replacement of NO_2 by H in activated compounds by NADH occurs via an electron-transfer chain mechanism as demonstrated by the influence of free radical inhibitors and scavengers on the reaction.[48] Evidence for a stepwise path of hydride equivalent transfer in the oxidation of MA by 2,3-dicyano-1,4-benzoquinone was provided by Lai and Colter[49] when they found the *N*-methylacridinyl radical was trapped by 2-methyl-2-nitrosopropane. Even PrNADH has been shown to react (although the kinetics are somewhat complex) with ferricyanide, presumably through a one-electron transfer mechanism.[50] In view of our earlier findings[51,52] that flavin radicals (whose reaction with NADH model compounds is exothermic) do not undergo detectable one-electron transfer with dihydronicotinamides, the aforementioned observations of one-electron transfer may be explained as follows: if the redox potential of the oxidant is high enough to form the putative nicotinamide radical intermediate, it will presumably do so; otherwise hydride transfer from dihydronicotinamides to less powerful one-electron or two-electron oxidants will follow a two-electron pathway—that of direct H^- transfer. A correlation of kinetic and secondary isotope effects and reaction rates with the redox potential[53] for various NADH model reactions would elucidate the factors governing such a dual mechanistic pathway for hydride equivalent transfer.

Acknowledgment. This work was supported by grants from the National Institutes of Health and the National Science Foundation. M.F.P. expresses appreciation to the Natural Sciences and Engineering Research Council of Canada for a postdoctoral fellowship. We thank James Wu for his experimental assistance.

Registry No. NADH, 18940-08-4; MAI, 948-43-6; PrNAD·I, 5463-59-2; TFA, 434-45-7; PhNAD-I, 87412-97-3; MeOPhNAD·I, 87412-98-4; MePhNAD·I, 87412-99-5; CF_3PhNAD·I, 87413-00-1; CNPhNAD·I, 87413-01-2; QNAD·I, 17260-82-1; BzNADH, 952-92-1; H,D-BzNADH, 17750-30-0; D,D-BzNADH, 60172-94-3; QNADH, 17260-79-6; H,D-QNADH, 79798-57-5; D,D-QNADH, 83077-37-6; MeOPhNADH, 87413-02-3; H,D-MeOPhNADH, 87413-03-4; D,D-MeOPhNADH, 87413-04-5; MePhNADH, 87413-05-6; D,D-MePhNADH, 87432-52-8; CF_3PhNADH, 83077-40-1; D,D-CF_3PhNADH, 83077-41-2; CNPhNADH, 87413-06-7; D,D-CNPhNADH, 87413-07-8; MA, 4217-54-3; H,T-PrNADH, 87413-08-9; PrNADH, 17750-24-2; D,D-PrNADH, 60764-07-0.

(48) Ono, N.; Tamura, R.; Kaji, A. *J. Am. Chem. Soc.* **1980**, *102*, 2851–2852.
(49) Lai, C. C.; Colter, A. K. *J. Chem. Soc., Chem. Commun.* **1980**, 1115–1116.
(50) Okamoto, T.; Ohno, A.; Oka, S. *J. Chem. Soc., Chem. Commun.* **1977**, 181–182.
(51) Powell, M. F.; Wong, W. H.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4604–4608.
(52) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 1014–1021.
(53) For example, see: Kellogg, R. M.; Piepers, O. *J. Chem. Soc., Chem. Commun.* **1982**, 402–404.

Effects of Hydration on the Dynamics of Deoxyribonucleic Acid

Michael Thang Mai, David E. Wemmer, and Oleg Jardetzky*

Contribution from the Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, California. *Received April 29, 1983*

Abstract: The effect of hydration on motions in the sugar California 94305 backbone of double-stranded DNA fragments 200 ± 50 base pairs in length has been examined by using ^{31}P NMR. It is shown that very little motion occurs in lyophilized DNA, but motions increase in amplitude with increasing hydration. Changes in the relaxation behavior with increasing hydration suggest that several different types of motion are present at different hydration levels, with the changes between them occurring at hydration levels that correlate with the successive hydration at various molecular sites previously described. In the hydration range of B-form DNA, the changes in the spectrum induced by increasing hydration are found to be similar to the changes induced by increasing temperature.

In the past several years, a number of experiments have shown that double-stranded B-forms DNA, both in solution and in ordered phases, undergoes some relatively fast motions in addition to the normal reorientation of a rigid cylinder.[1-9] Hogan and Jardetzky[1-3] and Bolton and James[5] reported correlation times of the backbone motions to be in the range of 1×10^{-9} s. Early, Feigon, and Kearns[4] showed that base pairs also show the effects of such motions. Opella, DiVerdi, and Wise[6,7] showed that ordered B-form DNA similarly showed motions of the sugar phosphate backbone. Optical measurements have additionally demonstrated internal flexibility for long DNA helicides in solution.[16,17] Keepers

(1) Hogan, M.; Jardetzky, O. *Proc. Natl. Acad. Sic. U.S.A.* **1979**, *76*, 6341–6345.
(2) Hogan, M.; Jardetzky, O. *Biochemistry* **1980**, *19*, 2079–2085.
(3) Hogan, M.; Jardetzky, O. *Biochemistry* **1980**, *19*, 3460–3468.
(4) Early, T. A.; Feigon, J.; Kearns, D. R. *J. Magn. Reson.* **1980**, *41*, 343–348.
(5) Bolton, P. H.; James, T. L. *Biochemistry* **1980**, *19*, 1388–1392.
(6) Opella, S. J.; Wise, W. B.; DeVerdi, J. A. *Biochemistry* **1980**, *20*, 284–290.
(7) DiVerdi, J. A.; Opella, S. J. *J. Mol. Biol.* **1981**, *149*, 307–311.
(8) Allison, S. A.; Shibata, J. H.; Wilcoxon, J.; Schurr, J. M. *Biopolymers* **1982**, *21*, 729–762.
(9) Keepers, J. W.; Kollman, P. A.; Weiner, P. K.; James, T. L. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5537–5541.
(10) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. J. *Am. Chem. Soc.* **1962**, *84*, 3843–3846.
(11) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. *J. Am. Chem. Soc.* **1963**, *85*, 387–391.
(12) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. *J. Am. Chem. Soc.* **1963**, *85*, 391–394.
(13) Dickerson, R. E.; Drew, H. R.; Conner, B. N.; Wing, R. M.; Fratini, A. V.; Kopka, M. L. *Science (Washington, D.C.)* **1982**, *216*, 475–485.
(14) Torchia, D. A.; Szabo, A. *J. Magn. Reson.* **1982**, *49*, 107–121.
(15) Mehring, M. "NMR Basic Principles and Progress"; Springer-Verlag: New York, 1978; Vol. II, pp 167–189.

0002-7863/83/1505-7149$01.50/0

Table I

D_2O per nucleotide	equivalent relative humidity[a]	T_1	NOE	$\sigma_{33} - \sigma_{11}$	σ_{11}	σ_{22}	σ_{33}
0	0	130	b	205	−85	−18	120
5	53	4.9	1.13	190	−82	−18	108
7	65	1.6	1.11	184	−82	−17	102
9	75	1.3	1.11	183	−81	−18	102
11	80	0.42	1.11	182	−83	−19	99
13	83	0.32	1.06	162	−71	−13	91
15	85	0.27	1.10	148	−62	−7	86
17	88	0.28	1.03	129	−59	−6	70
19	92	0.25	1.01	119	−57	−5	62
25	95	0.29	1.03	104	−50	−1	54
27	98	0.35	b	88	−42	0	46
30	c	0.45	1.03	<70			d
33	c	0.50	1.05	<30			d

[a] Values derived from Figure 1 of ref 10. [b] Not measured. [c] Approximately 99% relative humidity. [d] The line was not sufficiently anisotropic to assign tensor elements.

et al.[9] calculated that the DNA backbone should undergo rapid motions between local torsional minima, which do not couple along the chain or across the base pairs. Most measurements have shown that base-pair motions are slower and/or smaller in amplitude than motions of the backbone.[7-9]

Although the existence of motions in the DNA helix have been thoroughly discussed, many of the details have not yet been addressed. Only recently it was shown that in ordered phases the amplitude of the backbone motions are significantly affected by increasing temperature but the base motions are not.[7] Classical experiments by Falk and co-workers[10-12] showed that water of hydration changes the structure of double-stranded DNA and stabilizes the B form found in solution. This and the differences in ^{31}P longitudinal relaxation times among the ordered A, B, and lyophilized forms of DNA suggested that the degree of hydration had a considerable influence on the motions of the sugar phaosphate backbone of DNA. We have therefore measured the ^{31}P spectrum and relaxation time of a well-defined DNA sample as a function of hydration to clarify the effect of water molecules on the dynamics of DNA. Through measurement of both the width of the powder spectrum and the relaxation times we are provided with information about both the amplitude and frequency of the motions of the phosphate group.

Experimental Section

Calf thymus DNA was prepared and sized as previously described.[1] The material was free of single-strand ends and was prepared with 10% by weight sodium chloride. A sample of 94 mg of this DNA was dissolved in D_2O lyophilized, and then loosely packed into a 10-mm NMR tube under dry nitrogen. The tube was sealed with a septum cap and covered with parafilm to assure that it did not absorb moisture from the air. Successive stages of hydration were achieved by introducing D_2O (99.8% deuteration) into the top of the sealed tube with a microsyringe. The amount of D_2O added was determined both by the volume and by weighing immediately before and after addition of D_2O to the sample. The water added was put on the side of the tube, not directly on the DNA, and then allowed to equilibrate for 3 days at 303 K. At the end of the equilibration the water drop let introduced had always been absorbed by the DNA. NMR measurements were made on a modified Varian XL-100 spectrometer equipped with a Nicolet MONA accessory and a homebuilt decoupler and solid state/MAS probe. The probe was a single-coil double-resonance type with a 10-mm coil that would accept either a 10-mm tube or a MAS rotor assembly. The ^{31}P 90° pulse was 13 μs and about 8 G of decoupling field was used.

Results

The ^{31}P NMR parameters of a single DNA sample at various hydration states are given in Table I and corresponding spectra are shown in Figure 1. The line widths of lyophilized, A-form and B-form DNA have been previously reported,[15-17] and our measurements at the appropriate level of hydration, equivalent in relative humidity, are in agreement with them.

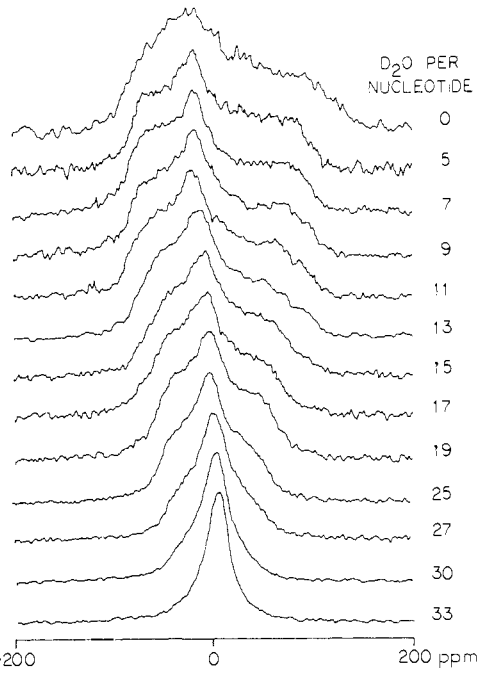

Figure 1. 40.5-MHz ^{31}P MNR spectra of a sample of double-stranded DNA at increasing hydration and 303 K. Spectra were taken by using a 90° pulse and strong proton decoupling, with an average of 20000 transients for each, taken with a delay of 5 times T_1. The spectrum of lyophilized DNA is the average of 15000 transients taken with cross polarization using a 1-ms contact time. 50-Hz line broadening was used in the data processing. Chemical shifts are referenced to external 85% H_3PO_4.

The spin–lattice relaxation time T_1 of lyophilized DNA was aproximately 130 s, with some variation among samples apparently corresponding to different amounts of residual water. Measurement of T_1 of a carefully dried sample using magic angle spinning gave a similar value. When the DNA had absorbed about five D_2O molecules per nucleotide, the T_1 dropped dramatically to about 5 s, indicating an increase in motion in the DNA. At this level of hydration all of the oxygen atoms of the phosphate group should be hydrated.[10-12] As more D_2O molecules were added, the T_1 continued to gradually decrease up to 11 D_2O molecules per nucleotide. At this level of hydration, all of the exposed hydration sites on the DNA are thought to be filled.[11,12] Beyond this the T_1 value plateaued until about 25 D_2O molecules per nucleotide, at which point it began to increase. The additional water at this stage fills the grooves of the DNA, and the spaces between molecules causing swelling to occur.[10-12]

The changes in the line width of the DNR with hydration are shown in Figures 2 and 3. There was a significant reduction in line width upon addition of the first five D_2O molecules, followed by a plateau up to 11 D_2O molecules per base pair. From 11 to 20 D_2O molecules there was a rapid decrease in line width, down to the values previously reported for the B form. Further increases in hydration brought about further reduction in the line width, with the line shape becoming more symmetric. The integrated ^{31}P NOE values upon saturating the protons are shown in Figure 4 for the same levels of hydration. These NOE values are small and the errors of the measurements could not really be determined. However, all of the values measured were positive so that an upper bound on the error of ca. ±0.5 could be estimated, and we can be confident that the enhancement values are nonzero at low hydration and decrease with increasing hydration.

Discussion

Although the measurements described here are not detailed enough to provide a complete description of the dynamic process, several features of the relaxation data make it possible to suggest

(16) Thomas, J. C.; Allison, S. A.; Apellof, C. J.; Schurr, J. M. *Biophys. Chem.* **1980**, *12*, 177–188.
(17) Millar, D. P.; Robins, R. J.; Zewail, A. H. *J. Chem. Phys.* **1982**, *76*, 2080–2094.

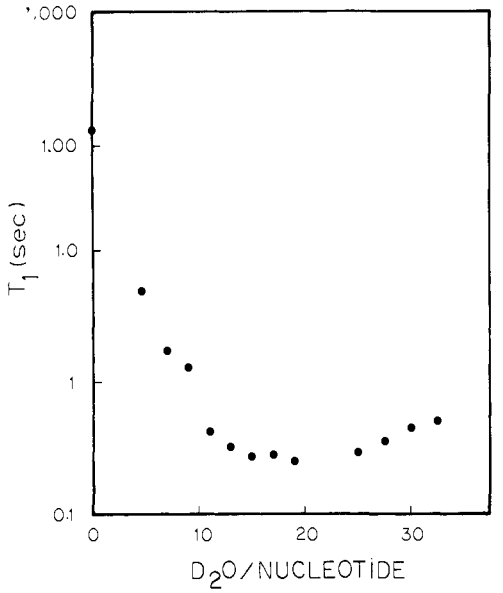

Figure 2. Effect of hydration on the transverse relaxation time of ^{31}P in double-stranded DNA. The integrated T_1 relaxation times were measured by using a saturation recovery sequence and a minimum of five different delays. Proton decoupling was used during collection of the FID but gated off between acquisitions. The T_1 values were calculted by using the standard Nicolet software, with the error of determination typically about 5% of the T_1 value. The sample was kept at 303 K during all measurements. The D_2O per nucleotide molar ratio was calculated by using 20 g/mol for D_2O and 340 g/mol for an average sodium nucleotide.

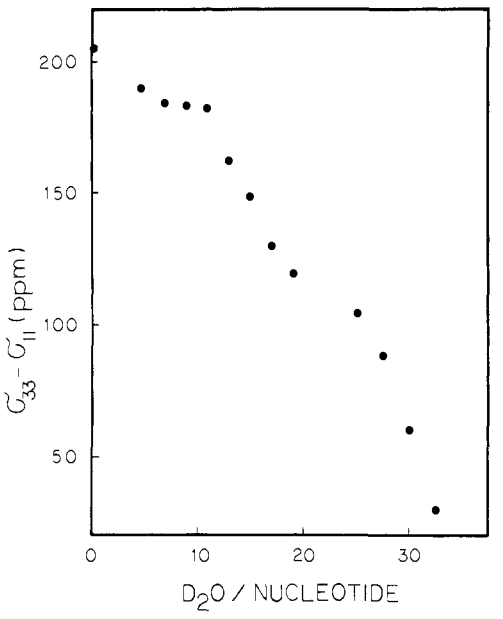

Figure 3. Effect of hydration on the line width of ^{31}P NMR spectra of DNA. Sample conditions are as in Figure 1. σ_{11} and σ_{33} were determined by eye from the discontinuities in the powder spectra. At high hydration the width reported is the full width at half-height of the line observed. Errors of determination are estimated to be approximately 5 ppm.

the range of frequencies and amplitudes involved. A relatively complete description of relaxation in ordered phases has been given recently.[14] The anisotropic nature of the problem complicates calculations significantly relative to solution problems. However, the qualitative dependence of the parameters T_1 and NOE on amplitude and frequency are not different from that found in solution. Fluctuations in either dipolar couplings or chemical shift, which arise from either the distance or orientation dependences of the couplings due to molecular motion, will give rise to short T_1 values only if the fluctuations are near the Larmor frequency

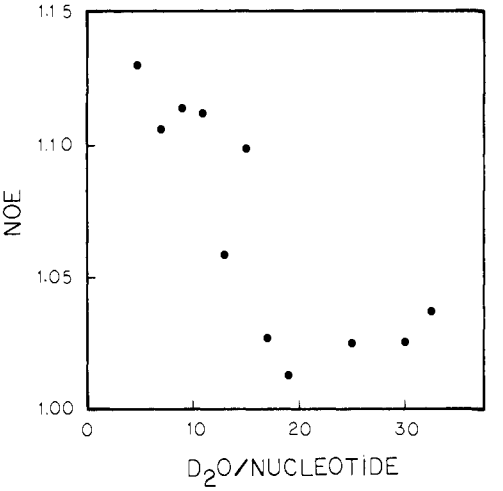

Figure 4. Effect of hydration on the observed NOE for ^{31}P in DNA. The NOE values have been measured as the ratio of the intergrated areas of spectra accumulated with and without preirradiation of the protons. Proton saturation was accomplished by using 90° pulses spaced by 2 ms, for a period equal to 5 times the measured T_1 for ^{31}P. Acquisition of transients with and without irradiation was alternated to minimize any spectrometer changes during the course of the measurements.

of the spin involved. For a significant nuclear Overhauser effect to occur, the fluctuations should occur at rates greater than the Larmor frequency (in angular units), and at least part of the relaxation must stem from dipolar coupling to the saturated spin (in this case protons). The effects of motion upon chemical shielding tensors are well-known and have been used for detailed studies of molecular reorientation processes.[15] If the motions occur at a rate greater than the chemical shift anisotropy (calculated in frequency units), then an average tensor will be observed, with principal values determined by the amplitude and direction of the motion. To acheive a large degree of averaging, a large amplitude of motion is required. If the motions are very slow, then no averaging will be observed, and in the intermediate range, averaging and broadening will occur.

The spectra and relaxation behavior observed suggest three different regimes of motion that occur. First, at low hydration, after addition of about five waters of hydration, a low-amplitude but high-frequency motion occurs. The low amplitude is indicated by the relatively small amount of averaging of the shielding tensor. The NOE that is observed indicates that the relaxation is, at least partially, coming from protons, which must be on the DNA since the added water was deuterated, and, in addition, that the frequency of the motion is relatively high. A reasonable possibility for this motion would be a small-amplitude but rapid conformational change in the backbone, such as a coupled sugar-phosphate twisting. Since only the phosphate motion is being monitored, no estimate of the amplitude of the sugar motion can be made. It is possible that some contribution to the relaxation is made by the deuterium and sodium dipolar couplings. As further water is added the amplitude of motion increases, as indicated by the further decrease in line width. The T_1 continues to drop, but the NOE decreases, suggesting that this additional motion has a frequency near enough the Larmor frequency to cause efficient relaxation but on the low side of the T_1 vs. frequency curve so that there is not a significant NOE. Previous studies have suggested that the B form is stable for relative humidities from 85% upward (corresponding to about 12–13 waters per nucleotide). The beginning of the rapid drop in line width may mark the conversion to the B form. The existence of motion in the B form has been pointed out previously.[7,18] However, regardless of the point of conversion, the dynamics of the B form change with hydration. From the degree of averaging of the shielding tensor at 20 waters per nucleotide, the motion must

(18) Shindo, H.; Wooten, J. B.; Peiffer, B. J.; Zimmerman, S. B. *Biochemistry* **1980**, *19*, 518–526.

correspond to an angular fluctuation of the phosphate of at least ±30°. This estimate is based on the minimum angular fluctuation required about the σ_{22} tensor axis, to provide the observed degree of averaging. If the axis of the fluctuation is not the σ_{22} axis, then the size of the fluctuation must be even larger. Since the features of the powder spectrum are not severely broadened as water is added, the motion must always be in the fast exchange limit, at a minimum frequency of ca. 10 kHz, and increasing in amplitude with additional water. The spectrum at 20 waters per nucleotide corresponds closely with that obtained for B-form DNA prepared by vapor-phase equilibration. The motions at this point may correspond to the approximately nanosecond time scale processes that are observed in solution and are attributed variously to strictly local[3,4] and a superposition of local and collective[8] motions. The line shape that occurs is consistent with a twisting motion about the long axis of the helix, assuming that σ_{22} is near the helix axis, expected from model compound studies. The angular fluctuation derived from the powder line shape is larger than that for the torsional modes derived from fluorescence measurements in solution. However, the NMR measurement is sensitive to slower motions than the optical measurements, and hence the amplitude will be larger if slow motions are present and their contribution is included in the total amplitude. Upon further addition of water, the averaging becomes even more drastic, with an essentially isotropically averaged line occurring by 33 waters per nucleotide. The relaxation behavior does not change significantly over this range, which indicates that the new motion is too slow to be efficient for relaxation, bracketing it to be in the range greater than ca. 10 kHz but less than 10 MHz. This motion is equivalent in effect to (and probably is the same as) the motion observed in B-form DNA upon raising the temperature. Opella et al.[7] did not observe averaging of the deuterium quadrupole coupling tensor of a base-attached hydrogen in the same sample for which the ^{31}P tensor averaging was observed. However, in a recent report[19] Valeutine and Opella concluded from partially averaged ^{15}N spectra that backbone motions also appear at the level of hydration of 5 water molecules per nucleotide. These motions must be internal since at this low level of hydration, tumbling of the DNA in the solvent would be extremely unlikely. The discrepancy between their ^{2}H and ^{15}N results remains unexplained, but given the low sensitivity of ^{2}H and the broad spectra observed, the initial partial averaging of the ^{2}H tensor could have been obscured by quadrupolar effects. It is clear from the findings reported here that hydration is essential to permit the type of internal motions in DNA which can be detected by NMR and that the amplitude of such motions increases with an increasing degree of hydration.

From the present work we have shown that several different kinds of motion occur in DNA at various levels of hydration. The measurements are thus far limited to ^{31}P and hence probe only the backbone of the DNA. Further measurements will be made on defined-sequence DNA with labels incorporated in various sites to further define the nature of these motions.

Acknowledgment. We thank Dr. M. Hogan for preparing the DNA used in these studies and for many helpful discussions on DNA dynamics. This research is funded by Grant GP23633, National Science Foundation Grant PCM 80-21899, and National Institutes of Health Grant RR00711.

(19) Valeutine, K.; Opella, S. J. In "Conversation in Biomolecular Stereodynamics"; Sarma, R. H., Ed.; Abstracts No. 17, p 42, 1983.

Ground States of Molecules. 62.[1] MINDO/3 and MNDO Studies of Some Cheletropic Reactions

Michael J. S. Dewar* and Lek Chantranupong[2]

Contribution from the Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712. *Received December 16, 1982. Revised Manuscript Received July 16, 1983*

Abstract: MNDO and MINDO/3 calculations are reported for the reverse cheletropic eliminations of nitrogen from *N*-(3-pyrrolinyl)nitrene and of carbon monoxide from 4-cyclopentenone and from tricyclo[5.2.1.0^{2,6}]deca-3,8-diene-5,10-dione, and the reverse Diels–Alder elimination of nitrogen from 3,6-dihydropyridazine. All these reactions are predicted to take place via very unsymmetrical transition states in which one of the breaking bonds is almost intact, leading to biradical-like species which then dissociate.

Introduction

While the mechanisms of cheletropic reactions[3] have been extensively discussed[4,5] in terms of qualitative MO theory, no adequate quantitative calculations for such reactions seem as yet to have been published. In order to be of any real chemical value in such connections, the calculations must not only be carried out by a procedure of adequate accuracy and reliability, but also with full geometry optimization, using a derivative optimization method and without making any assumptions.[6] Transition states must also be characterized by the McIver–Komornicki[7] procedure of calculating force constants and transition vectors. These requirements make calculations by adequate ab initio procedures prohibitively expensive for any but the simplest organic molecules.[8]

Meaningful calculations for the larger systems of direct interest to organic chemists can therefore be carried out at present only by some semiempirical procedure, and the only ones with any real claim to adequate accuracy currently available are the parametric methods developed by our group, i.e., MINDO/3[9] and MNDO.[10]

(1) For Part 60 in this series, see: Dewar, M. J. S.; Nelson, D. J. *J. Am. Chem. Soc.*, accepted for publication.

(2) Robert A. Welch Predoctoral Fellow.

(3) Woodward, R. B.; Hoffmann, R. "The Conservation of Orbital Symmetry"; Academic Press: New York, 1972.

(4) Dewar, M. J. S. *Angew. Chem.* **1971**, *83*, 859; *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 761.

(5) Dewar, M. J. S.; Dougherty, R. C. "The PMO Theory of Organic Chemistry"; Plenum Press: New York, 1975.

(6) Any assumptions concerning the geometry of the TS must be based on an assumed mechanism for the reaction. Any calculation making such assumptions will then tend to reproduce the assumed mechanism, right or wrong.

(7) McIver, J. W., Jr.; Komornicki, A. *J. Am. Chem. Soc.* **1972**, *94*, 2625; *Chem. Phys. Lett.* **1971**, *10*, 303.

(8) In order to obtain acceptable results, it is necessary to use a large basis set (of at least double-ζ quality) and to allow for electron correlation either by very extensive CI or some comparable procedure. See, e.g.: Komornicki, A.; Goddard, J. D.; Schaefer, H. F., III *J. Am. Chem. Soc.* **1980**, *102*, 1763.

(9) Bingham, R. C.; Dewar, M. J. S.; Lo, D. H. *J. Am. Chem. Soc.* **1975**, *97*, 1285, 1294, 1302.

(10) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899, 4907.

0002-7863/83/1505-7152$01.50/0