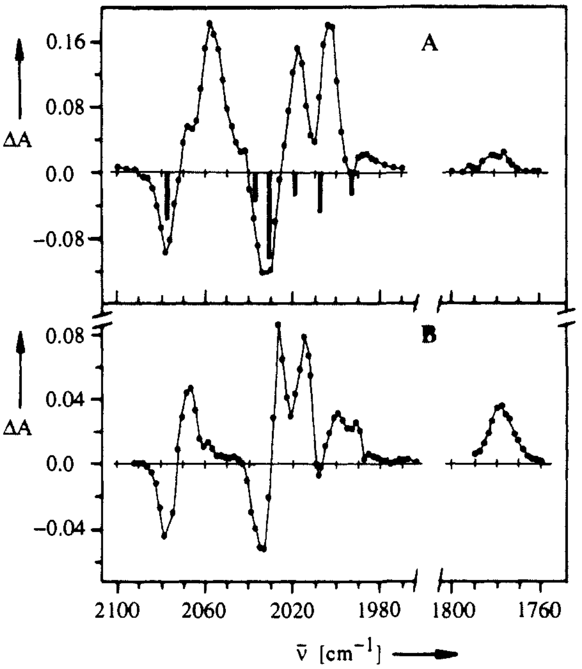

Figure 1. Transient ν_{CO} infrared difference spectra from flash photolysis (λ = 308 nm) of **1** (8×10^{-4} M) in cyclohexane at ambient temperature: (A) after ca. 3 μs under neat argon atmosphere (the vertical bars represent the ν_{CO} pattern of **1**); (B) after 100 μs in the presence of added ethene (ca. 10^{-2} M); note the difference in the scales.

mediately after the flash, **2** being too short-lived for detection. The decay of **3**, with nearly complete re-formation of **1**, occurs by first-order kinetics ($k = 8 \pm 3$ s^{-1}) independent of the amount of added ethene. From this we conclude that **3** already contains the ethene unit and thus represents an isomer of **1**, whereas **2** logically is identified as $Os_2(CO)_8$. Apparently, the (partial) conversion of **2** into **3** does not involve ethene in the rate-determining step, which leads us to postulate an intermediate X (Scheme I), presumably a CO-bridged isomer of **2**.

The ν_{CO} band pattern of **3** (Figure 1B) is in accord with a C_{2v} $(OC)_3Os(\mu\text{-}CO)_2Os(CO)_3$ skeleton, which requires five IR-active CO stretching vibrations in the terminal CO region (two A_1, two B_1, B_2)[6] and two bridging CO vibrations, one of which may be very low in intensity.[7] However, by analogy with the structure of $Os_2(CO)_9$[8] (and in view of the reluctance of third-row transition metals to support CO bridges), an alternative structure such as $(OC)_4Os(\mu\text{-}CO)Os(CO)_3(\eta^2\text{-ethene})$, with only one bridging CO, is equally plausible, if not even more likely. This structure would require more than five terminal CO bands, but it seems possible that weak bands are not observed or overlapping absorptions are not resolved.

For $Os_2(CO)_8$ (**2**), structures with D_{4h} or D_{4d} symmetry (two IR-active ν_{CO} modes: A_{2u}, E_u or B_2, E_1) can be excluded simply by the fact that three ν_{CO} bands are observed (Figure 1A), i.e., one band more than predicted. Although structures with D_{3h} or D_{3d} symmetry (three IR-active ν_{CO} modes: two A_2'', E' or two A_{2u}, E_u) would be in accord with the observed number of bands, they are unlikely because three bands with distinctly different intensities should be expected: the very strong E' or E_u mode, one A_2'' or A_{2u} mode of medium intensity (mainly involving the two axial CO groups), and one A_2'' or A_{2u} mode of low intensity (mainly involving the six equatorial CO groups). The D_{2h} structure shown in Scheme I, analogous to that assigned to the unbridged isomer of $Fe_2(CO)_8$,[7] is logical on the basis of the isolobal analogy between a d^8 ML_4 dimer and ethene.[9] Four IR-active CO stretching vibrations are expected for this geometry (two B_{1u}, B_{2u}, B_{3u}), which at first sight seems difficult to reconcile with the observed spectrum (Figure 1A). However, the one B_{1u} vibration mainly involving the axial CO groups and presumably located at high frequency should be distinctly weaker than the other modes and may be too weak to be observed. But we must not fail to note that the same reasoning would also apply to other structures composed of two C_{2v} $M(CO)_4$ subunits, such as D_{2d} (IR-active ν_{CO}: two B_2, two E) or C_{2h} (IR-active ν_{CO}: A_u, three B_u). Thus at present the latter two geometries cannot be excluded.

Low-temperature matrix experiments[10] complement the above studies in solution. Irradiation of **1** in an ethene-argon (1:5) matrix at 10-12 K with λ = 365 nm results in the exclusive formation of **2**, as indicated by the appearance of three terminal ν_{CO} bands at 2057, 2023, and 2006 cm^{-1} and no absorption in the bridging ν_{CO} region. Carbon monoxide is not detectable even after 50% conversion of **1**. It is noteworthy, in view of the fast re-formation of **1** in solution, that annealing of the matrix to 30 K leaves the ν_{CO} pattern of **2** essentially unchanged, apart from a small red shift of the maxima and some broadening of the band shapes. Photochemical reactions occurring upon irradiation into the electronic absorption of **2** (λ_{max} = 525 nm) are currently under investigation, which, in combination with a more detailed knowledge of the kinetics of **2** in solution, may provide a promising basis for the understanding of dinuclear reductive elimination and oxidative addition reactions[9b] in this and related species.

Acknowledgment. We are indebted to K. Fuchs and G. Klihm for their skilled technical assistance. Collaboration with Dr. H. Hermann in developing the instrumentation for time-resolved IR spectroscopy is gratefully acknowledged.

Registry No. **1**, 83705-05-9; **2**, 124442-24-6; **3**, 124602-64-8.

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[6] For comparison with the intensity pattern of various $Fe_2(CO)_6(\mu\text{-}X)_2$ compounds, see: Bor, G. *J. Organomet. Chem.* **1975**, *94*, 181-188.

[7] Fletcher, S. C.; Poliakoff, M.; Turner, J. J. *Inorg. Chem.* **1986**, *25*, 3597-3604.

[8] Moss, J. R.; Graham, W. A. G. *J. Chem. Soc., Dalton Trans.* **1977**, 95-99.

[9] (a) Hoffmann, R. *Angew. Chem.* **1982**, *94*, 725-739; *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 711-724. (b) Trinquier, G.; Hoffmann, R. *Organometallics* **1984**, *3*, 370-380.

[10] We have just been informed (Haynes, A.; Poliakoff, M.; Turner, J. J.; Bender, B. R.; Norton, J. R., submitted for publication) that matrix photolysis of $Os_2(CO)_9$ also yields $Os_2(CO)_8$ (the ν_{CO} data of which, in an argon matrix, agree with those reported in this paper within 1.1 cm^{-1}).

Direct Determination of the Partitioning of an Enzyme-Bound Intermediate

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In order to fully understand the basic principles by which enzymes function, it is important to be able to elucidate detailed free energy profiles for a variety of enzymatic reactions.[1-4] The nature of the partitioning of enzyme-bound intermediates is a particularly difficult problem, which generally must be addressed by indirect means. We now report the *direct* determination of the partitioning ratio of the dienol intermediate (**2**) in the 3-oxo-Δ^5-steroid isomerase (also called ketosteroid isomerase, KSI, EC 5.3.3.1)[5] catalyzed isomerization of 5-androstene-3,17-dione

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[1] (a) Nickbarg, E. B.; Knowles, J. R. *Biochemistry* **1988**, *27*, 5939. (b) Raines, R. T.; Sutton, E. L.; Strauss, D. R.; Gilbert, W.; Knowles, J. R. *Biochemistry* **1986**, *25*, 7142. (c) Albery, W. J.; Knowles, J. R. *Biochemistry* **1976**, *15*, 5627.

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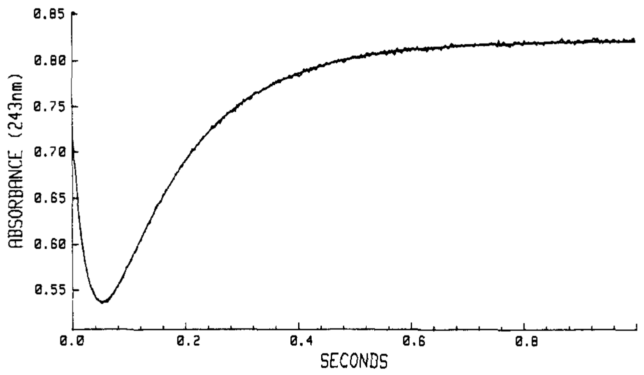
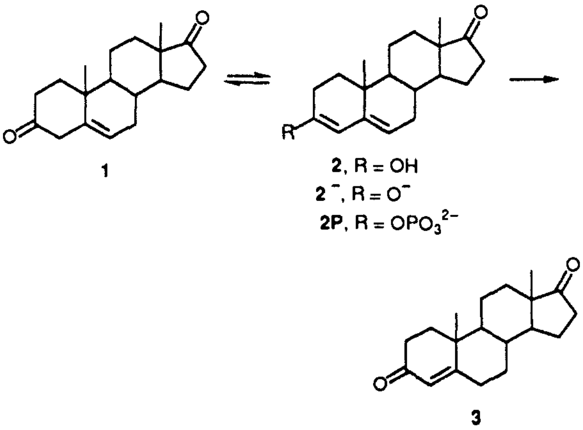


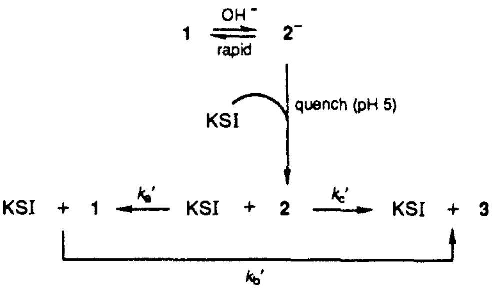
Figure 1. Absorbance change at 243 nm for the reaction of dienol **2** with KSI. Solutions of **2** (5×10^{-4} M in 20% methanol) and 1.0 N NaOH were mixed in a 1:1 ratio and allowed to age for about 0.5 s. This solution was then rapidly mixed with 5 volumes of a pH 4.7 acetate solution (400 mM acetate, 1% MeOH, 0.187 μM KSI) in the observation chamber of a stopped-flow spectrophotometer (final pH 5.1, 2.5% MeOH, 25.0 °C). The theoretical line is calculated from the following parameters: $k_a' = 24.0\ s^{-1}$; $k_b' = 6.66\ s^{-1}$; $k_c' = 10.4\ s^{-1}$.

(**1**) to 4-androstene-3,17-dione (**3**) (eq 1). This dienol partitions to form unbound reactant (**1**) in somewhat greater amounts than free product (**3**).



(1)

The dienol was generated in situ by the following series of reactions. Treatment of **1** with 0.5 M NaOH gives rapid equilibrium formation of the dienolate ion $\mathbf{2}^-$ ($t_{1/2} \simeq 35$ ms) followed by slower conversion of $\mathbf{2}^-$ to **3** ($t_{1/2} \simeq 6$ s).[6] Rapid quenching of $\mathbf{2}^-$ with mild acid, after a 0.5-s delay, gives the dienol **2**. When this process is carried out in a stopped-flow spectrophotometer with steroid isomerase in a mildly acidic buffer, the reaction of the dienol with the enzyme can be followed spectrophotometrically (eq 2).



(2)

Table I. Rate Constants for the Reaction of **2** with Steroid Isomerase[a]

[MeOH], %	k_a, $M^{-1}\ s^{-1}$	k_b, $M^{-1}\ s^{-1}$	k_c, $M^{-1}\ s^{-1}$	$k_c/(k_a + k_c)$, %
2.5	$(1.51 \pm 0.17) \times 10^8$	$(4.2 \pm 0.6) \times 10^7$	$(7.2 \pm 1.2) \times 10^7$	32.2 ± 2.2
3.3	$(1.52 \pm 0.13) \times 10^8$	$(3.9 \pm 0.4) \times 10^7$	$(7.0 \pm 1.2) \times 10^7$	31.4 ± 3.1

[a] The rate constants are defined in eq 3. Second-order rate constants are obtained from duplicate or triplicate runs, each consisting of 5–10 determinations, at enzyme concentrations of 0.1, 0.2, and 0.4 μM. pH values were between 5.0 and 5.1 for all runs (333 mM acetate). Enzyme concentrations were calculated by using a specific activity for pure enzyme of 52 000 units/mg.[11] Rates of ketonization in the absence of buffer were at least 100-fold slower than the observed enzyme-catalyzed rates. [b] Ratios of $k_c/(k_a + k_c)$ are obtained from individual runs and then averaged.

The reaction of the dienol with the isomerase was monitored at pH 5.2 under [S] ≫ [E] conditions at 243 nm, the isosbestic wavelength for **2** and **3**. A rapid drop in absorbance, followed by a somewhat slower increase, is observed (Figure 1). Since direct conversion of **2** to **3** would show no change in absorbance at the isosbestic wavelength, an intermediate species that absorbs much less strongly than either **2** or **3** must be formed. We interpret this kinetic behavior in terms of partitioning of **2** to **1** and **3**, where the initial drop in absorbance may be attributed to formation of the UV-transparent **1** and the subsequent rise is due to the enzyme-catalyzed conversion of **1** to **3**. This scheme can be analyzed in terms of two consecutive first-order processes (eq 3).[7] The experimental data were analyzed by nonlinear least-squares regression to give values for k_a, k_b, and k_c (Table I). The partitioning ratio, k_a/k_c, at pH 5, indicates that 68 ± 3% of the enzyme-bound dienol intermediate **2** is converted to unbound substrate (**1**) and ca. 32 ± 3% forms free product.

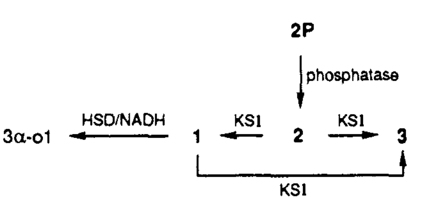
$$[\mathbf{1}]/[\mathbf{2}]_0 = A_1 e^{-(k_a'+k_c')t} + ([\mathbf{1}]_0/[\mathbf{2}]_0 - A_1)e^{-k_b't} \qquad (3)$$

$$\text{where } [\mathrm{X}]_0 = \text{initial concentration of X}$$

$$A_1 = k_a'/(k_b' - k_a' - k_c')$$

$$k_x' = k_x[\mathrm{E}] = (k_{cat}/K_m)_x[\mathrm{E}]$$

An independent estimate of the partitioning of the intermediate was obtained from generation of the dienol **2** by hydrolysis of the corresponding dienol phosphate **2P** catalyzed by sweet potato acid phosphatase in the presence of isomerase. 3α-Hydroxysteroid dehydrogenase (HSD) and NADH were added to irreversibly trap **1** as it was formed (eq 4). The amount of **3** was determined by HPLC analysis using various concentrations of HSD and constant isomerase concentration,[8] and the results were extrapolated to infinite [HSD]. The reaction of **2** with the isomerase (pH 5.0, 16 mM acetate, 1.7% MeOH) gives 35 ± 5% of **3**, in excellent agreement with the kinetic results (32 ± 3% of **3**).



(4)

The ability to monitor directly the partitioning of the enzyme–dienol intermediate opens up an attractive avenue for in-

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(5) For reviews, see: (a) Pollack, R. M.; Bounds, P. L.; Bevins, C. L. In *The Chemistry of Functional Groups, Enones*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1989; p 559. (b) Batzhold, F. H.; Benson, A. M.; Covey, D. F.; Talalay, P. *Adv. Enzyme Regul.* **1976**, *XIV*, 243.

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(7) It is assumed in this analysis that [S] (=42 μM) ≪ K_m for reaction of both **1** and **2** with the isomerase. K_m for **1** is ca. 300 μM;[5] K_m for **2** is not known, but the excellent fit of the data to the theoretical curves argues that K_m ≫ 42 μM. The value of $[\mathbf{2}]_0$, which is necessary to complete the kinetic analysis, was obtained from the absorbance drop when the dienolate ion was mixed with buffer in the absence of enzyme. The rate constant for the ketonization of the dienolate in the absence of enzyme under these conditions is 0.15 s^{-1}, about 80-fold slower than the slowest enzymatic rate measured.

(8) Isomerase concentrations of 0.01–0.03 μM, sufficient to trap >99% of the dienol **2** before it reacts with solvent, were used.

vestigations of the isomerase. Of immediate interest are the effects of site-directed mutagenesis on the individual steps of the reaction.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM 38155). We thank Mr. Jim Janc for carrying out some preliminary experiments, Dr. Joseph P. G. Mack for the isolation of hydroxysteroid dehydrogenase, and Professor Donald J. Creighton for several informative discussions.

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(9) The effects of site-directed mutagenesis of some of the residues implicated in the catalytic mechanism on the overall rate of catalysis have been reported.[10].

(10) Kuliopulos, A.; Mildvan, A. S.; Shortle, D.; Talalay, P. *Biochemistry* **1989**, *28*, 149.

(11) Talalay, P.; Benson, A. M. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1972; Vol. 6, pp 591–618.

Photoinduced Electron-Transfer Reactions of 1,4-Dimethyl- and 1,2,3,4,5,6-Hexamethylbicyclo[2.2.0]hexanes. Evidence for Boat Cyclohexane-1,4-diyl Radical Cation and Its Stereospecific Cleavage

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Cyclohexane-1,4-diyl radical cation has recently attracted considerable interest as a radical cation counterpart of the elusive cyclohexane-1,4-diyl diradical and also as an intermediate associated with possible catalysis of the Cope rearrangement by electron transfer.[1] As for its structure, preferential formation in a chair form has been observed upon ionization of both 1,5-hexadienes and unsubstituted bicyclo[2.2.0]hexane.[1b,e] Its cleavage to 1,5-hexadiene radical cation is thought to be thermodynamically unfavorable,[1,2] and the feasibility of the process in the substituted derivatives has remained unsubstantiated. Herein we report studies of the title reactions that provide evidence for the intermediate formation of hitherto unobserved *boat* cyclohexane-1,4-diyl radical cations and their stereospecific cleavage.

Photoinduced electron-transfer (ET) reactions of bicyclohexanes **1b**,[3] **1c**, and **7c**[4] (Scheme I) were investigated by irradiation of their solutions in acetonitrile in the presence of various acceptors.[5] Prerequisite initial ET from the bicyclohexanes to the excited singlet acceptors is supported by the exothermicity of the processes[6] and quenching of acceptor fluorescence by the former at near-diffusion-controlled rates (Table I).[7] The sensitized photolysis of **1c** was practically acceptor-independent and led to the stereospecific formation of *erythro*-(*E*,*E*)-diene **4c**[8,9] together with a small amount of hexamethylcyclohexene (**6c**) in 50–85% total yield (**4c**:**6c** = 86:14–93:7). DCA-sensitized photolysis of **1b** (*exo*-D:*endo*-D = ≥98:≤2) in acetonitrile afforded **4b** [*E*-D:*Z*-D = (83 ± 4):(17 ± 4)] in more than 80% yield.[10] On the other hand, the reaction of **7c** was strongly acceptor-dependent and the product compositions were found to be correlated with the reduction potentials ($E_{1/2}^{red}$) of acceptors (Figure 1).[12] Thus, **7c** was isomerized predominantly to **1c** by the acceptors whose $E_{1/2}^{red}$ is more negative than −0.8 V vs SCE,[13] while it afforded mainly **6c** when sensitized by the more readily reducible acceptors. In addition to **1c** and/or **6c**, *erythro*-(*E*,*Z*)-diene **11c**[9] was invariably obtained as the minor product, but *erythro*-*Z*,*Z* isomer **15c**[9] was not detected.

Table I. Calculated ΔG for Electron Transfer, Fluorescence Quenching Rate Constants, and Quantum Yields in Acetonitrile

substrate	acceptor	ΔG,[a] kcal/mol	k_q, $M^{-1}\,s^{-1}$	ϕ[b]
1b	DCA	−7	1.8×10^9	
1c	DCN	−16	10.0×10^9	0.034[d]
	DCA	−12	7.2×10^9	0.014[e]
	TCB	−39	16.7×10^9	0.069[d]
	TCA	−21	13.8×10^9	0.048[e]
	TCNQ[c]	−29		
7c	DCN	−17	10.7×10^9	0.067[d]
	DCA	−13	5.8×10^9	0.029[e]
	TCB	−40	16.2×10^9	0.12[d]
	TCA	−22	13.5×10^9	0.14[e]
	TCNQ[c]	−30		

[a] Calculated for ET from substrate to singlet excited acceptor by using the Weller equation.[6] [b] For the consumption of substrate. [c] The lowest excited singlet energy was estimated from the absorption spectrum. [d] At 313 nm. [e] At 366 nm.

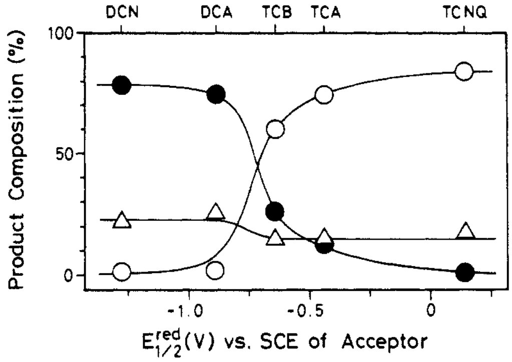

Figure 1. Correlation of product composition with the reduction potential of electron acceptor in the reaction of **7c**: (●) **1c**; (○) **6c**; (△) **11c**.

In the present reactions of **1b**,**c** and **7c**, the initial generation of cyclohexane-1,4-diyl radical cations is reasonably assumed. It should be noted that the ring strain inherent in this system is largely relieved by stretching of the central bond. The ionization of the parent **1a** (=**7a**) has, in fact, been shown to give **9a**.[1c] The

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(1) (a) Guo, Q.-X.; Qin, X.-Z.; Wang, J. T.; Williams, F. *J. Am. Chem. Soc.* **1988**, *110*, 1974. (b) Miyashi, T.; Konno, A.; Takahashi, Y. *Ibid.* **1988**, *110*, 3676. (c) Adam, W.; Grabowski, S.; Miranda, M. A.; Rubenacker, M. *J. Chem. Soc., Chem. Commun.* **1988**, 142. (d) Hasegawa, E.; Mukai, T.; Yanagi, K. *J. Org. Chem.* **1989**, *54*, 2053. (e) Williams, F.; Guo, Q.-X.; Bebout, D. C.; Carpenter, B. K. *J. Am. Chem. Soc.* **1989**, *111*, 4133. (f) Dai, S.; Pappas, R. S.; Chen, G.-F.; Guo, Q.-X.; Wang, J. T.; Williams, F. *Ibid.* **1989**, *111*, 8759.

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(3) The labeled **1b** was prepared by reducing 1,4-dimethyl(Dewar benzene) with diimine-d_2.

(4) Van Bekkum, H.; van Rantwijk, F.; van Minnen-Pathuis, G.; Remijnse, J. D.; van Veen, A. *Recl. Trav. Chim. Pays-Bas* **1969**, *88*, 911.

(5) The electron acceptors ($E_{1/2}^{red}$ vs SCE) employed are 1,4-dicyanonaphthalene (DCN, −1.28 V), 9,10-dicyanoanthracene (DCA, −0.89 V), 1,2,4,5-tetracyanobenzene (TCB, −0.65 V), 2,6,9,10-tetracyanoanthracene (TCA, −0.45 V), and tetracyanoquinodimethane (TCNQ, 0.13 V).

(6) Rehm, D.; Weller, A. *Ber. Bunsenges. Phys. Chem.* **1969**, *73*, 834; *Isr. J. Chem.* **1970**, *8*, 259. The $E_{p/2}^{ox}$ values vs SCE of **1b**, **1c**, **7c**, and **11c** in acetonitrile are 1.74, 1.50, 1.54, and 1.73 V, respectively. Irreversible anodic waves were observed in all cases.

(7) Emission ascribable to an exciplex was not observed.

(8) *E* denotes that the terminal substituent (R^1 = CH_3 or D) and main alkyl chain residue are on opposite sides of the double bond, and *Z* denotes that they are on the same side. The stereospecificity of the ring cleavage of **1c** was higher than 98%.

(9) Sinnema, A.; van Rantwijk, F.; de Koning, A. J.; van Wijk, A. M.; van Bekkum, H. *Tetrahedron* **1976**, *32*, 2269.

(10) Deuteriums in the recovered bicyclohexane had largely been retained in the exo positions even after 80% conversion. We suspect that the incomplete stereospecificity of the cleavage reaction of **1b** might be due to the configurational instability of **3b** rather than to the partial leakage of **2b** to **9b** prior to the cleavage.[11]

(11) Clark, T.; Nelsen, S. F. *J. Am. Chem. Soc.* **1988**, *110*, 868.

(12) The combined yields of the reaction products of **7c** were 50–85%.

(13) The secondary reaction of **1c** led to the formation of **4c**. The initial relative yield of **4c** was negligible.

0002-7863/90/1512-1998$02.50/0