84 (30), 69 (100), 57 (26), 55 (58), 53 (20), 45 (44), 43 (22), 43 (36), 41 (77); exact mass calcd for $C_{10}H_{17}O$ (M − Me) 153.1279, found 153.1282.

(*E*)- and (*Z*)-1-Phenyl-2-(1,2,2-trimethylpropylidene)cyclopropane (11*E* and 11*Z*). Reaction of triflate **5** (81% *E*:19% *Z*) in styrene–glyme mixture gave adducts **11*E*** and **11*Z*** in a 99.63 ± 0.01% *E*:0.37 ± 0.01% *Z* ratio (column A, 150 °C). Adduct **11*E*** and a sample enriched in adduct **11*Z*** for GC/MS analysis (**11*Z*** and **11*E*** in a 14% *Z*:86% *E* ratio) were obtained through use of column D at 150 °C. For **11*E***: IR (neat) 3025, 2962, 2865, 1758 (C=C), 1602, 1452, 1372, 1359, 753, 700 cm^{-1}; 300-MHz ^1H NMR ($CDCl_3$) δ 7.0–7.3 (m, 5, phenyl), 2.39 (m, 1, HCPh), 1.89 (m, 1, cyclopropyl), 1.74 (m, 3, $CH_3C{=}C$), 1.31 (m, 1, cyclopropyl), 1.19 (s, 9, *t*-Bu); mass spectrum, 200 (M^+, 12), 185 (18), 157 (24), 144 (44), 143 (87), 129 (67), 128 (26), 116 (55), 115 (35), 91 (27), 84 (100), 69 (91), 57 (27), 41 (67); exact mass calcd for $C_{15}H_{20}$ 200.1565, found, 200.1571. Adduct **11*Z*** was characterized through its mass spectrum which was virtually identical with that of **11*E***. For **11*Z***: mass spectrum, 200 (M^+, 13), 185 (18), 157 (27), 144 (43), 143 (87), 129 (68), 128 (23), 116 (51), 115 (41), 91 (28), 84 (100), 69 (98), 57 (25), 41 (61).

Analytical Reaction of Triflates 4*E* and 4*Z* in Styrene as Sole Solvent. Treatment of triflate **4** (70% *E*:30% *Z*) with base in styrene (no glyme present) for 1 day at −23 °C resulted in a 75.5% **3*E***:24.5% **3*Z*** ratio (column A, 140 °C).

Stability of Alkylidenecyclopropane 8*E*. Treatment of 17 mg (0.091 mmol) of **8*E*** under standard reaction conditions did not lead to isomerization or decomposition of **8*E*** as determined through GC analysis with an internal standard (tridecane).

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Kinetic and Thermodynamic Parameters for the Formation of 3,5,5-Trimethyl-2-oxomorpholin-3-yl (TM-3). A Negative Activation Energy for Radical Combination[1,2]

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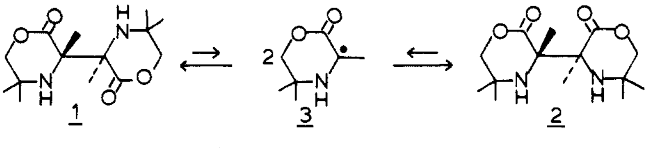
Abstract: Thermodynamic parameters for the bond homolysis of *meso*- and *dl*-bis(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**1** and **2**) to 3,5,5-trimethyl-2-oxomorpholin-3-yl (**3** TM-3) in ethanol, 1,2-dimethoxyethane, and benzene solvents and in ethanol solvent containing Na^+ and Mg^{2+} at an ionic strength of 0.3 μ are reported. The equilibrium constant varies by more than 6 orders of magnitude, 1.3×10^{-9}–5.8×10^{-16} mol/L, as a function of the medium, being largest in a polar solvent containing Mg^{2+}. The solvent effect is consistent with a polar radical structure most likely resulting from a captodative resonance interaction. In the solvent sequence ethanol to benzene, $\Delta H°$ and $\Delta S°$ vary from 21.5 to 35.9 kcal/mol and 24.1 to 50.6 cal/(mol·K), respectively. The free energy of formation of TM-3 is linearly related to the methyl hyperfine coupling constants and Kosower *Z* values, also consistent with the captodative resonance interaction. The effect of Mg^{2+} on radical concentration appears to be entropically derived. The kinetic parameters for bond homolysis of the *dl* dimer of TM-3 are also reported; $\Delta H^{\ddagger}$ varies from 20.4 to 28.1 kcal/mol, being smallest in polar solvent. The kinetic and thermodynamic parameters for bond homolysis give kinetic parameters for radical combination. In 1,2-dimethoxyethane and benzene solvents, $\Delta H^{\ddagger}$ for the radical combination is substantially negative, −6.1 and −7.8 kcal/mol, respectively. The negative enthalpies of activation are discussed in terms of the intermediacy of a H-bonded TM-3-TM-3 complex and in terms of a rapidly rising $-T\Delta S$ term.

We have reported that *meso*- and *dl*-bis(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**1** and **2**) exist in equilibrium with the persistent free radical 3,5,5-trimethyl-2-oxomorpholin-3-yl (**3**).[3] Radical **3**, to which we have assigned the acronym TM-3, is an important example of a class of radicals now described as merostabilized,[4] captodative,[5] or push-pull-stabilized.[6] Recent calculations suggest that TM-3 has an unusually high stabilization energy within this class of radicals.[7] The importance of TM-3 also stems from its effective use as a mild nontoxic reducing agent[8] for the study[9] and in vivo control[10] of the redox chemistry of quinone antitumor drugs such as adriamycin.



Earlier we reported enthalpies of bond homolysis of a mixture of the meso and *dl* dimers **1** and **2** as a function of solvent from measurements of TM-3 EPR signal intensities as a function of temperature.[3] These enthalpies have now been redetermined with improved instrumentation by double integration of the EPR signal relative to a spin concentration standard as a function of temperature in three solvents. The earlier measurements were qualitatively correct in terms of the observed effect of solvent on bond homolysis, but they underestimated the enthalpies most likely because of errors resulting from variation in the cavity quality factor *Q* as a function of temperature especially in lossy solvents such as ethanol[11] and errors associated with signal saturation. The

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Table I. Thermodynamic Parameters for Bond Hydrolysis of TM-3 Dimers (**1** and **2**)[a]

medium	K, mol/L	$\Delta G°$, kcal/mol	$\Delta H°$, kcal/mol	$\Delta S°$, cal/(mol·K)
ethanol	3.2×10^{-11}	14.3	21.5	24.1
σ	0.3×10^{-11}	0.1	0.7	2.1
1,2-dimethoxyethane	6.8×10^{-14}	18.0	30.1	40.1
σ	1.0×10^{-14}	0.1	0.6	1.9
benzene	5.8×10^{-16}	20.8	35.9	50.6
σ	0.7×10^{-16}	0.1	0.5	1.5
ethanol/Mg^{2+}[b]	1.3×10^{-9}	12.1	23.0	36.3
σ	0.3×10^{-9}	0.2	0.2	0.5
ethanol/Na^{+}[b]	7.0×10^{-11}	13.9	22.3	28.3
σ	0.4×10^{-11}	0.1	0.4	1.2

[a] Values of K and $\Delta G°$ are reported at 25 °C. [b] Ionic strength = 0.3 μ.

Table II. EPR Hyperfine Coupling Constants for TM-3

medium	temp, °C	$a_{H}^{CH_3}$, G	a_{N}, G	a_{H}^{NH}, G	ΔG (at T indicated), kcal/mol
ethanol	50	10.1	6.5	5.1	13.7
1,2-dimethoxyethane	60	11.4	6.2	3.8	16.6
benzene	80	12.1	6.2	2.8	18.0
ethanol/Mg^{2+}[a]	30	8.8	6.5	6.5	12.0
ethanol/Na^{+}[a]	40	9.8	6.4	5.2	13.4

[a] Ionic strength = 0.3 μ.

new thermodynamic data together with kinetic data for the bond homolysis of the *dl* dimer **2** yield interesting kinetic information on the combination of TM-3 radicals.

Results

The concentration of TM-3 in equilibrium with the meso and *dl* dimers **1** and **2** relative to standard solutions of di-*tert*-butyl nitroxide (DTBN) were measured by quantitative EPR spectroscopy in three solvents, ethanol, 1,2-dimethoxyethane, and benzene, and in ethanol in the presence of Mg^{2+} and Na^{+} as a function of temperature. DTBN was selected as the standard because it is stable, has the same spin multiplicity, has approximately the same g value, and gives an EPR signal with approximately the same line width as TM-3. The solvents were chosen because they span a large range of solvent polarity, and both TM-3 and DTBN are well-behaved in these solvents. The effect of Mg^{2+} relative to the effect of Na^{+} at the same ionic strength was investigated because of the reported stabilizing effect of Mg^{2+} on Kosower's pyridinyl radicals.[12] The equilibrium constants $K = [\text{TM-3}]^2/[\textit{meso} + \textit{dl}\ \text{dimer}] + \textit{dl}\ \text{dimer}]$ and the standard enthalpies, entropies, and free energies of formation are reported in Table I.

As noted earlier, the EPR hyperfine coupling constants for TM-3 are substituent-, solvent-, and temperature-dependent, and $a_{H}^{CH_3}$ appears to be inversely related to radical stability.[13] To test further this relationship, the hyperfine coupling constants were measured in the same solvents in which the equilibrium constants were measured (Table II). As anticipated, $a_{H}^{CH_3}$ values are inversely related to the free energy of formation. Complexation of Mg^{2+} with TM-3 is apparent from the two overlapping TM-3-type EPR signals observed in the presence of Mg^{2+} (Figure 1) and the intensity variation of the two as a function of the Mg^{2+} concentration. The equilibrium constants in Table I and the coupling constants in Table II for formation of TM-3 in the presence of Mg^{2+} are those for TM-3 complexed with Mg^{2+}.

The kinetics of bond homolysis of the *dl* dimer **2** as a function of temperature in the three solvents were determined by using the efficient radical-trapping agent diphenylpicrylhydrazyl (DPPH). In the presence of an excess of DPPH, bond homolysis follows clean first-order kinetics; TM-3 is quantitatively oxidized to 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**4**), and DPPH is reduced to diphenylpicrylhydrazine (**5**).[13] The rate constants and activation parameters for bond homolysis are reported in Table III.

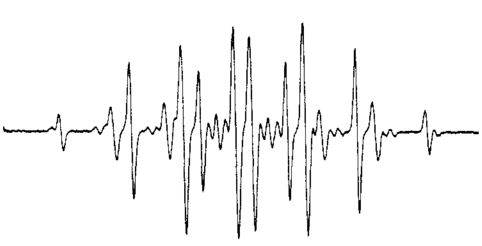
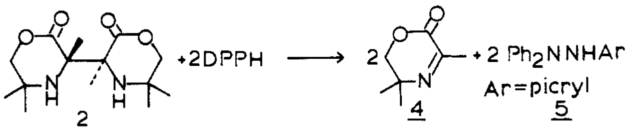


Figure 1. EPR spectrum of 9.4×10^{-2} M TM-3 dimer in ethanol solvent in the presence of 0.1 M magnesium perchlorate. The scan range equals a total of 60 G. The weaker signal is that from the uncomplexed radical. The hyperfine coupling constants for complexed and uncomplexed radical are reported in Table II and the g value for both radicals is 2.0036.



The thermodynamic and kinetic parameters in Tables I and III were used to determine the kinetic parameters for the combination of two TM-3 radicals to produce meso and *dl* dimers **1** and **2** and are reported in Table IV. An error inherent in the data in Table IV results from the determination of the equilibrium constants with mixtures of *meso* and *dl* dimers and the rate constants with the pure *dl* dimer. Because the activation energies for bond homolysis of the *meso* and *dl* dimers differ by less than 1 kcal/mol,[14] this error, not included in the standard deviations in Table IV, is small. A striking result in Table IV is the significant negative activation energies for radical combination in 1,2-dimethoxyethane and benzene solvents.

Discussion

The importance of captodative substituent stabilization of radicals has been actively debated in the literature. The captodative effect is defined as the extra stabilization resulting from the synergism of electron-donating and electron-withdrawing substituents. For cyano and methoxy substituents, experimental observations[15-17] and theory[18,19] estimate the extra stabilization at 0-4 kcal/mol in nonpolar solvent or in the gas phase.

In valence bond terms, the captodative effect is a measure of the contribution of the zwitterionic resonance structure **6**. The increase in the dimer-radical equilibrium constant by 5 orders of magnitude and the corresponding decrease of 14 kcal/mol in $\Delta H°$ of bond homolysis for the dimers in ethanol relative to benzene suggests that the radical is significantly more polar than the dimers. The higher polarity of the radical is consistent with an important contribution from the zwitterionic resonance structure **6**. Also, the solvent effects observed here suggest that the captodative stabilization energy of a radical in this class will in general be magnified by a polar solvent.

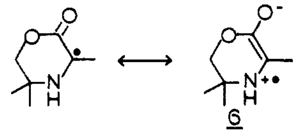


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Table III. Kinetic Parameters for Bond Homolysis of *dl* Dimer **2**[a]

solvent	k, s^{-1}	$\Delta G^‡$, kcal/mol	$\Delta H^‡$, kcal/mol	$\Delta S^‡$, cal/(mol·K)	A, s^{-1}	E_a, kcal/mol
ethanol	1.22×10^{-3}	21.4	20.4	−3.4	3.1×10^{12}	21.0
σ	0.16×10^{-3}	0.1	2.0	6.4	0.1×10^{12}	2.0
1,2-dimethoxyethane	7.84×10^{-6}	24.4	23.9	−1.6	8.2×10^{12}	24.6
σ	0.19×10^{-6}	0.1	1.8	5.5	0.2×10^{12}	1.8
benzene	3.35×10^{-7}	26.3	28.1	6.1	4.1×10^{14}	28.8
σ	0.14×10^{-7}	0.2	2.3	6.8	0.1×10^{14}	2.3

[a] Values of k and $\Delta G^‡$ are reported at 25 °C.

Table IV. Kinetic Parameters for Combintion of TM-3 Radicals[a]

solvent	k, L/(mol·s)	$\Delta G^‡$, kcal/mol	$\Delta H^‡$, kcal/mol	$\Delta S^‡$, cal/(mol·K)	A, s^{-1}	E_a, kcal/mol
ethanol	3.82×10^{7}	7.1	−1.1	−27.5	1.8×10^{7}	0.5
σ	0.52×10^{7}	0.1	2.0	6.5	0.1×10^{7}	2.0
1,2-dimethoxyethane	1.16×10^{8}	6.4	−6.1	−42.2	1.1×10^{4}	−5.5
σ	0.28×10^{8}	0.1	1.8	5.6	0.1×10^{4}	1.8
benzene	5.81×10^{8}	5.5	−7.8	−44.7	3.2×10^{3}	−7.2
σ	0.28×10^{8}	0.3	2.1	7.8	0.1×10^{3}	2.6

[a] Values of k and $\Delta G^‡$ are reported at 25 °C; all values are calculated from the data in Tables I and III.

If TM-3 is planar at the radical center, Fischer's theory[20] predicts that the EPR coupling constant $a_H^{CH_3}$ will be a useful measure of the free electron spin density at the 3-position. Viehe and co-workers[21] have assigned a planar geometry to a captodative carbon radical from the temperature dependence of $a_C^{CH_3}$. Assuming that delocalization leads to radical stabilization, $a_H^{CH_3}$ should then be an inverse measure of radical stability. Clearly the values of the methyl hyperfine coupling constants in Table II are directly related to the free energies and enthalpies of formation as a function of solvent and support the hypothesis that TM-3 is stabilized in polar solvent. Linear regression analysis of the data in Tables I and II in the absence of cations give eq 1 with a correlation coefficient of 0.9996. Equation 1 gives the

$$\Delta G = a_H^{CH_3}2.16 - 8.09 \qquad (1)$$

free-energy change at the temperature at which the coupling constant is measured. The correlation coefficient for eq 1 suggests that dimer–radical equilibrium constants in other solvents can be estimated from the methyl hyperfine coupling constant. Arnold and co-workers have related the magnitude of the benzylic hyperfine coupling constant of a series of benzyl radicals to the component of radical stability due to spin delocalization.[22]

In terms of standard solvent parameters, $\Delta G°$ correlates with Kosower Z values[23] moderately well. A plot of $\Delta G°$ vs. Z values follows eq 2 with a correlation coefficient of 0.992. The internal

$$\Delta G° = 34 - (0.25)Z \qquad (2)$$

consistency of eq 1 and 2 was tested by comparing $\Delta G°$ obtained from each in methanol and acetonitrile solvents. Equations 1 and 2 give values of $\Delta G°$ in acetonitrile solvent of 15 and 16 kcal/mol, respectively, and values in methanol solvent of 13 and 13 kcal/mol. Kosower Z values should address the specific solvent effects on the bond homolysis of TM-3 dimers because Z values are obtained from solvent effects on the energy of a charge-transfer absorption band and TM-3 is proposed to be stabilized by intramolecular charge transfer.

As with the captodative pyridinyl radicals,[12] the magnesium cation has a special effect on dimer–radical equilibrium relative to the sodium cation at the same ionic strength. The thermodynamic parameters in Table I suggest that the major difference resides in the entropy change. A reasonable explanation is that the dimer binds magnesium more tightly than does the radical possibly because it can function as a bidentate ligand. Tanaka and Ota report the binding of stannic ion to a thiocyano captodative-substituted carbon radical.[24] Semiquinone-type captodative radicals have been observed to bind alkaline earth[25] and Zn^{2+} and Cd^{2+} [26] The magnesium binding to TM-3 dimers will be the object of future studies because of its possible involvement in the redox chemistry of TM-3.

The kinetic parameters for bond homolysis in Table III are consistent with those observed for homolysis of the *dl* dimer in other solvents[13] and for homolysis of TM-3 dimer derivatives.[14] At the transition state for bond homolysis as defined by $\Delta G^‡$, the large entropy change has not been realized even though by the Hammond postulate the transition state is late. Hence, the radicals are still associated within a solvent cage at this point in the potential energy curve.

Substraction of the forward kinetic parameters from the thermodynamic parameters gives kinetic parameters for the combination of two TM-3 radicals. Of particular note are the significantly negative activation energies for radical combination in 1,2-dimethoxyethane and benzene solvents. Although these activation energies were determined indirectly, they appear to be negative beyond estimated experimental error. Furthermore, correction for the effect of a decrease in solvent viscosity as a function of temperature would make the activation energies even more negative.

Apparent negative activation energies coupled with apparent small A factors are commonly explained in terms of the reactants in equilibrium with an intermediate or complex which irreversibly forms the product as illustrated by eq 3. The rate of destruction

$$2\text{TM-3} \underset{k-1}{\overset{k_1}{\rightleftharpoons}} \text{complex} \xrightarrow{k_2} \text{dimer} \qquad (3)$$

of TM-3 with the steady-state approximation, applicable with $K = k_1/k_{-1}$ small, is then given by eq 4, which reduces to eq 5 if

$$\frac{d[\text{TM-3}]}{dt} = -k_1[\text{TM-3}]^2\left\{\frac{k_2}{k_{-1} + k_2}\right\} \qquad (4)$$

$k_{-1} \gg k_2$. The observed rate constant then is k_1k_2/k_{-1}, the observed activation energy $E_{a_1} + E_{a2} - E_{a_{-1}}$, and the observed A factor A_1A_2/A_{-1}. An apparent negative activation energy occurs when

$$\frac{d[\text{TM-3}]}{dt} = -\frac{k_1k_2}{k_{-1}}[\text{TM-3}]^2 \qquad (5)$$

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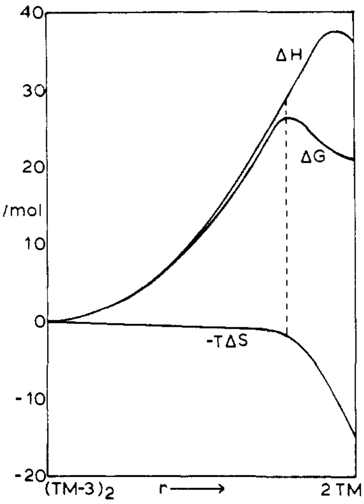
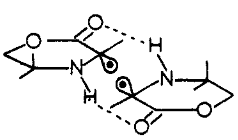
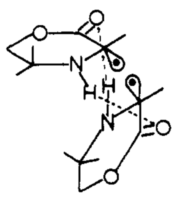


Figure 2. Possible ΔH, $-T\Delta S$ (298 K), and ΔG (298 K) profiles for the bond homolysis of the TM-3 dimer in benzene solvent which rationalize the negative enthalpy of activation for radical combination.

$E_{a_{-1}} > E_{a_1} + E_{a_2}$. A more complex rate expression is applicable if the dimerization equilibrium favors complex, but this in fact reduces to the second-order rate law if $4K[\text{TM-3}] < 1$.[27] Pertinent examples of reactions which show low activation energies and A factors are the Diels–Alder reaction of tetracyanoethylene with 9,10-dimethylanthracene,[28] the cycloaddition of halocarbenes to alkenes,[29] and the disproportionation of acyl nitroxides.[30] Charge-transfer and dipolar complexes have been proposed as possible intermediates. An intermediate complex in the combination of two TM-3 radicals might be two radicals intermolecularly hydrogen bonded as shown below. This type of complexation is anticipated to be more favorable in aprotic solvent where the larger negative activation energies are observed.





Recent calculations by Houk and co-workers[31] suggest that the most reactive halocarbenes do not form stable π-complexes with alkenes. They propose that an intermediate complex is not a necessary condition for a negative activation energy. A negative activation energy will also be observed for a single-step reaction in which $-T\Delta S$ is rising rapidly and ΔH is rising insignificantly or falling. With these enthalpy and entropy changes, the maximum in the free-energy change, which defines the transition state, can occur at a point at which the enthalpy is below that of the starting materials. The combination of TM-3 radicals in aprotic solvents appears to be dominated by the entropy change and, consequently, the Houk explanation for negative enthalpies of activation may also be applicable in this case, if the hydrogen-bonded complexes are not energy minima. An energy diagram illustrating this explanation for the negative enthalpy of activation is shown in Figure 2.

An argument in favor of the Houk explanation is the predicted magnitude of the activation energy $E_{a_{-1}}$ for dissociation of the intermediate complex. To achieve the observed negative activation energy, $E_{a_{-1}}$ would have to be greater than 8 kcal/mol in benzene solvent, a rather high activation energy for breaking two intermolecular hydrogen bonds.

Conclusion

The thermodynamic and kinetic parameters for bond homolysis of the meso and *dl* dimers of TM-3 are highly solvent-dependent, favoring radical formation in polar solvent. The solvent effect is consistent with a polar structure resulting from intramolecular charge transfer for the TM-3 radical as predicted by the captodative resonance interaction. The observed enthalpy of activation for combination of two TM-3 radicals is significantly negative, possibly because of the intermediacy of a TM-3–TM-3 complex or more likely a rapidly rising $-T\Delta S$.

In future experiments, direct measurement of the kinetics of radical combination will be attempted by using laser flash photolysis techniques. Kosower has shown that captodative pyridinyl radical dimers can be cleaved photochemically.[32] Possibly the TM-3 dimer can be cleaved with the 197-nm band of an ArF excimer laser or the 222-nm band of a KrCl excimer laser. If so, the absorption properties of TM-3 can be measured and the kinetics of the radical combination determined directly.

Experimental Section

Materials. The meso and *dl* isomers of bis(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**1** and **2**) were prepared by photoreductive dimerization of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**4**), and separation was achieved by alumina flash chromatography.[33] Radical dimer purity was checked by ^{1}H NMR spectroscopy and silica gel TLC, eluting with 10% methanol/90% methylene chloride (v/v). Di-*tert*-butyl nitroxide was prepared by reductive coupling of 2-methyl-2-nitropropane as described by Hoffmann.[34] Purity was established by silica gel TLC, eluting with 1% methanol/99% methylene chloride (v/v) and GLC on a 6-m 5% SE-30 on 60/80-mesh high-performance Chromasorb W column at 40 °C. All solvents were reagent grade (J. T. Baker or Fisher). Absolute ethanol was dried by distillation from magnesium ethoxide and stored over 4-Å molecular sieves. 1,2-Dimethoxyethane was refluxed with lithium aluminum hydride and distilled. Benzene was dehydrated with 4-Å molecular sieves. Anhydrous magnesium perchlorate was purchased from Mallinckrodt. Sodium perchlorate monohydrate (Fisher) was dehydrated on a hot plate. Both perchlorates were further dried overnight under a high vacuum and stored in a vacuum dessicator.

Quantitative EPR—Instruments and Methods. All EPR analyses were performed on a Varian Assoc. E-109E spectrometer equipped with a Varian EPR field/frequency lock and a Varian variable-temperature controller. The EPR cavity temperature was measured with a thermocouple attached to a Doric Trendicator Model 410 A. The analog output signal from the EPR was converted to a digital signal by a Fluke 8502A digital multimeter interfaced via an HP-IB bus to a Hewlett-Packard 85 computer. The computer software for data collection and reduction was written by G. Bishop and J. Olson and is reported elsewhere.[1]

Sample Preparation. Wilmad Glass Co. 705-PQ or 707-PQ matched quartz EPR tubes were used for all measurements. The apparatus used for preparation of oxygen-free samples consisted of the EPR tube attached perpendicular to a solvent reservoir with both the solvent reservoir and EPR tube perpendicular to a 9-mm tube for attachment horizontally via an Ultra Torr Union to a high-vacuum line. This geometry permitted transfer of the freeze–thaw–degassed solvent to the EPR tube by rotation of the apparatus while still on the vacuum line. The EPR tube size was selected to allow critical coupling of the cavity with the solvent in use. A mixture of the meso and *dl* isomers of bis(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**1** and **2**) in the ratio 1:2.2, respectively, corresponding to the equilibrium ratio in chloroform solvent,[14] was prepared. The solubility of the radical dimer mixture in each solvent was determined. The maximum amount soluble in the solvent was added directly into the EPR tube. The actual quantity was established from the difference in weight between the loaded and unloaded cell. The appropriate volume of solvent was added to the solvent reservoir. The cell was connected to the vacuum line, and the solvent was freeze(−196 °C)–pump(10^{-5} torr)–thaw(25 °C)–vibrate–degassed through five cycles. The degassed solvent was then allowed to flow into the EPR tube by rotating the apparatus, and the EPR tube was sealed with a torch. The final volume

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Table V. Equilibrium Constants for Bond Homolysis of TM-3 Dimers as a Function of Temperature and Medium

medium	[TM-3 dimers], M	temp, °C	*K*, mol/L	temp, °C	*K*, mol/L
ethanol	0.183	36.5	$(1.22 \pm 0.06) \times 10^{-10}$	58.6	$(7.62 \pm 0.25) \times 10^{-10}$
		47.8	$(4.19 \pm 0.11) \times 10^{-10}$		
1,2-dimethoxyethane	0.192	57.1	$(9.37 \pm 0.41) \times 10^{-12}$	76.6	$(1.21 \pm 0.04) \times 10^{-10}$
		67.4	$(3.74 \pm 0.10) \times 10^{-11}$		
benzene	0.0777	58.5	$(2.62 \pm 0.09) \times 10^{-12}$	76.5	$(4.32 \pm 0.09) \times 10^{-12}$
		67.7	$(1.13 \pm 0.02) \times 10^{-12}$		
ethanol/Mg^{2+}[a]	0.147	27.2	$(1.79 \pm 1.59) \times 10^{-9}$	49.2	$(2.41 \pm 2.21) \times 10^{-8}$
		37.7	$(6.41 \pm 3.70) \times 10^{-9}$		
ethanol/Na^{+}[a]	0.119	40.4	$(4.43 \pm 0.11) \times 10^{-10}$	59.4	$(3.42 \pm 0.07) \times 10^{-9}$
		51.5	$(1.51 \pm 0.02) \times 10^{-9}$		

[a] Ionic strength = 0.3 μ.

of solution was determined by using a height/volume calibration of the tube.

A gravimetrically determined amount of di-*tert*-butyl nitroxide (DTBN) was diluted by the solvent to obtain a concentration of free radical approximately equal to that of 3,5,5-trimethyl-2-oxomorpholin-3-yl (TM-3 radical). A suitable volume of the DTBN solution was added to the solvent reservoir of the apparatus described above. The sample was freeze–thaw–degassed and sealed as described.

EPR Tuning and Collection of Data. To facilitate reproducible tuning of the EPR, the analog meters for the detector reference arm current, the microwave frequency, the microwave power, and the cavity temperature were modified with transparent tape to eliminate parallax error. The EPR cavity was critically coupled for all measurements. The microwave frequency was not adjusted after the first tuning. The cavity was recoupled after each sample change. Most samples and standards loaded the cavity to the same extent; thus, major retuning was not generally necessary. The modulation amplitude was set at 0.8 G to achieve the largest signal amplitude for the TM-3 radical, the scan range at 100 G, and the gain to achieve an EPR signal of approximately 0.1 V. The linearity of the amplifier was established from a linear plot of doubly integrated strong pitch signal vs. gain with a correlation coefficient of 0.9999. The phase of the lock-in amplifier was adjusted for the maximum signal at the optimum modulation amplitude by first obtaining the minimum signal of a Varian strong pitch sample and then changing the phase by 90°. The low-pass filter (time constant) was set to reduce noise to approximately 10% of the signal. The scan rate was selected so that no peak distortion resulted, and the scan length was chosen to enclose the peak plus about 4 times the line width in the base line (generally 3.3–4.0 G for the TM-3 radical and 6.0–8.0 G for DTBN). The dwell time for digital aquisition was selected to allow the maximum number of data points for the scan time used. After a scan was completed, it was recorded on magnetic tape for reduction at a later time.

Power Saturation. The TM-3 radical was found to be more easily saturated than DTBN in equal radical concentrations; consequently, the TM-3 radical was used to measure power saturation. A temperature was selected where an adequate EPR signal was seen. The first EPR peak of the TM-3 radical was measured at power levels from 25 to 60 dB (200-mW maximum power). The doubly integrated areas were plotted vs. the square root of the microwave power.[35] The maximum power in the linear region was selected for analysis.

Equilibrium Measurements. Measurements were made at three temperatures approximately 10 °C apart, the lowest temperature being that at which power saturation was determined. Five spectra each for both DTBN and TM-3 radical samples were recorded at the lowest and highest temperatures and two spectra each were recorded for the intermediate temperature. This method of taking data is statistically ideal when fitting data to a linear equation if the linearity of the relationship is not in question. The first peak of the TM-3 radical signal was used for analysis, except in benzene, where a well-resolved center peak was used. The center peak of the DTBN signal was used for all analyses. The total signal areas where calculated by use of a multiplicative factor: 48 when the first peak of the TM-3 radical signal was doubly integrated, 16 when a center peak of the TM-3 radical signal was doubly integrated, and 3 when the center peak of the DTBN signal was doubly integrated. In all measurements, variation in the signal intensity across a complete scan resulting from differences in the Boltzmann, distribution appeared negligible and consequently was neglected.

Cavity Temperature. A matched EPR tube was filled with the appropriate solvent and inserted in the EPR cavity. Themocouple leads when then positioned in the tube such that the temperature in the center of the cavity was measured. The temperature was allowed to stabilize and a reading taken. The variation with time was approximately ±0.1 °C. Calibration of the thermocouple was checked in ice–water and boiling water and found to be accurate to ±0.1 °C.

Double Integration of the EPR Signals. The software was written by Bishop and Olson[1] following the method of Wyard.[36] A least-squares base-line correction was used initially and after the first integration. A base-line correction range was entered and was typically 5% of the entire spectrum at each end. Calibration factors for the scan range and rate were included in the software. A correction for the Lorentzian line shape was included but not used because the line shapes were Gaussian in character. In ethanol solvent in the presence of magnesium perchlorate (0.3 μ), TM-3 displayed two overlapping EPR signals, one due to complexed and the other to uncomplexed radicals as shown in Figure 1. The peaks due to the two radicals were not well-resolved; the negative portion of the first derivatives of the TM-3 signal overlapped with the positive portion of the first derivative of the $Mg^{2+}\cdots$TM-3 signal. Consequently, the HP 85 computer was used to compute the area of the complexed radical signal by first artificially creating the full peak, using just the negative portion which was resolved, assuming the peak was symmetrical, and then doubly integrating. This method was internally checked by subtracting the created peak from the original peak to yield the uncomplexed peak which was subsequently double integrated. The doubly integrated area of the full spectrum was then compared to the sum of the areas of the two artificially created signals. Excellent agreement was found by using this method. The results of the equilibrium constant measurements are summarized in Table V.

Errors. The measurements of the equilibrium constants contained three major sources of error: instrumental variability, sample preparation accuracy, and error associated with the use of a constant factor to derive the total area. The instrumental errors and errors associated with the constant factor are reflected in the scatter of experimental data. The wet chemistry error, however, is not directly reflected in the data. Results from independent measurements and duplicate analyses indicated that the error due to wet chemistry was about 5%. Since this error is involved in the preparation of both the standard and the sample, the total error is $(0.05^2 + 0.05^2)^{1/2} = 0.07$ or 7%. The non-wet chemistry errors reported in Table I were derived from the standard deviations of the regression analysis. The errors for the free energies were derived by standard propagation of errors techniques from the errors in the equilibrium constants.

Kinetics—Instruments and Methods. A Hewlett-Packard 8450A diode array UV–vis spectrometer interfaced via a serial interface to a Hewlett-Packard 85 computer was used for data collection. Samples in Pyrex cuvettes were thermostated in a specially constructed insulated cell holder[37] connected to a Lauda RC3 Model B-2 circulator. The temperature was monitored with a Doric Trendicator Model 410 A.

Sample Preparation. Approximately 5 mg of the *dl* isomer of the TM-3 dimer was dissolved in 100 mL of nitrogen degassed methylene chloride cooled to 0 °C in an ice bath. A suitable aliquot (0.5–1.0 mL) was added to the cuvette compartment of a two-compartment cell[37] via a syringe. The cell was placed in an ice bath and the methylene chloride evaporated with a stream of nitrogen. Approximately 5 mg of dipicrylphenylhydrazyl (DPPH, Aldrich) was dissolved in 100 mL of the solvent to be investigated, and the solution was mixed well for 5 min in an ultrasonic bath. Into the solution compartment of the cell was added 3.0 mL of this solution. The cell was connected to a high-vacuum line with a 9 mm Ultra Torr Union, and the DPPH solution was freeze(−196 °C)–pump(10^{-5} torr)–thaw(25 °C)–vibrate–degassed through five cycles and sealed with a torch. The solution compartment of the cell was

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Table VI. Rate Constants for Bond Homolysis of *dl* Dimer **2** as a Function of Temperature and Solvent

solvent	temp, °C	k, s^{-1}	temp, °C	k, s^{-1}
ethanol	24.9	$(1.12 \pm 0.01) \times 10^{-3}$	45.9	$(1.18 \pm 0.2) \times 10^{-2}$
	33.4	$(3.65 \pm 0.03) \times 10^{-3}$		
1,2-dimethoxyethane	38.9	$(4.71 \pm 0.05) \times 10^{-5}$	60.9	$(6.43 \pm 0.03) \times 10^{-4}$
	49.2	$(1.97 \pm 0.01) \times 10^{-4}$		
benzene	50.6	$(1.65 \pm 0.03) \times 10^{-5}$	70.8	$(2.29 \pm 0.01) \times 10^{-4}$
	60.7	$(5.35 \pm 0.06) \times 10^{-5}$		

wrapped with aluminum foil to assist heat transfer, and the cell was placed in the thermostated cell holder. The cell was temperature equilibrated for 7 min, the DPPH solution was vigorously mixed with the TM-3 dimer in the cuvette compartment, and the spectrometer program was started. The procedure was performed at three temperatures approximately 10 °C apart for each of the three solvents. The absorbance at infinity was obtained by immersing the cell in a constant-temperature bath at 70 °C until no further reaction was discernible by visible spectroscopy.

Data Collection and Reduction. The HP-8450 spectrometer was preprogrammed prior to analysis. Initially a balance measurement was performed on a sample of pure solvent and stored in memory. The λ_{max} of DPPH was determined to be 516 nm; consequently, the average absorbance from 514 to 518 nm was measured. A data point was collected every 10 s, and after 400 data points, the data were transferred to memory. The data were later transferred to the HP 85 and stored on magnetic tape. The data were analyzed by least-squares fitting of the data to first-order kinetics plotting, $-\ln (A_t - A_\infty)$ vs. time with the HP 85. The slope was the rate constant for bond homolysis, Table VI. The free energy of activation was calculated from k, and the other activation parameters were obtained from standard least-squares analysis of $\ln k$ and $\ln (k/T)$ vs. $1/T$ plots. All values are reported at 25 °C. Errors expressed are standard deviations from the least-squares analysis.

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Phospholipid Inverted Micelles. Autoxidation Kinetics and ^{31}P NMR Exchange Rates

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Abstract: Autoxidation in phospholipid reverse micelles in benzene and chlorobenzene is initiated by a water-soluble initiator azobis(2-amidinopropane) (ABAP) hydrochloride inserted into the aqueous core and inhibited when micelles containing the water-soluble antioxidant 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (Trolox) are added. Autoxidation of egg phosphatidyl choline (EPC) is studied quantitatively in water–benzene by using ABAP and Trolox in the aqueous cores. The oxidizability value ($k_p/2k_t^{1/2} = 0.023$ M$^{-1/2}$ s$^{1/2}$) obtained is similar to that of EPC in bilayers. Autoxidation initiation and inhibition were also observed between saturated, dipalmitoylphosphatidyl choline (DPPC) reverse micelles containing ABAP and unsaturated, dilinoleoylphosphatidyl choline (DLPC) reverse micelles containing Trolox in the aqueous cores in benzene. Similar results were obtained when the locations of the ABAP and Trolox were switched. Kinetic studies indicate the oxidizability of DLPC in these reverse micelles to be 0.092 M$^{-1/2}$ s$^{1/2}$. Absolute rate constants for propagation, k_p, and termination, $2k_t$, determined for DLPC in reverse micelles in benzene gave values of k_p similar to that in homogeneous solution while the $2k_t$ is an order of magnitude smaller. The exchange rates and lifetimes of phospholipids in DLPC reverse micelles, containing Pr^{3+} shift reagent in the aqueous cores, were measured by ^{31}P NMR magnetization transfer techniques. The micelle to micelle phospholipid exchange lifetime for DLPC (27 ms) is similar to its monomer to micelle lifetime (34 ms), whereas for DPPC the latter lifetime is twice as long. Certain preparations of reverse micelles in nonprotic organic solvents exhibit an instability and they break up after several hours.

Phospholipids are known to form inverted (reverse) micelles especially in nonprotic organic solvents in the presence of water.[1] Interest in such inverted micelles has developed because of the polar core's catalytic effects and the idea that they provide a model for processes in biomembranes[2] including trans bilayer transport[3] and fusion.[4] Various reviews reflect the attention given to ionic reactions in inverted micelles.[2,5,6] Comparatively little is known of the effect of inverted micelles on *free radical* reactions. Free radical lipid peroxidation is related to important pathological events[7-10] resulting in current interest in the free radical autoxidation in such microenvironments as micelles and bilayers[11-14] as models for biomembranes. There is also related interest in the

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