on phase purity, elapsed time, temperatures, pressures, and a variety of mechanical stresses.[14,21,22] Further isotopic substitutions are under study to examine other possibilities of phonon exchange.

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Theoretical Study of the Low-Lying Triplet and Singlet States of Diradicals: Prediction of Ground-State Multiplicities in Cyclic Analogues of Tetramethyleneethane

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The two frontier orbitals of tetramethyleneethane (TME) are close in energy, and as a result, TME has low-lying singlet (S) and triplet (T) states.[1] The energies of these states depend strongly upon the value of the dihedral angle, α, between the planes of the two allyl groups.[2,3] Recent CI calculations[3] show that, although the S state lies below the T state for the planar ($\alpha = 0°$) and perpendicular ($\alpha = 90°$) structures, the T state at its potential minimum ($\alpha \approx 50°$) is about 1 kcal/mol more stable than the S state. This can explain the observation of an EPR spectrum and a linear Curie plot, due to the T state, upon photolysis of a TME precursor in a low-temperature matrix.[4]

Here, we report the results of calculations of the S–T gaps of **1–6**, in which the TME group is incorporated into ring systems, thereby precluding large deviations from planarity. On the basis of the results for TME, this should favor the S state. On the other hand, the resonance or hyperconjugative interactions present in the ring systems could favor the T state.

1   2   3

4   5   6
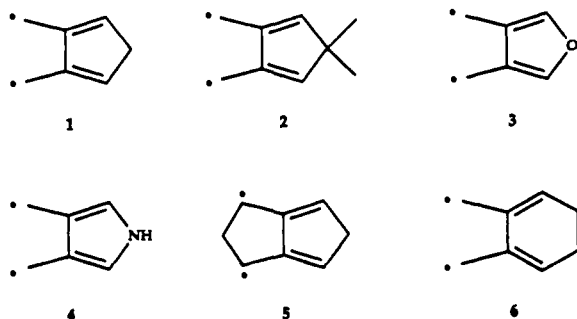
The theoretical method used for **1–6** is identical to that employed[2,3] for TME. The geometries of the S and T states were optimized[5] using both the MCSCF(2,2)/3-21G[6] and MCSCF-

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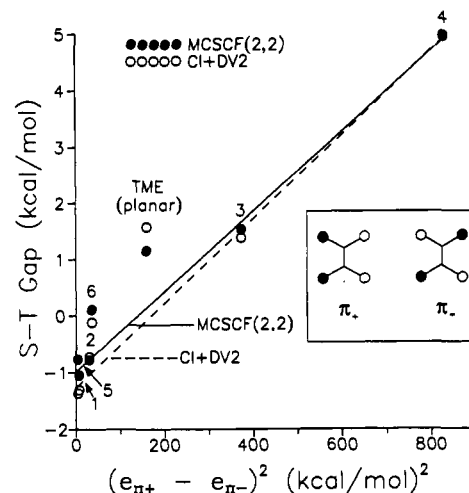

Figure 1. MCSCF(2,2) and CI+DV2 S–T energy gaps vs $(\epsilon_{\pi+} - \epsilon_{\pi-})^2$ for **1–6** and planar TME. Straight-line fits of the data for **1–5** have been included.

(6,6)/3-21G procedures,[7] assuming D_{2h} and C_2 symmetry for **5** and **6**, respectively, and C_{2v} symmetry for **1–4**. CI calculations were carried out at the MCSCF(6,6)/3-21G optimized geometries using MCSCF(6,6) MO's[8] generated from a "DZP" basis set (3s2p1d on the carbon atoms[9a] and 2s on the hydrogen atoms[9b]). The CI calculations employed one and two reference configurations for the T and S states, respectively, and permitted all single and double excitations from the valence orbitals. A Davidson-type[10] correction (DV2) was added to the CI energies to correct approximately for size-consistency errors.

Both the MCSCF(2,2) and CI calculations predict T ground states for **1**, **2**, and **5** and S ground states for **3**, **4**, and planar TME.[11] For **6**, the S–T gap is predicted to be less than 0.2 kcal/mol in magnitude, favoring the T state in the CI calculations and the S state in the MCSCF(2,2) calculations. The CI orderings agree with experiment in those cases for which the multiplicity of the ground state has been determined. For example, T ground states have been reported for **2**[12] and **6**,[13] and an S ground state has been reported for **3**.[14]

The splitting energy between the π_+ and π_- frontier orbitals (shown schematically in Figure 1) is particularly important in determining whether a diradical has an S or a T ground state.[1] At zero orbital splitting, the ground state should be a triplet, and for sufficiently large orbital splittings, it will be a singlet. For planar TME, the π_+ orbital lies about 0.5 eV below the π_- orbital.[15]

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(15) The orbital energies are from ROHF calculations on the triplet states, using the Q_{aa} matrix in the Columbus MCSCF program and employing the MCSCF(6,6) optimized geometries.

In **1**, **2**, and **5**, hyperconjugative interactions destabilize the π_+ orbital relative to the π_- orbital, causing the frontier orbitals to become nearly degenerate and leading to T ground states. In **6**, the π_+ and π_- orbitals are destabilized to nearly the same extent by hyperconjugative interactions, and it is the twisting of the TME group that decreases the π_+,π_- splitting and leads to a T ground state. In **3** and **4**, the resonance interactions with the heteroatoms are sufficiently large that the π_+ orbital is "pushed" above π_-, leading to orbital splittings greater than that in planar TME and resulting in S ground states.

In a two-orbital model, the S–T splitting can be expressed as

$$\Delta E = E_T - E_S = J_{12} - 0.5(J_{11} + J_{22}) + 0.5(\epsilon_1 - \epsilon_2)^2/K_{12} \quad (1)$$

where ϵ_1 and ϵ_2 are the frontier orbital energies and J and K represent the Coulomb and exchange interactions, respectively, and where it has been assumed that $K_{12} \gg |\epsilon_1 - \epsilon_2|$. This expression is derived from eqs 16 and 18 of ref 1b.

Figure 1 plots the MCSCF(2,2) and CI+DV2 S–T gaps vs $(\epsilon_{\pi+} - \epsilon_{\pi-})^2$ as well as straight-line fits of the data for the five-membered-ring systems, **1–5**. Although eq 1 is derived for a two-electron model, the scatter of the data points from the straight-line fits is small. The 0.4–1.0 kcal/mol deviations of the points for TME and **6** from the straight lines is due, in part, to the non-planarity of **6** and to the variations of the exchange integrals across the series.

This study shows that, for a series of diradicals such as **1–5**, the S–T splittings display an approximate quadratic dependence on $(\epsilon_{\pi+} - \epsilon_{\pi-})$, even when calculated employing methods that recover electron correlation. Since the trends in the orbital splittings can be interpreted in terms of orbital interactions, simple MO calculations can be used to design diradical systems with desired S–T gaps.

Hammerhead Ribozyme Tertiary Folding: Intrinsic Photolabeling Studies

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The class of hammerhead ribozymes includes artificial RNA constructs which, when properly designed, exercise endonuclease activity on their RNA substrate.[1] In this case, the ribozyme–substrate complex (R–S) forms a hammerhead-shaped secondary structure containing the conserved nucleotides which have been identified in various self-cleaving RNAs, in viroids, and in satellite RNAs of plant viruses.[2] It is likely that the cleavage reaction requires a well-defined tertiary structure for R–S whose determination will demand much research effort.[3] In this respect, we

Scheme I. Design of the 35-mer Ribozyme R and Its 14-mer Substrate S according to Ref 1b[a]
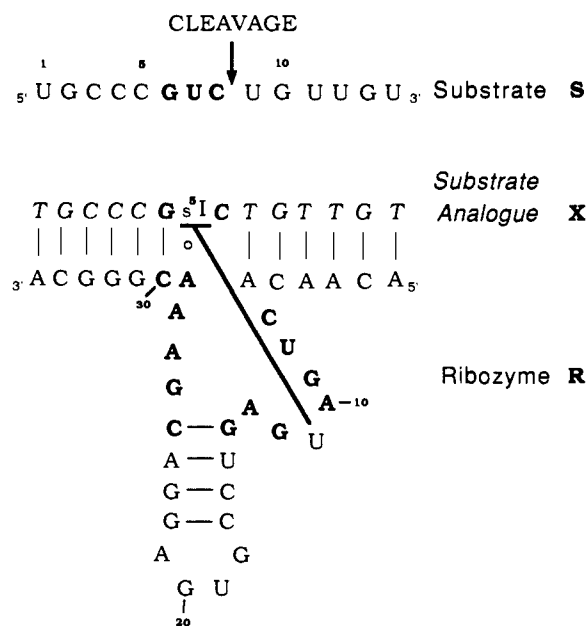
[a] R contains a 24-nucleotide sequence in common with the (+) strand of the satellite RNA of tobacco ringspot virus (sTobRV RNA), i.e., the consensus sequences and stem III. S corresponds to a conserved sequence, between residues 107 and 121, of the U5 region in the long terminal repeat (LTR) of HIV RNA. The photoactivable DNA substrate analogue X is sequentially identical to S except for 2′-deoxy-6-thioinosine at position 7 and thymidine in place of uridine. These oligonucleotides were chemically synthesized. Standard Watson–Crick base pairs are represented by —. The O symbol between ds⁶I and A represents for the nonstandard base pair possibly formed between these residues. The kinetic data for the cleavage reaction of S were found to be $k_{cat} = 25$ min⁻¹ at pH 8 (50 mM Tris-HCl), 37 °C, 25 mM Mg²⁺.

have formed a hammerhead domain between a 35-mer ribozyme (R) and a 14-mer substrate (S) exhibiting high catalytic activity (Scheme I). Reported herein is our exploration of the tertiary folding of this system by application of a labeling technique which makes use of the photo-cross-linking properties of the newly designed intrinsic probe, 2′-deoxy-6-thioinosine (ds⁶I).

The preliminary photochemical studies, performed with thymidylyl-(5′-3′)-2′-deoxy-6-thioinosine (1) (Tpds⁶I),[4] showed the remarkable capacity of ds⁶I to undergo covalent bonding with a pyrimidine residue. When 1 was exposed to UV light (366 nm), the major photoproduct 2 (λ_{max} 271 nm) was formed in good yield (Scheme II). Structure 2 was established after examination of the spectral data. The molecular formula $(C_{20}H_{25}N_6O_{10}PS)$ resulted from HR FAB MS. The ¹H NMR spectrum of the (6–6) adduct 2 displays at 5.64 ppm a characteristic signal due to the H_6 of its saturated Tp unit. Such a chemical shift is consistent with that of the corresponding proton in related (6–4) pyrimidine pyrimidinone photoproducts.[5] Other key NMR arguments were

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