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The Molecular Association of Tetracaine with Ethylpalmitate: An Infrared Spectroscopic Study*

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The molecular association between a local anesthetic, tetracaine, and a model lipid, ethylpalmitate, has been investigated by infrared spectroscopy. In carbon tetrachloride solution, tetracaine associates with ethylpalmitate through hydrogen bonding between the N–H (tetracaine) and C=O (ethylpalmitate) groups, the enthalpy of hydrogen bond formation being −18 kJ/mol. The role of the N–H group, common to many local anesthetics, is discussed in terms of its ability to form hydrogen bonds which could disrupt the hydrogen bond systems already existing in biomembranes.

KEY WORDS: Tetracaine / Local anesthetic / Infrared spectra / Molecular association / Hydrogen bond / Ethylpalmitate /

INTRODUCTION

The current interest in the structure and function of biomembranes has stimulated wide interest in related areas, such as membrane-drug interactions. Anesthetics and their interaction with model and natural membranes are now actively studied by a variety of techniques.[1~3] A comprehensive review on this subject has been published by Seeman.[1]

Anesthetics are known to alter the permeability of biological[4] and model membranes[5] and, while drug-protein interactions are likely to play an important role, it is widely accepted that anesthetics interact strongly with the membrane lipids.[1,2,6] However, despite an intensive ongoing research effort, the mode of action at the molecular level of both general and local anesthetics is still far from understood. Besides, due to the complexity of intermolecular interactions that can occur in biomembranes, overall relationships such as that established between anesthetic potency and lipid solubility, are compatible with a variety of different modes of action at the molecular level.

Consequently, it seemed to us of interest to look for specific interactions between a typical anesthetic and a well defined model system. We choose the widely used local anesthetic tetracaine, while ethylpalmitate served as the model compound for the membrane lipids. Local anesthetics usually contain strongly associative groups,

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and contrary to general anesthetics, have a strong tendency to self-associate. Recently, Sandorfy and co-workers[3,7~10] have shown that there is a striking parallelism between the hydrogen bond breaking ability and the potency of a variety of anesthetics. In this IR study we have paid particular attention to the hydrogen bonding properties of the N–H group in tetracaine, as this is a typical local anesthetic derived from *p*-aminobenzoic acid.

EXPERIMENTAL

Materials. High purity tetracaine was purchased from Sigma Chem. Corp. and dried over P_2O_5 under vacuum, prior to being used. Ethylpalmitate was obtained from Sigma and carbon tetrachloride was Fisher spectrograde. All solutions were prepared immediately before measuring the spectra.

Spectra. A Perkin-Elmer 180 grating spectrometer and standard techniques were used to study the concentration-dependent self-association of tetracaine. The spectral resolution was 2 cm^{-1}. All other studies were performed with a Nicolet 7199 Fourier transform infrared spectrometer, equipped with a deuterated triglycine sulfate detector. Typically, one hundred interferograms were co-added using an optical velocity of 0.267 cm/sec with a maximal optical retardation of 0.25 cm. The 16 K interferograms were zero filled once, apodized with a Happ-Genzel apodization function and Fourier transformed to yield a spectral resolution of 4 cm^{-1}. Temperature control was achieved by flowing a thermostated ethanol/water mixture through a hollow cell mount, the temperature being monitored with a copper-constantan thermocouple located against the edge of the cell windows. Spectra were processed according to the techniques described previously.[11,12]

RESULTS AND DISCUSSION

The self-association of tetracaine

In order to quantitate the molecular association of tetracaine with ethylpalmitate, the intermolecular self-association of tetracaine had to be investigated first under identical conditions. The infrared spectra of tetracaine in carbon tetrachloride solutions contain two major concentration-dependent features which correspond to the N–H and C=O stretching vibrations. In the C=O stretching region there are two bands 14 cm^{-1} apart, at 1710 cm^{-1} and 1696 cm^{-1}, due to the free and associated tetracaine, respectively. The N–H stretching region is illustrated in Fig. 1 and consists of two main bands about 50 cm^{-1} apart, one at 3434 cm^{-1} due to the free tetracaine and a second one around 3384 cm^{-1}, characterizing the associated tetracaine. There is an isosbestic point at 3426 cm^{-1}; however, the band maxima shift slightly with concentration for both the monomeric and the associated species. These shifts to lower frequency of the band maxima with increasing tetracaine concentration represent a typical band overlapping effect, but may also reflect (i) a change in the existing ratios of various associated oligomers such as dimers and trimers, or (ii) various

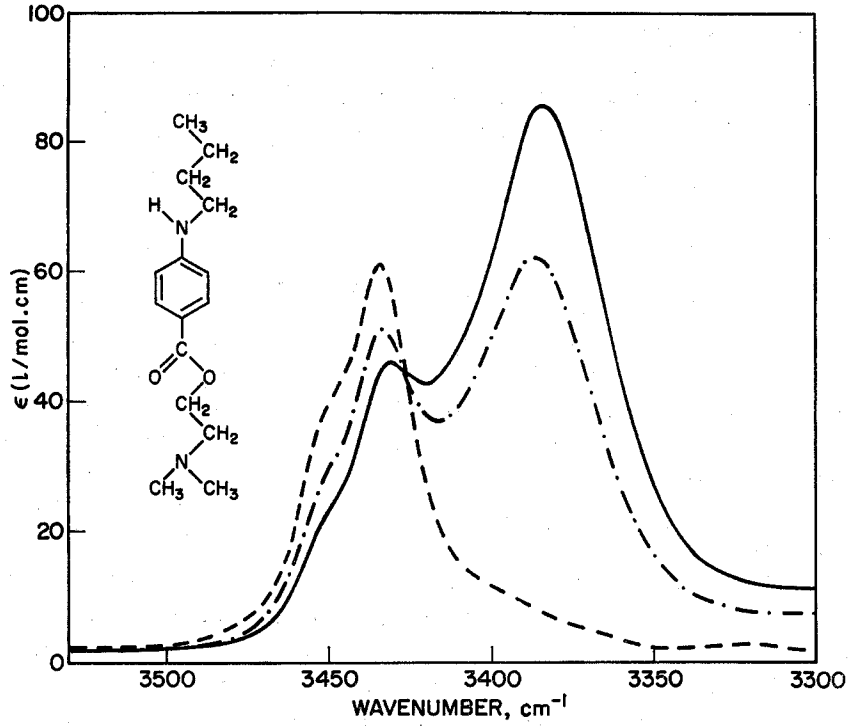

Fig. 1. Concentration dependent self-association of tetracaine. Room temperature infrared spectra of tetracaine in CCl_4 in the N–H stretching region (Perkin-Elmer 180) illustrated for three different concentrations: 0.9 M (——), 0.45 M (-·-·-) and 0.018 M (---). *Inset*: Structural formula of tetracaine.

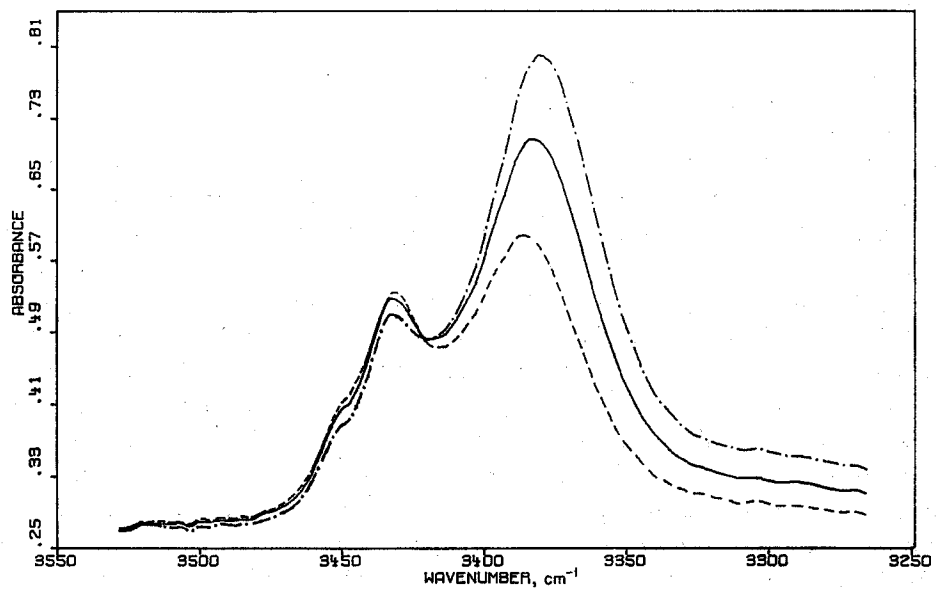

Fig. 2. Temperature dependent self-association of tetracaine. FT–IR spectra of 0.51 M tetracaine in CCl_4, illustrated for three different temperatures: --- (45°C), —— (20°C) and -·-·- (5°C).

association types such as N–H··O=C (ester), N–H··N (tertiary), or N–H··N–H. The shoulder band at 3450 cm^{-1} is due to a combination tone and does not interfere with the evaluation of the self-association process. As shown by the traces in Fig. 1, at a concentration of 0.018 M, almost all tetracaine molecules exist as free monomers. At this concentration the homo- or self-association is reduced to a minimum and it becomes possible to study the hetero-association with a suitable acceptor or donor in excess.

The self-association of tetracaine was also studied as a function of temperature. Fig. 2 shows the temperature-dependent change in the degree of association of a 0.510 M tetracaine solution in CCl_4. At 5°C the band area ratio of associated *vs.* free tetracaine, is 6: 1. With increasing temperature the hydrogen bonded species start to dissociate, and at 45°C this ratio is reduced to 2.5: 1. As in the case of the concentration-dependent equilibrium in Fig. 1, there is an isosbestic point around 3425 cm^{-1}.

From the above temperature dependence one could derive thermodynamic data characterizing the self-association of tetracaine if a simple monomer-dimer equilibrium is assumed. However, since oligomers are likely to be involved in this equilibrium,[13,14] we have not done so.

As to the nature of the self-association of tetracaine, this is clearly intermolecular and is certain to involve N–H··O=C (ester) associations, as deduced from the C=O stretching mode. However, N–H··N (tertiary) and N–H··N–H associations may not be excluded.

The hetero-association of tetracaine with ethylpapmitate

In order to avoid interference from the self-association, a tetracaine concentration of 0.018 M was chosen according to the results from the preceding section. The selection of such a low tetracaine concentration is reasonable since anesthetics do act at very low concentrations.

The complex formation, *via* hydrogen bonding, between this tetracaine and excess palmitate is reflected in Fig. 3. The free N–H stretching vibration of tetracaine gives rise to the already familiar band at 3433 cm^{-1}, whereas a new band appears at 3401 cm^{-1}, clearly due to the hydrogen bonded complex. Since palmitate has a band at around 3455 cm^{-1} due to an overtone of the C=O stretching mode, a single beam spectrum of 0.5 M palmitate in CCl_4 was used as the background in this region.

Although there is no simple relationship between the frequency shift and the enthalpy change,[14] from the experimentally observed frequency shift of 32 cm^{-1} it may be concluded that the hydrogen bond between tetracaine and palmitate is weaker than that found for the self-association in concentrated tetracaine solutions, where $\Delta\nu = 50$ cm^{-1}.

As to the nature of the association between tetracaine and palmitate, this must involve a N–H··O=C (ester) association, since the ester group in palmitate can act only as a proton acceptor.

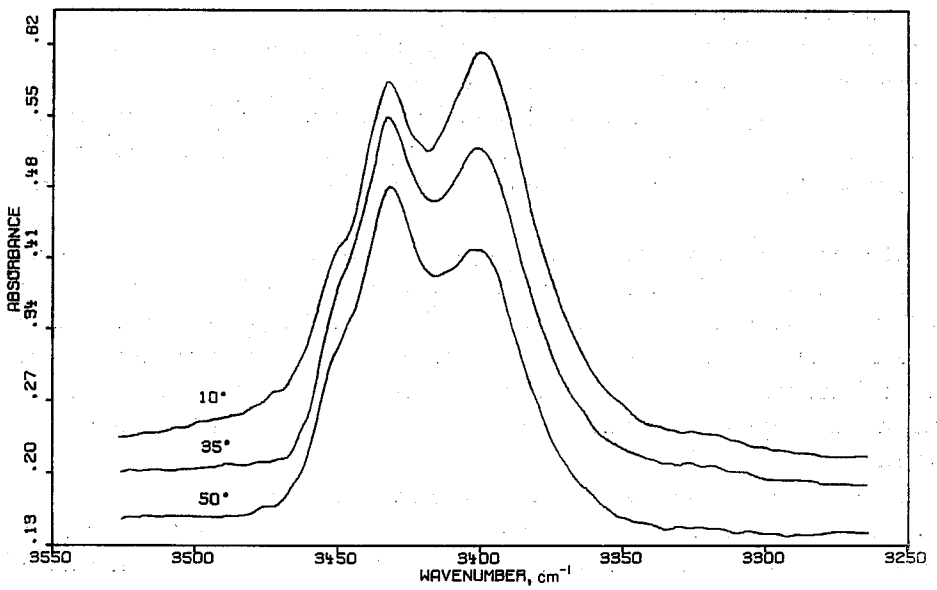

Fig. 3. Complex formation between 0.018 M tetracaine and excess (0.5 M) ethylpapmitate in CCl_4 as a function of temperature. FT-IR spectra in the N-H stretching region obtained in a 3 mm thick CaF_2 cell.

Thermodynamic data

In order to derive thermodynamic parameters characterizing the complex formation between tetracaine and ethylpalmitate, we have performed a temperature dependent study of this equilibrium. From the three representative spectra in Fig. 3 it is evident that with increasing temperature the free N-H band of tetracaine increases in intensity at the expense of the bound N-H component. To determine the enthalpy change involved between two such states, normally the peak heights or band areas are determined for the two bands for a series of temperatures. It is assumed that these are proportional to the number of molecules in each state.[15] Therefore the two overlapping N-H bands, centered around 3433 and 3401 cm^{-1} were first fitted using the simplex method of Nelder and Mead.[16] The band areas, obtained from Lorentzian-type curves, were then analysed according to the following equation:

$$K=\frac{[T_b]}{[T_f][P]}$$

where $[T_f]$ and $[T_b]$ represent the free and bound tetracaine, and $[P]$ the palmitate concentration. If A_f and A_b are used to denote the integrated intensities of the free and bound N-H bands, l the cell length, and κ_f and κ_b the corresponding absorption coefficients: $A_f=\kappa_f[T_f]l$ and $A_b=\kappa_b[T_b]l$. Thus, the equilibrium constant is:

$$K=\frac{A_b}{A_f}\cdot\frac{\kappa_f}{\kappa_b}\cdot\frac{1}{[P]} \quad \text{or,} \quad \ln K=\ln\frac{A_b}{A_f}+\ln\frac{\kappa_f}{\kappa_b}-\ln[P]$$

Assuming that the absorption coefficients κ_f and κ_b are temperature-independent and

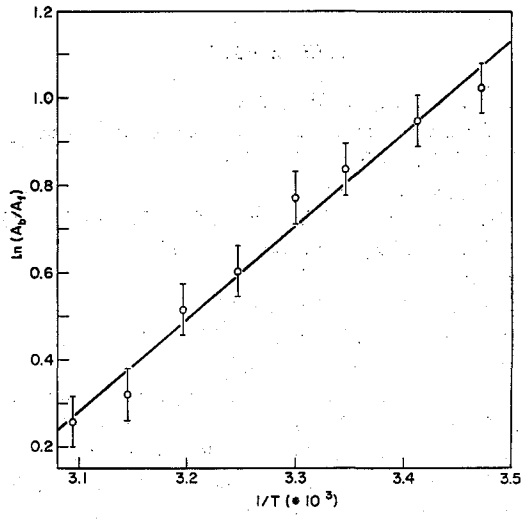

Fig. 4. Van't Hoff plot for the association of tetracaine with ethylpalmitate as obtained from the temperature-dependent change in band area for the free (A_f) and bound (A_b) components of the N–H stretching band.

that the palmitate concentration, due to its large excess, is constant, it is now possible to construct a simple van't Hoff plot for the equilibrium between these two states. This is shown in Fig. 4 which gives a plot of ln (A_b/A_f) *vs.* $1/T$ for eight different temperatures between 10 and 50°C. From the slope of this plot, a van't Hoff $\Delta H°$ of -18 ± 2 kJ/mol is obtained for the complex formation of tetracaine with excess palmitate. This is a fairly large enthalpy change, despite the relatively small frequency shift of 32 cm^{-1}. The enthalpy difference itself is typical of hydrogen bond formations.

CONCLUDING REMARKS

This IR spectroscopic study shows that local anesthetics such as tetracaine have a strong tendency to associate in concentrated solutions, but that hetero-association occurs at concentrations where little or no self-association is observed. The importance of a free N–H group in local anesthetics has been pointed out earlier;[17] however, the present study provides direct evidence that at very low concentrations the N–H group of tetracaine is capable of forming hydrogen bonds with suitable proton acceptors. This preferred hetero-association is likely to occur between the low concentration anesthetics and the lipid component, always present in excess in cell membranes, thus possibly disrupting the existing hydrogen bonds. Although this is a highly speculative supposition, it is in good agreement with the theory of anesthesia and hydrogen bonding put forward by Sandorfy[3,10,18] and with the location of anesthetics in model membranes as demonstrated by a recent ^{2}H-NMR study.[19]

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