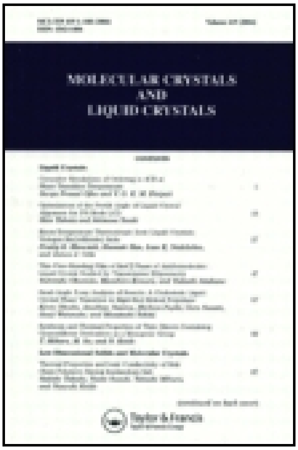

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# The Influence of Lamellar Liquid Crystalline Media in the Hydrolysis of 4-Substituted Benzylidene t-Butylamine N-Oxides

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# The Influence of Lamellar Liquid Crystalline Media in the Hydrolysis of 4-Substituted Benzylidene *t*-Butylamine *N*-Oxides

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Cationic lamellar liquid crystalline morphologies decrease the rate of hydrolysis of a series of 4-substituted benzylidene *t*-butylamine *N*-oxides compared to aqueous solutions. Rate constants for the aqueous acid hydrolysis decrease as the electron releasing effect increases and they are linear when plotted against $\sigma^+$-substituent constants. Rho-values indicate a less sensitive reactivity series than the benzoic acids and a more sensitive reactivity in lamellar solvents. Reaction rates for the series in the latter solvent parallel the hydrophobic substituent constants. Three different lamellar morphologies were identified in the aqueous surfactant systems.

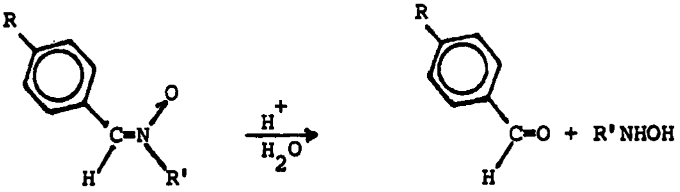
## INTRODUCTION

The fundamental structure of biological membranes is a bilayer arrangement of lipid molecules. These molecules organize themselves with the polar parts shielding the hydrocarbon chains from the aqueous environment surrounding the membrane. Such membranes arrange molecules in their different compartments, provide suitable microenvironments and allow for the controlled transport of solutes. The term bilayer has usually been reserved for the lamellar liquid crystalline arrangement commonly referred to as the smectic mesophase.[1]

The simplest functional membrane model having a lamellar morphology is a vesicle prepared from synthetic surfactants.[2] These vesicles are characterized as smectic mesophases of synthetic surfactant bilayers containing entrapped water.

We have recently reported the influence of two different lamellar liquid crystalline solvents on chemical reactions as due to an electrostatic effect imposed upon the reaction by the lamellar morphology and to partitioning of the substrate between water and the hydrocarbon regions.[3] We have extended this work to include a comparison of the rate studies of a homologous series of reactions in the lamellar mesophase and the aqueous systems.

An investigation of the acid hydrolysis of the substituted homologs of benzylidene *t*-butylamine *N*-oxide has not been reported. In this work the reactions include the acid hydrolysis of 4-substituted benzylidene *t*-butylamine *N*-oxides as shown in the following equation:



R = H, F, $CH_3$, $C_2H_5$, $CH_3O$; R' = t-$C_4H_9$.

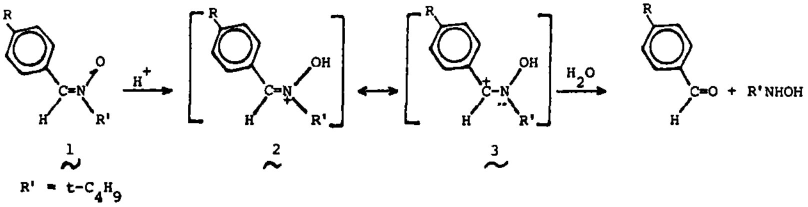
We wish to compare the electrostatic influence of the polar substituents in the lamellar and aqueous media and to correlate rates of hydrolysis with the partitioning of the substrate between the aqueous and the hydrocarbon regions. Finally, we wish to report an investigation of the different morphologies in the two different lamellar mesophases by electron and light microscopy. The first lamellar mesophase is aqueous dodecylammonium chloride[4] (DdAC) and the second is an aqueous mixed system of molar quantities of DdAC, and didodecyldimethylammonium chloride (DdDMAC).[3] The cationic lamellar solvents were selected to minimize surface reactions of the protonated intermediates during the hydrolysis study.

Although the lamellar liquid crystalline solvents and water at the same pH are both highly structured fluids due to hydrogen bonding, a major difference is the anisotropy of the former system.

## RESULTS AND DISCUSSION

In the series shown in Table I, we have found that the rate constants for the aqueous hydrolysis decreases as the electron release effect increases. For example, the rate constant is largest for the unsubstituted compound and decreases progressively with the methoxy substituent the smallest and an overall change of about five times.

These results are in harmony with the mechansim originally suggested for the acid hydrolysis of the parent compound[5] and outlined in the equation below.



The addition of water to the protonated intermediate **2** or **3** is the rate determining step. Our results support this conclusion as the rate of addition would be expected to change as the inductive effect within the aromatic ring changes.

We expect to include electron attracting groups as this work progresses because these substituents will increase the rate of hydrolysis and probably show a bell-shaped [H$^+$]-rate profile[7] as demonstrated by the parent compound.[5]

Micellar effects of anionic surfactants enhance the rate of hydrolysis due to binding of intermediate **2** or **3**, to the negatively charged micelles while cationic micelles do not affect the rates at the CMC.[6] These effects appear to be consistent with an electrostatic interpretation.

The changes in rate constants for the hydrolysis reactions parallel the change in the dissociation constants for 4-substituted benzoic acids[8] containing the same polar substituents, except for the fluoro-

TABLE I

Rate constants for the hydrolysis of 1 in aqueous solution

| 4-Substituent | H | F | $C_2H_5$ | $CH_3$ | $CH_3O$ |
|---|---|---|---|---|---|
| Rate[a] · $10^4$sec$^{-1}$ | 33.74 | 25.69 | 18.19 | 15.61 | 6.67 |

[a] Pseudo first-order at pH 1.00.

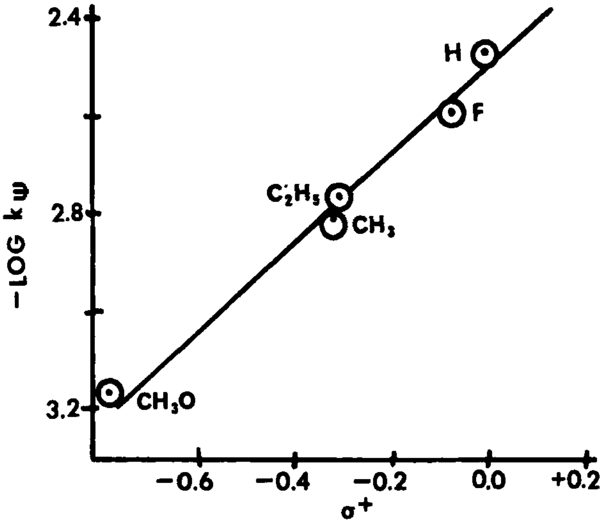


FIGURE 1 Relation between rate constants for 1 in aqueous solution and $\sigma^+$-substituent constants.

atom, and the correlation of the rate constants with $\sigma^+$-substituent constants[7] is shown in Figure 1. Least square analysis yields a value of $\rho = 0.85$ for the aqueous hydrolysis indicating a less sensitive reactivity series than the benzoic acids.

A linear relationship suggests a possible mechanism for the operation of this electrical effect is through the $\pi$-electrons in the unsaturated system present in the benzene ring. This effect reflected in the rates of hydrolysis is expected to be more pronounced in less polar solvents.[9]

Rate constants for the first three members of the homologous series determined in cationic lamellar liquid crystalline solvents are one-tenth those in the aqueous system and they also suggest a linear relationship with $\sigma^+$-substituent constants as shown in Table II. The slope of the line

TABLE II

Rate constants for hydrolysis of 1, in lamellar solvent, and $\sigma^+$-substituent constants

| R | H | F | $CH_3$ |
|---|---|---|---|
| $\log k_\psi$[a] | −3.701 | −3.860 | −4.10 |
| $\sigma^+$ | 0 | −.07 | −.31 |

[a] Pseudo first-order at pH 1.00.

which defines $\rho$ is more positive suggesting that changes in the 4-substituents show greater sensitivity in the lamellar than in the aqueous solution.

It is certainly true that the variation in $\rho$-values between the reactions in the lamellar and the aqueous systems may be the consequence of other factors. For example, different polar substituents change the partitioning of the substrate which results in different kinetic consequences. Therefore, the measured rate constants are the sum of the rates in the aqueous and hydrocarbon regions and the amount of substrate divided between these regions is expressed as a partition coefficient.

In the case of **1**, (R═H) we have found the partition coefficient in *n*-octanol-water is eleven suggesting a preference for the hydrocarbon region.[3] The hydrophobic component is based on the compound's partition coefficient, a measure of how the molecules distribute themselves between two immiscible phases and the Hansch analysis uses *n*-octanol-water as the solvent.[10] The hydrophobicity effects of the 4-substituents of **1** can be empirically evaluated by the substituent parameter $\pi$.[11] These values are determined from the partition coefficients of a series of compounds partitioned in an octanol-water system.

A linerar free-energy model relating structure activity of biological compounds was developed by Hansch.[11] An equation developed from this work is very similar to the Hammett equation

$$\log \frac{P_{SX}}{P_{SH}} = \rho\pi(x)$$

where $P_{SH}$ and $P_{SX}$ represent the partition coefficients of $SH$ and $SX$ respectively; $\pi(x)$ represents the hydrophobic substituent constant, i.e. the contribution of substituent $x$ to the lipophilicity of structure $SH$ when $X$ replaces an $H$ atom in $SH$, and $\rho$ reflects the characteristics of the solvent pair used in determining the partition coefficient. Standardization of the immiscible solvent system to *n*-octanol-water so that $\rho = 1.00$ allows the $\pi(x)$ values to be calculated.

A comparison of the rate of hydrolysis of the first three members of the series shown in Table II, and the $\pi(x)$-values calculated for 4-substituents in aromatic compounds[11] is shown in Figure 2. This linear relationship suggests that partitioning of the first three members of the series between the aqueous and hydrocarbon environment apparently parallels partitioning between water and octanol.[12] Since the overall rate of hydrolysis for each member of the series is a function of the rate in the aqueous layer and in the hydrocarbon region, it may not be sur-

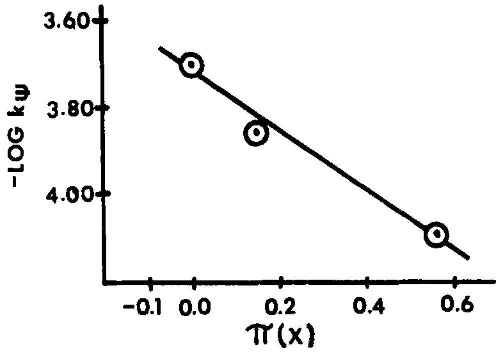


FIGURE 2 Relation between rate constants for 1 in lamellar solvent and the hydrophobic substituent constants.

prising that the rate constants are related to the partitioning of the substrate.

The major factor in solution and partition is the energy necessary for a solvent to accommodate a solute and in the case of water the displacing of small unit clusters may make this possible.[13] However, with the aqueous lamellar mesophase accommodation is more difficult because of the different morphologies usually present. Microscopic confirmation of layer structures in the lamellar mesophase was inferred from the observation of confocal regions and oily streaks shown previously.[3] The electron micrograph of aqueous DdAc in Figure 3, shows a multiwalled vesicle with a diameter of 116 μm. The operational criteria that multilamellar vesicles are those which appear dark by phase-contrast microscopy[14] could not be checked as no structures were visible in these solutions. Single-chain lipids have been considered incapable of forming stable lipid bilayers in water at low concentration,[15] however, a wide variety of saturated single-chain amphiphiles are capable of forming lipid vesicles.[14] Examples of multi-walled vesicles are those formed in aqueous phospholipid preparations reported by Dervichian.[16]

Mixed surfactant solutions were examined by Zeiss Normarski differential interference microscopy and the much larger vesicles with a diameter of 3171 μm are shown in Figure 4. These appear to contain water on the inside from the light appearance of the center. Also, the electron micrographs of these vesicles appear completely dark probably caused by the migration of the phosphotungstic acid used as the dye into the center. The presence of larger and many more vesicles is not surprising in the mixed system since surfactants containing two

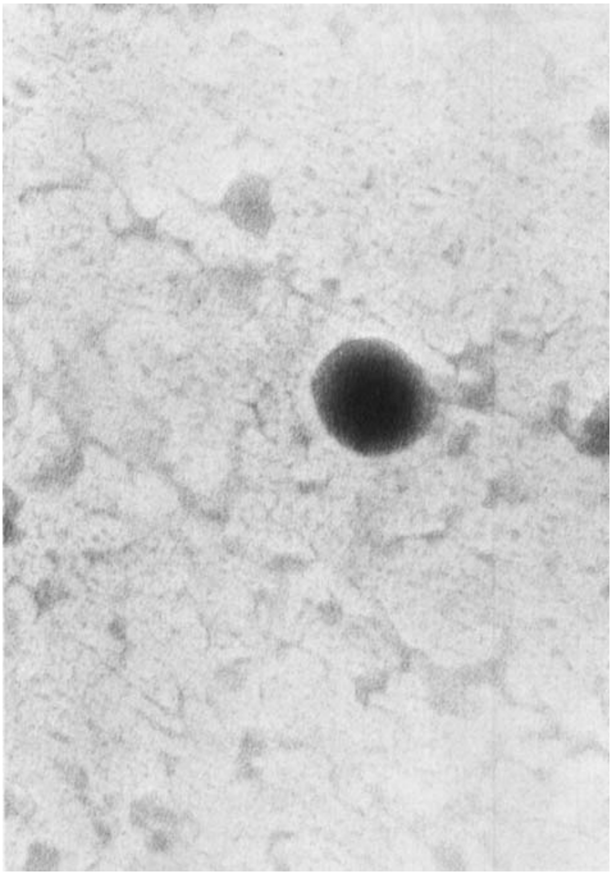


FIGURE 3 Electron micrograph of multiwalled vesicle of DdAC solution. 190,000x

hydrocarbon chains are more prone to form vesicles. Aqueous didodecyldimethyl-ammonium bromide is reported to contain them.[17]

Also present in this same system are several examples of tubules shown in Figure 5 by Normarski microscopy. These structures probably arise from cylindrical micellar aggregates. It is interesting to note that a similar examination of aqueous DdAc samples failed to reveal any lamellar morphologies.

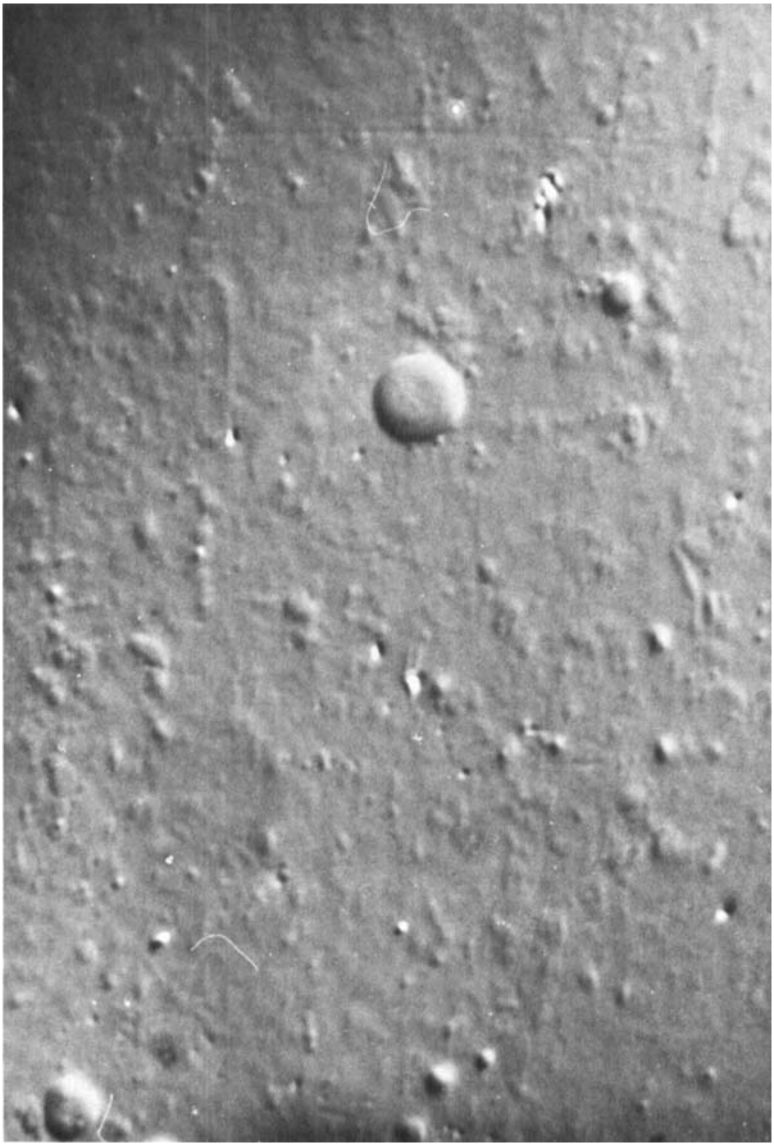


FIGURE 4 Nomarski Differential Interference Micrograph of vesicles in aqueous mixed surfactant system. 4,100x

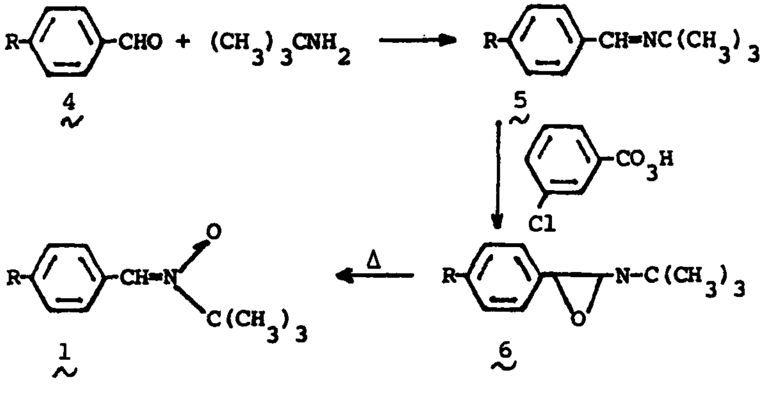
FIGURE 5 Nomarski Differential Interference Micrograph of tubules in aqueous mixed surfactant system. 4,100x

Electrostatic repulsion between the similarly charged probes and vesicle surfaces causes the probes to locate in the middle of the aqueous interiors.[2] Therefore, it is probable that protonated intermediates **2** or **3** may penetrate the vesicle surface and locate at the center of the aqueous interiors. This may also be the case with tubules and in either case the rate constants would be altered. The linear relationship shown in Figure 2 suggests this type of partitioning is not important; however, this point must await the determination of the hydrophobic substituent constants for this series in *n*-octanol-water by reverse phase chromatography.

We conclude from this work that the rate of hydrolysis in the lamellar liquid crystalline system is dependent upon: (a) partitioning of the substrate between the aqueous and hydrocarbon regions, (b) the electrostatic effect of the surfactant aggregate upon intermediates, **2** and **3**, and (c) the morphology of the lamellar systems.

## SYNTHESIS

The nitrones **1** with R=H or $CH_3$ were prepared by oxidation of the anils **5** using Pew's procedure[18] followed by thermal isomerization of the resulting oxazirane **6** by the method of Emmons.[19]



However, we found it difficult to use this method to prepare the Et and MeO analogs. The major problem was contamination of the intermediates **5** and **6** with the aldehyde **4** either as a result of incomplete reaction or hydrolysis of the anil **5**. Separation of the aldehyde from either intermediate was difficult. When R=OMe, neither distillation or repeated recrystallizations gave the anil free of the aldehyde. Even when a pure sample of the anil was used in the oxidation to the oxazirane **6**, molecular sieves had to be added to the reaction mixture to obtain the oxazirane free from the aldehyde indicating that the anil is highly sensitive to moisture when R = Et or MeO. A small amount of the oxazirane **6** (R=MeO) with mp 144–148°C was isolated but this was not sufficient material to continue with this synthetic approach.

We found it easier to obtain the pure nitrones by treating the aldehydes with *t*-butylhydroxylamine using Emmons method for preparing the nitrone **1** with R=$NO_2$.[19] Preparation of a fresh batch of the hydroxylamine from 2-methyl-2-nitropropane using the method of Beckman[20] proved to be more convenient than neutralization of the commercially available salt. We did not attempt to purify this material before use because of its instability. Crude yields varied from 26–66% but no attempt was made to maximize the yield.

The nitrones, **1** in which R=Et or MeO were isolated in purified yields of 6–13%, much lower than that obtained for R=$NO_2$ (55%). However, any unreacted aldehyde in the crude product could be removed by vacuum distillation and the remaining nitrone purified further by recrystallization.

## EXPERIMENTAL

Melting points were determined using a Thomas-Hoover Mel-temp apparatus (Arthur H. Thomas Co.) and are corrected. All thin-layer chromatograms were obtained using 2.5 × 10 cm × 250$\mu$ Anal-Tech sillica gel Unplates® as the adsorbent, $CHCl_3$ as the solvent and UV light as the detector. A Perkin-Elmer model 283 (ir, $CHCl_3$) and a Varian EM-360 (nmr, TMS internal standard in $CDCl_3$) were used as analytical tools. Assignment of the C=N absorptions in ir spectra of the nitrones were made on the basis of comparison with spectra for the corresponding aldehydes. Elemental analyses were obtained from Spang Microanalytical Laboratories, Ann Arbor, Michigan.

## PREPARATION OF NITRONES

*Benzylidene t-butylamine-N-oxide*, **1** (*R=H*) was recrystallized from *n*-hexane, mp 72–73°C. An nmr spectrum was identical to that reported in previous studies.[5]

*4-Methylbenzylidene-t-butylamine-N-oxide*, **1** (*R*=$CH_3$). The anil **5** was isolated in a yield of 41% with bp 88–89° (1.0 mm). The crude nitrone was recrystallized from petroleum ether (bp 30–60°C) to give purified material in a 55% yield: mp 68–70°C; IR,† 1610 (weak, Ar), 1580 (weak, C=N) cm$^{-1}$ and no C=O and NMR δ8.12 (d, 2, J = 8 Hz, ArH *ortho* to C=N), 7.44 (d, 2, J = 8 Hz, ArH *ortho* to $CH_3$), 2.3 (s, 3, $ArCH_3$) and 1.5 (s, 9, *t*-butyl).

*Anal.* Calcd. for $C_{12}H_{17}NO$: C, 75.35; H. 8.96. Found: C, 75.71; H, 8.51.

*t-Butylhydroxylamine.* To a vigorously stirred soln of 2-methyl-2-nitro-propane (26.0 g, 0.25 moles, Aldrich) and $NH_4Cl$ (8.8 g, 0.16 moles) in 120 ml $H_2O$ cooled in an ice-salt bath was added Zn dust (42.0 g, 0.64 g atoms) within 1 hr at a temperature of 10–20°C. After stirring for 2 hr, the insoluble material was removed by filtration and washed thoroughly with $H_2O$. The filtrate was neutralized (pH 7.0) with concd HCl, water removed (Rotovap), $CHCl_3$ added to the remaining solid and insoluble material removed by filtration. The solvent was removed from the filtrate (Rotovap) and the remaining material stored *in vacuo* until it was used. Then it was added to benzene, filtered to remove some additional insoluble solid and solvent removed from the filtrate (Rotovap) to give 14.6 g (65.8%) of *t*-butylhydroxylamine; IR 3600-2500 (broad OH, NH) and 1600 (weak, NH) cm$^{-1}$.

*4-Methoxybenzylidene-t-butylamine-N-oxide,* **1** *(R=MeO).* A soln of 7.0 g (~79 mmoles) of *t*-butylhydroxylamine and anisaldehyde (5.4 g, 40.0 mmoles) in 20 ml benzene was refluxed 18 hr using a Dean-Stark trap. The solvent was removed (Rotovap) and the remaining liquid distilled at 104–120° (1.4 mm) to remove unreacted anisaldehyde. The remaining material (3.2 g, 38.5%) was recrystallized three times from ligroine (bp 60–80°) with Norit treatment in the first recrystallization to give 1.1 g (13.2%) of the purified nitrone, **1** (R=MeO): mp 96–98°C, tlc one spot, $R_f = 0.05$ (aldehyde's major spot $R_f = 0.53$, minor spot $R_f = 0.02$); IR‡ 1610 (strong Ar), 1580 (weak, Ar), 1570 (weak, C=N), 1510 (strong, Ar) cm$^{-1}$ and no C=O and NMR δ8.30 (d, 2, J = 8 Hz, Ar *ortho* to C=N), 7.43 (s, 1, CH), 6.90 (d, J = 8 Hz, 2, Ar *ortho* to MeO), 3.74 (s, 3, MeO) and 1.57 (s, 9, *t*-butyl).

*Anal.* Calcd. for $C_{12}H_{17}NO_2$: C, 69.53; H, 8.27. Found: C, 69.75; H, 7.77.

*4-Ethylbenzylidene-t-butylamine-N-oxide,* **1** *(R=$C_2H_5$).* This material was prepared in the same manner as the methoxy compound. Vacuum

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†IR absorptions for tolualdehyde: 1700 (strong, C=O) and 1610 (medium, Ar) cm$^{-1}$.

‡IR for anisaldehyde: 1600, 1580, 1510 (all strong, Ar) and 1690 (C=O) cm$^{-1}$.

distillation of the crude product gave unreacted 4-ethylbenzaldehyde at 85°C (3 mm) followed by the nitrone at 170°C (0.5 mm). Recrystallization of the nitrone from ligroine (bp 60–80°C) gave purified material, **1** ($R{=}C_2H_5$) in a yield of 6.0%: mp 62–64°C; tlc, one spot, $R_f = 0.11$ (4-ethylbenzaldehyde; major spot, $R_f = 0.62$ and minor spot, $R_f = 0.05$); IR† 1610 (weak, Ar), 1580 (weak, C=N) $cm^{-1}$ and no C=O and NMR $\delta$8.22 (d, 2, J = 8 Hz, Ar *ortho* to C=N), 7.51 (s, 1, CH=N), 7.22 (d, 2, J = 8 Hz, Ar *ortho* to alkyl), 2.65 (q, 2, J = 7 Hz, $CH_2$), 1.57 (s, 9, *t*-butyl) and 1.20 (*t*, 3, J = 7 Hz).

*Anal.* Calcd, for $C_{13}H_{19}NO$: C, 76.05; H, 9.33. Found: C, 76.30; H, 9.00.

## PREPARATION OF SURFACTANTS

Didodecyldimethylammonium chloride (DdDMAC) was prepared from the corresponding bromide[3] and the purification of dodecylammonium chloride (DdAc) was described previously.[4] The lamellar phase of the mixed surfactant was prepared in small batches and used immediately. DdDMAC, 5.0 g, 0.012 M and water 20.00 g, 1.111 M were mixed, heated 78–85° with stirring to give a smooth paste and cooled to 55–60°. DdAc 1.5 g, 0.011 M was added and the mixture heated again to 85–90° with stirring. The mixture was heated with stirring for about ten minutes below the boiling point until it was optically clear and then cooled to room temperature. The solution was adjusted to a pH 1.00 by the addition of concentrated hydrochloric acid using a Fisher Accumet pH Meter with an expanded scale, equipped with a Corning combination electrode. The addition of hydrochloric acid causes a precipitate to form which was dissolved by reheating. These solutions remain optically clear over periods of months. The microscopic classification of these solutions as lamellar liquid crystalline was outlined previously.[3]

## KINETIC STUDIES

Kinetic measurements of the hydrolysis of I, R=H, was followed by the disappearance of the peak at 290 $\mu$m in the thermostated curvettes

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†IR for 4-ethylbenzaldehyde (Eastman Kodak Co.): 1700 (strong, C=O) and 1610 (medium, Ar) $cm^{-1}$

at 25.0 ± 0.05° with a Gilford recording spectrophotometer. The reaction was initiated by the injection of 10 μl sample of an aqueous solution of 1 into a 1 cm curvette followed by stirring to insure homogeneous solution. The pseudo first-order plots are linear to greater than 90% reaction and reproducibility is within 5%. The procedure was repeated for the other four derivatives except for the different $\lambda_{max}$ values which are as follows: R=F, 285, nm; R=$C_2H_5$, 300 nm; R=$CH_3$, 294 nm; R=$CH_3O$, 310 nm. Correlation coefficients for Figures 1 and 2 were 0.99 and 0.97 respectively. Table II was 0.97.

## ELECTRON MICROSCOPY

A solution containing 37 mg of DdAc and 3.5 ml of deionized water was sonicated for ten minutes to give a homogeneous solution. An aliquot of 1.0 ml of solution plus 1.0 ml of 1% phosphotungstic acid were mixed and carefully placed on a 150 mesh copper grid covered with formvar. The solvent evaporated at room temperature and the residue was examined after cooling the microscope with liquid nitrogen. The electron microscope used was a JEOL 120u with an accelerating voltage of 120 kV.

The mixed surfactant solution containing 12 mg of the lamellar mixture plus 2.0 ml of ionized water was sonicated 10 minutes to give a homogeneous solution. The Nomarski differential interference microscopy was done on a drop of solution between a slide and cover slip by a Zeiss Universal Microscope.

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