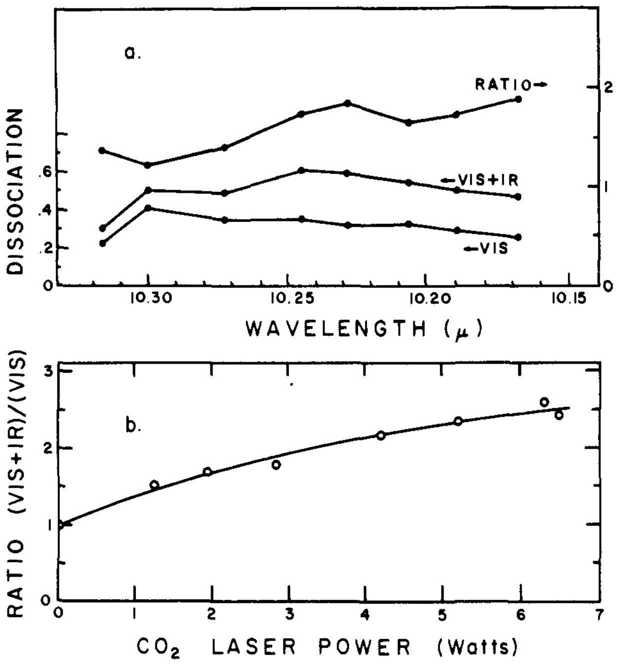

Figure 2. (a) Wavelength dependence of photodissociation. The lower two plots are the dissociation (−ln P/P_0, where P and P_0 are the parent ion signals, respectively, with and without light) for visible light only and for combined visible and IR irradiation (left-hand scale). The upper plot (right-hand scale) is the ratio of these (pressure 1.5 × 10^{-7} Torr). (b) Plot of the ratio of two-laser photodissociation to visible-laser-only dissociation as a function of IR laser power (pressure 5 × 10^{-8} Torr).

absorbs the first visible photon to put it 2 eV or more above the ground state, the IR pumping slows its subsequent relaxation and maintains it in the region of pumping/relaxation balance.[15] The probability of the ion remaining excited long enough for absorption of another visible photon is thus greatly increased, enhancing the dissociation rate.

Quantitative computer modeling of the two-laser photodissociation process suggests that the pressure dependence, visible intensity dependence, visible pulse rate dependence, and IR intensity dependence can be successfully accounted for within this model. These comparisons will be described in a fuller publication. The kinetic model applied here, involving sequential photon absorption and stochastic relaxation, is not fundamentally new, being a variety of the master-equation formalism.[16,17] However, it seems useful to point out that, at modest IR light intensities, this model leads to a (markedly non-Boltzmann) population of ions in dynamic equilibrium in an energy region part way between the ground state and the dissociation threshold.

The implications of this experiment for study of the properties of vibrationally excited species and vibrational relaxation processes are interesting. Steady low-level IR pumping of species like $C_6H_5I^+$ results in a stable steady-state population of ions with large and fairly uniform vibrational excitation. Two-photon visible photodissociation provides a successful probe for exploring some of the characteristics and dynamic behavior of these vibrationally excited ions. We are pursuing this approach in more depth and for other ions.

Acknowledgments. The support of the donors of the Petroleum Research Fund, administered by the American Chemical Society, of the National Science Foundation, and of the U.S. Air Force Geophysics Laboratory is gratefully acknowledged.

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(9) At low pressures, where the pulse rate is much faster than the collisional relaxation rate, this should be fully equivalent to using a continuous laser as in previous work, since the ion averages out the energy input of many laser pulses between collisions assuming the sequential photodissociation mechanism of ref 3–5. At high pressures, when relaxation of excited ions between pulses is appreciable, pulsed-laser excitation differs from CW excitation: this point will be treated in detail in a forthcoming publication.
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(15) It seems likely that some ions will occupy this energy region even in the absence of visible light. However, ions within ~0.3 eV of the ground state lie below the "quasi-continuum" and are expected to be pumped relatively slowly by IR radiation, so that visible light may well be necessary to prevent a substantial population from remaining near the ground state.
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Received December 18, 1979

Exceptional Reactivity of the Bicyclo[2.2.1]heptene Double Bond

Sir:

Norbornene shows two exceptional characteristics in its reactivity: high preference for exo reaction and increased rate constants in all addition reactions including cycloadditions.

The exo selection was ascribed to a favorable transition state conformation (torsional effect)[1] or to a steric hindrance of endo attack.[2] The size of exo/endo ratios render both explanations doubtful. Inagaki, Fujimoto, and Fukui[3] applied the "orbital mixing rule" to norbornene and deduced "nonequivalent orbital extension", i.e., greater exo than endo lobes for the π HO; this change of hybridization (see formula **1**) was made responsible for preferential exo addition of electrophilic reagents.[3]

The increased reactivity of norbornene as a dienophile or dipolarophile compared with simple cycloalkenes was interpreted by the release of ring strain in the transition state.[4] The heat of hydrogenation of norbornene exceeds that of cyclohexene by 6 kcal mol^{-1}.[5] The 1,3-dipolar cycloadditions of benzonitrile oxide,[6] diazomethane,[7] and phenyl azide[8] to norbornene are 6100-, 5400-, and 5700-fold faster than those to cyclohexene, corresponding to $\Delta\Delta G^{\ddagger}$ = 4.7-5.1 kcal mol^{-1}. On the other hand, in the last decade evidence for *early* transition states of concerted cycloadditions has accumulated,[9-11] i.e., only a fraction of the 6 kcal mol^{-1} (roughly the difference between the strain energies of norbornene and norbornane) can be released in the transition state.

Table I. Heats of Formation ($\Delta H_f°$) and Strain Energies of Cycloalkenes and Cycloalkanes[a]

	cycloalkene			cycloalkane			
	exptl	calcd		exptl	calcd		
	$\Delta H_f°$	$\Delta H_f°$	strain	$\Delta H_f°$	$\Delta H_f°$	strain	Δ strain
cyclohexene	-1.08^b	−1.88	2.76	-29.50^b	−29.53	2.61	−0.15
bicyclooctene **2**	$+4.88^c$	+5.59	15.15	-23.75^b	−22.41	14.64	−0.51
bicycloheptene **1**	$+20.6^d$	+19.40	23.20	-12.42^b	−12.84	18.46	−4.74
bicyclohexene **3**		+51.41	49.45		+13.95	39.49	−9.96
tricyclooctene **4**		+49.86	52.82		+13.45	43.91	−8.91

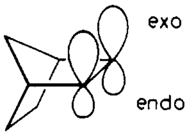
[a] In kcal mol^{-1} calculated with MM2. [b] J. D. Cox and G. Pilcher, "Thermochemistry of Organic and Organometallic Compounds", Academic Press, London, 1970. [c] Reference 26. [d] Reference 5.

Table II. Rate Constants of Concerted Cycloadditions to Cyclic and Bicyclic Alkenes[a]

1,3 dipole or diene	reaction conditions[b]	cyclohexene	bicyclooctene **2**	norbornene (**1**)	tricyclooctene **4**	bicyclohexene **3**
2,4,6-trimethylbenzonitrile oxide (**5**)	CCl_4, 25, 10^6	1.2	5.7	3150	2300	2280
4-nitrophenyl azide	DMF, 50, 10^5		1.3	112	126	
diazomethane	DMF, 25, 10^7	0.4		2020	960	
C-p-nitrobenzoyl-*N*-phenylnitrone (**6**)	dioxane, 25, 10^7		5.9	6340	6420	1010
dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**)	dioxane, 25, 10^3	1.27	113	11700	5370	

[a] In L mol^{-1} s^{-1}. [b] Solvent, °C, k_2.

We have compared the cycloaddition rates of norbornene (**1**) with those of models **2–4** in which, due to symmetry, nonequivalent orbital extension cannot occur. The results demonstrate that norbornene owes its high reactivity only partially to the strain release; the larger portion of $\Delta\Delta G^{\ddagger}$ stems from a factor "x" which must be related to the contrasting symmetry properties of **1** compared with those of **2–4.**



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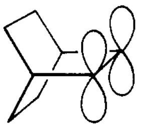

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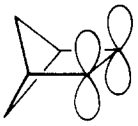

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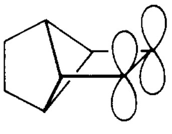
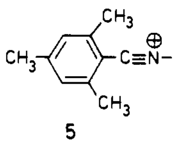


4

A knowledge of the strain energies of the cycloalkenes and cycloalkanes is mandatory for a quantitative understanding of the facts. Thermochemical data on bicyclo[2.1.1]hexene (**3**)[12] and the more easily accessible tricyclo[3.3.0.0^{2,6}]octene (**4**)[13] are lacking, whereas bicyclo[2.2.2]octene (**2**)[14] surpasses cyclohexene by 1.2 kcal mol^{-1} in the heat of hydrogenation.[5] The improved force field for molecular mechanics calculations, MM2,[15] was extended to alkenes.[16] The agreement between the calculated and experimental $\Delta H_f°$ values of cyclohexene, **1**, **2**, and the corresponding saturated compounds (Table I), as well as several cycloalkenes and cycloalkanes containing four-membered rings,[18] indicates that the results for **3** and **4** in Table I are trustworthy.

The loss of ring strain in going from the cycloalkene to the cycloalkane is, for bicyclohexene **3** and its tricyclic derivative **4**, twice as large as for norbornene (**1**). Would the rate increase of norbornene over cyclohexene in cycloaddition reactions originate only from strain release in the transition state, then one would expect the cycloadditions of **3** and **4** to show a value of $\Delta\Delta G^{\ddagger}$ which is twice as large as that for **1.**



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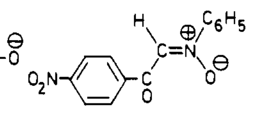

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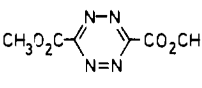

7

We have measured the rate constants for four 1,3-dipolar cycloadditions and one Diels-Alder reaction (with inverse electron demand).[19] Instead of reacting 10^3–10^4 times faster than norbornene (**1**), the rate constants of bicyclohexene **3** and tricyclooctene **4** are similar or somewhat smaller than those of **1** (Table II). Thus, release of strain energy in the transition state can only be partially responsible for the high rate constants of norbornene (**1**). The additional rate increase is attributed to the mentioned factor "x".

The numerical evaluation of the two factors rests on the simplifying assumptions that the strain release is percentagewise the same for the transition states of additions to **1–4**, that the steric requirements are equal, and that the rate enhancement of **3** compared with **2** fully originates from strain release in the transition state. For the cycloadditions of the various reagents of Table II, 27–49% of the $\Delta\Delta G^{\ddagger}$, observed between **1** and **2**, are accounted for by strain release in the transition states of norbornene cycloadditions. Factor "x" would be responsible for 1.2–2.9 kcal mol^{-1} of the experimental $\Delta\Delta G^{\ddagger}_{2-1}$.

Nonequivalent orbital extension, i.e., a change of hybridization at the olefinic carbon atoms,[20,21] would constitute a fitting interpretation of factor "x". It should be mentioned, however, that neither MNDO[24] nor MINDO/3 calculations[25] of norbornene corroborate Fukui's effect. In a conceivable alternative, the phenomenon would lower the energy of the transition state of exo cycloaddition.

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(19) All of the cycloadditions of Table II were first studied on a preparative scale. The reactions proceed with high yields and the adducts have been isolated. Their properties, analyses, and spectral data will be published elsewhere.

(20) According to Fukui et al.,[3] hyperconjugation phenomena are responsible for the large exo lobes of the π HO of **1**. The accompanying change of hybridization at the olefinic carbon atoms suggests that the phenomenon of nonequivalent orbital extension is general for all π MO's of **1**.

(21) Diazomethane cycloadditions are HO(diazomethane)–LU(olefin) controlled,[22] whereas the other 1,3-dipolar cycloadditions of Table II receive contributions from both HO–LU interactions (Sustmann's type II).[23] The Diels–Alder reaction of the tetrazine dicarboxylate **7** is LU(diene)–HO(dienophile) controlled. The ionization potentials (cyclohexene, 9.18; **1**, 8.97; **2**, 9.05; **4**, 8.63 eV) do not provide a key to the understanding of the rate sequences.

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Received January 21, 1980

Spectroscopic Studies on Cobalt(II) Metallothionein: Evidence for Pseudotetrahedral Metal Coordination

Sir:

The elucidation of the spatial structure of the metal binding sites in metallothionein, a widely occurring metal- and sulfur-rich protein, is indispensable for the understanding of the proposed involvement of this protein in metabolism, homeostasis and detoxification of zinc and other metals.[1] The most characteristic features of mammalian metallothioneins are the presence of seven atoms of Zn and/or Cd and of 20 cysteinyl residues (Cys) per chain weight of 6100. The position of these residues in the amino acid sequence is extremely preserved in evolution.[2] In contrast to most other metal thiolate proteins for which Cys-X-Y-Cys metal-binding (X, Y = amino acid residues other than Cys) sequences are typical,[3] mammalian metallothioneins contain seven Cys-X-Cys sequences.[2]

There is unambiguous evidence that all 20 cysteinyl residues participate in metal thiolate coordination.[4a,5] From the Cys/metal ratio and from complexometric titration studies and charge measurements, it has been inferred that the minimum metal binding unit of metallothionein contains as a rule three thiolate ligands and one or possibly more as yet unidentified nonprotogenic ligands. Based on sequence information and arguments of stoichiometry, each zinc and other bivalent metal ion is thought to be bound to the protein through mercaptide bonds with a Cys-X-Cys chelating structure and, in all but one case, also with an additional cysteinyl residue brought into position by appropriate tertiary structure folding.[4b] Recent ^{1}H NMR studies have established the existence of a compact and well-defined structure of metallothionein.[6]

In the present work, the coordination geometry of the metal binding sites of metallothionein is explored by means of optical and magnetooptical spectroscopy using the cobalt(II)-substituted protein. Replacement of the metal by cobalt(II) has been commonly employed in the study of zinc metalloenzymes and metalloproteins.[7] Because of their similarity, such a substitution does not measurably alter the overall protein conformation and often preserves the catalytic function of the enzyme. Cobalt(II) is particularly suitable as an environmental probe owing to its paramagnetism and the sensitivity of its d–d transitions to changes in coordination geometry.[7]

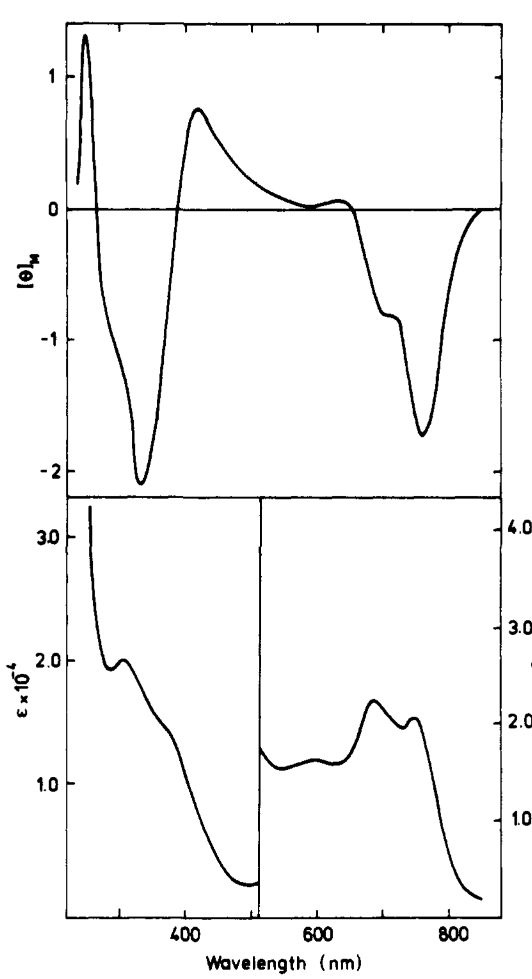

Figure 1. Magnetic circular dichroism (top) and absorption spectra (bottom) of cobalt(II) metallothionein. Protein concentration was 1.85×10^{-4} M in 0.1 M Tris-HCl. Measurements were performed using a path length of 1 cm or 1 mm. Molar absorptivity, ϵ, and magnetic ellipticity, $[\theta]_M$, are referred to the mol wt 6100 of the peptide chain of metallothionein. The natural ellipticity has been subtracted from the total ellipticity measured in the magnetic field before normalizing to unit field (magnetic field employed: 40 kG).

Metallothionein 1A was isolated from horse kidney according to Kojima et al.[4b] Cobalt(II) metallothionein containing 6 to 7 g-atoms of cobalt(II)/mol was prepared by the addition of 7 mmol of $CoSO_4$ to 0.7 mmol of metal-free protein (apometallothionein) obtained by dialyzing native metallothionein 1A against three changes of 0.1 M HCl and subsequent neutralization of the sample to pH 8 by Trizma base [tris(hydroxymethyl)aminomethane]. All preparative steps and measurements were carried out under nitrogen. The excess of cobalt(II) ions was removed by stirring with Chelex 100 followed by filtration. At neutral pH cobalt(II) metallothionein has a green color.

Figure 1 (bottom) shows the electronic absorption spectrum of cobalt(II) metallothionein. In the visible region broad bands with maxima at 600, 682, and 743 nm are discernible with molar absorptivities of ϵ 1560, 2220, and 2170 M^{-1} cm^{-1}, respectively. Similar spectra have also been reported for in-

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