polydodecamer at a specific site in each repeating unit (Figure 1a, open arrows). The restriction endonuclease *Fnu*DII catalyzes blunt-ended cleavage at 5'-d(CGCG) sites, three of which overlap in each repeating unit (Figure 1a) of the polydodecamer: two possess m^7dG residues (shaded), whereas one is native (unshaded). *Fnu*DII cleavage took place only at the unmodified site, thereby generating the Dickerson/Drew dodecamer, 5'-d(CGCGAATTCGCG).[12-14] The presence and amount of m^7dG in the dodecamer were confirmed by nucleoside composition analysis, and its location within the sequence was confirmed by specific cleavage at the m^7dG site with piperidine.[14]

In thermal denaturation experiments, the duplex half-melting transition (T_m) of the Dickerson/Drew dodecamer was lowered slightly by the presence of m^7dG (Table I). However, analysis in terms of $\Delta G°$, $\Delta H°$, and $\Delta S°$ revealed that, at room temperature, duplex stability in 5'-d(CGCGAATTCGCG) is indistinguishable from that in 5'-d(CGCGAATTCGCG). Moreover, the enthalpy ($\Delta H°$) of duplex formation was made significantly more favorable by the presence of m^7dG, suggesting that the m^7dG·dC base-pair is more stable than dG·dC.[15] The less favorable entropy change ($\Delta S°$) for denaturation of 5'-d(CGCGAATTCGCG) is more difficult to interpret, but may arise from effects on solvation and stacking interactions.[16] Whatever their origins, the small magnitude of these thermodynamic effects strongly suggests that m^7dG does not significantly perturb duplex DNA structure. By way of comparison, N^6-methylation of adenines in DNA reduced T_m by 6-18 °C,[17] yet was shown by X-ray crystallography to have a negligible effect on duplex structure.[18]

We have found that m^7dG is stable for days in duplex DNA at 25 °C (half-life > 1000 h; K.E.-N., unpublished), which contrasts with the reported instability of the free nucleoside.[5,10] This observation leads us to conclude that the probe moiety in dimethyl sulfate[7] and template-directed[8] interference footprinting is m^7dG and not a product of its decomposition. Furthermore, these findings are consistent with the observed persistence of m^7dG residues in genomic DNA.[19] Repair proteins specific for m^7dG are ubiquitous[20,21] although poorly understood mechanistically. The modified dodecamer reported here now provides a homogeneous substrate for studies of recognition and repair by these proteins.

The methodology reported herein should be useful for generating specifically modified DNA molecules that could not be accessed by prior methods.[22]

Supplementary Material Available: Detailed experimental procedures, polyacrylamide gel electrophoretic analysis of reaction products, and an HPLC trace showing the results of nucleoside composition analysis on 5'-d(CGCGAATTCGCG) (6 pages). Ordering information is given on any current masthead page.

(12) This synthesis has been carried out on a ~5-mg scale using overproduced enzymes; this scale is sufficient to service NMR and X-ray crystallography studies.

(13) The 5'-OH version of the Dickerson dodecamer can be obtained readily and on a large scale by treatment with calf intestinal alkaline phosphatase.[14]

(14) Further analytical and experimental details are available in the supplementary material.

(15) Abdulnur, S. F.; Flurry, R. L., Jr. *Nature* **1976**, *264*, 369-370.

(16) Ishida, T.; Doi, M.; Ueda, H.; Inoue, M.; Schledrick, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 2286-2294.

(17) Ono, A.; Ueda, T. *Nucleic Acids Res.* **1987**, *15*, 219-232.

(18) Frederick, C. A.; Quigley, G. J.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J.; Rich, A. *J. Biol. Chem.* **1988**, *263*, 17872-17879.

(19) Karran, P.; Hjelmgren, T.; Lindahl, T. *Nature* **1982**, *296*, 770-773.

(20) Nakabeppu, Y.; Kondo, H.; Sekiguchi, M. *J. Biol. Chem.* **1984**, *259*, 13723-13729.

(21) O'Connor, T. R.; Laval, F. *EMBO J.* **1990**, *9*, 3337-3342. Male, R.; Helland, D. E.; Kleppe, K. *J. Biol. Chem.* **1985**, *260*, 1623-1629. Chakravarti, D.; Ibeanu, G. C.; Tano, K.; Mitra, S. *J. Biol. Chem.* **1991**, *266*, 15710-15715.

(22) We thank Stan Tabor and Richard Kolodner for gifts of Sequenase 2.0 and T4 DNA ligase, respectively; Larry McLaughlin for the use of his T_m apparatus; and A. M. MacMillan for helpful discussions. This work was supported by the NSF Presidental Young Investigators Program, the Searle Scholars Program, and the Sloan Foundation, in addition to gifts from Hoffmann-La Roche, Pfizer, Bristol Myers-Squibb, and Eli Lilly.

A [4 + 3] Transition State for a [4 + 2] Cycloaddition. A New Secondary Orbital Interaction in Diels-Alder Reactions

Daniel A. Singleton

Department of Chemistry, Texas A&M University
College Station, Texas 77843
Received April 17, 1992

Recent reports from this laboratory have detailed the exceptional and unusual characteristics of Diels-Alder reactions of vinylboranes.[1] We describe here some surprising results of ab initio calculations of transition structures for these reactions which shed light on the unusual reactivity and selectivity of vinylboranes and suggest the importance of a new secondary orbital interaction for Diels-Alder reactions in general.

Fully optimized endo and exo transition structures for the reactions of butadiene with vinylborane and vinyldimethylborane were located in ab initio RHF and MC-SCF[2] calculations as shown in Table I. The most striking result of these calculations is the [4 atom + 3 atom] character of the endo transition structures (**1-3**). In each case, C_1 is closer to the boron atom (B) than to C_6. The bonding of C_1 and B in **1-3** is apparent from positive Mulliken overlap populations and pyramidalization of C_1 toward B instead of C_6.[3] In sharp contrast, the exo transition structures (**4** and **5**) are [4 + 2] in character: C_1 is closer to C_6 than to B, and the C_1-C_4-C_5-C_6 dihedral angles are only -5.9° and -4°, compared to 19.8°, 18.0°, and 16.7° for **1-3**, respectively.

Although the activation energies at the 6-31G*//3-21G level are high by ~25 kcal, the predicted endo selectivity and *relative* reactivity of vinyldimethylborane correlate well with experimental observations. Calculations correctly predict that butadiene should react faster with vinyldimethylborane than with acrolein ($\Delta\Delta G^{\ddagger}_{calcd}$ = 2.9 kcal,[4] $k_{rel}^{298} \approx 40$[5]), but slower than with maleic anhydride ($\Delta\Delta G^{\ddagger}_{calcd}$ = -1.6 kcal,[6] $k_{rel}^{298} \approx 0.06$[7]) or Lewis acid-acrolein complexes,[8] despite no allowance for solvent and entropy effects or zero-point energies. The calculations also predict well the endo selectivity of vinyldimethylborane ($\Delta\Delta G^{\ddagger}_{calcd}$ = 2.3 kcal, endo:exo ≈ 95:5 with piperylene). These results suggest that the structural factors responsible for the relative reactivity and endo selectivity of vinyldimethylborane are reflected in the calculations (Chart I).[9]

The preference for endo [4 + 3] and exo [4 + 2] transition structures seems fairly rigid. When the C_3-C_4-C_5-C_6 dihedral angle of the exo vinylborane transition structure was fixed at 15°, the resulting [4 + 3]-like structure (after reoptimization) was 3.3 kcal higher in energy at the RHF-3-21G level. An attempt to obtain a [4 + 2]-like endo transition structure by fixing the C_3-C_4-C_5-C_6 angle at 57° failed; considerable C_1-B interaction

(1) (a) Singleton, D. A.; Martinez, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 7423. (b) Singleton, D. A.; Martinez, J. P.; Watson, J. V. *Tetrahedron Lett.* **1992**, *33*, 1017. (c) Singleton, D. A.; Martinez, J. P. *Tetrahedron Lett.* **1991**, *32*, 7365. (d) Singleton, D. A.; Martinez, J. P.; Watson, J. V.; Ndip, G. M *Tetrahedron*, in press.

(2) MC-SCF calculations were carried out with CASSCF wave functions with CI expansions derived from four electrons in the four π-like HOMO, NHOMO, LUMO, and NLUMO orbitals. For an MC-SCF calculation on a Diels-Alder reaction, see: Bernardi, F.; Bottoni, A.; Field, M. J.; Guest, M. F.; Hillier, I. H.; Robb, M. A.; Venturini, A. *J. Am. Chem. Soc.* **1988**, *110*, 3050.

(3) No intermediate after the transition state could be found at the RHF/3-21G level.

(4) Loncharich, R. J.; Brown, F. K.; Houk, K. N. *J. Org. Chem.* **1989**, *54*, 1129-1134.

(5) See ref 1a,d and Blankenburg, B.; Fiedler, H.; Hampel, M.; Hauthal, H. G.; Just, G.; Kahlert, K.; Korn, J.; Mueller, K. H.; Pritzkow, W. *J. Prakt. Chem.* **1974**, *316*, 804-16.

(6) Singleton, D. A. Unpublished data.

(7) See ref 1a,d and Craig, D.; Shipman, J. J.; Fowler, R. B. *J. Am. Chem. Soc.* **1961**, *83*, 2885.

(8) (a) Birney, D. M.; Houk, K. N. *J. Am. Chem. Soc.* **1990**, *112*, 4127. (b) For example experimental rates, see: Bonnesen, P. V.; Puckett, C. L.; Honeychuck, R. V.; Hersh, W. H. *J. Am. Chem. Soc.* **1989**, *111*, 6070.

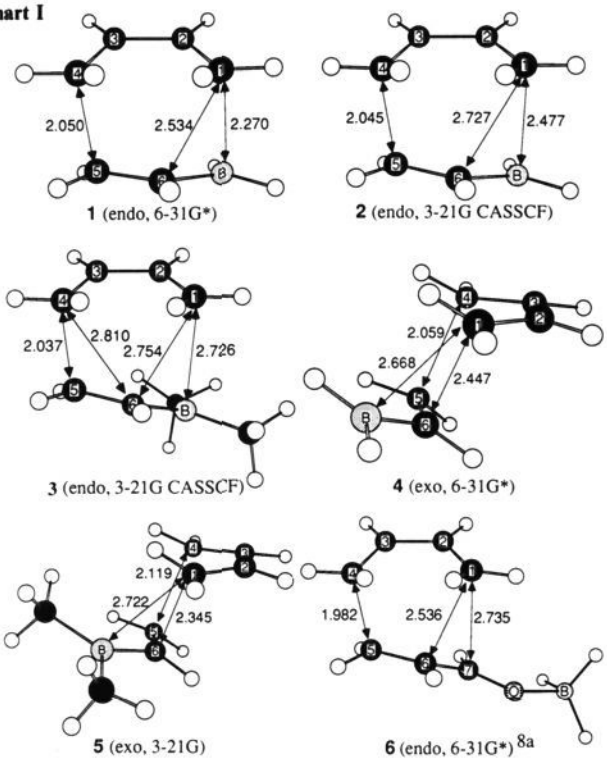
(9) For discussions of the correlation of ab initio calculations and experimental reactivities for Diels-Alder reactions, see: (a) Houk, K. N.; Loncharich, R. J.; Blake, J. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1989**, *111*, 9172. (b) Houk, K. N. *Pure Appl. Chem.* **1989**, *61*, 643.

0002-7863/92/1514-6563$03.00/0

Table I. Absolute and Activation Energies of Transition Structures for Diels-Alder Reactions with Butadiene

dienophile	absolute energies (activation energies) 6-31G*//3-21G	6-31*//6-31G*	CASSCF 3-21G
vinylborane, endo	−258.153 502 (35.0)	−258.153 755 (34.9)	−256.781 874
vinylborane, exo	−258.145 864 (39.8)	−258.146 123 (39.7)	
dimethylvinylborane, endo	−336.244 239 (39.0)		−334.455 069
dimethylvinylborane, exo	−336.240 575 (41.3)		

Chart I

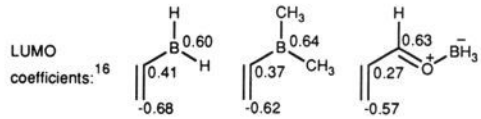


[8a]

remained as measured by a C_1-B distance of 2.25 Å.

There is little charge separation in these structures. By a Mulliken population analysis in the 6-31G*//3-21G calculations, only 0.06 *e* is transferred to vinyldimethylborane from butadiene. The charges on C_2 and C_3 are unchanged from the starting butadiene (to 0.01 *e*). This is consistent with the striking lack of substituent effects on the rate of these reactions.[1b,d] Despite high asynchronicity, particularly in the CASSCF structures (**2** and **3**), there is little biradical character; using Jensen's definition,[10] the biradical characters for **2** and **3** are only 0.074 and 0.077, respectively.

As with previous ab initio calculations with activated dienophiles,[4,8a,9a] there is no support in these calculations for a Woodward-Hoffman type "secondary orbital interaction"[11] of C_2 with B. However, the bonding interaction of C_1 with B defines a new type of secondary orbital interaction. Notably, this "[4 + 3] interaction" may be discerned in the highest level transition structure calculated by Houk and Birney[8a] for the reaction of butadiene with acrolein·BH_3 (**6**).[12] Although in **6** there is clearly greater interaction of C_1 with C_6 than with the carbonyl carbon C_7, a significant interaction of C_1 with C_7 is suggested by a positive Mulliken overlap population (0.011, this study), a C_1-C_4-C_5-C_6 angle of 17°, and a partial pyramidalization of C_1 toward C_7. No such interaction can be discerned in the corresponding exo transition structures.



A [4 + 3] transition structure can be rationalized from an FMO analysis. Because the largest coefficients of the LUMO of vinylboranes[13] are on the boron and the terminal carbon, the greatest initial overlap of the diene HOMO with the vinylborane LUMO can occur in a [4 + 3] fashion. The LUMO of acrolein·BH_3 is similar. The intriguing dichotomous behavior of endo and exo transition structures in adopting [4 + 3] versus [4 + 2] character is more difficult to understand, but it suggests that [4 + 3] interactions play an important role in the endo selectivity of some Diels-Alder reactions. This will be the subject of a future paper.

Acknowledgment. We thank the NIH, The Robert A. Welch Foundation, and the Texas A&M Supercomputer Center for research support and Ken Houk for a helpful discussion.

Supplementary Material Available: Calculational procedures and final geometries and energies for all calculations (4 pages). Ordering information is available on any current masthead page.

(10) Jensen, F. *J. Am. Chem. Soc.* **1989**, *111*, 4643.

(11) (a) Woodward, R. B.; Hoffmann, R. *The Conservation of Orbital Symmetry*; Verlag Chemie: Weinheim, Germany, 1970. (b) Houk, K. N. *J. Am. Chem. Soc.* **1973**, *95*, 4092. (c) Houk, K. N.; Strozier, R. W. *J. Am. Chem. Soc.* **1973**, *95*, 4094. (d) Salem, L. *J. Am. Chem. Soc.* **1968**, *90*, 553.

(12) An alternative secondary orbital interaction of C_3 with the oxygen atom of acrolein (see ref 11d) was noted by Houk and Birney in ref 8a for the endo s-cis transition structure. This interaction cannot be present with vinylboranes or s-trans dienophiles.

(13) Program MOPAC QCPE 455. The "outer" components of the LUMO coefficients in ab initio calculations with a 3-21G basis set display a similar trend.

A Cytochrome *c* Oxidase Reactivity Model: Generation of a Peroxo-Bridged Iron/Copper Dinuclear Complex

Alaganandan Nanthakumar, M. Sarwar Nasir, and Kenneth D. Karlin*

Department of Chemistry
The Johns Hopkins University
Baltimore, Maryland 21218

Natarajan Ravi and Boi Hanh Huynh

Department of Physics
Emory University
Atlanta, Georgia 30322

Received May 1, 1992

Cytochrome *c* oxidase (C*c*O) is a terminal respiratory protein complex which catalyzes the four-electron four-proton reduction of O_2 to water.[1] It possesses an array of metal ion sites, including a dinuclear (porphyrin)iron-copper complex, seen to be critically involved directly in O_2 binding, reduction, and proton pumping. This center consists of a heme a_3 and Cu_B which in the oxidized resting state are strongly spin coupled and EPR silent, with a Cu···Fe distance thought to be less than 5 Å.[1e] The linkage of the O_2-reduction process to proton translocation allows the energy released to be stored as a pH gradient and membrane potential for subsequent ATP synthesis.

Inorganic modeling of this active site dinuclear complex can be very helpful in elucidating aspects of structure, associated spectroscopy, and mechanism of O_2 reduction. Most of the activity associated with such chemistry has been directed at resting-state enzyme models and the generation of mixed-metal iron(III)-Cu(II) complexes having oxo, imidazolato, sulfur-containing, or

* Author to whom correspondence should be addressed.

(1) (a) Babcock, G. T.; Wikström, M. *Nature* **1992**, *356*, 301-309 and references cited therein. (b) Chan, S. I.; Li, P. M. *Biochemistry* **1990**, *29*, 1-12. (c) Malmström, B. G. *Chem. Rev.* **1990**, *90*, 1247-1260. (d) Capaldi, R. A. *Annu. Rev. Biochem.* **1990**, *59*, 569-596. (e) Scott, R. A. *Annu. Rev. Biophys. Biophys. Chem.* **1989**, *18*, 137-158.

0002-7863/92/1514-6564$03.00/0