at temperatures up to 300 °C. This is consistent with a large isomerization barrier (26.6 kcal mol^{-1}, MP2/6-31+G*//6-31+G*) as predicted by Squires and co-workers.[11]

The methodology described herein provides a means to examine areas that have traditionally been difficult to address in the gas phase. The regio- and stereospecificity of the technique should enable many new carbanions to be prepared. Efforts toward this end will be reported in due course.

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The Radical Cation of Benzvalene: An ESR and ENDOR Study[1]

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Tricyclo[3.1.0.0^{2,6}]hex-3-ene (**1**) is one of the reactive valence isomers of benzene, usually named benzvalene.[2] It has an interesting structure, as it contains an olefinic double bond strongly interacting with a bicyclo[1.1.0]butane moiety. A few years ago, Roth and co-workers[3] reported on nuclear polarization effects (CIDNP) which were observed upon treatment of **1** with photoexcited chloranil in nitromethane-d_3 at 243 K. These effects have been ascribed to the intermediately formed radical cation $\mathbf{1}^{\bullet+}$. In the present work, **1**[4] was ionized by γ-rays (^{60}Co source) in a CF_3CCl_3 matrix at 77 K. The thus generated radical cation gave rise, at 115 K, to the ESR spectrum shown at the top of Figure 1 ($g = 2.0029 \pm 0.0001$). Its well-defined hyperfine pattern consists of a two-proton triplet spaced by 2.790 ± 0.005 mT, each component being further split by 0.835 ± 0.005 mT into another two-proton triplet. A third hyperfine splitting of 0.158 ± 0.002 mT, also attributed to two protons, is masked by the large width (0.3 mT) of the ESR lines, but it is revealed by the corresponding ENDOR spectrum reproduced at the bottom of Figure 1. Apart from the absorption in the 23-27-MHz region, associated with the coupling constants of 2.790 and 0.835 mT, this spectrum exhibits signals symmetrically spaced about $\nu_H = 14.56$ MHz, the frequency of the free proton.[5] These signals represent the perpendicular and parallel features of the coupling constant of 0.158 mT at $\nu_H \pm 2.61$ MHz and $\nu_H \pm 1.45$ MHz where $^1/_2|A_\perp| = 2.61$ MHz and $^1/_2|A_\parallel| = 1.45$ MHz.[6]

An INDO calculation,[7] based on a MNDO-optimized geometry[8] of $\mathbf{1}^{\bullet+}$, yields +2.804, −0.707, and −0.147 mT for the coupling constants $a(H_{bb})$, $a(H_{et})$, and $a(H_{al})$ of the bicyclobutane-bridgehead (bb), ethylenic (et), and allylic (al) protons, respectively. The excellent agreement with the experimental findings leaves no doubt that the observed ESR and ENDOR spectra arise from the unrearranged radical cation $\mathbf{1}^{\bullet+}$. Moreover, not only can the coupling constants unequivocally be assigned to the protons H_{bb}, H_{et}, and H_{al} in the individual positions of $\mathbf{1}^{\bullet+}$, but also a positive sign is clearly indicated for $a(H_{bb})$ and a negative one for $a(H_{et})$ and $a(H_{al})$.

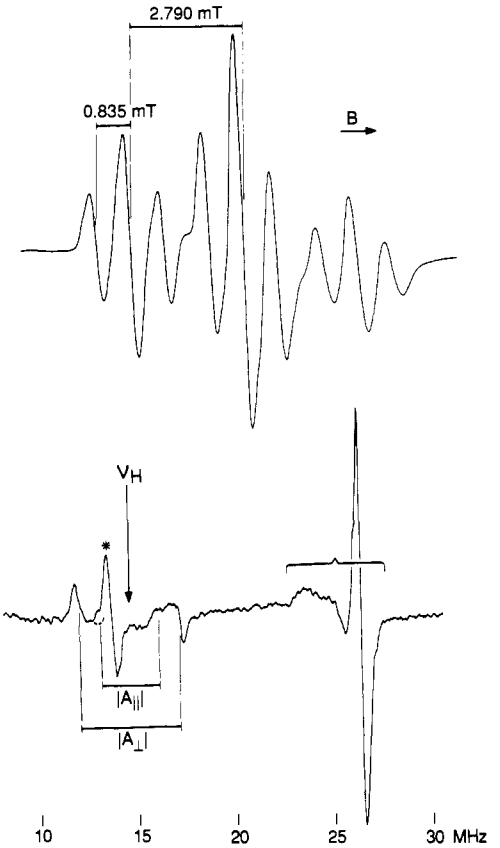
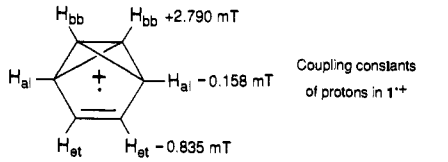


Figure 1. Top: ESR spectrum of γ-irradiated benzvalene (**1**) in a CF_3CCl_3 matrix; temperature, 115 K. Bottom: corresponding proton ENDOR spectrum. The ENDOR signals in the 23-27-MHz region, marked by a brace, are associated with the coupling constants of 2.790 mT [$^1/_2a(H) - \nu_H$] and 0.835 mT [$\nu_H + {}^1/_2a(H)$]; they overlap and have not been analyzed in detail. The signal denoted by an asterisk arises from ^{19}F nuclei of the matrix; it obscures the low-frequency feature of $|A_\parallel|$ which is drawn by a dotted line.



The hyperfine data for $\mathbf{1}^{\bullet+}$ are fully consistent with the previously described[3] CIDNP effects. Thus, the moderately enhanced absorption by H_{et} and the very strong emission by H_{bb} closely correspond to the negative, middle-sized $a(H_{et})$ and to the positive, large $a(H_{bb})$, respectively. Likewise, the failure to observe a CIDNP effect for H_{al} is in line with the small $|a(H_{al})|$.

The singly occupied orbital of $\mathbf{1}^{\bullet+}$ is best represented as the HOMO of ethene with a substantial delocalization into the bicyclobutane moiety.[2,9] Accordingly, $a(H_{et})$ has a negative sign, required for protons directly attached to π-centers,[10] and its absolute value is comparable to those found for such protons in the

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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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