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.

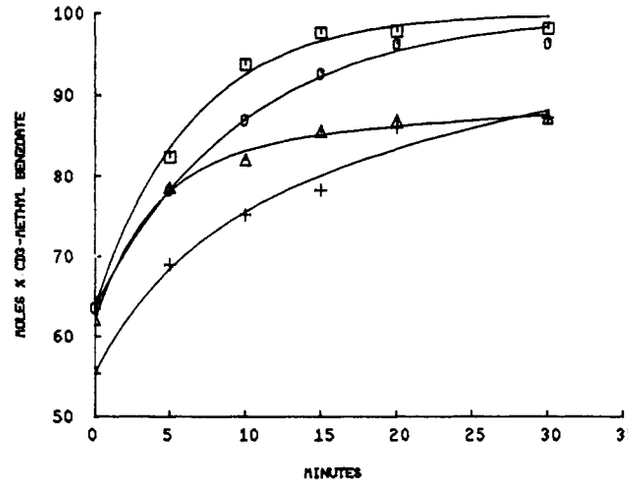

Figure 1. Time course experiments to measure the rate of deuterium incorporation in diazomethane. Key: +, system 1; △, system 2; ○, system 3; □, system 4. Curves were drawn using a computer program assuming a first-order two-compartment model (systems 1 and 2) and a first-order one-compartment model (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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Registry No.—Dibenzoylcystine, 25129-20-8.

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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

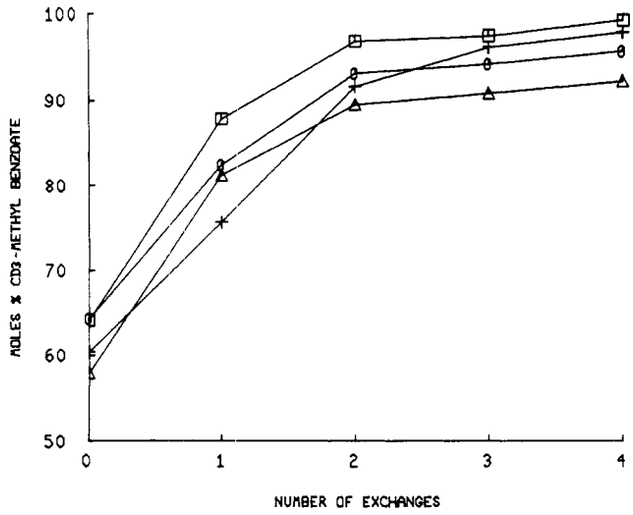

Figure 2. Multiple exchange experiments. Experimental points are connected. Key: +, system 1; △, system 2; ○, system 3; □, system 4.

function reflecting separate rates for exchange and solubility.

Multiple exchanges were effected by mixing fresh aqueous solutions in systems 1–4 in a microgenerator, separating the phases after 15 min, and either reacting the diazomethane with benzoic acid-*O*-*d* or mixing with additional aqueous phase. An exchange time of 15 min was chosen as a compromise between enrichment and yield of diazomethane. Highest enrichments were observed in systems 1 (97.7 mol % D_3) and 4 (99.1 mol % D_3) as shown in Figure 2. The lower isotopic enrichment observed with system 3 was probably due to the hygroscopic nature of the catalyst and the difficulty of preparing a D_2O solution with 99% deuterium.

Yields were somewhat variable, but using the precautions of keeping all solutions and flasks cold and basic, 25–35% of theoretical yield was obtained after five exchanges. For unexchanged diazomethane, 50–60% yield was obtained. Use of phase transfer catalysts did not significantly alter yields. We have used the methods described to exchange diazomethane generated on a larger scale (50–100 mmol) with similar enrichments and yields.

Experimental Section

Low resolution electron ionization mass spectra were recorded with a Finnigan 3200 quadrupole GC–MS. Samples were introduced via the gas chromatograph using a 10% Apolar 10c on 100/200 mesh gas Chromosorb Q (2 m × 2 mm) column at a temperature of 145 °C. Data were obtained by selected ion recording from *m/e* 134–144 ($M^{+\cdot}$). Precise isotopic enrichments were calculated by comparing labeled vs. unlabeled methyl benzoate using LABDET, a program in the NIH-EPA Chemical Information System.[9] Prior to all series of experiments, glassware was washed with D_2O and heated to 200 °C for 12 h. Anhydrous solvents were partitioned with D_2O prior to use.

Phase Transfer Catalysts. Cetyltrimethylammonium bromide (CTAB) was purchased from Aldrich. Hexadecyltributylphosphonium bromide[8] (HDTPB) was prepared by heating 1-bromohexadecane (10 g, 0.06 mol) and tri-*n*-butylphosphine (12.2 g, 0.06 mol) at 60–70% for 3 days. The resulting solid was filtered and recrystallized from hexane. The product was freeze dried giving the salt in 63% yield: mp 53–54 °C (lit.[8] mp 54 °C). A solution of 5% NaOD (Aldrich, 99 + atoms % D) and 5% quaternary ammonium or phosphonium salt in D_2O was used in all exchanges.

Benzoic Acid-*O*-*d*. Monodeuterated benzoic acid was prepared by exchanging benzoic acid five times with excess methanol-*O*-*d* (Merck, 99.7 atoms % D). Generally, for each series of experiments the final stock solution of benzoic acid-*O*-*d* in methanol-*O*-*d* (150 mg/10 mL) was equally divided between five screw capped tubes (Kimax) and the solvent was removed with dry nitrogen. The residue was redissolved in diethyl ether (1 mL).

Diazomethane-d_2. Partially deuterated diazomethane was prepared in a diazomethane microgenerator by the action of 40% NaOD on *N*-methyl-*N*-nitrosoguanidine (13 mg) as described by Fales and Jaouni.[7a] The product was trapped in ice-cooled diethyl ether (3 mL) over a 30-min period.

(a) Time Course Study. Following generation of diazomethane the apparatus was opened and 2 mL of the ice-cooled aqueous catalytic solution was transferred into the trap. After resealing the microgenerator the two phases were mixed by intermittent shaking over 30 min. At various time intervals (0, 5, 10, 15, 20, and 30 min) aliquots of the diazomethane–diethyl solution were transferred to ice-cooled benzoic acid-*O*-*d* solutions and capped. After a further 10 min at room temperature excess diethyl ether was removed with dry nitrogen and the residue was dried under vacuum.

(b) Multiexchange Study. Same procedure as for (a). Each exchange was allowed to proceed for 15 min with intermittent shaking. After this time the aqueous phase was withdrawn by pipet and replaced with fresh catalytic solution. Following the final exchange, the ethereal diazomethane solution was transferred to the reaction tube as outlined.

Registry No.—CD_2N-2, 14621-84-2; CH_2N_2, 334-88-3; NaOD, 14014-06-3; D_2O, 7789-20-0; HDTPB, 14937-45-2; 1-bromohexadecane, 112-82-3; tributylphosphine, 998-40-3; benzoic acid-*O*-*d*, 1005-01-2; benzoic acid, 65-85-0.

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Approaches to the Mitomycins: A Novel Pyrrole Photooxidation Product

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The photooxidation of pyrroles has been studied thoroughly in the past decade and a half.[2] The oxidation has been of some use in the introduction of the angular oxygen function in mitomycin-like molecules.[3] The accepted mechanism of the reaction has featured the photosynthesis of singlet oxygen which then attacked pyrrole. The transient endoperoxide **2**, which is initially formed, then fragments to yield a variety of products. One important proposed pathway involves cleavage of the O–O bond of **2** to yield 5-hydroxypyrrolines **3**, while a second postulated route requires bimolecular nucleophilic opening of the endoperoxide to yield **4a** or a unimolecular opening to yield peroxy isopyrrole **4b** which is trapped by nucleophiles.[4] In either event, products such as **5** and **6** are obtained. In addition it has been suggested that either endoperoxide **2** or hydroperoxide **4b** can rearrange to a dioxetane **7** which can then fragment to ring-cleaved products. In this note, we wish to describe a product of pyrrole photooxidation that has hitherto been unobserved and which might require reconsideration of currently accepted mechanisms.

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