rate, confirming that the yellow color of flavin does not disappear. The products were analyzed by using high-pressure LC (Shimadzu LC-3). Four peaks appeared, which were identified to be 4-chlorobenzoic acid, 4,4′-dichlorobenzoin, 4,4′-dichlorobenzil, and 4-chlorobenzaldehyde by using each authentic sample. The sum of these four compounds was always better than 70%. The details of the treatment of the reaction products and the product analysis were described previously.[8,9]

Kinetics. The kinetic measurements of the flavin oxidation of aldehydes were carried out at 30 °C under anaerobic (N_2) conditions in 3 vol % aqueous ethanol. A Thunberg cuvette was used to provide the anaerobic reaction conditions. The progress of the reaction was monitored spectrophotometrically by following the disappearance of each absorption maximum of flavin: 448 nm for MeFl, 447 nm for 10-DodFl, and 438 nm for Fl-T. The detailed procedure was described previously.[8,9]

Acknowledgment. We thank Professor T. Kunitake (Kyushu University) and Professor M. Koike (Nagasaki University School of Medicine) for helpful discussions. We also thank Mr. Y. Hara and Miss M. Sato for technical assistance.

Registry No. 4-Chlorobenzaldehyde, 104-88-1; 3-(*r*-hydroxypropyl)-10-dodecylisoalloxazine, 79828-14-1; 3-(*r*-bromopropyl)-10-dodecylisoalloxazine, 79828-15-2; HxdT, 75066-49-8; MeFl, 79839-37-5; 10-DodFl, 79828-16-3; Fl-T, 79828-17-4; PyCHO, 872-85-5.

Solvent Effects on Rates of Free-Radical Reactions. Addition of the *p*-Aminobenzenethiyl Radical to Styrene[1]

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Contribution from the Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, Katahira-2, Sendai, 980 Japan. *Received July 22, 1981*

Abstract: Absolute rate constants (k_1) for addition of the *p*-aminobenzenethiyl radical (p-$NH_2C_6H_4S\cdot$) to styrene in 26 kinds of solvents have been determined by the flash photolysis technique. The relative rate constants for the reverse reactions and the relative equilibrium constants have also been estimated. A large bathochromic shift of the transient absorption maxima of p-$NH_2C_6H_4S\cdot$ was observed with an increase in solvent polarity, which suggests the stabilization of p-$NH_2C_6H_4S\cdot$ by the solvation of polar solvents. The k_1 values decreased largely with solvent polarity, whereas the relative reverse rate constants were practically invariant. This suggests that the solvated thiyl radicals in the reactant lose many of the solvent molecules in the transition state. In the plots of log k_1 vs. empirical solvent polarity parameters such as Dimroth–Reichardt's $E_T(30)$, a linear relationship was observed for most solvents. Solvents having hydrogen-bonding ability and electron-accepting ability accelerate the rates, and alkylamines reduce the rates; the solvent polarity is a main factor governing the solvent effect on the reaction and the specific interactions also play important roles.

An extensive study has revealed that the medium affects the reactivity and selectivity of the free-radical reacitons.[2] The solvent effects in atom transfer, disproportionation, and addition of the free radicals have mainly been interpreted in terms of specific interactions between the free radicals and solvents such as electron donor–acceptor interaction[3-5] and hydrogen bonding.[6,7] The electron donnor–acceptor complexes between halogen atoms and π-electron donors have been directly detected by means of the flash-photolysis technique by Strong.[8] The importance of sovlent polarity in the free-radical reactions was first pointed out by Walling and Wagner.[7] The effects of solvent polarity on the hyperfine structures of the ESR spectra of stable free radicals[9-12] suggested that large solvent polarity effects would be anticipated in the free-radical reactions if polarizable free radicals are chosen. We will report in this paper our finding that the introduction of the amino group in the para position on the benzenethiyl radical makes the thiyl radical polarizable and that both the absorption bands of p-$NH_2C_6H_4S\cdot$ and the absolute addition rate constants of p-$NH_2C_6H_4S\cdot$ to styrene, which can be determined by the flash-photolysis technique,[1,13] vary greatly with the change of solvent polarity.

Results and Discussion

Assignment and Solvent Effect of the Absorption Band of p-$NH_2C_6H_4S\cdot$. Figure 1 shows the transient absorption spectra observed by the flash photodecomposition of $(p\text{-}NH_2C_6H_4)_2S_2$ in various solvents. These absorption bands were ascribed to p-$NH_2C_6H_4S\cdot$ since the same absorption band was observed by the photolysis of *p*-aminobenzenethiol in each solvent. A similar spectrum was reported by Thyrion.[14]

The bathochromic shift of the absorption maxima and the broadening of the bandwidths were observed with an increase in solvent polarity. Both the shift and broadening correlate roughly to some empirical parameters of solvent polarity such as Kosower's Z values[15] and Dimroth-Reichardt's $E_T(30)$ values,[16] but definite

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(1) Evaluation of Addition Rates of Thiyl Radicals to Vinyl Monomers by Flash Photolysis. 4. For part 3 see: Ito, O.; Matsuda, M. *J. Am. Chem. Soc.* **1981**, *103*, 5871.

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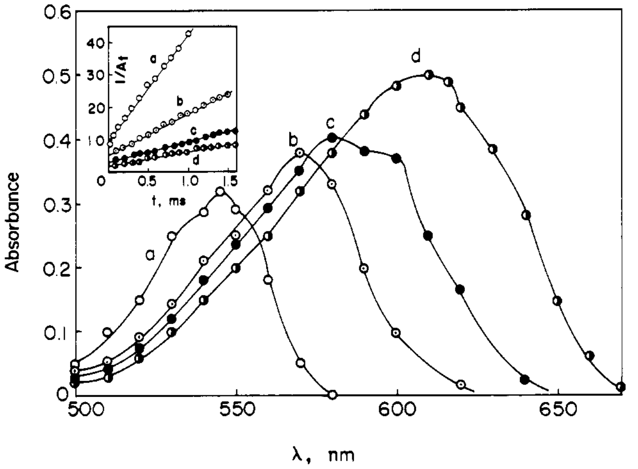
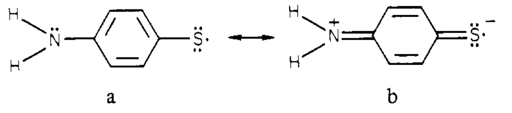


Figure 1. Solvent effect on the absorption band of p-$NH_2C_6H_4S\cdot$; transient absorption spectra observed at 30 μs after the flash photolysis of $(p\text{-}NH_2C_6H_4)_2S_2$ (2.0×10^{-5} M) in (a) cyclohexane, (b) toluene, (c) tetrahydrofuran, and (d) dimethyl sulfoxide at 23 °C. Insert: Second-order plots for decays of the transient absorption bands at each peak. A_t refers absorbance at time t.

correlations could not be obtained because the absorption bands show a round peak in polar solvents. Both the bathochromic shift and broadening might be rationalized in terms of valence bond theory by considering the relative contribution of the two main mesomeric structures (a and b) in eq 1 to the actual electronic structure. Since similar spectral changes were not observed with



(1)

p-aminobenzenethiol, the above findings may be characteristic of the interactions between the radical and solvents.

Second-order kinetics of the decay of p-$NH_2C_6H_4S\cdot$ (insert in Figure 1) indicates that the recombination reaction occurred predominantly; the slopes yield $2k_r/\epsilon$ (k_r refers to the rate constant for recombination and ϵ to the molar extinction coefficient of p-$NH_2C_6H_4S\cdot$). The k_r/ϵ values decrease with an increase in solvent polarity ($k_r/\epsilon = 1.7 \times 10^5$ in cyclohexane, 7.5×10^4 in toluene, 3.0×10^4 in tetrahydrofuran, and 2.5×10^4 cm s^{-1} in dimethyl sulfoxide); the k_r values may decrease in the order above even if the ϵ values increase with solvent polarity as much as the initial absorbances shown in Figure 1.[17] Increase in the contribution of the mesomeric structure b may reduce the k_r value by the repulsion of the electric charge on the sulfur atom and/or by the stabilization of the radical in polar solvents.

Determination of Rate Constants. The low reactivity of p-$NH_2C_6H_4S\cdot$ toward molecular oxygen was confirmed since decay kinetics of p-$NH_2C_6H_4S\cdot$ was not affected by the addition of oxygen to the solution. Figure 2 shows the decay curves of p-$NH_2C_6H_4S\cdot$ after flash in ethyl acetate. Curve 2b shows the decay trace in the presence of styrene without oxygen in solution; the decay rate was only slightly affected compared with that without styrene (curve a). On the addition of both styrene and oxygen (curves c–f) the decay rates increased and decay kinetics changed from second order to first order (insert in Figure 2). The decay rates increased with increases in the concentrations of both styrene and oxygen. Under these conditions the broad absorption band having double peaks at 570 and 595 nm decayed without modifying the shape of the absorption band (Figure 3); this indicates that any species other than p-$NH_2C_6H_4S\cdot$ is not included in the broad band.

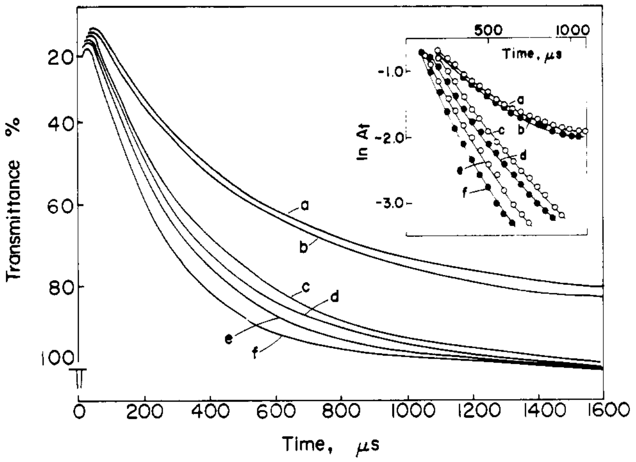


Figure 2. Oxygen concentration effect on the decay curves of p-$NH_2C_6H_4S\cdot$ in the presence of styrene in ethyl acetate (at absorption peak 578 nm): (a and b) in degassed solution without styrene (a) and with styrene (b) (0.46 M); (c–f) variation in partial pressure of oxygen (p_{O_2}) in the presence of styrene (0.46 M), (c) $p_{O_2} = 0.07$, (d) $p_{O_2} = 0.11$, (e) $p_{O_2} = 0.18$, and (f) $p_{O_2} = 1.0$. Insert: First-order plots of the decay curves above.

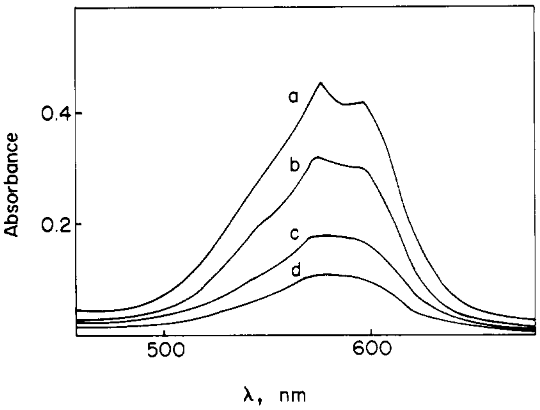


Figure 3. Shape of absorption band of p-$NH_2C_6H_4S\cdot$ in ethyl acetate in the presence of both styrene (0.46 M) and oxygen ($p_{O_2} = 1.0$): (a) 50 μs, (b) 150 μs, (c) 250 μs, and (d) 500 μs after flash.

The findings above suggest that the reaction of p-$NH_2C_6H_4S\cdot$ toward styrene is reversible and that oxygen acts as a selective radical trap to the product radicals. The anti-Markovnikov addition reaction may be presumed for the reaction of p-$NH_2C_6H_4S\cdot$ toward styrene (Scheme I), since the anti-Markovnikov addition

Scheme I

$$p\text{-}NH_2C_6H_4S\cdot + CH_2{=}CHPh \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} p\text{-}NH_2C_6H_4SCH_2C\cdot HPh \xrightarrow[k_2]{O_2} \text{peroxy radical} \quad (2)$$

products were identified by Oswald et al. for the reactions of the benzenethiyl and toluenethiyl radicals toward styrene;[18] high reactivity of oxygen toward carbon-centered radicals was also reported.[19]

Under experimental conditions, both $[CH_2{=}CHPh]$ ($\sim 10^{-3}$–0.5 M) and $[O_2]$ (($\sim 5 \times 10^{-4}$)–10^{-2} M) were in large excess compared with $[p\text{-}NH_2C_6H_4S\cdot]$ ($\sim 10^{-5}$ M);[17] thus decay kinetics of p-$NH_2C_6H_4S\cdot$ obey a pseudo-first-order relationship with respect to $[CH_2{=}CHPh]$ under constant oxygen concentration. The first-order rate constants, k_I, could be estimated from the slopes of the first-order plots (insert in Figure 2). When the strict linear first-order plots were not observed, the k_I values could

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[17] The k_r values may be smaller than the diffusion-controlled limit. By assuming that the k_r values in polar solvents are ca. 10^7 M^{-1} s^{-1} and those in nonpolar solvents are ca. 10^8 M^{-1} s^{-1}, we can calculate the ϵ values to be ca. 10^3 cm^{-1} M^{-1} and then the concentration of p-$NH_2C_6H_4S\cdot$ after the flash is ca. 10^{-5} M.

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Table I. The Absorption Peaks (λ_{max}) of p-$NH_2C_6H_4S\cdot$, the Addition Rate Constants (k_1) to Styrene, the Relative Reverse Rate Constants ($k_{-1}/k_2[O_2]_s$), and the Relative Equilibrium Constants ($Kk_2[O_2]_s$) in Various Solvents at 23 °C[a]

no.	solvent	λ_{max}, nm	$k_1 \times 10^{-4}$, $M^{-1}\ s^{-1}$	$k_{-1}/k_2[O_2]_s \times 10$, $M^{-1}\ s^{-1}$	$Kk_2[O_2]_s \times 10^{-6}$, $M^{-1}\ s^{-1}$
1	cyclohexane	545	21.7	0.74	2.93
2	benzene	570	5.88	0.68	0.87
3	toluene	570	5.00	0.48	1.04
4	ethylbenzene	570	6.33	0.54	1.17
5	mesitylene	570	5.12	0.44	1.16
6	anisole	575	2.00	0.52	0.45
7	fluorobenzene	570	4.35	0.47	0.93
8	o-dichlorobenzene	578	2.63	1.34	0.20
9	benzonitrile	580	1.12	1.34	0.08
10	acetonitrile[b]	610	<0.4		
11	dioxane	577	1.74	0.92	0.19
12	tetrahydrofuran	580	1.04	0.68	0.15
13	ethyl acetate	578	1.00	0.72	0.14
14	ethyl benzoate	578	1.03	1.08	0.10
15	N,N'-dimethylformamide[c]	610	0.07		
16	dimethyl sulfoxide[c]	610	0.03		
17	3-pentanol[b]	610	~0.2		
18	carbon tetrachloride	550	20.0	0.90	2.22
19	chloroform	570	3.93		
20	dichloromethane	577	1.86		
21	1,2-dichloroethane	577	1.05		
22	pyridine	600	0.33	1.32	0.03
23	triethylamine	575	1.38	0.83	0.17
24	tributylamine	575	2.13	0.87	0.24
25	dibutylamine	580	0.67	0.96	0.07
26	butylamine[c]	600	0.07		

[a] Estimation error may be within ±10%. The concentrations of styrene used were 10^{-3}–0.5 M, and the pressures of oxygen were 50–760 mmHg. [b] Accurate measurements were not made because of low solubility of styrene. [c] The reverse rate constants were not observed because of the small dependence of k_I upon oxygen concentrations.

be estimated by the graphic methods[20,21] or by the computer simulation method.[22] The k_I values observed can be expressed as eq 3 by applying the steady-state approximation with respect to [p-$NH_2C_6H_4SCH_2C\cdot HPh$] in eq 2. Dependence of $k_I/[CH_2{=}CHPh]$ upon $[O_2]$ can be expressed as eq 4.[13]

$$k_I/[CH_2{=}CHPh] = k_1\{1 - k_{-1}/(k_{-1} + k_2[O_2])\} \quad (3)$$

$$[CH_2{=}CHPh]/k_I = 1/k_1 + k_{-1}/(k_1k_2[O_2]) \quad (4)$$

Figure 4 shows the plots of eq 4 in various solvents; fairly good linear relationships were obtained for $[CH_2{=}CHPh]/k_I$ vs. the reciprocal of the partial pressure of oxygen ($1/p_{O_2}$); p_{O_2} is proportional to the oxygen concentration in solution. The intercepts and the slopes yield $1/k_1$ and $k_{-1}/k_1k_2[O_2]_s$, respectively; $[O_2]_s$ refers to the oxygen concentration of oxygen-saturated solution. The rate constants of the reverse reactions (k_{-1}) and the equilibrium constants ($K = k_1/k_{-1}$) were estimated in the forms of $k_{-1}/k_2[O_2]_s$ and $Kk_2[O_2]_s$, respectively. These values are summarized in Table I. The k_1 values are ascribed to the addition rate constants since the k_1 value in cyclohexane agrees with an extrapolated rate constant from a linear line of the Hammett plot for the addition reactions of the substituted benzenethiyl radicals such as p-CH_3O, p-CH_3, and p-Cl toward styrene.[18,23,24] Invariance of the reaction mechanism will be confirmed by examining the linear free-energy relationship in the next section. The variations in the k_1 values with solvent change are larger than the reported ones for the nitroxide radical.[25]

The $[O_2]_s$ values are calculated to be ca. 1×10^{-2} M from Henry's law, using the Ostwald coefficients reported in the literature.[26] Thus, the k_{-1}/k_2 values of ca. 10^{-4} M can be calculated.

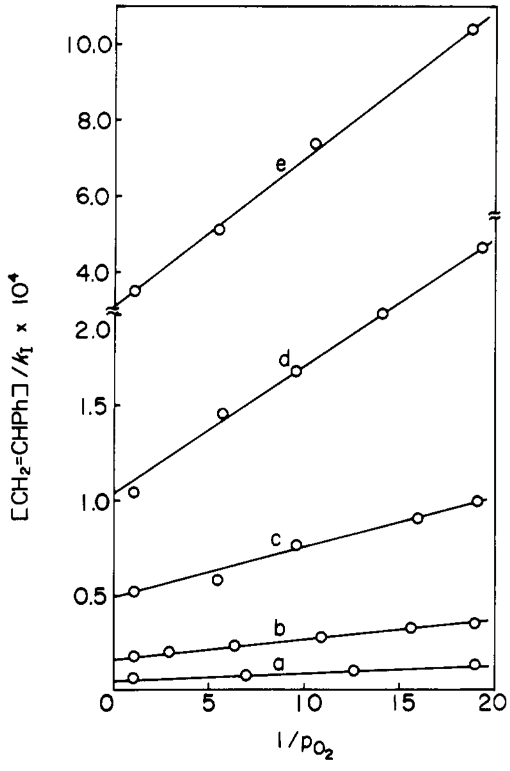

Figure 4. Dependence of reciprocal of the second-order rate constants ($[CH_2{=}CHPh]/k_I$) upon reciprocal of partial oxygen pressure: (a) in carbon tetrachloride, (b) in benzene, (c) in anisole, (d) in ethyl acetate, and (e) in pyridine.

Since the k_2 value may be invariant with changing solvent polarity,[27] the k_{-1} values of ca. $10^5\ s^{-1}$ can be calculated by substituting $k_2 \simeq 10^9\ M^{-1}\ s^{-1}$.[19] The K values in the range of $\sim 10^{-2}$–1 M^{-1} were calculated in a similar manner. The K values are a

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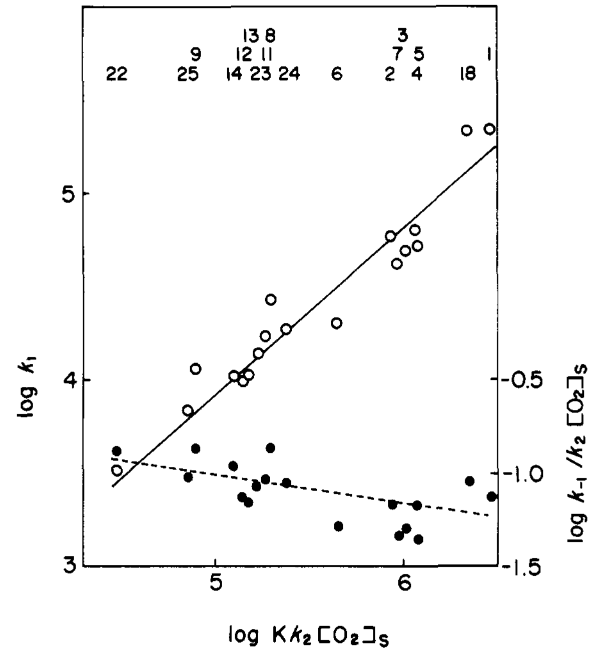

Figure 5. Plots of log k_1 or log $k_{-1}/k_2[O_2]_s$ vs. log $Kk_2[O_2]_s$; numbers refer to solvents in Table I. Slope for log k_1 is +0.9 and that of log $k_{-1}/k_2[O_2]_s$ is ca. −0.1.

measure of the thermodynamic stabilities of p-$NH_2C_6H_4S\cdot$ with changing solvent plarity since the change in stabilities of p-$NH_2C_6H_4SCH_2C\cdot HPh$ may be small.[27]

Solvation and Desolvation Processes. In order to understand the linear free-energy relationship with changing solvent polarity, the logarithms of the rate constants (log k_1 and log $k_{-1}/k_2[O_2]_s$) were plotted against the logarithms of the equilibrium constants (log $Kk_2[O_2]_s$) as depicted in Figure 5. A fairly good linear dependence of log k_1 upon log $Kk_2[O_2]_s$ having a slope of +0.9 was seen, whereas changes of log $k_{-1}/k_2[O_2]_s$ were rather random and a small negative slope (ca. −0.1) was obtained. From the linear relationships we can exclude the possibility that the reaction mechanism changes from the addition reaction to other reactions such as electron transfer and ionic reactions, though the difference in the k_1 values between dimethyl sulfoxide (3×10^2) and cyclohexane (2.17×10^5 M^{-1} s^{-1}) is large enough to suspect the interchange of the reaction mechanism.

The k_1 values decrease with an increase in the bathochromic shift of the absorption maxima of p-$NH_2C_6H_4S\cdot$: $k_1 = \sim 2$–2.2×10^5 M^{-1} s^{-1} for λ_{max} ~545–550 nm, $k_1 = \sim 3$–6×10^4 M^{-1} s^{-1} for λ_{max} ~570 nm, $k_1 = \sim 0.7$–2.6×10^4 M^{-1} s^{-1} for λ_{max} ~ 577–580 nm, and $k_1 \lesssim 3 \times 10^3$ for $\lambda_{max} \gtrsim 600$ nm. A similar correlation was found for the $Kk_2[O_2]_s$ values. These findings suggest that the solvent effect on the thermodynamic stability of p-$NH_2C_6H_4S\cdot$ in the reactant is an important factor in determining the solvent effect in the reactivity. Small variations in the $k_{-1}/k_2[O_2]_s$ values may indicate that the potential energy of the transition state is rather insensitive to solvent polarity since the solvent effect on p-$NH_2C_6H_4SCH_2C\cdot HPh$ may be small. Therefore, we can deduce an important conclusion that the polar solvents surrounding p-$NH_2C_6H_4S\cdot$ in the reactant are desolvated in the transition state. This result agrees with that for the hydrogen abstraction of the *tert*-butoxy radical reported by Walling and Wagner.[7] The desolvation process in the transition state suggests that the transition state is less polar or less polarizable than the reactant. We have pointed out in the earlier paper[23] that the polar effect in the reactivities of p-$XC_6H_4S\cdot$ toward styrene is mainly determined by the polar effect in the thermodynamic stabilities of p-$XC_6H_4S\cdot$ and that the contribution of the polar resonance structures (e.g., [p-$XC_6H_4S^-$, $CH_2{=}CHPh^{+}\cdot$]) to the transition state is rather small. Especially, the introduction of a strong electron-donating substituent such as p-NH_2 to the benzenethiyl radical reduces the contribution of the polar resonance structure. The small negative slope for log $k_{-1}/k_2[O_2]_s$ may suggest the slight polarity of the transition state.

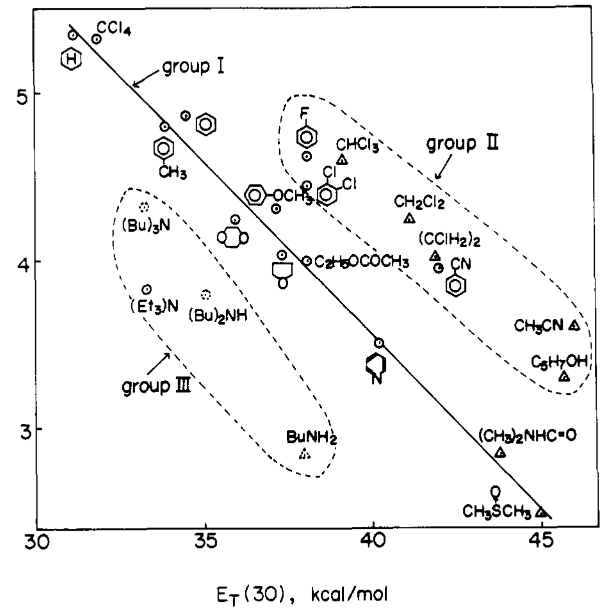

Figure 6. Plots of log k_1 vs. Dimroth–Reichardt's $E_T(30)$ values: ○, the k_1 values were estimated from eq 4; △, the k_1 values were estimated from $k_1/[CH_2{=}CHPh]$ in oxygen-saturated solution; broken circle and triangle mean that these $E_T(30)$ values were estimated from similar amines.

Relation with Solvent Polarity Parameter. The relationships between the log k_1 values and empirical parameters of solvent polarity were examined; Figure 6 shows a plot of log k_1 vs. Dimroth–Reichardt's $E_T(30)$ values.[2c,16] The points can be divided into three groups: group I contains solvents fitting a strict linear relationship, group II, which deviates upward from the line of group I, contains polar alkyl and aryl halides, nitriles, and alcohols, and group III, which deviates downward from the line of group I, contains alkylamines. A similar classification was realized for the plots of Kosower's Z values[15] and Reddoch–Konishi's $\mu\rho/M$ values (μ is dipole moment, ρ density, and M molecular weight of solvent).[11] Group I contains many kinds of solvents such as saturated hydrocarbons, aromatic hydrocarbons, ethers, esters, amides, and sulfoxides; the linear relationship observed in group I indicates that the solvent polarity is a main factor of the solvent effects in the addition reaction.[28,29] In group II aromatic halides and nitriles having electron-acceptor ability reduce the electron density on p-$NH_2C_6H_4S\cdot$; thus the contribution of mesomeric structure b may be reduced, which results in both an increase in the reactivity and a decrease in the bathochromic shift. Alcohols and alkyl chlorides may solvate p-$NH_2C_6H_4S\cdot$ with the hydrogen-bonding interactions, which results in a decrease in the electron density of p-$NH_2C_6H_4S\cdot$. On the other hand, alkylamines in group III may donate the lone-pair electrons to the positive center of resonance structure b and may increase its participation, which results in a decrease in the reactivity.[30,31]

In conclusion, a large solvent effect on the addition rate constants of p-$NH_2C_6H_4S\cdot$ to styrene can be interpreted as follows:

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(28) The linear relation in group I suggests that the interchange in the reaction mechanism does not occur within the group.

(29) The slope of group I (−0.206) is smaller than the value (0.248) for the S_N2 Menshutkin reaction between triethylamine and ethyl iodide which forms aminimum ion and iodide anion: Abraham, M. H.; Greelier, P. L. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1735.

(30) In the radical polymerization of vinyl acetate, the propagation rate observed in ethylbenzene by the rotating sector method was ca. 3 times faster than that in toluene in spite of similar properties of both solvents (Yamamoto, T.; Yamamoto, T.; Mito, A.; Hirota, M. *Nippon Kagaku Kaishi* **1979**, 408); in the case of p-$NH_2C_6H_4S\cdot$ such a difference was not observed.

(31) A drastic difference between the vinyl acetate propagation rates in ethyl acetate and in ethyl benzoate was reported by Kamachi et al. (Kamachi, M.; Lia, D. J.; Nozakura, S. *Polym. J.* **1979**, *11*, 921). A large difference was not observed in our reaction systems; in the case of p-$NH_2C_6H_4S\cdot$ specific interaction was not observed in the aromatic π-electron donors such as mesitylene and anisole.

The solvent molecules which solvate $p\text{-}NH_2C_6H_4S\cdot$ are desolvated from the radical in the transition state. The solvent polarity is a main factor in governing the solvation ability of $p\text{-}NH_2C_6H_4S\cdot$. In addition specific interactions are present in the hydrogen-bonding solvents, π-electron-accepting solvents, and alkylamines.

Experimental Section

p-Aminobenzene disulfide was prepared by the air oxidation of the corresponding thiolate anion and recrystallized from aqueous ethanol.[32] Styrene and solvents were distilled before use. The flash photolysis apparatus was of standard design; the half-duration of the xenon flash lamps (Xenon Corp. N-851C) was ca. 10 μs. The transient absorption spectra were measured by a multichannel photodetector (Union Co. LTD.). The measurements were performed in a cylindrical cell (optical path = 10 cm) at 23 ± 1 °C. The oxygen concentration in solvent was controlled by dissolving oxygen under an appropriate pressure after degassing up to 10^{-4} torr; the oxygen concentrations were calculated from Henry's law.

Registry No. Styrene, 100-42-5; $p\text{-}NH_2C_6H_4S\cdot$, 31053-95-9; $(p\text{-}NH_2C_6H_4)_2S_2$, 722-27-0.

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Determination of Proline Ring Nonplanarity from Proton Spin–Spin Coupling Constants: Applications to Two Cyclic Pentapeptides

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Abstract: A complete analysis of the 600-MHz proton nuclear magnetic resonance spectra of the proline residues in two cyclic pentapeptides, *cyclo*(D-Phe-Pro-Gly-D-Ala-Pro), I, and *cyclo*(Gly-Pro-Gly-D-Ala-Pro), II, has been performed. The proline spectral data have been treated by using a new correlation of vicinal coupling constants and ring nonplanarity. It is shown that if the vicinal coupling constants obey a population-weighted Karplus curve of the type $^3J = K_0 + K_1\langle\cos\theta\rangle + K_2\langle\cos^2\theta\rangle$, where the angular brackets indicate population-weighted averages, then the relation $^3J_c + {}^3J_{c'} + 2({}^3J_t + {}^3J_{t'}) = 6K_0 + 3K_2$ must be satisfied. The experimental data on the eight methylene pairs in I and II fit this relation within experimental error, yielding $6K_0 + 3K_2 = 40.0 \pm 0.3$ Hz (standard deviation). Two functions, $\hat{\chi}$ and $\tilde{\chi}$, of measured coupling constants are defined. The first, $\hat{\chi}$, is derived from $\langle\cos\chi\rangle$ and is a measure of the magnitude of twist in the particular C–C bond but does not give information about the sign of χ. The second, $\tilde{\chi}$, is derived from $\langle\sin\chi\rangle$ and indicates, by its sign, preference for positive or negative χ. Equality of χ from the two measures implies a single nonplanar form of the ring, while large $\hat{\chi}$ and small $\tilde{\chi}$ values indicate a ring interconverting between two conformers, the Ramachandran A and B forms. Each of the model compounds contains one proline ring of each kind. The results indicate that one of the rings is subject to motional constraints in the crystal which are removed in solution.

Very high field NMR provides a means of analyzing complex spin systems such as the seven-spin system found in the imino acid proline. Proline rings are of intrinsic interest for their impact on the conformations of peptides and proteins. The cyclic side chain of proline limits the possible dihedral angles of the peptide backbone and causes a bend to occur in the peptide chain. Prolines occur with a very high frequency in reverse turns in peptides and proteins.[2,3]

A further aspect of the proline ring of importance in peptide and protein structure is its puckering motion. Ramachandran et al.[4] noted in an examination of numerous X-ray diffraction structures of peptides and proteins that the proline ring is usually nonplanar in crystals. They classified the conformations encountered as A and B, respectively, according to whether the γ ring carbon was out of plane on the opposite side as the proline carbonyl group (exo) or on the same side (endo) (see Figure 1). Ramachandran A and B forms can also be characterized by $\chi^1 < 0$ for A and $\chi^1 > 0$ for B. Evidence for rapid endo–exo interconversion of proline rings in peptides in solution has been obtained from measurements of ^{13}C longitudinal relaxation times (T_1's);[5-8] longer T_1's have been interpreted in terms of increased mobility due to puckering. A number of small peptides containing prolines have been examined in solution by high-resolution proton NMR spectroscopy.[9-12] Partial or complete analysis of the spin systems was performed. A search was then made for a single conformer which would yield coupling constants matching the observed ones, based on Karplus curves.[13] This method should work well when a single conformer is present, but the presence of two or more rapidly interconverting conformations may either

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