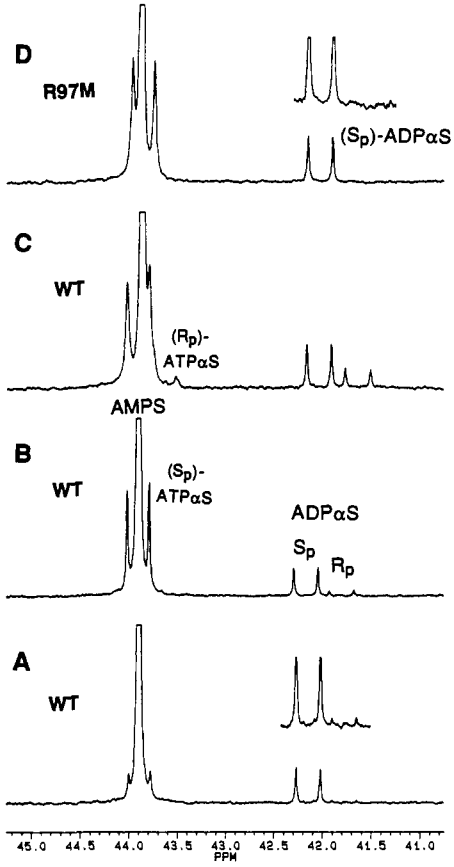

Figure 2. ^{31}P NMR spectra showing the conversion of AMPS to ADPαS (and subsequent conversion to ATPαS). Only the P_α resonances are shown. (A–C) WT AK, after 9%, 17%, and 29%, respectively, of AMPS has reacted. (D) R97M, at 27% conversion. Spectra were obtained after addition of 100 mM EDTA and 150 μL of triethylamine to optimize for the detection of ADPαS. Except for sample C, no (R_p)-ATPαS was detectable even under other conditions, optimized for the detection of ATPαS (spectra not shown). The intensities of various components described in the text were measured by cutting and weighing from greatly expanded spectra. The assignments of different species have been confirmed by mixing with known compounds and agree with previous reports.[5,10] The starting reaction mixture (600 μL) consisted of 22 mM AMPS, 75 mM ATP, and 80 mM $MgCl_2$, in a 50 mM Tris buffer containing 50 mM KCl, 2.5 mM EDTA, and 15% D_2O, pH = 7.8. The broadband decoupled spectra were obtained at 121.5 MHz with a pulse width of 45°. The acquisition time was 1.5 s, the repetition time was 2 s, and ca. 23 000 transients were accumulated for each spectrum. The FID was processed with 2.0-Hz exponential multiplication.

These were accomplished by ^{31}P NMR analysis as shown in Figure 2. Spectra A, B, and C are the reaction mixtures of WT after 9%, 17%, and 29%, respectively, of AMPS has been converted to products. The minor isomer, (R_p)-ADPαS, is clearly detectable in these spectra and constitutes 5%, 15%, and 28%, respectively, of the total ADPαS, or 0.27%, 0.54%, and 1.7%, respectively, of the starting AMPS. In D, 27% of AMPS has been converted to products by R97M, but *no* (R_p)-ADPαS can be detected. If there was no change in stereospecificity, sample D should have consisted of more than 0.54% but less than 1.7% of the R_p isomer relative to the starting AMPS (the 1.7% of spectrum C could be an overestimation since the reaction has passed equilibrium). If we take a conservative value of 1%, the R_p isomer in D should have been 3–4 times that in A. Since the signal/noise ratios in D and A are comparable and the signal/noise ratio of (R_p)-ADPαS in A is ca. 3, *formation of the R_p isomer has decreased at least 10-fold in D.* These results and analysis indicate *an enhancement of stereospecificity in R97M relative to WT*, i.e., the A to B equilibrium has been shifted to A in R97M, as also indicated in Figure 1.

In terms of molecular events at the active site, the two arginine side chains appear to "compete" for the nonbridging sulfur and/or oxygen. Such competing interactions should start at the AK·AMPS binary complex and persist through the transition state. The balance between the two competing interactions results in the observed stereospecificity in WT, which is shifted one way or the other upon removal of one of the two interactions. Since the major conformer has been perturbed upon R44M mutation, Arg-44 appears to "win" over Arg-97 in orienting the phosphorothioate. The molecular detail of such interactions, however, remains to be established.

Ab Initio Study of Spiropentadiene, C_5H_4

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Received July 22, 1991

In a recent paper, Billups and Haley[1] reported the synthesis of spiropentadiene, the simplest small-ring, spiro-connected cycloalkene. Because of its high endothermicity, it was not possible for these investigators to characterize spiropentadiene structurally or spectroscopically. A previous ab initio theoretical study of spiropentadiene has been reported,[2] but was carried out at the Hartree-Fock (HF) level of theory with small basis sets. In our recent work we have been investigating basis set, correlation, and geometry effects on the stabilities of hydrocarbons and carbocations.[3] We now extend this work to the prediction of the structure, vibrational frequencies, and standard heat of formation of spiropentadiene.

The structure of spiropentadiene has been optimized with D_{2d} symmetry constraints at second-order many-body Møller-Plesset perturbation theory[4-9] [MBPT(2) ≡ MP2] using the 6-31+G(d,p) basis set.[10-13] Harmonic vibrational frequencies were computed to ensure that this structure is a local minimum, to obtain both zero-point and thermal vibrational energies, and to predict the vibrational spectrum. A single-point calculation at fourth-order perturbation theory including triple excitations [MBPT(4)] was carried out at the optimized MP2/6-31+G(d,p) geometry using the much larger Dunning correlation-consistent polarized valence triple-ζ basis set (cc-pVTZ).[14] The cc-pVTZ basis set contains two sets of first polarization functions and a single set of second polarization functions on each atom. The MP2/6-31+G(d,p) structure optimization and frequencies calculations were done using the Gaussian 90 system of computer programs.[15] The cc-pVTZ

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Table I. Harmonic MP2/6-31+G(d,p) Frequencies (cm^{-1}) and IR Intensities (km/mol) of Spiropentadiene and Corresponding Frequencies in Cyclopropene

spiropentadiene					cyclopropene[a]
mode	sym	intensity	freq	assignment[b]	freq
ν_{16}	E	38	376	inter-ring tilt	
ν_7	B_1		410	inter-ring twist	
ν_{15}	E	32	522	CH wag (asym)	821
ν_4	A_1		711	C-C (sym, in-phase)	1193
ν_{14}	E	27	832	C-C, mixed with CCH (asym)	808
ν_5	A_2		845	CH wag (sym, in-phase)	557
ν_6	B_1		857	CH wag (sym, out-of-phase)	
ν_{11}	B_2	2	937	CCH (sym, out-of-phase)	959
ν_3	A_1		1035	CCH (sym, in-phase)	
ν_{13}	E	14	1112	CCH, mixed with C-C (asym)	1063
ν_{10}	B_2	109	1509	ring deformation (out-of-phase)	
ν_2	A_1		1621	C=C (in-phase)	1717
ν_9	B_2	141	1647	ring breathing (out-of-phase)	
ν_{12}	E	4	3288	C-H (asym)	3346
ν_1	A_1		3325	C-H (sym, in-phase)	3392
ν_8	B_2	3	3329	C-H (sym, out-of-phase)	

[a] Not all of the modes of cyclopropene are given, but only those which can be identified with corresponding modes in spiropentadiene. [b] Abbreviations: sym, the two ends of each ring move symmetrically; asym, the two ends of each ring move antisymmetrically; in-phase, the two rings change in the same way; out-of-phase, the two rings change in opposite ways; wag, bending out of the plane of the corresponding ring; A-B, single-bond A-B stretch; A=B, double-bond A-B stretch; ABC, in-plane bend of the ABC angle.

perturbation energies were calculated with the ACES II program.[16] The large size of the cc-pVTZ basis [206 contracted functions for C_5H_4, compared to 115 for the 6-31+G(d,p) basis] necessitated the utilization of spatial symmetry to make the MBPT(4) calculation feasible, and this requirement could only be met with the ACES II program.

In our recent study,[3] we have used the energy of the hydrogenolysis reaction as a convenient method of evaluating the stabilities of hydrocarbons and carbocations relative to H_2 and CH_4, and using the cc-pVTZ basis set at the MBPT(4) level, we have obtained computed values in agreement with experimental data to about 2 kcal/mol. The hydrogenolysis energy of C_5H_4 is the energy of the reaction

$$C_5H_4 + 8H_2 \rightarrow 5CH_4 \quad (1)$$

The electronic contribution to the enthalpy of this reaction (ΔH^{298}) has been obtained from the MBPT(4) calculation and the corresponding calculations on H_2 and CH_4. Zero-point and thermal vibrational contributions have been evaluated using the scheme described in ref 3. The remaining thermal terms have been evaluated classically. We will employ the computed hydrogenolysis energy of C_5H_4 and the experimental heat of formation of CH_4 to predict the standard heat of formation of spiropentadiene.

Spiropentadiene, with D_{2d} symmetry, has two perpendicular three-membered rings sharing a central carbon atom (C_1). Its MP2/6-31+G(d,p) structure has a C_1–C_2 single bond length of 1.480 Å, while the C_2–C_3 double bond length is 1.328 Å. The single bond is shorter than the MP2/6-31+G(d,p) C_1–C_2 single bond in cyclopropene, 1.508 Å, while the double bond is longer than the double bond in cyclopropene, 1.304 Å. Thus, there is less distinction between the C–C bond lengths in spiropentadiene. The C_2–C_1–C_3 angle in spiropentadiene is 53.3°, which is larger than the corresponding angle of 51.2° in cyclopropene. Corresponding C–H bond lengths in spiropentadiene and cyclopropene are similar at 1.078 and 1.074 Å, respectively. However, the C_2–C_3–H angle of 146.6° in spiropentadiene is smaller than the corresponding angle of 149.9° in cyclopropene. An indication of the strain at the carbon shared by the two rings in spiropentadiene is also evident from comparing the C_2–C_1–C_3 angle with the H–C_1–H angle in cyclopropene, which is 114.0°. The severely strained C_5H_4 structure is associated with a highly endothermic compound.

Table II. Total Hartree–Fock and Correlated Energies (hartrees) of H_2, CH_4, and C_5H_4 and Standard Heat of Formation (kcal/mol) of C_5H_4

Total Energies

molecule	Hartree–Fock	MBPT(2)	MBPT(3)	MBPT(4)
H_2	−1.132 989	−1.164 642	−1.170 242	−1.171 693
CH_4	−40.213 442	−40.411 666	−40.429 937	−40.437 564
C_5H_4	−191.509 674	−192.290 705	−192.310 264	−192.358 607

Standard Heat of Formation of C_5H_4

Hartree–Fock	MBPT(2)	MBPT(3)	MBPT(4)
+181.3	+154.2	+171.1	+157.4

The harmonic frequencies of spiropentadiene are reported and characterized in Table I. In point group D_{2d}, only B_2 and E vibrational excitations are IR active, and their intensities are also given in Table I. Asymmetrical motions (modes in which the two ends of each ring move antisymmetrically) in C_5H_4 always lead to doubly degenerate E modes. The C—C and C=C stretches mix as ring deformations and ring breathing modes, but only when out-of-phase, i.e., when the two rings move in opposite ways. The C—C stretch and the CCH bend mix only in the asymmetric case. The frequencies of the analogous modes in cyclopropene are also listed in Table I, but the comparison is not always straightforward for various reasons, including the fact that different masses are moving in the two molecules. The ring modes in C_5H_4, which mix C—C and C=C, cannot be related simply to C_3H_4, where there is essentially no such mixing. The mixing of CCH bending and C—C stretching modes is common in both molecules. The in-phase (i.e., the two rings move symmetrically) C=C stretch in C_5H_4 occurs at a lower frequency (1621 cm^{-1}) than the corresponding mode in C_3H_4 (1717 cm^{-1}). This is consistent with the longer C_2–C_3 bond is spiropentadiene. However, the symmetric C–C single bond stretch in C_5H_4 also occurs at a lower frequency (711 cm^{-1}) than in C_3H_4 (1193 cm^{-1}) even though the C–C bond is shorter in C_5H_4. The high-frequency C–H stretches in C_5H_4 occur at lower frequencies than the corresponding stretches in C_3H_4. It is also interesting to note that the C–H symmetric wags at 845 and 857 cm^{-1} and the asymmetric wag at 522 cm^{-1} in C_5H_4 exchange in C_3H_4 with the symmetric wag at 557 and the asymmetric wag at 821 cm^{-1}.

The HF, MBPT(2), MBPT(3), and MBPT(4) total energies computed with the cc-pVTZ basis set at the optimized MP2/6-31+G(d,p) geometries for C_5H_4, CH_4, and H_2 are reported in

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Table II. From these data, the standard heat of formation of C_5H_4 may be calculated at the various levels of theory. The computed MBPT(4) electronic hydrogenolysis energy of C_5H_4 at 0 K is -285.9 kcal/mol. The zero-point vibrational energy change for this reaction is 49.7 kcal/mol. The thermal vibrational energy and other thermal terms contribute -8.2 kcal/mol to the reaction enthalpy at 298 K. Thus, the computed value of ΔH^{298} for the hydrogenolysis of C_5H_4 is -244.4 kcal/mol. This value and the experimental standard heat of formation of CH_4 of -17.4 kcal/mol[17] lead to a predicted standard heat of formation of C_5H_4 of 157.4 kcal/mol. This value is more than twice the experimental heat of formation of 66.2 kcal/mol for cyclopropene[17] and is consistent with the anticipated high endothermicity of spiropentadiene.

Acknowledgment. All calculations were performed on the Cray Y-MP8/864 computer at the Ohio Supercomputer Center. We thank R. J. Bartlett and J. D. Watts for making the latest version of the ACES II program available and for assistance with its use and the Ohio Supercomputer Center for providing the computer time required for these calculations.

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General Approach to the Synthesis of Short α-Helical Peptides

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Received August 5, 1991

The availability of stable, short α-helical peptides would provide useful frameworks for the design of biologically active small molecules as well as models for probing aspects of protein folding. Several approaches have recently been reported for stabilizing α-helical peptides including incorporation of salt bridges,[1] metal chelates,[2] or amide bonds[3] that bridge the i and $i + 4$ positions; incorporation of amino acids with high helix propensity[4] and helix caps;[5] and the formation of amphiphilic helix bundles.[6] One of the most successful strategies to date exploits the disulfide-bridged framework of the bee venom peptide, apamin, to generate chimeric α-helical peptides.[7] We now report the synthesis of short peptides containing a two-turn α-helix stabilized by a single intramolecular disulfide bond bridging the i and $i + 7$ residues (Scheme I). An eight-residue peptide containing this bridge shows high helicity in water at 0 and 60 °C.

Scheme I

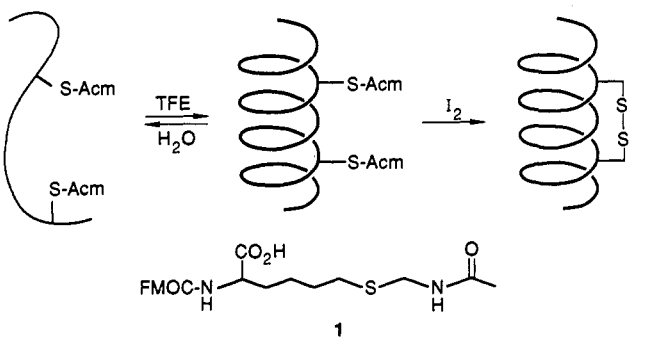

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Table I. $[\theta]_{222nm}$ for Peptides **2-5** (15-30 μM) in Water (0.1% TFA) at 0 and 60 °C[a]

	peptide	0 °C $-[\theta]_{222}$	0 °C f, %	60 °C $-[\theta]_{222}$	60 °C f, %
2	Ac-A-A-A-(D)1-K-A-A-A-A-K-(L)1-A-A-A-K-A-NH$_2$				
	oxidized	29 600	99	15 800	53
	protected	8400	28	5100	25
3	Ac-(D)1-K-A-A-A-A-K-(L)1-NH$_2$				
	oxidized	21 000	105	13 300	59
	protected	3100	16	2500	13
4	Ac-A-E-(D)1-A-A-A-K-F-L-(L)1-A-H-A-NH$_2$				
	oxidized	29 000	105	16 800	48
	protected	7000	25	4500	16
5	Ac-A-N-E-A-A-D-(D)1-I-A-Y-L-K-Q-(L)-T-K-NH$_2$				
	oxidized	31 100	104	14 900	49
	protected	8900	30	4500	15

[a] $-[\theta]_{222}$, mean residue ellipticity (degree·cm^2/dmol) of peptides at 222 nm. $f = [\theta]_{obsd} - [\theta]_O/([\theta]_{max} - [\theta]_O)$ = the fraction of helix. $[\theta]_{obsd}$, $[\theta]$ observed from a previous column. $[\theta]_O = 0 \pm 500$ deg·cm^2/dmol. $[\theta]_{max} = ((n - 4)/n)[\theta]_\infty$ = the maximal mean residue ellipticity value for chain length where n = the number of residues and $[\theta]_\infty = -40000$ deg·cm^2/dmol.[17]

Molecular modeling studies[8] indicated that incorporation of the deprotected forms of the D and L enantiomers of amino acid **1** at the i and $i + 7$ positions of an α-helix, respectively, could lead to intramolecular disulfide bond formation with little perturbation on helix conformation. Moreover, disulfide bonds can be formed under mild conditions, in the presence of many functional groups, and in a variety of solvents known to favor α-helix formation. The D and L forms of *N*-Fmoc-*S*-(acetamidomethyl)-2-amino-6-mercaptohexanoic acid (**1**) were synthesized via conversion of the ε-amino group of D- and L-N^α-Boc-lysine to a pyridinium salt (prepared from the corresponding pyrillium salt[9]) and subsequent displacement with 4-methoxybenzyl mercaptan. Removal of the protecting groups with TFA and reprotection with the acetamidomethyl (Acm) and Fmoc groups afforded fully protected **1**. Optical purity was confirmed by NMR analysis of the L-Ala-*O*-methyl ester derivatives.

Peptides **2-5** (Table I) were chosen as models to investigate the helix-stabilizing potential of an intramolecular disulfide spanning eight residues of a peptide. Peptide **2** is derived from a previously reported alanine-rich peptide[4] and contains two lysine residues that prevent intermolecular aggregation and increase water solubility. Peptide **3** is an eight amino acid truncated version of **2**, and peptide **4** incorporates essential residues of the C-peptide of ribonuclease A.[10] Peptide **5** is derived from a moth cytochrome C-peptide sequence that binds class II MHC molecules.[11] Peptides were synthesized using solid-phase methodology, protecting groups (with the exception of the Acm group) were removed with reagent K, and the deprotected peptides were purified

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