time of 12 h. At this resolution, individual VCD features with the same contours as the absorption bands are observed for all the modes. At 4-cm^{-1} resolution, band contours for the more isolated bands were reproduced, but the individual contributions from the three modes near 1050 cm^{-1} were not resolved. However, the signal-to-noise ratio was considerably higher at the lower resolution (6144 AC and 384 DC scans for each enantiomer). The signs of the VCD intensities calculated by Lowe et al. agree with the experimental observations at 1-cm^{-1} resolution. In particular, the VCD couplets due to the modes at 1356 (*A*), 1300 (*B*) cm^{-1}, and at 1141 (*B*), 1061 (*A*) cm^{-1} are prominent in both the experiment and calculation.

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TICT Fluorescence Emission Dependence on Excitation Wavelength for Ethyl *p*-(Dimethylamino)benzoate in Supercritical Trifluoromethane

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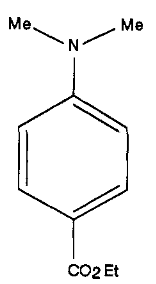
Supercritical fluids[3,4] have only recently been recognized[5,6] for their utility in probing solvent effects on photophysical phenomena. The alluring feature of a supercritical fluid is that a minor perturbation, such as a small change in pressure in the vicinity of the critical point, affords a large change in the density-dependent bulk solvent properties such as dielectric constant and viscosity. Uniquely, then, solvent effects can be probed without change of solvent. We report here the use of supercritical media to examine the highly polarity-dependent formation of the twisted-intramolecular-charge-transfer (TICT)[7] state of ethyl *p*-(dimethylamino)benzoate (**1**).



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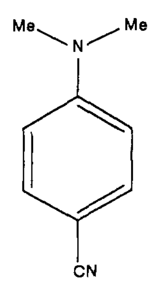

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(6) Kajimoto, O.; Futakami, M.; Kobayashi, T.; Yamasaki, K. *J. Phys. Chem.* **1988**, *92*, 1347. Kajimoto demonstrated that the fluorescence and absorbance behavior of *p*-(dimethylamino)benzonitrile (**2**) is a useful probe of solute–solvent clustering in supercritical CHF_3. The use of the supercritical medium as a probe of TICT photophysical behavior was a secondary concern. Johnston previously demonstrated, both experimentally and theoretically, that UV absorbance is a very effective tool for examining solute–solvent interactions in supercritical media. See: Kim, S.; Johnston, K. P. *Ind. Eng. Chem. Res.* **1987**, *26*, 1206.

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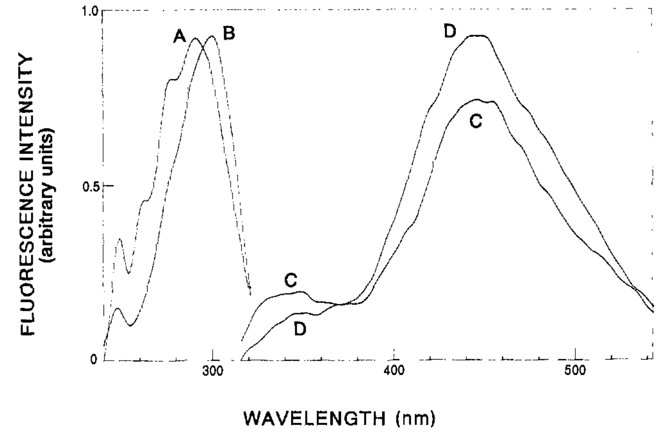

Figure 1. Luminescence dependence on excitation wavelength for ester **1** (10^{-6} M) in CHF_3 at 28 °C and 102.0 bar. Curve A: excitation spectrum monitored at 350 nm; maximum 291 nm. Curve B: excitation spectrum monitored at 434 nm (intensity divided by a factor of five); maximum 300 nm. Curve C: emission spectrum for excitation at 282 nm; maxima 350 and 444 nm. Curve D: emission spectrum for excitation at 298 nm; maximum 446 nm. Curves C and D are corrected for relative absorbance efficiency. Data collected in ratio mode.

We studied the steady-state fluorescence behavior of **1** in CHF_3 (T_c = 25.9 °C, P_c = 46.9 bar) at 28 °C and several pressures ranging from 44.9 to 136.0 bar. The ester **1** was purified by column chromatography and sublimation, and CHF_3 was deoxygenated by freeze–pump–thaw techniques (< 10 ppm O_2) and freed of weakly fluorescent impurities by passage through an in-line activated carbon filter.

Representatively, Figure 1 depicts the luminescence behavior of ester **1** (10^{-6} M) in CHF_3 at 28 °C and 102.0 bar. The emission profile was a strong function of excitation wavelength for all pressures studied at 28 °C. The excitation spectra, monitored at the planar (curve A) and TICT (curve B) emission maxima, were decidedly nonsuperimposable. Excitation at 282 nm enhanced emission from the short wavelength planar state (curve C), whereas excitation at 298 nm enhanced emission from the long wavelength TICT state (curve D). Similar excitation wavelength dependence was observed for ester **1** in CHF_3 at 50 °C but not at 70 °C. Control experiments showed that ester **1** is stable under our experimental conditions.

This dependence was not observed in normal liquid solvents such as *n*-C_6H_{14}, CH_2Cl_2, $CHCl_3$, and CH_3CN; nor was it observed in the supercritical media CO_2 (35 °C) and C_2H_6 (36 °C) at the many pressures examined. The absence of an excitation wavelength dependence under these conditions indicates that the anomalous dependence in CHF_3 was not due to the presence of an impurity in our sample of ester **1**.

Kajimoto[6] did not report emission dependence on excitation wavelength for the closely related *p*-(dimethylamino)benzonitrile (**2**) in supercritical CHF_3 at 50 °C. We confirmed the absence of excitation wavelength dependence for **2** at 50 °C in CHF_3 and further report that this dependence is absent at 28 °C. These experiments indicate that the presence of an excitation wavelength dependence for **1** in CHF_3 is not due to an impurity in CHF_3 and also suggest that the ester functionality in **1** plays a key role in this dependence phenomenon.

The possibility that deposition of microcrystalline **1** on the emission window or aggregation of **1** in solution could account for the observed excitation energy dependence can be discounted. First, windows of dissimilar materials, both quartz and sapphire, gave identical results. Second, our sample-loading technique precluded initial window deposition. Third, ester **1** is substantially more soluble in CHF_3 than in the nonpolar CO_2 and C_2H_6. If aggregation or deposition were to occur, it would most likely do so in the solvents in which **1** is less soluble.

Several instances[8–11] of luminescence dependence on excitation

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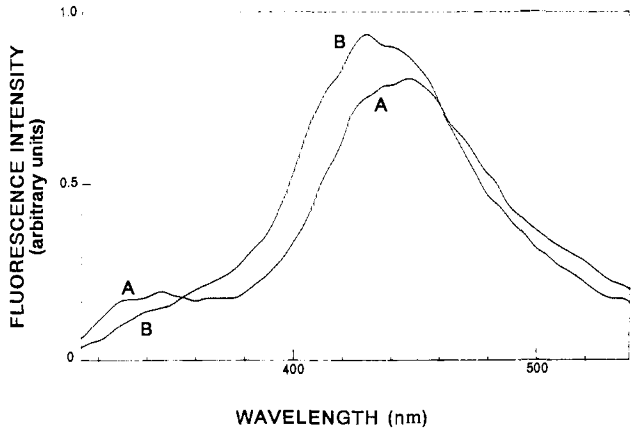

Figure 2. Luminescence dependence on pressure for ester **1** (10^{-6} M) in CHF_3 at 28 °C with excitation at 282 nm. Curve A: emission spectrum at p = 102.0 bar; maxima 350 and 446 nm. Curve B: emission spectrum at p = 46.3 bar; maximum 430 nm. Curves A and B are corrected for changes in relative absorbance efficiency.

energy have been reported. Explanations for these anomalous phenomena generally include factors such as the dependence of intersystem crossing rate on excitation energy, the formation of different solvation sites at low temperatures, and the existence of different conformers in the ground state. Since the $n\pi^*$ state of the ester **1** is high in energy,[7] it is unlikely that phosphorescence from the triplet can account for our observation that low-energy excitation causes enhanced TICT emission. In rigid media, the existence of different solvation sites or different conformers are satisfactory explanations for the anomalous behavior of closely related TICT-forming systems.[10,11] However, we observed excitation energy dependence in fluid densities of CHF_3 ranging from gas-like to liquid-like. We suggest that the excitation dependence for **1** in CHF_3 at 28 °C may be related to differential hydrogen bonding of the ester functionality. An equilibrium distribution of at least two species which differ in the extent to which the ester functionality is hydrogen-bonded would rationalize our observations.

Figure 2 depicts the emission of **1** in CHF_3 at 28 °C as a function of pressure. An increase in pressure and, therefore, an increase in the dielectric constant of the medium,[3] led to a decrease in the intensity and a red-shift of the long wavelength TICT emission. This was accompanied by an increase in the intensity and no shift in the short wavelength planar emission. Normally, with an increase in solvent polarity the TICT band both shifts to the red and gains in relative intensity.[7] The observed decrease in TICT intensity with increasing pressure suggests that the increase in viscosity of the medium with pressure[3] has an effect on the kinetics of relaxation to the TICT species on the excited-state surface. In fact, this TICT emission intensity dependence on pressure represents further experimental verification of the "twist hypothesis", which dictates hydrodynamic control[7] in the dual fluorescence of **1**.

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Biosynthesis of Vitamin B_6: Incorporation of D-1-Deoxyxylulose

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We have shown[3] that the C_8 skeleton of pyridoxol (vitamin B_6) is derived in toto from the carbon atoms of glucose and, furthermore, that the only two carbon–carbon bonds of the pyridoxol skeleton that are formed de novo in the course of its biosynthesis from glucose are the bonds C-2,3 and C-4,5. This finding confirmed inferences that were drawn from earlier tracer experiments,[4–7] which showed that the C_3 units of pyridoxol, C-3,-4,-4′ and C-5′,-5,-6, were derived from intact triose phosphate generated from glucose by the normal glycolytic sequence and that the C_2 unit, C-2′,-2, of pyridoxol was generated from one such triose phosphate by loss of a terminal carbon atom. We now present evidence that an intact pentose derivative, D-1-deoxyxylulose (**2**), gives rise to the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol (**3**), i.e., of the unit generated from the C_2 plus one of the C_3 precursors.

In separate experiments cultures of *Escherichia coli* B WG2 were incubated, as described earlier,[4] with D-glucose as the general carbon source, in the presence of D-1-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose[8] (**2**) (experiment 1) and L-1-deoxy[1,1,1-$^2H_3$,-(*RS*)-5-$^2H_1$]xylulose[8] (experiment 2), respectively. Pyridoxol hydrochloride was isolated from each culture after addition of natural abundance pyridoxol hydrochloride (2.5 mg) as carrier and purified by column and thin-layer chromatography, followed by high vacuum sublimation.

The ^2H NMR spectra of the isolated samples of pyridoxol hydrochloride (ca. 1.7 mg in 50 μL of methanol, saturated solution) were recorded on a Bruker AM 500 spectrometer (Figure 1).

The spectra of the two samples (Figure 1 (parts B and D)) were different.

The spectrum of the sample of pyridoxol hydrochloride in methanol, from the *E. coli* B WG2 culture incubated with deuteriated L-1-deoxyxylulose (experiment 2) (Figure 1D), was identical with the natural abundance deuterium spectrum of the solvent (Figure 1E). Evidently, deuterium from this substrate had not been incorporated into pyridoxol.

The spectrum of the sample of pyridoxol hydrochloride, obtained from the incubation with deuterium-labeled D-1-deoxyxylulose (experiment 1) (Figure 1B), showed three signals. One of these, at δ 2.54 ppm, is readily assignable to the C-methyl group, C-2′

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(8) D-1-Deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (**2**) was synthesized in six steps from D-arabinose, by a published method.[9] The product **2** was obtained, in the final step of the synthesis, by acid hydrolysis of D-1-deoxy-3,5-*O*-benzylidene[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (**1**), whose purity and deuterium content was determined by ^1H and ^2H NMR spectroscopy: ^1H NMR (CCl_4) δ (ppm) 3.95 (1 H, H-4), 4.06 (0.4 H, H-5β), 4.20 (0.6 H, H-5α), 4.25 (1 H, H-3), 5.60 (1 H, PhC*H*); ^2H NMR (CCl_4) δ (ppm) 2.26 (3 ^2H, CD_3), 4.09 (0.61 ^2H, D-5β), 4.23 (0.43 ^2H, D-5α); ^2H content at the C-methyl group 100%; at 5β 61%; at 5α 43%; ratio: ^2H at CD_3/^2H at (5β plus 5α) = 3/(1 × (0.61 + 0.43)) = 3:1.04. L-1-Deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose was obtained analogously, from L-arabinose. In each of the two experiments, a culture of *E. coli* B WG2 was incubated in the presence of D-glucose as the general carbon source with the deuterium-labeled samples of 1-deoxyxylulose. Expt. 1: D-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (1 g), D-glucose (1 g). Expt. 2: L-1-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (0.7 g), D-glucose (0.7 g).

