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AGGREGATION OF AMPHIPHILIC MOLECULES IN WATER. I. α-PHENYLETHYLAMINE: ^{1}H and ^{13}C NMR STUDY

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The concentration dependence of the ^{1}H and ^{13}C NMR chemical shifts in D_2O and in $CDCl_3$ solutions were determined for (±)-α-phenylethylamine (I). Aqueous solutions of (−)-I and (+)-I, 50% enantiomerically enriched in (−)- and (±)-2, 2, 2,-trifluoro-1-phenylethanol and the (+)- and (−)-*N*-formyl derivatives of I, were also studied. ^{1}H nuclear Overhauser enhancements were used to check the conformations of the solutes at various concentrations and ^{1}H T_1 values were used to monitor the changes in molecular tumbling in solutions. The results are interpreted in terms of a spontaneous aggregation of solute molecules in water, with the possible determination of the critical micelle concentration. The time-dependent splittings in the ^{1}H NMR spectra suggest further, more detailed, studies of the structures of the aggregates and the possibility of chiral recognition in water.

INTRODUCTION

Substantial experimental data have been accumulated which show that parallel stacking of aromatic molecules is the preferred orientation of their interaction in organic solvents. This applies to the complexes of electron-donating and electron-accepting molecules,[1,2] intercalation of aromatic rings into biological molecules[3,4] and the orientation of substituted benzenes in the liquid crystalline matrix.[5]

On the other hand, molecular clusters of amphiphilic molecules in water have attracted much attention recently,[6,7] owing to their importance in membrane mimetic chemistry, where their formation mimics the spontaneous organization encountered in membranes of living tissues. In addition there have been attempts to elucidate the conditions of spontaneous racemate resolution in water,[8] a problem related to chiral recognition.[9,10]

As regards the above, few data exist[4,11] concerning the means of association of small aromatic amphiphiles in aqueous solutions. In this paper we present the results of a preliminary study concerned with the ^{1}H and ^{13}C NMR chemical shift dependence on increasing concentration of aromatic amphiphiles in water. It is shown that the aggregation of small amphiphilic molecules such as α-phenylethylamine exhibits interesting new spectral properties, especially as the neat compound does not form a nematic phase.[5] Monitoring the ^{13}C chemical makes possible the determination of the critical micelle concentration (cmc), whereas the observation of extra splittings in the ^{1}H NMR spectra should open the way for a more detailed study of the structure of these aggregates.

EXPERIMENTAL

Three model molecules were chosen, i.e. (±) and (−)-α-phenylethylamine, [(±)-**I** and (−)-**I**], 2, 2, 2-trifluoro-1-phenylethanol (**II**) and (±)- and (−)-*N*-formyl-α-phenylethylamine [(±)-**III** and (−)-**III**], owing to the rigidity of their hydrophobic part and the hydrogen-bonding ability of the polar functional group.

The measurements were performed on a Bruker AC 250-MHz NMR spectrometer in 5-mm sample tube using commercially available D_2O. The ^{1}H NMR spectra were routinely obtained under the same experimental conditions using a 45° pulse width, 3·3-s acquisition time and 32K memory with no extra delay

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between pulses. The ^{13}C NMR spectra were obtained on the same solutions using a 30° pulse width, 0·8-s acquisition time, 3-s delay between pulses and 32K memory. The ^{1}H and ^{13}C chemical shifts were measured using the spectrometer frequency parameter (*SR*) as reference. Aliquots of 2 μl of **I** and **II** were added under recorded time intervals to the D_2O solution in the NMR sample tube. A stock solution of **III** in pyridine-d_5 was used as this solvent does not prevent micelle formation.

The discussion below will concentrate on (±)-**I** as similar results were obtained with the other two compounds.

The measurements were performed on (±)-**I** and (−)-**I** in order to obtain information on the differences in the aggregation of racemic and optically active compounds. For the sake of comparison, (±)-**I** was also measured in chloroform solution in the same range of concentrations. In each case, the final concentration was increased until the cloud point was reached in aqueous solutions and the occurrence of other phases was monitored by observation of broad spectral resonances displaced to lower frequencies with respect to the relevant resonances in solution. The conformation of the functional group was monitored by nuclear Overhauser enhancement (NOE) experiments showing proximity of the *ortho* aromatic positions and the methine proton in the functional group. These measurements (see Table 4) were performed using a Bruker standard program for acquiring two different FIDs used for obtaining NOE difference spectra. Multiplet components were irradiated separately with low power for *ca* 10s using 32 cycles of 8 scans, a 15-s delay between pulses and a 3-s acquisition time to satisfy conditions for steady-state experiment. Samples were not degassed. The changes in molecular tumbling were checked by measurements of ^{1}H longitudinal relaxation times (T_1). Specific data for chloroform and aqueous solutions are presented in Table 4. Measurements were made using standard Bruker software for inversion–recovery pulse sequences using a 50-s delay between pulses and variable delays ranging from 0·1 to 20-s to cover the full range of signal recovery.

RESULTS AND DISCUSSION

Tables 1 and 2 present the ^{13}C chemical shift data of aqueous solutions of (±)-**I** and (−)**I**, respectively. The plot of the ^{1}H NMR chemical shift of the methyl group in (±)-**I** vs concentration in D_2O is shown in Figure 1(A) and the ^{13}C chemical shift data from Tables 1 and 2 are plotted in Figures 1(B) and 2. The same kind of concentration dependence was observed for the C-*i* resonances (See Tables 1 and 2), whereas the C-α, C-*o*, C-*m* and C-*p* resonances behave slightly differently.

Taking into account the well established preference of aromatic molecules to interact by means of superimposition in a parallel fashion,[1–5] we propose that the molecules under study aggregate in water by means of stacking of several molecules, giving rise to parallel arrangements of phenyl rings in a rod-like micelle aggregate (Figure 3). The rotation of the whole molecule against its close neighbour creates the possibility of hydrogen bond formation of the type N ··· H ··· N. The set of these bonds forms a helical pattern, hiding the hydrophobic phenyl rings in a micelle interior. This spontaneous process stems from the hydrophobic effect, which is a driving force of aggregation of amphiphiles in water.[4]

The ^{13}C NMR data for the methyl resonance of (±)-**I** show a minimum at *ca* 100 mM. The same data plotted against concentration expressed as $(mM)^{-1}$ or log (mM) give straight lines intersecting at *ca* 125 mM. We suggest that this concentration represents the cmc value for racemic α-phenylethylamine in water. The change in the direction of the ^{13}C chemical shift

Table 1. Concentration dependence of the ^{13}C chemical shifts of (±)-α-phenylethylamine (**I**) in D_2O

Concentration (mM)	δ(^{13}C) (ppm)					
	CH_3	CH	C-*o*	C-*p*	C-*m*	C-*i*
39·05	24·113	50·458	126·114	127·376	129·042	147·524
77·72	24·070	50·462	126·100	127·438	129·024	146·877
116·00	24·049	50·464	126·117	127·416	129·048	146·922
153·92	24·104	50·481	126·136	127·417	129·067	147·001
191·46	24·126	50·467	126·130	127·404	129·062	147·054
228·64	24·146	50·463	126·116	127·399	129·048	147·097
265·45	24·149	50·463	126·116	127·398	129·047	147·096
301·92	24·170	50·474	126·145	127·426	129·076	147·150
338·03	24·188	50·475	126·146	127·406	129·076	147·168
373·80	24·199	50·464	126·118	127·379	129·048	147·158
409·24	24·206	50·462	126·097	127·358	129·028	147·175
444·33	24·206	50·462	126·097	127·358	129·028	147·175

Table 2. Concentration dependence of the ^{13}C chemical shifts of (−)-α-phenylethylamine (**I**) in D_2O

Concentration (mM)	δ(^{13}C) (ppm)					
	CH_3	CH	C-*o*	C-*p*	C-*m*	C-*i*
39·05	24·068	50·448	126·109	127·389	129·032	146·980
77·72	24·127	50·425	126·097	127·359	129·026	147·096
116·00	24·166	50·425	126·077	127·340	129·011	147·176
153·9	24·186	50·426	126·079	127·342	129·016	147·216
191·46	24·208	50·425	126·096	127·339	129·028	147·231
228·64	24·224	50·427	126·094	127·340	129·029	147·253
265·45	24·225	50·424	126·077	127·324	129·028	147·250
301·92	24·242	50·425	126·077	127·320	129·025	147·271
338·03	24·243	50·424	126·078	127·320	129·007	147·272

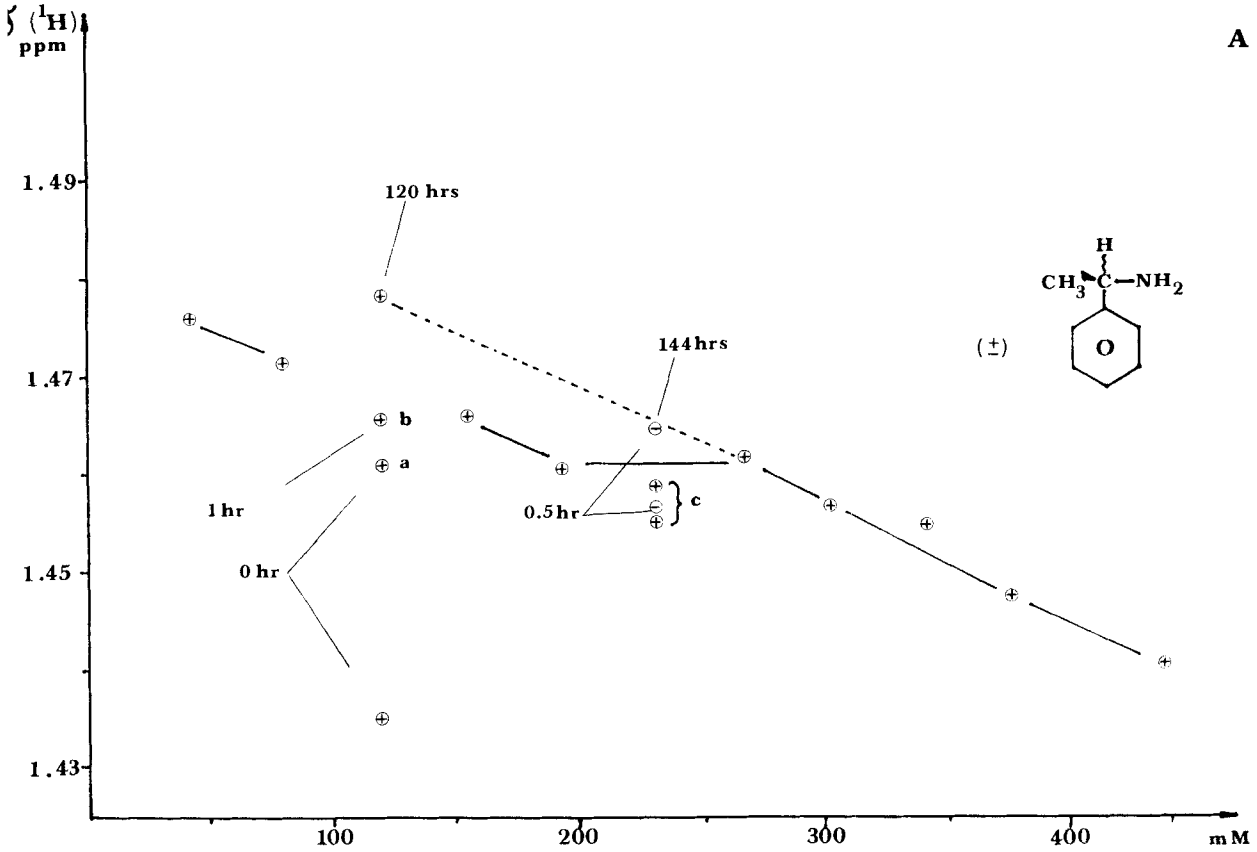


Figure 1. (A) Concentration dependence of the δ(^1H) of the methyl group in (±)-**I** in D_2O. (a) Splitting shown in Fig. 4; (b) chemical shift *ca* 1 h after addition of solute to old micellar solution; (c) splittings observed immediately after addition of solute

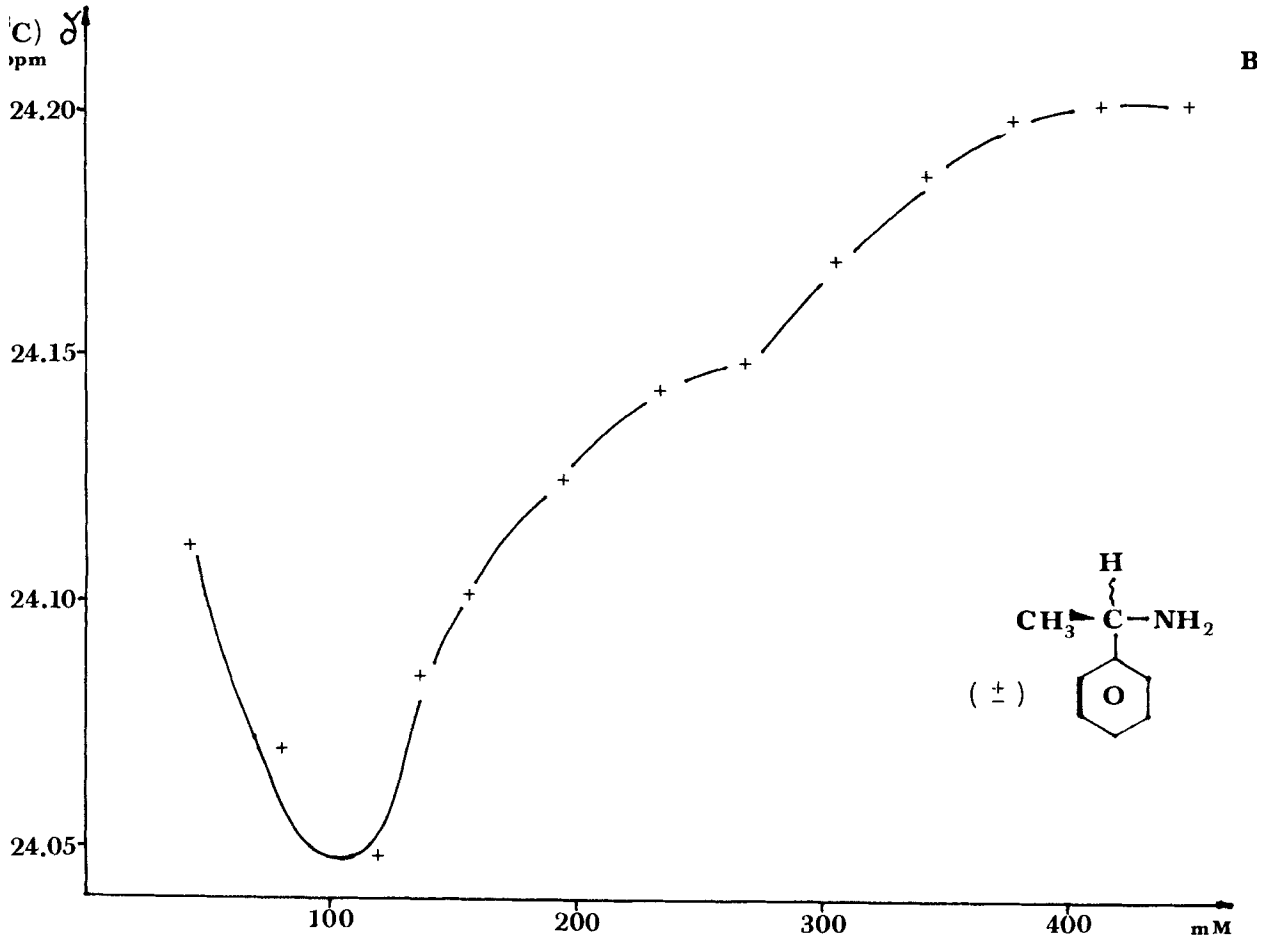


Figure 1. (B) Concentration dependence of $\delta(^{13}C)$ of the methyl group in (±)-**I** in D_2O

dependence vs concentration seen at *ca* 100 mM in Figure 1(B) may be related to changes in the intermolecular association in solution. In dilute solutions (below 100 mM), premicellar interaction between opposite enantiomers may be preferred whereas at higher concentrations micellization is favoured. Support for this is given by the absence of a similar minimum with optically active (−)-**I** (Figure 2). The plots of $\delta(^{13}C)$ vs concentration expressed as $(mM)^{-1}$ or log (mM) are straight lines which intersect with the abscissa at a concentration of 40 mM. This suggests that (−)-**I** starts to aggregate at a lower concentration than does (±)-**I** . The chemical shifts of each plotted ^{13}C resonance of (±)-**I** in $CDCl_3$ in both situations are almost invariant above 125 mM. We conclude that the observation of the ^{13}C chemical shift of **I** in D_2O solutions reveals the spectral process which could be assigned to the intermolecular process of aggregation.

Other spectral parameters, i.e. the nuclear Overhauser enhancement and ^1H T_1 data, given in Table 3 and 4, respectively, give independent evidence supporting the above view. The most obvious conclusion from the NOE data (see Table 3) is that, after irradiation of the methine proton in the functional group, the aromatic resonance gives a significantly larger NOE in water than in chloroform solution. This result is consistent with our model of molecular aggregation in water (see below) because an intermolecular hydrogen bond is most easily formed if the methine proton in the monomer eclipses the *ortho* aromatic proton, whereas this conformation need not be favoured in chloroform solution.

Another argument can be deduced from the relaxation data. It is easily seen (see Table 4) that the ratio of T_1 values for methyl group and aromatic resonances (*ca* 1 : 2) in $CDCl_3$ solutions is not changed if the concentration of the solute is increased nearly tenfold. This indicates that the molecules are tumbling isotropically

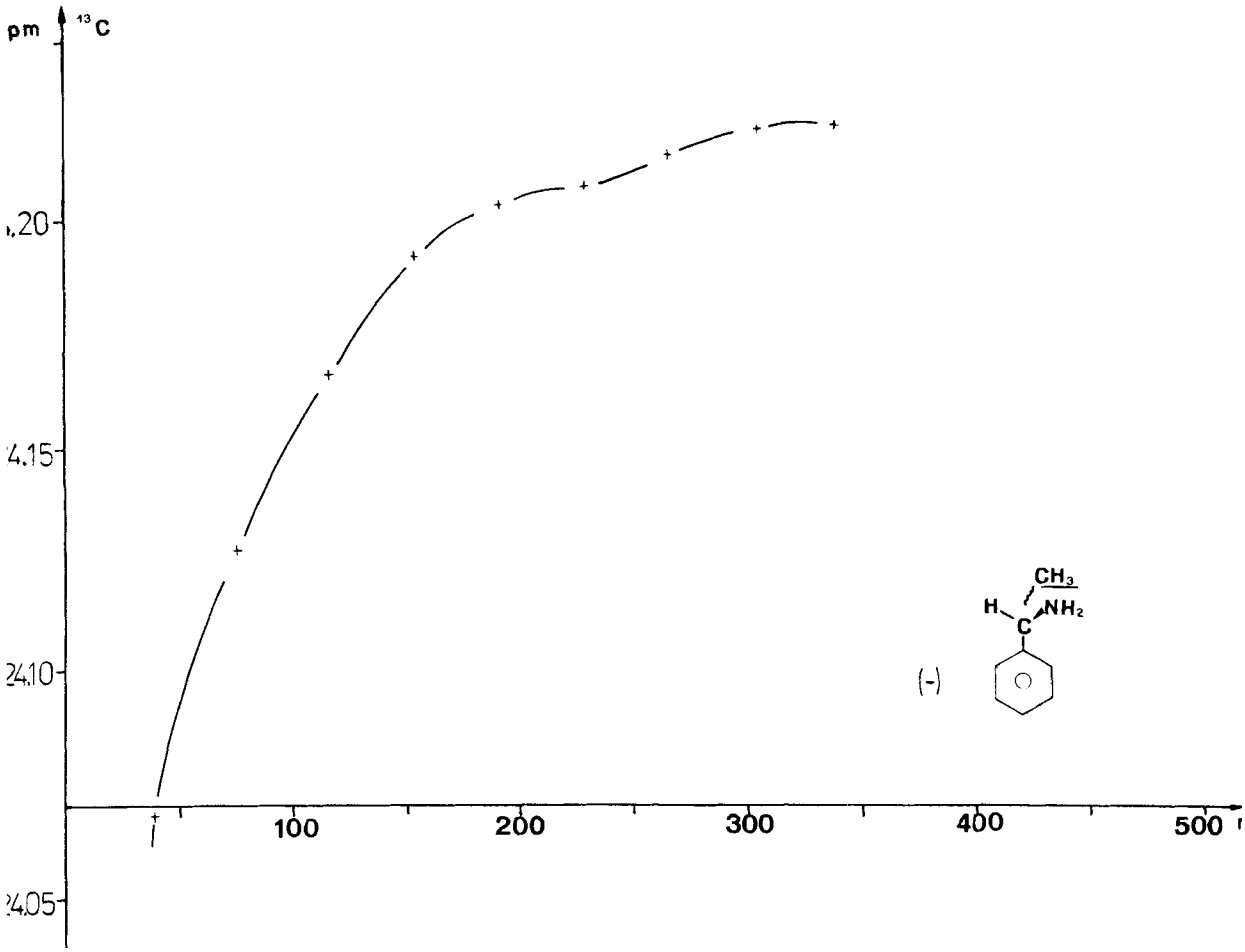


Figure 2. Concentration dependence of $\delta(^{13}C)$ of the methyl group in (−)-I in D_2O

regardless of concentration. On the other hand, the stacking of molecules in water introduces anisotropy in the molecular tumbling, which changes the rotational correlation times to different extents in various parts of the molecule and hence the ratio of T_1 values for methyl group aromatic resonances are now in the range 1 : 3–1 : 4. This type of relationship between longitudinal relaxation times and anisotropic motion is particularly well established for aromatic molecules.[12]

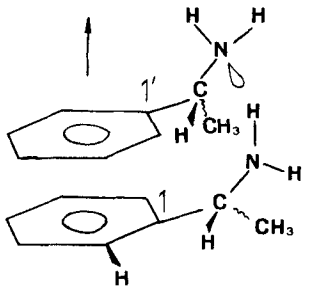


Figure 3. Proposed model of the intermolecular stacking of α-phenylethylamine molecules in water involving hydrogen bonding

The relaxation data reveal another important effect. It is easily seen that the T_1 values (see Table 4) are a maximum at concentrations of *ca* 260 mM in aqueous solution. This maximum coincides with the deflection on the curves of the concentration dependence of the ^1H and ^{13}C NMR chemical shifts which also occurs at a solute concentration of *ca* 260 mM [see Figures 1(A) and (B) and 2]. We relate these spectral changes to the appearance of the secondary aggregation process in solution. According to the two-step model[13] of longitudinal relaxation in micelles, several slow and fast motions on different time scales contribute to various extents to the overall relaxation. Moreover, in the present case, the relaxation of the monomer should

Table 3. Nuclear Overhauser enhancements (NOE) in amphiphiles studied in $CDCl_3$ and D_2O solutions[a]

Compound	Solvent	Concentration (mM)	Signal irradiated	NOE (%) CH_3	C-αH	NH_2	CH_{ar}
(●)-**I**	$CDCl_3$	116	C-αH		—	−9·2	+1·07
			NH_2		−22·0	—	0
	$CDCl_3$[b]	116	C-αH			−2·3	+1·0
			NH_2		−10·4		+0·7
	D_2O[b]	116	CH_3		−10·1		+4·1
			C-αH	+1·8			+7·9
(−)-**III**	D_2O	66	C-αH	+12·1	—		+4·2
			CH_3	—	+17·7		+3·8
(±)-**III**	D_2O	112·5	C-αH	+1·2	—		+5·3
			CH_3	—	+25·0		0

[a] Unless indicated otherwise, the measurements were performed on a Bruker AC 250 instrument.
[b] Measurements performed on a Bruker AM 500 NMR spectrometer.

Table 4. ^1H T_1(±0·04 s[a]) relaxation measurements at different concentrations of amphiphiles in $CDCl_3$ and D_2O solutions

Compound	Solvent	Concentration (mM)	Parameter	Aromatic resonances				$CDCl_3$	C-αH		NH_2	CH_3	
(±)**I**	$CDCl_3$	39·1	δ(ppm)	7·34	7·33	7·255	7·250	7·27	4·12	4·10	1·52	1·40	1·37
			T_1(s)	5·09	5·04	7·06	5·44	11·39	4·27	4·21	3·52	2·42	2·46
	$CDCl_3$	301·9	δ(ppm)	7·33	7·32	7·317	7·315		4·12	4·09	1·51	1·39	1·37
			T_1(s)	5·13	5·18	5·23	5·21		4·33	4·35	2·55	2·49	2·47
								HDO					
	D_2O	39·1	δ(ppm)	7·51	7·50			4·38	4·18	4·15		1·46	1·43
			T_1(s)	5·77	5·67			17·44	5·03	5·09		1·59	1·57
	D_2O[b]	265·5	δ(ppm)	7·50	7·49	7·48			4·14	4·12		1·44	1·42
			T_1(s)	6·45	6·52	6·81			5·87	5·84		1·71	1·62
	D_2O	265·5	δ(ppm)	7·50	7·497	7·494	7·47	4·83	4·13	4·11		1·43	1·41
			T_1(s)	6·19	5·34	5·35	6·16	15·53	5·50	5·50		1·64	1·90
	D_2O[c]	409·2	δ(ppm)	7·47	7·46	7·41		4·83	4·11	4·08		1·42	1·40
			T_1(s)	5·17	5·69	6·47		18·97	4·99	4·95		1·49	1·43
						COH[e]							
(−)-**III**	D_2O[d]	195	δ(ppm)	7·43	7·41	8·10	8·07	4·83	5·03	5·00		1·57, 1·54[e], 1·50, 1·47[e]	
			T_1(s)	5·24	5·25	6·57	10·22	16·16	4·13	4·14		1·12, 1·13[e], 1·15, 1·19[e]	

[a] T_1 calculation using the standard Bruker program gave a fit of individual points better than the indicated standard deviation.
[b] Solution was degassed by bubbling pure nitrogen through capillary for 4 h before measurements.
[c] Concentration close to saturation of aqueous solution.
[d] Solution prepared from stock solution as described in footnote a in Table 3.
[e] Minor and major rotamers along the amide bond are cited in that order.

contribute significantly, owing to the high cmc value. Hence at lower concentrations the effect of lowering the solution viscosity may influence the motions contributing to relaxation, whereas at higher concentrations the aggregation can significantly lengthen the effective correlation time, leading to a shortening of T_1 values.

The ^1H NMR data for the methyl resonance in (±)-**I** shown in Figure 1 require special attention. Two features of the ^1H NMR spectra are noticeable, i.e. a low-frequency shift is usually observed and time-dependent splittings are characteristic for a range of concentrations where micelle formation may be assumed (above 100 mM). The low-frequency shift is consistent with the situation usually encountered when small molecules are trapped and partially oriented in a lyotropic liquid crystal mesophase.[14]

The splittings of the ^1H resonances shown in Figure 1(A) for (±)-**I**, with indications of their time dependence, are reproduced in Figure 4 together with similar phenomena found in the ^1H NMR spectra of (−)-**I** and enantiomerically enriched **I** [50% e.e. in the (−)-*S* enantiomer]. It is worth mentioning that the same splittings, as regards the intensities and non-equivalences, are observed for the methine proton

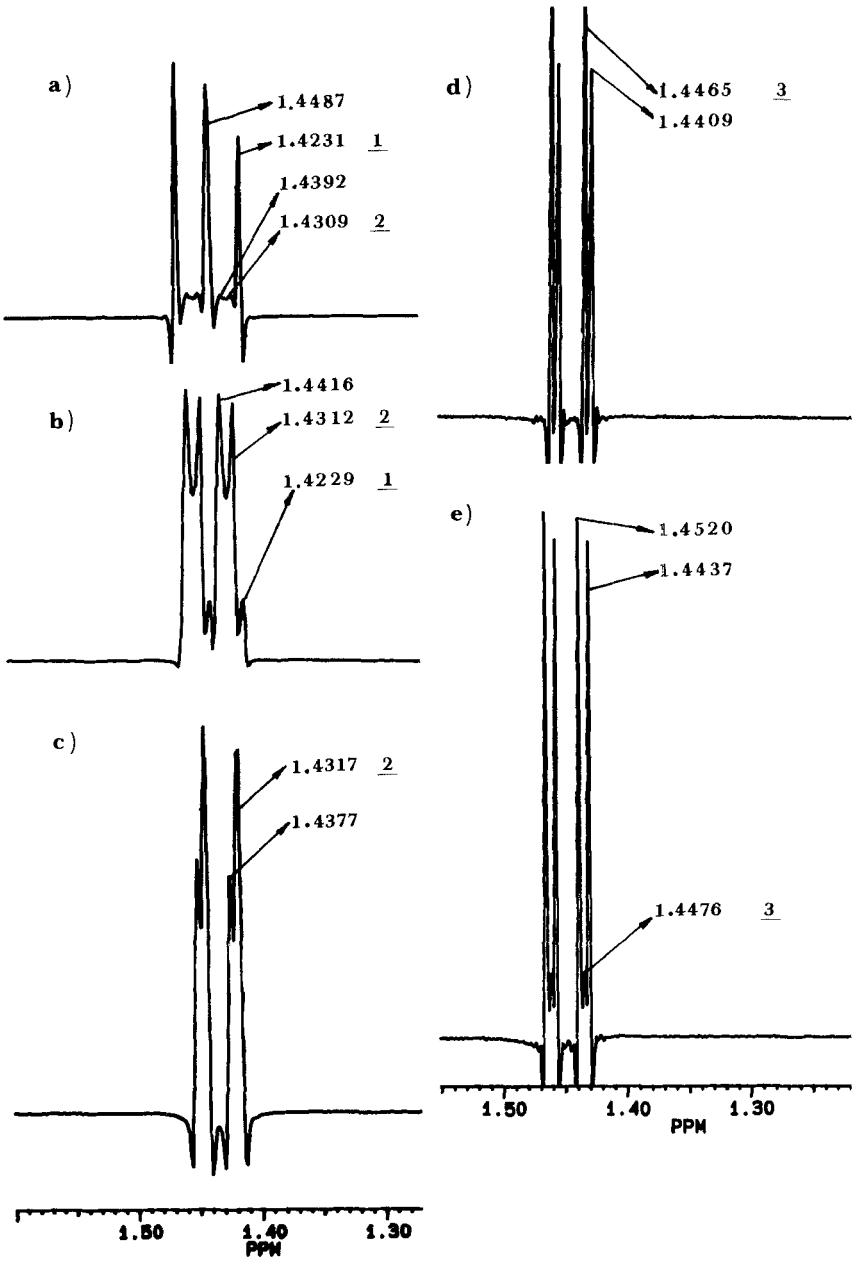
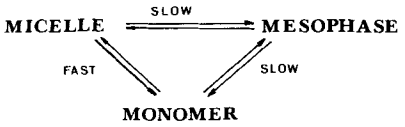


Figure 4. Time-dependent splittings in the ^1H NMR of the methyl group resonance of I in D_2O solution. All spectra were resolution enhanced. Chemical shifts of each doublet are marked at the low-frequency component. Chemical shifts differing by less than 1·5 ppb were considered to be identical and are numbered 1–3 (see text). All spectra were recorded on rotating samples (20 cps). (a) 153·9 mM solution of (±)-I monitored immediately after addition of solute to a matured micelle; (b) 191·5 mM solution of (−)-I monitored 24 h after addition of solute to an old micelle solution; (c) 301·9 mM solution of I 50% enantiomerically enriched in the (−)-enantiomer monitored 24 h after dissolving the solute in pure water; high-frequency component appeared after 24 h; (d) 265·5 mM solution of (±)-I monitored immediately after addition of solute to an old micellar solution; (e) spectrum of the same sample as in (d) but recorded 0·5 h later.

resonance. Owing to the complexity of the aromatic portion of the spectrum it was not possible to observe the phenomenon for the aromatic protons. Comparison of the spectra (a), (b) and (c) in Figure 4 shows that some of the resonances (numbered 1–3) are found in all the spectra regardless the concentration and enantiomeric composition. Further, the intensities of these signals change in a regular manner with increasing concentration, i.e. resonance 2 is enhanced whereas resonance 1 is decreased. This spectral process may reflect the situation in solution shown in Scheme 1. The constant chemical shift (i.e. 2) represents the mesophase which does not exchange rapidly with the other species in bulk solution, whereas the other two species in the scheme, owing to fast exchange, give rise to an averaged chemical shift at different concentrations. The time scale is visualized in spectra (d) and (e) in Figure 4, which show a decrease in resonance 3 within 0·5 h after addition of a new portion of solute to an old micellar mixture.



Scheme 1

Spectra (a), (b) and (c) in Figure 4 show an additional feature with respect to the enantiomeric composition of the samples. The high-frequency doublet in the racemic mixture shown in spectrum (a) is not present in spectrum (b), which contains the pure (−)-enantiomer of **I**. Further, the 50% enantiomeric excess of (−)-**I** can be linked to the intensities found in spectrum (c), especially as species 2 is present in all three mixtures. This suggests further possibilities for a more detailed study of chiral recognition in lyotropic aqueous mixtures.

A detailed interpretation of the spectral processes described above requires several phenomena to be considered.

Experimental conditions such as spinning may be involved, since the measurements were done with a superconducting magnet where the direction of the magnetic field is collinear with the spinning axis. The direction of the short-range orientation of molecules in solution tends to be perpendicular to the applied field.[14,15] However, owing to the loss of resolution, it was not possible in the present case to observe the splitting in a non-spinning sample.

Since the new portions of solute were added to an old micellar solution, the observed splitting could reflect an initial non-equilibrium state which reaches the equilibrium after some time. This would imply the existence of two species in solution, e.g. a micelle and pseudophase, which exchange slowly on the NMR time scale [see Scheme 1 and the time dependence of the ^{1}H NMR spectra in (d) and (e) in Figure 4] and which have different magnetic susceptibilities owing to the large difference in molecular shape.

Perturbation of the initial order in a solution created by aggregation on application of a magnetic field should also be considered. It is known that the magnetic field tends to unbind the helical structure in a cholesteric mesophase and orient the principal vector of molecular alignment perpendicular to its direction, the process being characterized by a relaxation time measured in hours.[14,16]

The situation in which monomeric solute molecules would exhibit residual dipolar coupling due to time averaging over environments in bulk solution and in a highly orienting pseudo-phase of liquid crystals can be disregarded because of the different spectral pattern which would be observed.[16]

Finally, the present observations could be related to the situation in which a nematic phase gives rise to self-induced non-equivalence[17] of a solute by means of the built-in screw sense of molecular aggregates.[9]

CONCLUSIONS

Small amphiphilic molecules are useful models for the study of intermolecular processes in water by NMR. Evidence has been obtained which indicates the micellization of α-phenylethylamine to be a spontaneous process in aqueous solution at concentrations above 100 mM. The observed time-dependent splittings in the ^{1}H NMR spectra require further investigation for their unambiguous assignment to a physical process in solution.

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