

spectrum showed two Soret peaks absorbing at 437 to 439⁷ and 417 m μ , corresponding to two distinct complexes. The spectrum was critically dependent upon the method of mixing the solutions. In our experiments, protoheme (1% in 2.50 M pyridine) was diluted 12.5 times with a solution of 3.75 M pyridine, and then the resulting solution was further diluted (10 times) with an aqueous solution of sodium dithionite (0.02 M) that was saturated with CO. In the absence of CO, a different pair of Soret peaks (433 and 420 m μ) was observed, and the α, β absorbancies were quite different from those of the CO complexes. Similar pairs of Soret peaks were observed when pyridine was replaced by equivalent quantities of either *n*-butylamine or imidazole (Table I).

Table I. Absorption Maxima (m μ) of Reduced Protoheme Complexes

Base	—Monomer state—		—Associated state—	
	Base-base	Base-CO	Base-base	Base-CO
Butylamine (0.3 M)	422	420	442	450
	528	537	532	548
	557	568	563	580
Imidazole (0.5 M)	408	417	434	442
	538	545		
	565	577		
Pyridine (0.37 M)	420	418	433	437-439
	527	540	530	543
	558	568	563	570

Increase in the concentration of heme decisively changed the proportion of the CO complexes (Table II) in favor of the complex providing the long-wave-

Table II. Effect of Heme Concentration on Reduced Protoheme-CO Complexes

Concentration of heme, M $\times 10^6$	OD associated form/OD monomer form ^a		
	Pyridine	<i>n</i> -Butylamine	Imidazole
0.32	0.33		
0.64	0.63		
1.22	1.10	0.58	
2.02	1.27	0.97	0.69

^a Ratios of the respective Soret peaks (Table I).

length Soret peak. Thus, association of hemes was strongly indicated as the cause of this absorption. The tendency to association was only slightly affected by the ligand (pyridine > *n*-butylamine > imidazole \cong CO), suggesting that most of the association energy derived from a direct heme-heme interaction.

The relative proportion of the two forms of the hemo-chrome-CO complexes was affected by the same substances that convert P-450 into P-420. Thus, in general the complex providing a long-wavelength Soret peak was converted to the form that absorbed at around 420 m μ in the presence of sodium deoxycholate (1 mg/ml) and organic reagents such as alcohols, while the reverse change was effected by an increase in salt concentration. In addition, the effect of alcohols became more pronounced as the alkyl chain increased in length. The pyridine-heme-CO complex was almost completely converted to the associated form by 0.1 M KCl, while, however, increase concentrations of salt resulted in a

(7) The position of this Soret peak was slightly dependent on the mixing method.

pronounced and specific hyperchromicity of the 439-m μ absorbancy.

The tendency of sodium deoxycholate and alcohols to dissociate the reduced protoheme-CO complexes while increased ionic strength favors association both suggest that the association energy in these complexes derives from a hydrophobic interaction of a pair of hemes which are oriented to maximize lipophilic overlap.

The relationship between the two types of hemo-chrome-CO complex is very similar to that between the CO complexes of P-450 and P-420. Thus, this model is in agreement with evidence that the anomalous spectral properties of reduced P-450 may arise from a direct contact of the hemes of the same or separate subunits which can be specifically oriented by the cellular membrane. In addition, either an imidazole group of histidine or, particularly, an ϵ -amino group of lysine is indicated as a protein ligand for the hemes of P-450 and P-420 in the reduced state. This is consistent with our recent evidence⁸ that the epr spectrum of oxidized P-450 can be attributed to a single heme coordinated by a sulfhydryl group and either histidine or lysine, and that the sulfhydryl interaction is lost on reduction.

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Oriented Smectic Liquid Crystal Solutions

Sir:

The orientation of molecules dissolved in nematic liquid crystal solvents has been exploited in studies of the anisotropy in nuclear and electron spin, electronic, and vibrational interactions;¹ it has also been shown that molecules can be oriented in mixtures of cholesteric liquid crystals which have been "compensated" to a nematic structure.²

An ordered smectic solution would offer the possibility of arbitrary control of the direction of orientation, and would therefore be of great use in nmr experiments.^{1a} Despite this advantage, no results obtained with smectic solutions have been reported.³ It has commonly been thought that the nmr lines obtained from smectic solutions would be too broad to be useful.⁴ Nevertheless, since pure smectic materials have been oriented,⁵ one may conclude that the preparation of ordered solutions should be possible. The purpose of this communication is to report the confirmation of this conclusion, and some of the interesting properties of oriented smectic solutions.

A 20 mole % solution of 1,1,1-trifluorotrichloro-

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(2) E. Sackmann, S. Meiboom, and L. C. Snyder, *ibid.*, **89**, 5981 (1967).

(3) G. R. Luckhurst, *Quart. Rev.* (London), **22**, 179 (1968). Since the submission of this letter, P. D. Francis and G. R. Luckhurst (*Chem. Phys. Letters*, **3**, 213 (1969)) have reported electron spin resonance spectra obtained in a smectic solution.

(4) S. Meiboom and L. C. Snyder, *Science*, **162**, 1337 (1968).

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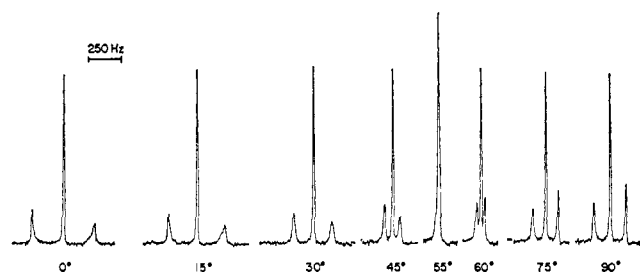


Figure 1. ^{19}F nmr spectrum of 1,1,1-trifluorotrichloroethane oriented in a smectic liquid crystal solvent at 50° . The spectra were measured at several angles with respect to the initial direction (0°) along which the sample was oriented.

ethane (TTE) in *p*-(*p*-2-*n*-propoxyethoxybenzylidene-amino)acetophenone⁶ was used. Differential scanning calorimetry and microscopic studies show unequivocally that the liquid crystal solvent exhibits only a smectic mesophase.⁶ The sample was oriented by cooling from the isotropic melt in the presence of a magnetic field, and ^{19}F magnetic resonance was used to detect and measure the orientation of TTE.

A single sharp resonance is obtained when the sample is in the isotropic state ($>63^\circ$). Upon cooling into the smectic phase, the intensity of this line decreases, and a diffuse triplet appears. As the temperature is lowered, the outer peaks move away from the center, and the triplet structure becomes more sharply defined. At 50° , the spectrum labeled 0° in Figure 1 is obtained. The triplet is characteristic of the anisotropic dipolar splitting among three equivalent spin $1/2$ nuclei located at the vertices of an equilateral triangle, and is observed only when the triangle (in this case, the FFF plane) is oriented.⁷

In the analysis which follows, it is assumed that upon cooling the solvent achieves orientation with its optic axis parallel to the external field, and ordering of the solute follows as a result of solute-solvent interactions. Since the same ordering mechanism is presumed to be operative in nematic solutions, the separation between the outer peaks in the TTE triplet can be written^{1a}

$$\Delta\nu = 3\gamma_{\text{F}}^2\hbar|S_{\alpha}|/2\pi r_{\text{FF}}^3 \text{ Hz} \quad (1)$$

where γ_{F} is the fluorine gyromagnetic ratio, and r_{FF} is the interfluorine distance. S_{α} describes the orientation of the symmetry axis of TTE (along the C-C bond) with respect to the external field, and is given by

$$S_{\alpha} = (3 \cos^2 \alpha - 1)S_0/2 \quad (2)$$

where α is the angle between the magnetic field and the smectic optic axis. S_0 is a measure of the ordering of the solute by the oriented solvent.

It is clear from the spectra in Figure 1 that the outer lines are not sharp as in nematic solutions; this is interpreted as a distribution in orientations of the TTE molecules.⁸ The validity of eq 1 and 2 can be checked by measuring $\Delta\nu$ as a function of α . This is done by turning the nmr tube, which is equivalent to rotating the sample about an axis perpendicular to the external field direction (for the resulting spectra, see Figure 1). As the sample is turned, the outer lines move in toward the

(6) The author thanks Mr. R. J. Cox, of the IBM San Jose Laboratory, for making some of this material available, and Dr. I. Haller of this laboratory for the results of his phase-transition studies.

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(8) Studies of possible orientation distributions consistent with the observed spectra are currently being made.

center and, at 55° , coalesce under the center peak. At 90° , the splitting is exactly one-half that measured at 0° , and the intensities confirm that the outer lines have switched position relative to the center. All these observations are consistent with the angular dependence expressed in eq 2, and the splitting between the satellite maxima follows the relation

$$\Delta\nu_{\text{max}} = 230(3 \cos^2 \alpha - 1) \text{ Hz} \quad (3)$$

From eq 1, an assumed molecular geometry,⁹ and the splitting at $\alpha = 0^\circ$, S_0 is found to be 0.0148. This is similar in magnitude to ordering factors for TTE in nematic solutions.¹⁰

Perhaps the most useful property of smectic solutions is that the external field is no longer necessary to maintain the orientation, once the sample has been prepared. As an illustration, the sample was taken out of the gap, and kept at 50° for 96 hr without any observable change in the orientation. In conclusion, it is felt that smectic solvents may prove to be the most versatile liquid crystal matrix for orienting molecules, and that the advantage of field-independent orientation in the bulk may be realized in a wide variety of experiments designed to measure phenomena in which molecular ordering plays a role.

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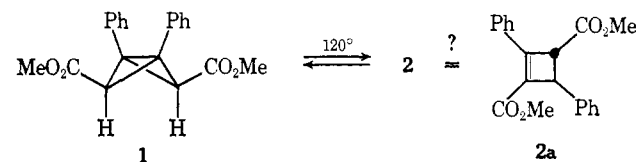
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Dimethyl 1,3-Diphenylbicyclobutane-2,4-dicarboxylates

Sir:

In 1966 the thermal equilibration of dimethyl 1,3-diphenylbicyclobutane-2,4-dicarboxylate (**1**) and dimethyl 2,4-diphenylcyclobutene-1,3-dicarboxylate (**2a**) was reported.¹ Several facts led us to question the structure (**2a**) assigned to the isolated compound (**2**),



mp $118-119^\circ$. Among these were the observations that the equilibrium only slightly favored the presumably much more stable isomer (**2a**),^{1a,2} and that the two unique protons in **2** have identical chemical shifts (δ 4.26, CHCl_3 , TMS).^{1b} A third objection to structure **2a** is mechanistic, considering the contortions that **1** must undergo during the isomerization. It has been suggested³ that structure **2b** better accounts for the experimental data; however, it is difficult to see why **2b**

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