radical cations of cycloalkenes[11] where a considerable part of the spin population is transferred from the ethenic π-system to the formally saturated moiety. As the protons H_{al} lie in the nodal plane of the π-orbital, hyperconjugation is not effective for $a(H_{al})$, and consequently, spin polarization has to account for the small absolute value and the negative sign of this coupling constant. By contrast, the arrangement of H_{bb} relative to the ethenic π-system in a W or zig-zag fashion[12] is such that extensive delocalization of the spin population occurs onto these protons, resulting in a large and positive $a(H_{bb})$. The pertinent electron-spin transfer can also be referred to as homohyperconjugation or through-bond coupling.[13]

As might be expected, visible irradiation[14] of $\mathbf{1}^{\bullet+}$ in a CF_3CCl_3 matrix at 77 K led to a partial transformation of the ESR spectrum of $\mathbf{1}^{\bullet+}$ (Figure 1) into that of the benzene radical cation.[15]

In conclusion, we have proved that the radical cation of benzvalene does not rearrange spontaneously in a rigid matrix at low temperatures and that it can be fully characterized by its hyperfine data under these conditions with the use of ESR and ENDOR spectroscopy.

Note Added in Proof. Subsequent experiments indicated that $\mathbf{1}^{\bullet+}$ isomerizes to the benzene radical cation not only photolytically but also thermally. The rearrangement is almost complete in a CF_3CCl_3 matrix at 135 K.

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Novel ^1H Nuclear Magnetic Resonance Assignment Procedure for RNA Duplexes

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A novel sequential resonance assignment pathway is presented which aids in the assignment of RNA duplexes and also allows for identification of A-form helices in nucleic acids. With the advent of improved methods for the synthesis of milligram quantities of RNA,[1] NMR structural studies of RNA oligomers have become feasible, and studies have recently been performed on systems ranging from simple helices and hairpins[2,3] to more complex structures such as pseudoknots[4] and catalytic RNAs.[5-7]

The first step in NMR structural studies is resonance assignment. The standard sequential resonance assignment procedure, which was originally developed for proteins and subsequently applied to DNA oligomers,[8] is often sufficient for assignment of small nucleic acids, but for larger systems resonance overlap in the 2D NMR spectra becomes a significant problem. This is especially true for RNA systems where the H2'-H5' sugar protons normally resonate in a very narrow region of the spectrum (between 3.5 and 5 ppm). In addition, the lack of sufficient useful starting points in the sequential resonance assignment procedure often severely limits the size of RNA molecules that can be studied by NMR. Thus resonance assignment of RNA presents a much more complicated problem than the assignment of proteins or DNA oligomers. For RNAs, the starting points for resonance assignment of imino protons normally involve identification of G imino proton to C H5 NOEs in GC base pairs and U imino to A H2 NOEs in AU base pairs, and the starting point for sugar-base proton assignments often involves A H2 to H1' NOEs.[8] In this report we describe a novel proton resonance assignment pathway for RNA helices which employs 2D NOE spectra recorded in H_2O and involves identification of NOE connectivities between base-paired G imino protons and sugar C1' protons. This resonance assignment pathway is extremely useful in providing starting points for the more standard sequential resonance assignment procedures and will also serve as a probe for identification of the A-form type helices normally found in RNAs.

The assignment pathway is illustrated for the 15-mer RNA hairpin shown in Figure 1A. Figure 1B schematically shows the well-known G imino proton-C amino proton-C H5 NOE connectivity which is routinely used as a starting point in the resonance assignment of DNA or RNA helices. On the basis of this assignment pathway one would predict a single cross peak for every base-paired G imino proton in the imino to H1'/H5 region of a 2D NOE spectrum recorded in H_2O. However, for the spectrum of the RNA hairpin shown in Figure 1C, one instead sees three cross peaks for every base-paired G imino proton in the H1'/H5 region. By comparison of the 2D NOE spectrum recorded in H_2O with a previously assigned 2D NOE spectrum recorded in D_2O,[9] these extra cross peaks in the H_2O spectrum are readily assigned. As expected, one of the three cross peaks for each set arises from the G imino proton to C H5 interaction. The other two cross peaks arise from interactions between the G imino and C1' protons. Comparison with the spectrum recorded in D_2O reveals that there is a cross peak for the G imino proton and the H1' of the sugar on the same strand 3' to the G (as indicated by solid lines in Figure 1) as well as a cross peak for the G imino proton and the H1' of the sugar on the opposite strand, 3' to this base pair (as indicated by the dashed lines).

These additional cross peaks arise from a NOE pathway involving G amino protons as illustrated in Figure 1B. In an A-type helix the G imino proton is close to the hydrogen-bonded G amino proton (2.3 Å), whereas the non-hydrogen-bonded G amino proton is close to both the 3' H1' on the same strand (3.4 Å) and the 3' H1' on the opposite strand (4.3 Å).[10]

Figure 2 shows this resonance assignment pathway applied to a larger system, which consists of a hammerhead 34-mer RNA enzyme that forms a complex with a 13-mer DNA substrate.[7] The RNA-DNA complex contains one RNA hairpin and two RNA-DNA helices (Figure 2A). We have previously assigned most of the imino protons in helices I and II of this complex using standard techniques,[7] but to further probe the three-dimensional structure of this molecule, assignment of the sugar-base protons is required. However, this is an extremely challenging problem due to severe spectral overlap in the 2D spectrum. Figure 2B shows the imino to H1'/H5 region of the 2D NOE spectrum in 90% H_2O for this

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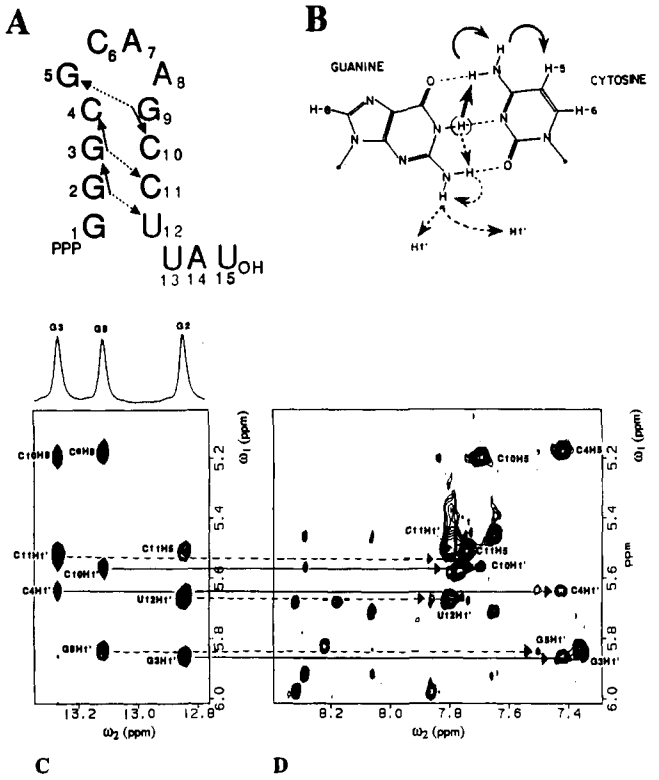

Figure 1. (A) Secondary structure model of the RNA hairpin. The hairpin contains four base pairs closed by a tetranucleotide loop and also has a trinucleotide 3′ dangling end. Arrows denote the intrastrand G imino proton to H1′ NOE connectivities (solid arrows) and the cross-strand G imino to H1′ NOE connectivities (dashed arrows) as discussed in the text. (B) Schematic of a GC base pair with the solid arrows showing the G imino–C amino–C5 proton connectivities and the dashed arrows showing the G–imino–G amino–C1′ proton connectivities used in resonance assignments. (C) Contour plot of a portion of the 2D NOE spectrum recorded in 90% H_2O at 5 °C showing part of the imino proton to the H1′/H5 region. The assigned imino proton region of the 1D NMR spectrum is shown on top of the contour plot. The spectrum was recorded by using a 1331 water suppression acquisition pulse[12] with 10K spectral width in both dimensions and a 400-ms mixing time. A homospoil pulse was applied in the mixing time to help eliminate residual transverse magnetization from the water protons. Ninety-six scans were taken with 4096 complex data points in the t_2 dimension and 300 complex FIDs in the t_1 dimension. The spectrum was apodized with a 65° shifted sine bell window function in both dimensions and zero-filled to 1K data points in t_1. In order to eliminate base-line roll caused by the 1331 water suppression pulse, a ninth degree polynomial base-line correction was applied to the imino proton region of the spectrum after Fourier transformation in t_2. (D) Contour plot of a portion of the 2D NOE spectrum recorded in D_2O at 5 °C showing the aromatic to H1′/H5 region. The arrows between parts C and D connect assigned cross peaks in D with cross peaks in C, with line styles corresponding to the arrows used in A. The spectrum was recorded with a 5K spectral width in both dimensions and a 400-ms mixing time. Ninety-six scans were taken in the t_2 dimension and 256 complex FIDs in t_1. The spectrum was apodized as in A, except that no base-line correction was performed.

system, and as observed for the RNA hairpin described above, every G imino proton in this region shows a set of three cross peaks to the H1′/H5 region. It is important to note that this cross peak pattern exists not only for the RNA helix II but also for helix I, which implies that this RNA–DNA helix also adopts an A-form type helix.

The cross peaks to the downfield-shifted C1′ protons, indicated by the arrows in Figure 2B, are extremely useful for resonance assignment in this system. These cross peaks arise from DNA C1′ protons, which tend to resonate more downfield than RNA C1′ protons and therefore now provide a number of specific starting points for the assignment of the proton resonances on the DNA strand. The resonance assignment for T9 H1′ was confirmed by the standard T methyl proton–H6–H1′ resonance pathway (unpublished results). The other imino proton–H1′ cross

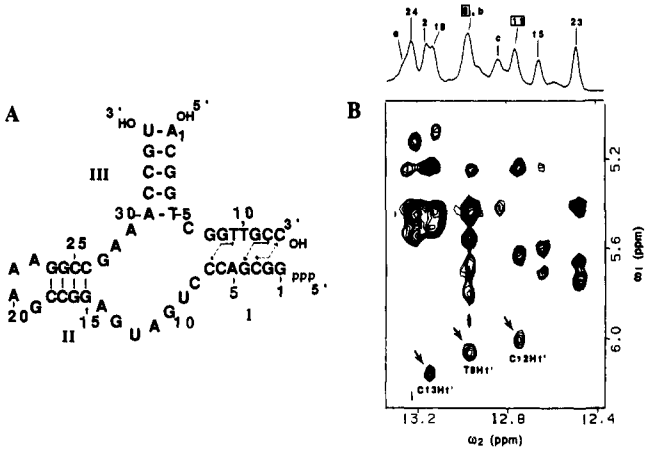

Figure 2. (A) Secondary structure model of a 34-mer hammerhead RNA enzyme base paired to a 13-mer DNA substrate. The RNA and DNA strands are numbered separately, and helices are defined by Roman numerals. Helix formation in II and III is indicated by solid lines between nucleotides (with these left out in helix I for clarity). For helix I, the G imino–C1′ proton connectivities discussed in the text are indicated by the solid or dashed arrows as defined in Figure 1. (B) The imino to aromatic proton region of the 2D NOE spectrum recorded at 15 °C of this RNA–DNA complex. Arrows indicate G imino–DNA C1′ cross peaks. The 1D NMR spectrum of this imino proton region is plotted above with the resonance assignments. Resonances involving imino protons on the DNA strand are boxed (letters indicate unassigned imino proton resonances[7]). The spectrum was recorded with a 200-ms mixing time with 128 scans in t_2 and 300 t_1 increments. Other data acquisition and apodization parameters were as described in the legend to Figure 1C.

peaks are being used as starting points for assignment of the rest of the DNA strand. The results presented here clearly show the usefulness of this novel resonance assignment procedure in RNA systems.

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Unprecedented Isomerization of an Aryl Nitrosyl Organometallic Complex to Its Arylimido Oxo Analogue[1]

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Most transformations involving organometallic nitrosyl complexes result in the nitrosyl ligand remaining intact in the transition metal's coordination sphere.[2] A notable exception to this generalization involves the class of reactions summarized in eq 1 in which Cp′ = η^5-C_5H_5 (Cp) or η^5-C_5Me_5 (Cp*); M = Mo or W; R = alkyl[3a] or aryl.[3b] Reactions 1 thus result in the formal loss

$$Cp'M(NO)R_2 \xrightarrow[-{}^{\bullet}RNO^{\bullet}]{O_2} Cp'M(O)_2R \qquad (1)$$

of RNO upon treatment of the precursor complexes with molecular

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