Experimental Section

Sample Preparation. Uridine was purchased from Aldrich Chemical Co. (Milwaukee, Wis.), and cytidine 5′-monophosphate was purchased from Sigma Chemical Co. (St. Louis, Mo.). These compounds were used without further purification. Typically, samples were prepared as aqueous solutions containing either 100 mg per mL or 200 mg per mL of the nucleoside or nucleotide, with or without equimolar sodium bisulfite. Concentrated HCl or sodium hydroxide (8 M) was used to obtain specific pH values. Sufficient solid potassium chloride was added to maintain the final ionic strength at a fixed value of 1.0; pH measurements were carried out at 30 °C with an IL Delta Matic pH meter (Perkin-Elmer).

NMR Measurements. All carbon-13 spectra were measured on samples in 8 mm sample tubes. A capillary tube containing 20% of 1,4-dioxane and 80% of deuterium oxide was used as a lock signal and an external reference. The observed chemical shifts were converted to the Me_4Si scale using δ(C − dioxane) = 67.40 ppm. Spectra were recorded on a Varian CFT-20 (16K) spectrometer equipped with a single side-band crystal filter for signal to noise ratio improvement and a Sykes diskette unit for storage. All ^{13}C NMR spectra were measured corresponding to a 4000 Hz (200 ppm) spectral width in 4096 data points.

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A Carbon-13 Nuclear Magnetic Resonance Study of Dibenzoylcystine Gels

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Gels are semirigid colloidal systems rich in liquid. Protoplasm ranks as the most widespread example of this peculiar state of matter. Two not entirely distinct theories have been advanced to explain gelation. The first, championed by Bradford[1] in the 1920's, maintains that the sol-to-gel transformation is a type of crystallization (the gel consisting of two phases composed of microcrystalline forms surrounded by water[2]). Alternatively, a gel may be formed by noncrystalline aggregates which cross-link in solution so as to entrain the dispersing medium in the capillary spaces between them.[3,4]

Dibenzoylcystine (I) is conspicuous among those organic substances that gel (e.g., agar, gelatin, poly(2-hydroxyethyl methacrylate), etc.) because of its simple structure.[5,6] Dibenzoylcystine is unique for another reason; a stiff hydrogel is produced by only 3×10^{-3} M disperse phase! In contrast to gelatin which associates in solution,[7,8] dibenzoylcystine seems to be a "fibrillary crystalline gel".[9] We describe herein an examination of dibenzoylcystine gels by ^{13}C NMR spin–lattice relaxation times (T_1).

Table I. Carbon-13 Spin–Lattice Relaxation Times in Seconds (T_1) of Dibenzoylcystine Gels at 37 °C

conditions	physical state	T_1 ortho	T_1 para
0.62 M in Me_2SO	liquid	0.35	0.16
0.30 M in Me_2SO	liquid	0.49	0.16
0.30 M in 10% D_2O–Me_2SO	soft gel	0.41	0.14
0.30 M in 20% D_2O–Me_2SO	thick gel	0.41	0.15
0.30 M in 20% D_2O–Me_2SO (sonicated)	thick gel	0.42	0.16

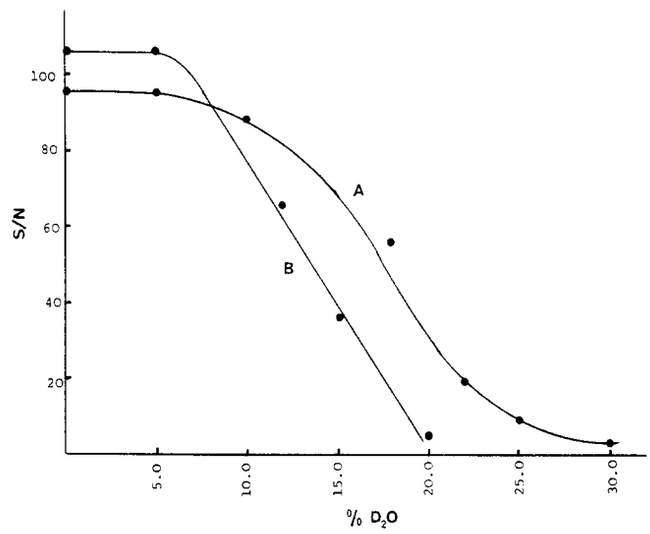
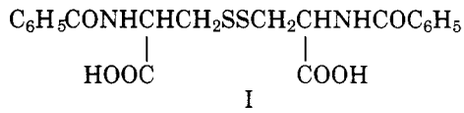


Figure 1. ^{13}C NMR signal-to-noise values for 0.32 M (curve A) and 0.63 M (curve B) dibenzoylcystine in Me_2SO with varying concentrations of D_2O at 37 °C using a constant number of transients (10 000 for A and 1000 for B).



I

T_1 studies provide information on molecular motion without the need for an external probe.[10,11] If a gel-forming compound associates in solution akin to surfactants, then T_1 values should decrease only modestly (two- to five-fold) relative to the monomeric state.[12] If a clear gel behaves as a two-phase system, then the ^{13}C NMR line widths and T_1's should be affected dramatically.[13] It is the purpose of this note to differentiate these alternatives with gels of a simple organic substance.

Since dibenzoylcystine in water does not form clear gels at the relatively high concentrations required for T_1 measurements, we used a dimethyl sulfoxide–water solvent system. A 0.30 M solution of I in pure Me_2SO is a fluid liquid; adding 10% water (v/v) produces a transparent semisolid gel. Further quantities of water (20%) give a thick white material. Thus, the gel consistency can be varied continuously by regulating the water content. The following T_1 values in seconds were found for 0.62 M I in pure Me_2SO at 37 °C: carbonyls (2.2 and 2.3); methine (0.15); C_1, C_2, C_3, and C_4 of aromatic ring (2.3, 0.35, 0.35, and 0.16, respectively).[14] These are quite ordinary values for an organic molecule the size of I.[15] In Table I we tabulate T_1 values for two aromatic carbons of I in Me_2SO-d_6 with and without D_2O. It is seen that gelation, in contrast to micellization,[12] need not alter the T_1's. Dibenzoylcystine apparently exists in two *non-exchanging* states: (a) a monomeric species which possesses normal T_1 values and line widths and (b) an aggregate whose ^{13}C resonances are broadened to the point of unobservability by ^{13}C–^1H dipolar

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interactions associated with long correlation times. If this conclusion is correct, then one would expect the signal-to-noise ratio (S/N) of the ^{13}C NMR spectra to decrease as added D_2O diminishes the monomer concentration. This is found to be the case (Figure 1). The point at which the S/N begins its precipitous decline depends on the concentration of I. Furthermore, soft gels form at D_2O levels where there is no decrease in S/N. Thus, *both* theories for gelation, mentioned above, may have merit. The soft gels, induced by low water concentrations, behave as if they are one phase as far as we can determine by ^{13}C NMR. Solid dibenzoylcystine with broad resonances predominates in the stiffer more opaque gels. Presumably, the increased viscosity and opacity of the gels at higher water concentrations is related to the appearance of a solid phase. In any event, soluble monomer entrapped within the microcrystalline network neither exchanges with the fibrillar species (on the NMR time scale) nor experiences difficulty moving about. Since the gelatinizing properties of I are destroyed by replacing the -S-S- linkage with $-CH_2-CH_2-$ or -CH=CH-,[9] the C-S-S-C dihedral angle[16] of 90° probably plays a key role in the formation of the molecular fibers.

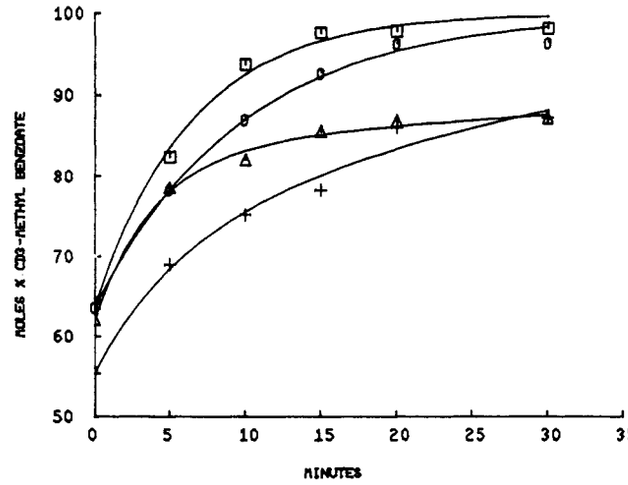

Figure 1. Time course experiments to measure the rate of deuterium incorporation in diazomethane. Key: +, system 1; △, system 2; ○, system 3; □, system 4. Curves were drawn using a computer program assuming a first-order two-compartment model (systems 1 and 2) and a first-order one-compartment model (systems 3 and 4).

Experimental Section

Spin-lattice relaxation time measurements on decoupled ^{13}C resonances were carried out with a Varian CFT-20 spectrometer using the inversion-recovery method.[17] A typical experiment included a pulse width of 24 μs (calibrated), pulse delay $\geq 4T_1$, 10 points per run, and 1000–2000 accumulations per point. The T_1 values (accurate to ±15%) are sufficiently small that degassing of the samples was not necessary. Signal-to-noise ratios were estimated from the height of the ortho-carbon peak divided by the height of the noise. Dibenzoylcystine was prepared by benzoylation of L-cystine, mp 190–192 °C (lit.[5] 190–192 °C). A small amount of benzoic acid impurity was removed by filtration from hot water.

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Preparation of Highly Enriched Diazomethane-d_2

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Several reports of preparations of deuterated[2,3a] or tritiated diazomethane[4] have appeared in the literature, but none of the available methods are suitable for routine preparation of highly enriched (>98%) material. The need for single isotopically labeled species as internal standards for quantitative mass spectral assays has led us to examine techniques for producing deuterated diazomethane (CD_2N_2) of 99% isotopic purity. We wish to report a convenient phase transfer catalyzed method using a diethyl ether solution of CH_2N_2 with NaOD and D_2O.

Diazomethane may be labeled by generation from labeled precursors,[5] nonlabeled precursors in the presence of labeled solvents and base,[3a] or by subsequent exchange with D_2O.[6] McManus et al.[5] used generation from labeled hydrazine and chloroform to obtain 92% labeled CD_2N_2 with yields of 10–20%. They had attempted direct NaOD/D_2O exchange for two 30-min periods as previously reported by others[6] but experienced the loss of 95% of the original diazomethane. Probably the most widely used method is that of Campbell[3a] who reacted nondeuterated nitrosamide precursors with deuterated solvent and base to effect a 50–60% yield of CD_2N_2 with from 83 to 97% deuterium depending upon the ratio of reagents to precursor. Fales et al. used this method in a convenient micro apparatus[7b] and reported 71% deuteration (50% CD_3) of methyl benzoate in 60% yield. Facile exchange of diazomethane protons requires that the glass apparatus, solvents, etc., be proton free or back exchanges will result in lowered isotopic yields for any of the above procedures.

Trying to improve upon the method of Campbell, we felt that diazomethane could be enriched by multiple exchanges with D_2O. We investigated the use of several solvent mixtures and phase transfer catalysts[8] to promote rapid exchange with minimum decomposition: system 1, diethyl ether (3 mL)/5% NaOD in D_2O (2 mL); system 2, diethyl ether:THF (1:1, 3 mL)/5% NaOD in D_2O (2 mL); system 3, diethyl ether (3 mL)/5% hexadecyltributylphosphonium bromide (HDTPB)–NaOD in D_2O (2 mL); system 4, diethyl ether (3 mL)/5% cetyltrimethylammonium bromide (CTAB)–NaOD in D_2O (2 mL).

Figure 1 summarizes several time course experiments. Equilibration was reached in 20–30 min with each of the four solvent systems tested with the phase transfer catalysts promoting higher incorporation of deuterium more rapidly. The low solubility of NaOD and D_2O in diethyl ether limits contact of diazomethane with exchange media in the absence of phase transfer catalysts. The curves describing systems 1 and 2 plateau before reaching the theoretical enrichment if all the D_2O were accessible for exchange and are best fit with a