylate. This total ionization has the effect to separate planar bilayer sheets from each other. They do neither roll up to form rods or scrolls nor do they disintegrate to form spherical micelles.

Upon addition of more sodium hydroxide or chloride, the planar monolayers are not salted out as one might have expected. They rather condense to form monohydrated bilayer scrolls, which then can be isolated in solid form, e.g. by lyophilization. The rise of ionic strength leads to curvature and subsequent carboxylate hydration and intralayer hydrogen bonds, not to interlayer aggregation and precipitation. Charge repulsion does not lead at first to curvature, as anticipated by Thiele,[4] but to layer separation.

The assembly processes are presumably kinetically controlled: the hydrogen-bonded 1:1 complex precipitates rapidly in the form of three-dimensional crystallites, the better water-soluble soaps are slowly salted out to form crystals with isolated bilayers. More sodium hydroxide or sodium chloride then weakens the repulsion effects and causes a rolling-up of the sheets or curvature. The curved bilayer is then stabilized by

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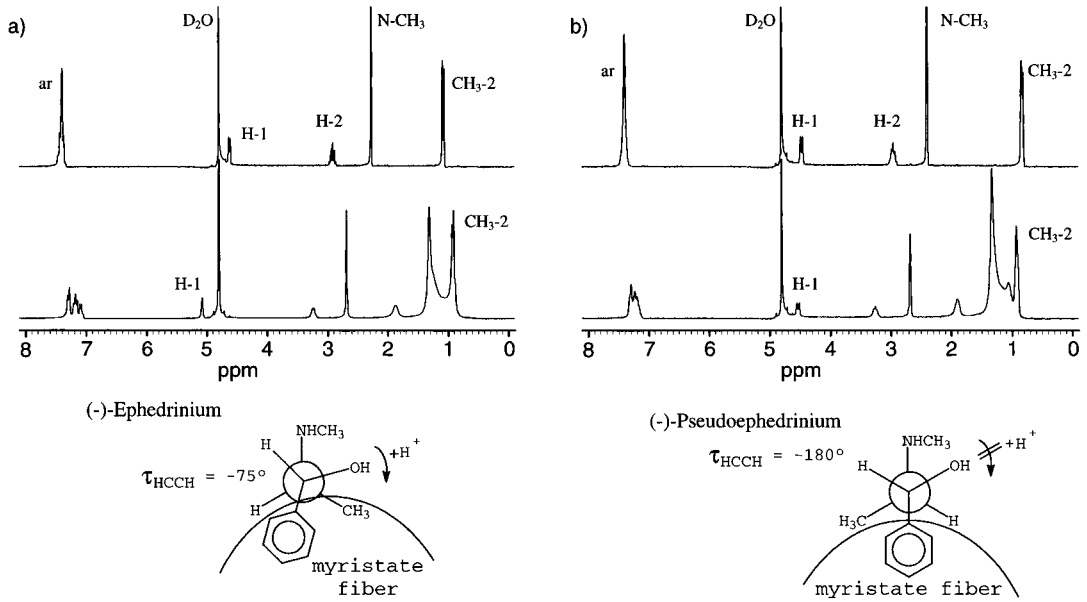

Figure 3. ^{1}H-NMR spectra of (a) (−)-ephedrine and (b) (−)-pseudoephedrine in D_2O. Top: free base (20 mM). Bottom: myristate fiber (20 mM base + 20 mM myristic acid). The assumed conformational changes and the position of the myristate fiber surfaces are indicated in Newman projections (see Discussion).

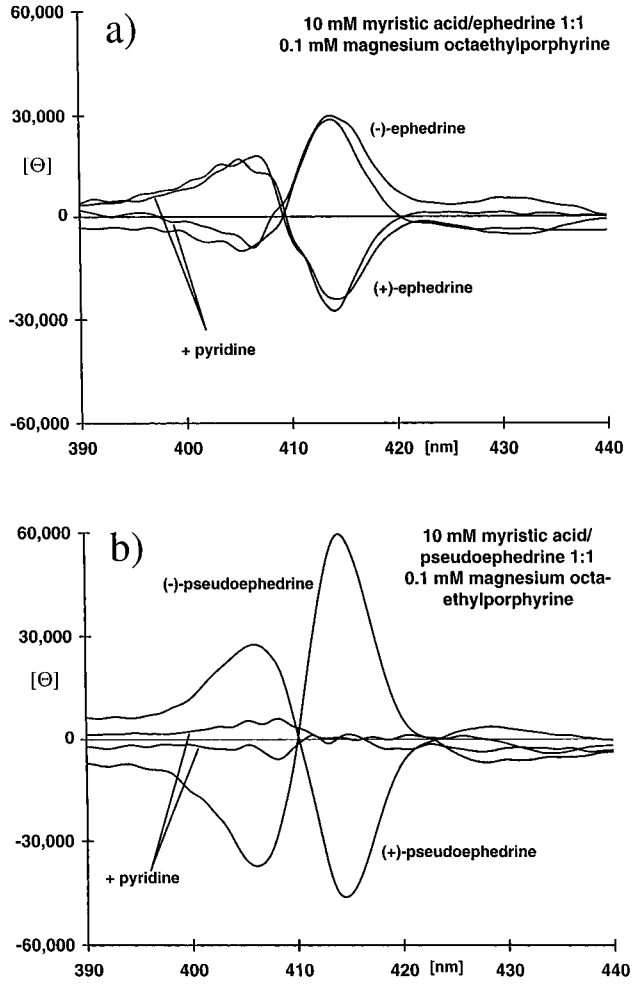

Figure 4. CD spectra of viscoelastic solutions of myristic acid with (a) ephedrinium and (b) pseudoephedrinium counterions containing 0.1 mM magnesium octaethylporphyrin before and after addition of 10 mM pyridine.

filling the voids with one water molecule, which also provides hydrogen bridges between carboxylate pairs. The fact that these fibers are indefinitely stable in aqueous, viscoelastic solutions presumably depends on the presence of spherical micelles, which are in equilibrium with the fibers and dissolve crystallites. A similar effect has been described earlier for chiral gluconamide fibers, which are stable for months in the presence of SDS micelles and crystallize rapidly from pure gels.[19]

The liquid fibers of ephedrinium myristate are, however, of more interest to supramolecular architecture because they are chiral and allow the integration of a porphyrin. The upfield shifts of ephedrinium ^{1}H-NMR signals upon fiber formation indicate a total dissolution of the phenyl rings in the hydrocarbon region of the micellar fibers. In the case of ephedrinium myristate the 2-methyl group proton signal is shifted upfield by the same effect. In pseudoephedrinium myristate, on the other hand, a downfield shift occurs, which probably means that the 2-methyl group is in the polar surface region. Another difference between ephedrine and pseudoephedrine in the mixtures with myristic acid lies in the conformational change of the (−)-ephedrinium HCCH *synclinal* arrangement upon protonation. The torsion angle changes from −60° to −75° (^3J = 6 to 3 Hz), whereas the *anti* conformation of HCCH in (−)-pseudoephedrine (^3J = 9 Hz) does not change. The same behavior has been observed in aqueous solutions upon titration with acetic or hydrochloric acid.[20,21] The *synclinal* ammonium and hydroxyl groups are thought to rotate apart in the ephedrine case, whereas in pseudoephedrine, this is not possible because of repulsive interactions between phenyl and the 2-methyl group (Figure 3). The shifts in the NMR spectra can thus be related to the dissolution of the ephedrines in myristate fibers and the simultaneous protonation of both nitrogen bases.

The ephedrinium−carboxylate interaction seems to be more rigid than the one of the Ψ-diastereomer. This is shown by the dissolution of MgOEP and its reaction with pyridine. Ephedrinium myristate gives the smaller CD effect, and this is *not* disturbed by pyridine interaction. In pseudoephedrines, on the other hand, the CD effect is much stronger and it is wiped out by pyridine. Another hint for the possible arrangement of the ephedrine diastereomers within the fibers comes from the

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crystal structure of ephedrinium−2-tetradecyloxirane-2-carboxylate.[22] In this structure, the myristate chains are interdigitated, and the phenyl ring is located above of the alkyl chain's end. A similar situation should prevail in the myristate fibers, if one considers intermolecular −OH···O═C hydrogen bonds as observed in the crystal structures[22,23] as an important contribution to fiber stabilization.

The fluid character of the ephedrinium myristate fibers as opposed to all the other chiral ammonium salts, which precipitate as sheets, is certainly caused by the benzene units, which make interactions between the oligomethylene chains slippery and prevent local crystallization. In this respect, the structure of the ephedrinium myristate fibers is closely related to the well-known cetyltrimethylammonium salicylate fibers. The replacement of salicylic acid by chiral AMP anions, however, did not lead to fluid fibers. Binding interactions between the adenine units presumably lead to some stacking and solidification of the fibers.

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