compared with the 1.3 value deduced by Dervan and Santilli[6] at 712 K for tetramethylene derived from *cis*-3,4,5,6-TP-*3,4-d*$_2$. Others have found ratios of ethene-*1,2-d*$_2$ isomers from CB-*1,2-d*$_2$ at or near 1:1.[5,9]

For an entropy-locked diradical, the data lead, via k_{fl}/k_g ratios, to relative $\Delta G^{\ddagger}$ estimates for fragmentation versus ring closure of tetramethylene (Table I). The change in $\Delta\Delta G^{\ddagger}$ over the 693–1048 K range is negligible; the apparent increase in $\Delta\Delta G^{\ddagger}$ at 1130 °K may even be an artifact (see above).

Stereospecifically labeled substrates, shock tube techniques, and TDL analyses used in combination offer great promise in studies of the detailed thermal chemistry of simple hydrocarbons. Shock tube heating provides upward extension of temperatures, and avoids wall-catalyzed reactions. The TDL system eliminates spectral interferences; with planned system improvements, order of magnitude gains in precision should be realized. The present results, a preference for **3** from **1** and the absence of a significant temperature dependence for the k_f/k_g ratio, suggest either a very short lifetime for the diradical or a conservation-of-momentum effect as has been probed with the aid of trajectory calculations.[24]

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Novel Three-Dimensional NMR Techniques for Studies of Peptides and Biological Macromolecules

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Two-dimensional (2D) NMR[1] has succeeded to become the method of choice for the elucidation of biomolecular structure in solution.[2] The combination of J coupling correlation techniques, such as COSY and TOCSY, with cross-relaxation measurements, such as NOESY and ROESY, allows the determination of the three-dimensional structure of medium size proteins and nucleic acid fragments of molecular weight up to about 15 000.

Apart from possibly unfavorable relaxation times for large macromolecules, the molecular size limitation is primarily caused by spectral overlap in 2D frequency space. It is the purpose of this communication to demonstrate that the introduction of a third frequency dimension provides additional resolving power and exhibits considerable promise for future extensions of NMR structure elucidation methods.

Three-dimensional spectroscopy is a straightforward extension of 2D spectroscopy. The free induction decay is recorded as a function of the time variable t_3 with two independently incremented time parameters t_1 and t_2. The three time periods are separated by two coherence transfer processes. The large set of $N_1 \times N_2$ experiments, where N_1 and N_2 are the sample numbers in t_1 and t_2, respectively, necessarily leads to unacceptably long performance times when, for example, the full 10-ppm frequency range of protons were to be covered in all three dimensions. For this reason, we propose to limit sampling to restricted volumes of actual interest in 3D frequency space by application of frequency-selective pulses.

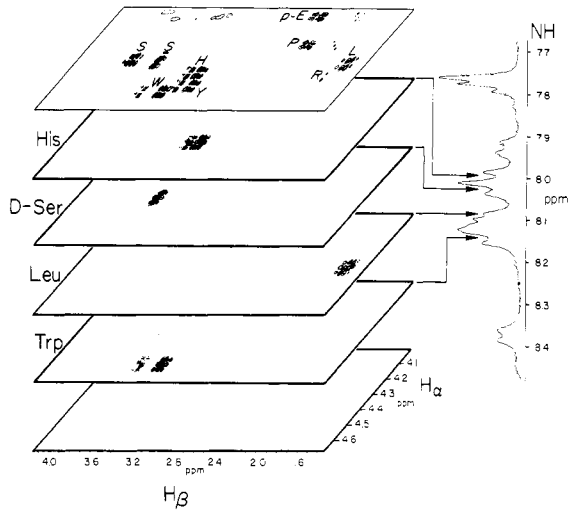

Figure 1. 3D COSY–COSY spectroscopy: On top is shown the $C_\alpha H$–$C_\beta H$ cross-peak region of a 300-MHz soft-COSY experiment of 70 mM buserilin in DMSO-d_6. Four 2D cross sections of the 3D spectrum parallel to the $\omega_2(C_\alpha H)$ and $\omega_3(C_\beta H)$ axes are shown at NH frequencies of His, D-Ser, Leu, and Trp, respectively. The 3D data matrix of dimension 96 × 96 × 2048 points, recorded in 48 h, was zero-filled to 256 × 256 × 4096 points prior to the 3D Fourier transformation requiring 7 h on an ASPECT 1000 computer. The spectral width is 500 × 500 × 3000 Hz. Contours are drawn for positive and negative intensities.

It is sufficient to apply selective excitation preceding the two evolution periods t_1 and t_2. Nonselective pulses may precede the detection period, and selective detection in t_3 is only required in the case of limited data storage.

3D spectra can be obtained by various combinations of coherence transfer processes. In a COSY–COSY experiment, for example, both transfers involve the scalar J couplings (homonuclear and heteronuclear transfers can be arbitrarily combined[3]). In a NOESY–COSY experiment, on the other hand, the first transfer involves cross relaxation while the second is effected by J couplings. Combinations involving ROESY,[4,5] TOCSY, multiple quantum excitation, or relayed transfer are also conceivable.

We demonstrate the features of 3D spectra by experiments on the linear nonapeptide buserilin, pyro-Glu-His-Trp-Ser-Tyr-D-Ser-Leu-Arg-Pro-NHCH$_2$CH$_3$ (p-E-H-W-S-Y-S-L-R-P-NHEt). Figure 1 shows on top the moderately crowded $C_\alpha H$–$C_\beta H$ cross-peak region of a 2D soft-COSY[6] spectrum, obtained with the pulse sequence $(\pi/2)^{(C_\alpha H)}$– t_1 – $(\pi/2)$ – t_2 consisting of a selective and a nonselective pulse. The 3D COSY–COSY sequence with the pertinent coherence transfer NH → $C_\alpha H$ → $C_\beta H$ leads to a 3D spectrum with $(\omega_1,\omega_2,\omega_3) = (\omega_{NH},\omega_{C_\alpha H},\omega_{C_\beta H})$. It requires the pulse sequence

$$(\pi/2)^{(NH)}\text{–}t_1\text{–}(\pi/2)^{(NH)}(\pi/2)^{(C_\alpha H)}\text{–}t_2\text{–}(\pi/2)\text{–}t_3$$

By displaying 2D cross sections of the 3D spectrum taken at fixed ω_1 frequencies, it is possible to spread the 2D $C_\alpha H$–$C_\beta H$ cross-peak region according to the NH chemical shifts as is visualized in Figure 1. In this case each cross section contains only one cross peak that characterizes a particular amino acid residue by the three chemical shifts of NH, $C_\alpha H$, and $C_\beta H$ protons. The COSY–COSY experiment visualizes two-step relayed coherence transfer whereby the relay spin is uniquely identified in contrast to 2D relay experiments.[7]

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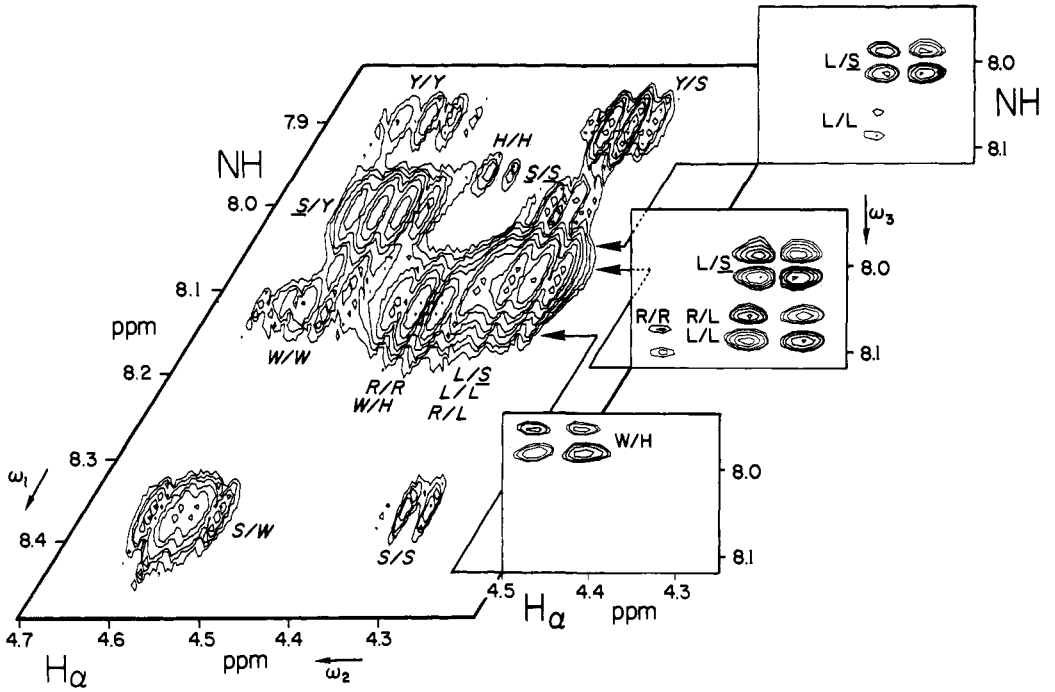

Figure 2. 3D NOESY-COSY spectroscopy: The top spectrum shows the NH-$C_\alpha H$ cross-peak region of a 300-MHz soft-NOESY experiment on buserilin. Same acquisition and processing parameters as for the 3D COSY-COSY.) The spectrum was recorded in 96 h. The three inserted blocks give cross sections parallel to $\omega_2(C_\alpha H)$ and $\omega_3(NH)$ axes of a 3D NOESY-COSY experiment. Peaks are labeled with the one-letter codes of the amino acids forming origin and destination of coherence transfer. Contours are drawn for positive and negative intensities.

A soft-NOESY spectrum, again of buserilin, showing the NH-$C_\alpha H$ cross-peak region is given in Figure 2. Severe overlap of intra- and interresidue NOE cross peaks makes the interpretation difficult. In particular the overlap of the peaks $NH^{(R)}$-$C_\alpha H^{(R)}$ and $NH^{(W)}$-$C_\alpha H^{(H)}$ as well as of $NH^{(R)}$-$C_\alpha H^{(L)}$, $NH^{(L)}$-$C_\alpha H^{(L)}$, and $NH^{(L)}$-$C_\alpha H^{(\underline{S})}$, should be noted. The NOESY-COSY experiment involving the coherence transfer processes

$$NH \xrightarrow[\text{inter}]{\text{intra}} C_\alpha H \xrightarrow{\text{intra}} NH$$

provides a spread in the third dimension by the intraresidue J correlation of $C_\alpha H$ and NH protons. The pulse sequence reads $(\pi/2)^{(NH)}$-(π)-Δ-t_1-$(\pi/2)$-τ_m-$(\pi/2)^{(C_\alpha H)}$-t_2-(β)-t_3. The -(π)-Δ-refocusing element removes peak shape distortions. Three cross sections parallel to ω_2 and ω_3 through the 3D spectrum are included in Figure 2. Because the NH resonances of R and H, and of L and S, do not overlap, it is possible to separate all mentioned coincidences except for the peaks $NH^{(R)}$-$C_\alpha H^{(L)}$(R/L) and $NH^{(L)}$-$C_\alpha H^{(L)}$(L/L) that overlap even in the 3D spectrum, because $NH^{(R)}$ and $NH^{(L)}$ are almost isochronous. However, the small L/L + R/L cross peaks in the 2D section (a) compared to (b) indicate that the L/L correlation is much weaker than the R/L correlation. A more complete separation of the R/L and L/L cross peak can be achieved in a different part of the full 3D spectrum, involving the transfer

$$C_\alpha H \xrightarrow{\text{NOE}} NH \xrightarrow{J} C_\alpha H$$

that is not displayed here.

Selective 11.9-ms Hermite-shaped pulses[8] were used. 3D absorption peakshapes are obtained by independent time proportional phase incrementation (TPPI) of all pulses preceding the first and second mixing sequences, respectively.

The results obtained with 3D NMR spectroscopy applied to a small peptide are encouraging in view of application to medium-size proteins where more severe overlap among cross peaks calls for additional resolution.

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First Example of Organic Reaction Implying the Axial Ligand in Metalloporphyrin Series. Synthesis, Characterization of 4-Substituted (Tetrazolato)indium(III) Porphyrin Complexes, and Molecular Structure of (4-Phenyltetrazolato)-(2,3,7,8,12,13,17,18-octaethylporphinato)indium(III)

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Organic azides are powerful reagents in heterocyclic chemistry and have been the focus of a considerable number of papers.[2,3] On the contrary, chemistry of metal coordinated azides is scarce, and few reported works demonstrate that the reactivity of such ligands is comparable to organic azides.[4-8] The biological interest

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