Multiple Hartmann-Hahn Coherence Transfer in Nuclear Magnetic Resonance Spectroscopy

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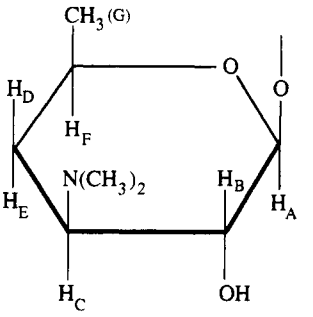
We propose a new NMR method for establishing that certain designated protons in a molecule are linked together by scalar coupling in a chain. A chosen proton is excited selectively, and its coherence is transferred to its neighbor by frequency-selective homonuclear Hartmann-Hahn mixing.[1,2] This new coherence is then propagated from proton to proton in a stepwise manner, finally being monitored as an absorption-mode signal at the last site in the chain, with negligible responses elsewhere in the spectrum. This provides direct evidence of connectivity. In practice, as many as six consecutive Hartmann-Hahn transfers can be achieved, the equivalent of a seven-dimensional shift correlation experiment. The practical limit is set by signal attenuation through spin-spin relaxation during the "spin-lock" periods. The technique is largely unaffected by overlap and may therefore be used to simplify overcrowded spectra. It differs from the uncontrolled diffusion of coherence characteristic of total correlation spectroscopy (TOCSY[3] or HOHAHA[4]) in that the coherence follows a single, predefined path and is not dissipated in other branches of the coupling network; this allows the coherence to be followed over a larger number of steps.

The initial excitation of the chosen proton (I) uses a shaped band-selective pulse that generates uniform intensity, pure absorption mode signals.[5] Hartmann-Hahn transfer to a second proton (S) is implemented by applying two continuous spin-lock radiofrequency fields at the appropriate chemical shift frequencies (ν_I and ν_S) for a period of $1/(J_{IS})$ (in seconds). These fields, B_1 and B_2, of equal intensity, are generated as two interleaved "DANTE" sequences,[6] one with pulses of constant phase, the other with the phase incremented in small, equal steps to give the effect of an offset in frequency.[7,8] The Hartmann-Hahn condition is made very selective ($\gamma B_1/2\pi$ comparable with J_{IS}) so that coherence transfer only occurs when the spin-lock frequencies are close to the respective chemical shifts ν_I and ν_S. After the first transfer, a new absorption mode signal appears at site S but all other NMR signals vanish. This S-spin coherence may be transferred to a third site (R) by applying simultaneous spin-lock fields at ν_S and ν_R for a period $1/(J_{SR})$. This coherence may then be propagated through further couplings, generating a signal from the last proton in the chain. An eight-step cycle alternates the phase of the initial soft pulse, the first and last spin-lock fields, and the receiver.

Chart I



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Multiple stepwise coherence transfer is demonstrated in Figure 1 for the 400-MHz proton spectrum of one of the six-membered rings (Chart I) of erythromycin. This was chosen as an example of a crowded spectrum. Two propagation paths were implemented:

$$\begin{array}{l} A \rightarrow B \rightarrow C \rightarrow D \rightarrow E \\ \qquad\qquad\quad \downarrow \\ \qquad\qquad\quad F \rightarrow G \end{array}$$

The coupling J_{EF} was too small to sustain appreciable coherence transfer. Note that the individual spin multiplets are faithfully reproduced in the pure absorption mode, esssentially unaffected by overlap with the intense methyl resonances. These results provide unequivocal evidence for the coupled proton chains A-B-C-D-E and A-B-C-D-F-G.

In applications where spin-spin splitting is the important parameter, a closer examination of the fine detail of individual multiplets is possible, unencumbered by overlapping resonances. Figure 2 shows the results of coherence transfer along the chain $CH-CH_2-CH_3$, illustrating how multiplets from the methyl group

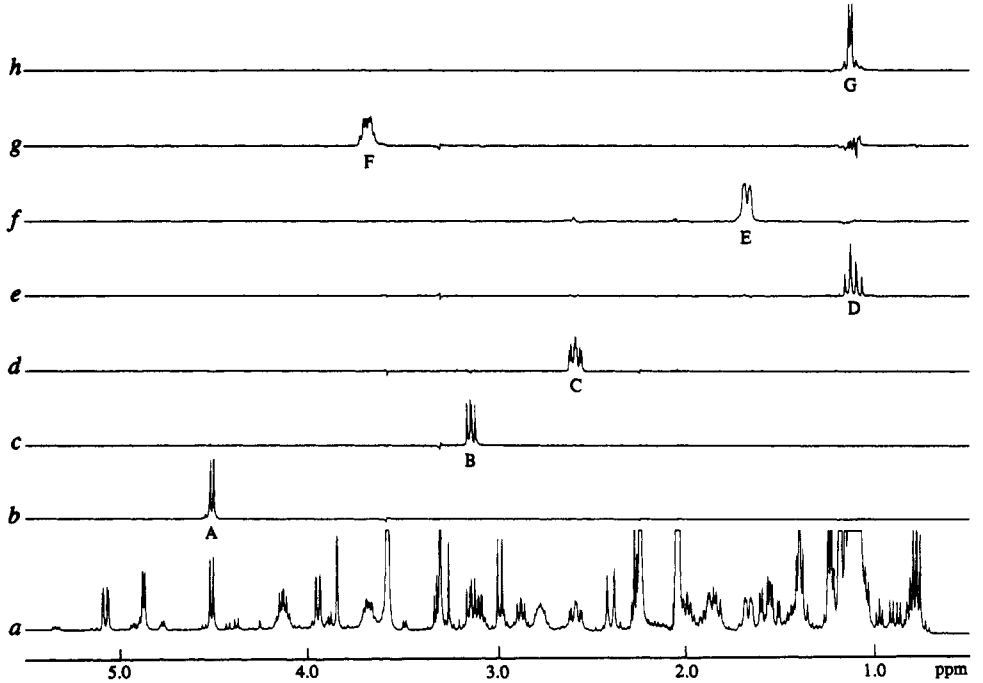

Figure 1. (a) The 400-MHz proton spectrum of erythromycin. (b) Selective excitation of proton A. (c) Hartmann-Hahn coherence transfer A → B. (d) A → B → C. (e) A → B → C → D. (f) A → B → C → D → E. (g) A → B → C → D → F. (h) A → B → C → D → F → G.

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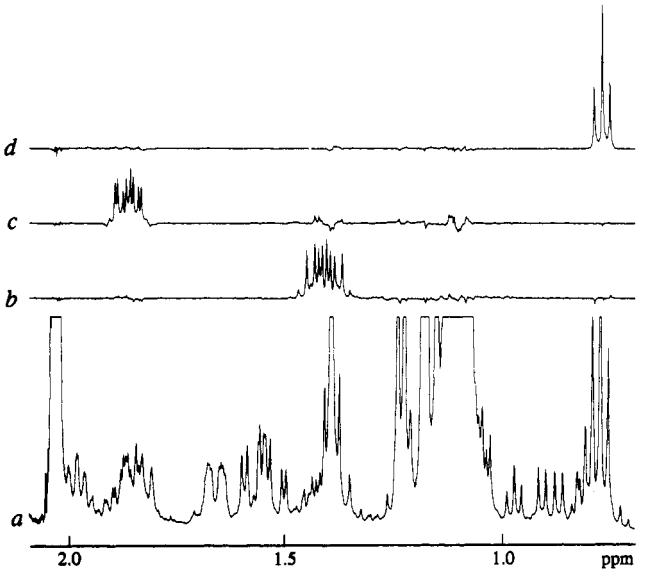

Figure 2. (a) A crowded region of the erythromycin spectrum. (b) Hartmann–Hahn coherence transfer from a methine proton to one of the adjacent (inequivalent) methylene protons. (c) A two-step transfer to the other methylene proton. (d) A three-step transfer to the adjacent methyl group. All three multiplets are obscured by overlap in the conventional spectrum a.

and the two inequivalent methylene protons may be salvaged from an overcrowded region of the erythromycin spectrum.

Several useful elaborations of this technique are possible. Chain branching or ring formation can be identified by suitably directing the flow of coherence. A nonselective "INEPT" transfer[9] from protons to carbon-13 at the end of the sequence enables an analogous step-by-step assignment of the carbon-13 spectrum.[10]

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Design, Synthesis, and Crystal Structure of a Pyrrolinone-Based Peptidomimetic Possessing the Conformation of a β-Strand: Potential Application to the Design of Novel Inhibitors of Proteolytic Enzymes

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The design and synthesis of nonpeptidal peptidomimetics have emerged as a synergistic enterprise spanning organic, bioorganic, and medicinal chemistry,[1] driven by the quest for improved pharmacodynamic properties such as oral bioavailability and biological half-life.[2] There is mounting evidence that hydrogen bonding involving the amide backbones of peptide hormones and their receptors is not required for receptor binding or activation,[3] as demonstrated by the classic peptidomimetic morphine[4] and other ligand mimetics[5] lacking the amide scaffolding.[6] In contrast, convincing crystallographic evidence indicates that H-bonding involving the amide backbones plays a critical role in the binding of peptidal inhibitors to proteolytic enzymes.[1c,7] Because they must mimic both the β-strand conformations and, at least in part, the H-bonding capabilities of their peptide counterparts, the design of nonpeptidal enzyme inhibitors is considerably more challenging than the creation of mimetics of peptidal hormone-receptor ligands.

Interactive computer modeling[8a] suggested that a series of 3,5-linked pyrrolin-4-ones (e.g., **1**) would adopt a backbone conformation mimicking a β-strand (Figure 1). Moreover, a conformational search[8b] indicated that the peptidal side chains appended to the 5-positions could assume an orientation axial to the heterocyclic ring (Figure 2). In the calculated low-energy conformers, the pyrrolinone rings fix the dihedral angles analogous to ψ and ω in a peptide, and gauche steric interactions of the side chains with the neighboring pyrrolinone rings constrain rotations corresponding to φ. The crystalline methyl ester of an equine angiotensinogen fragment [i.e., H-Leu-Leu-Val-Tyr-OMe (**2**)] exists as a parallel β-pleated sheet.[9] Comparison with our model

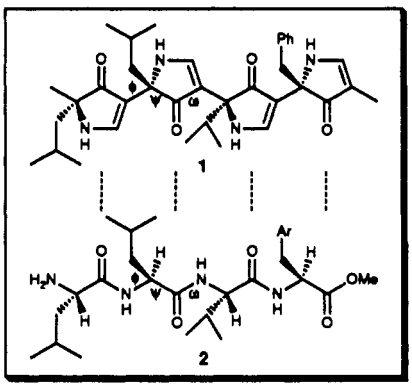

Figure 1. Correlation of the pyrrolinone backbone and side chains in **1** with tetrapeptide **2**. Dotted lines show the alignment of the carbonyl groups.

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