(24) For a discussion of the nuclear Overhauser effect and its chemical applications, see J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect", Academic Press, New York, N.Y., 1971.

(25) F. W. Nader, *Tetrahedron Lett.*, 1591 (1975).

(26) The following results were obtained upon irradiation at the phenyl resonance of **2**: at $-10\,^\circ$C, enhancement of H(2) = $6 \pm 2\%$; at 80 $^\circ$C, enhancement of H(2) = $3 \pm 3\%$.

(27) At $-25\,^\circ$C the H(2) proton of **2** resonates at 5.349 ± 0.005 ppm and at 80 $^\circ$C H(2) resonates at 5.315 ± 0.005 ppm. The 0.034 ± 0.007 ppm difference between these two values is probably not significant since the chemical shift of the 4,6-CH_3 doublet of **2** shows a 0.02 ± 0.01 ppm variation over this same temperature range.

(28) H. Keller, E. Langer, and H. Lehner, *Monatsh. Chem.*, **107**, 949 (1976).

(29) E. N. Klimovitskii, L. K. Yuldasheva, A. N. Vereshchagin, G. N. Sergeeva, and S. S. Debeleya, *Bull. Acad. Sci. USSR*, **24**, 979 (1975).

(30) The technique of reaction calorimetry has met with mixed success when applied to conformational problems. The ΔH° for 5-methyl-1,3-dioxane and the chair–boat enthalpy difference in 1,3-dioxane have been determined by this method [R. M. Clay, G. M. Kellie, and F. G. Riddell, *J. Am. Chem. Soc.*, **95**, 4632 (1973)] but others have commented on the experimental difficulties associated with the technique [K. Pihlaja, R. Keskinen, and A. Nikkila, *Bull. Soc. Chim. Belg.*, **85**, 435 (1976)].

(31) The standard deviation from the mean (root mean square deviation) was determined for each series of measurements of a particular quantity and this value was propagated to give the errors reported for all thermochemical and equilibration data. See H. Margenau and G. M. Murphy, "The Mathematics of Chemistry and Physics", Van Nostrand, Princeton, N.J., 1968, pp 504–515.

(32) The ΔH_{fus} value for **1** is in line with heats of fusion which have been determined for other aromatic compounds [see D. R. Stull, E. F. Westrum, Jr., and G. C. Sinke, "The Chemical Thermodynamics of Organic Compounds", Wiley, New York, N.Y., 1969]. A few representative values of ΔH_{fus} (kcal/mol) follow: Benzene, 2.351; naphthalene, 4.536; biphenyl, 4.44.

(33) G. N. Lewis and M. Randall, "Thermodynamics", 2nd ed, revised by K. S. Pitzer and L. Brewer, McGraw-Hill, New York, N.Y., 1961, pp 419–448.

(34) N. Davidson, "Thermodynamics", McGraw-Hill, New York, N.Y., 1962, pp 169–210.

(35) The contribution to ΔS° from differences in the overall rotational entropy (ΔS°_{OR}) for axial and equatorial 2-phenyl-1,3-dioxane is evaluated from

$$\Delta S^\circ_{OR} = R[\ln Q_{OR} \text{ (equatorial)} - \ln Q_{OR} \text{ (axial)}]$$

and Q_{OR} is given by (ref 33)

$$Q_{OR} = (0.014837/\sigma)\,(I_x\,I_y\,I_z)^{1/2}\,T^{3/2}$$

where $I_x\,I_y\,I_z$ are the reduced principal moments of inertia (in units of amu Å^2), T is the absolute temperature, and σ is the external symmetry number ($\sigma = 1$ for the isomeric 1,3-dioxanes).

(36) H. D. Rudolph, H. Dreizler, A. Jaeschke, and P. Windling, *Z. Naturforsch. A*, **22**, 940 (1967), and references cited therein.

(37) Tables of entropy due to internal rotation as a function of barrier height within the harmonic oscillator model are found in K. S. Pitzer, "Quantum Chemistry", Prentice-Hall, Englewood Cliffs, N.J., 1954, pp 457–467.

(38) N. L. Allinger and D. Y. Chung, *J. Am. Chem. Soc.*, **98**, 6798 (1976).

(39) K. Pihlaja and S. Luoma, *Acta Chem. Scand.*, **22**, 2401 (1968).

(40) We note that similar arguments have been proposed to intuitively account for the conformational behavior of some 2-phenyl-1,3-dithianes [H. T. Kalff and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **85**, 467 (1966)]. Our observations lend support to these arguments.

(41) E. L. Eliel and F. W. Nader, *J. Am. Chem. Soc.*, **92**, 584 (1970).

(42) K. Pihlaja and P. Ayras, *Acta Chem. Scand.*, **24**, 531 (1970), and references cited therein.

(43) (a) There is, apparently, a sign error in ref 19 and the isomer having an axial 2-phenyl, equatorial 2-methyl conformation is favored at equilibrium. (b) The report of a predominance of axial 2-isopropyl, equatorial 2-methyl isomer at equilibrium (ref 20) has been withdrawn; H. Lehner, private communication to E. L. Eliel (July 1977).

(44) See Table V.

(45) U. Berkert, *Tetrahedron*, **33**, 2237 (1977).

Solvent Participation in Reactions. 2.
Reactions of the Cystamine Anion Radical

O. I. Mićić,*[1a] M. T. Nenadović,[1a] and P. A. Carapellucci[1b]

Contribution from the Boris Kidrič Institute of Nuclear Sciences, Vinča, 11001 Beograd, P.O. Box 522, Yugoslavia, and the Jet Propulsion Laboratory California Institute of Technology, Pasadena, California 91103. Received June 23, 1977

Abstract: First-order decomposition of the anion radical $RSSR^-$ ($RSSR^- \rightarrow RS\cdot + RS^-$) formed in cystamine solutions was studied by pulse radiolysis. The rate constants and activation energies of this reaction were determined in sucrose, glycerol, and ethanol aqueous solutions. Solvent cage effects seem to be important in more viscous media and determine the rate of separation of products. The reaction rate of electron transfer from the $RSSR^-$ radical to oxygen ($RSSR^- + O_2 \rightarrow RSSR + O_2^-$) was also determined in the same solutions. This reaction is affected by dielectric properties of solutions and enhanced in polar media.

Introduction

The kinetics of electron transfer reactions in aqueous solutions of alcohols is complex owing to ion–solvent interactions.[2a] Very often these solutions undergo structural and dielectric changes which may affect the reaction rates of reacting species.[2b] In this work the effect of composition of aqueous solutions of glycerol, sucrose, and ethanol on reactions of the cystamine anion radical was studied by observing the unimolecular decomposition reaction

$$RSSR^- \rightarrow RS\cdot + RS^- \qquad (1)$$

and electron transfer to an oxygen molecule,

$$RSSR^- + O_2 \rightarrow RSSR + O_2^- \qquad (2)$$

The cystamine anion radical, $RSSR^-$, was formed by pulse radiolysis of cystamine (RSSR) solutions in reactions with solvated electrons.

$$RSSR + e_s^- \rightarrow RSSR^- \qquad (3)$$

Cystamine (2,2'-dithiobisethylamine) dihydrochloride, $Cl^-NH_3^+CH_2CH_2SSCH_2CH_2NH_3^+Cl^-$,[3] is convenient for this study since it reacts very fast with hydrated electrons ($k_3 = 4.2 \times 10^{10}$ dm^3 mol^{-1} s^{-1})[4] and the concentration of $RSSR^-$ anion radicals can be measured accurately by pulse radiolysis. The anion radical has high molar absorptivity (λ_{max} 410 nm, a_{410} 9000 dm^3 mol^{-1} cm^{-1})[4,5] and the absorption peak is well separated from those of other radicals formed in cystamine solutions.[6]

Experimental Section

A pulse technique was used to measure the decay rates of the cystamine anion radical at 410 nm. This technique has been described previously.[7] Concentrations of several micromoles of $RSSR^-$ radicals were produced in the samples by irradiation with 20-ns pulses from a Febetron 707 (Field Emission Corp.) electron accelerator. The ab-

Table I. Rate Constants of Reaction 1 and 2 for Various Aqueous Sucrose, Glycerol, and Ethanol Solutions at 19 ± 1 °C

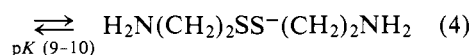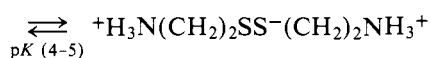| Nonaqueous component	Mole fraction X_1	η 20 °C,[d] cP	ϵ[d]	$k_1 \times 10^{-5}$, s^{-1}	$(E_a)_1$, kcal/mol	O$_2$ present in solution, dm^{-3} mol $\times 10^3$	$k_2 \times 10^{-8}$, dm^3 mol^{-1} s^{-1}
Sucrose	0	1.005	80.38	3.75	9.60	1.26	7.70
	0.006	1.33	78.04	3.56		1.05	7.46
	0.009	1.67	75.00	3.40		0.96	
	0.013	1.91	75.45	2.97		0.89	6.18
	0.022	3.20	72.64	2.44		0.79	6.17
	0.034	6.07	69.45	1.62	9.75	0.68	4.59
	0.050	16.00	65.88	0.56	11.20	0.60	4.37
	0.073[a]	62.90	61.80	0.20	13.80	0.50	4.30
Glycerol	0.034	1.65	76.30	3.44		1.30	
	0.075	2.50	71.77	2.64	10.7	1.06	6.74
	0.115	3.90	68.76	1.65		0.86	6.58
	0.164	5.90	65.63	1.09	15.8	0.76	6.50
	0.227	10.30	62.03	0.76		0.67	5.23
	0.313	24.20	57.06	0.29	17.9	0.60	5.00
	0.370	35.80	54.70	0.09		0.58	3.86
	0.439[b]	64.00	52.27	0.049	21.4	0.57	2.60
Ethanol	0.043	1.50	76.60	3.18		1.36	8.00
	0.080	2.13	68.63	2.96		1.32	8.50
	0.150	2.66	64.50	2.68		1.57	7.24
	0.220	2.89	56.49	1.70		1.80	5.75
	0.245	2.88	53.00	1.55			
	0.280[c]	2.83	50.38	1.43		2.32	4.62

[a] 60% sucrose. [b] 80% glycerol. [c] 50% ethanol. [d] From ref 9.

sorbed doses are in the range 0.5-1 krad/pulse. The experimental technique for the determination of activation energies was calculated from the slopes of Arrhenius plots (ln k against inverse of the absolute temperature in K). The reproducibility of the activation energies was within 10%.

Cystamine dihydrochloride (Sigma Chemical Co.), sucrose glycerol, and ethanol (Merck and BDH, analytical grade) were used without further purification. All solutions were prepared with triply distilled water immediately before use. The solution pH was adjusted with phosphate buffers at a concentration of 5 × 10^{-3} mol dm^{-3}. Deaerated solutions were prepared by bubbling with argon. Oxygen concentrations for all samples were determined by gas chromatography using a Perkin-Elmer 154 DG apparatus and a molecular sieve column.

Results

In diluted aqueous solutions the values for the rate constants of reaction 1 and their dependence upon the pH of the solutions were found to be in fairly good agreement with previously published data.[4,5] k_1 depends on the pH of solutions since the RSSR$^-$ radical follows the equilibrium[4]

$$^+H_3N(CH_2)_2SSH(CH_2)_2NH_3^+$$

$$\underset{pK\ (4-5)}{\rightleftarrows} {}^+H_3N(CH_2)_2SS^-(CH_2)_2NH_3^+$$

$$\underset{pK\ (9-10)}{\rightleftarrows} H_2N(CH_2)_2SS^-(CH_2)_2NH_2 \quad (4)$$

The value of k_1 is lowest in neutral solutions where RSSR$^-$ radicals exist in the form $^+H_3N(CH_2)_2SS^-(CH_2)_2NH_3^+$. In acid and alkaline solutions k_1 is higher by more than a factor of 3. In alkaline solution the value of k_1 follows the pK_a (8.69 and 9.16)[8] for deprotonation of amino groups in cystamine. In this study the decay kinetics of RSSR$^-$ radicals was examined as a function of pH in aqueous solutions of sucrose (60%), glycerol (80%), and ethanol (50%) and the pH dependence was found to be the same as that in pure water, i.e., no effect of the presence of the cosolvent was observed. The absorption spectrum of the cystamine anion radical is also independent of the presence of sucrose, glycerol, or ethanol and there is no shift in the absorption maximum. In the pH range of 5.5-8.5, the decay kinetics of RSSR$^-$ radical was inde-

pendent of pH for all solutions; pH was kept constant at 7 in all our experiments.

Reactions 1 and 2 were carried out in aqueous glycerol (0-80% glycerol, or 0 ≤ X_1 ≤ 0.44, where X_1 is the mole fraction of the nonaqueous component), in aqueous sucrose (0-60% sucrose or 0 ≤ X_1 ≤ 0.073), and in aqueous ethanol solutions (0-50% ethanol or 0 ≤ X_1 ≤ 0.3). The values of the rate constants of reactions 1 and 2 and the activation energies for reaction 1 are presented in Table I. Table I also contains the data for viscosities, η, dielectric constants, ϵ, and oxygen solubility.

Cystamine concentration was constant at 1 × 10^{-3} mol dm^{-3} in all our experiments. Sucrose, glycerol, and ethanol did not compete with it to a great extent for solvated electrons. Other radicals formed in aqueous glycerol,[10] sucrose,[11] and ethanol[12] solutions did not interfere with determination of the RSSR$^-$ decay at 410 nm.

The RSSR$^-$ decay rate observed at 410 nm in the presence of higher RSSR concentrations has shown the existence of the reaction between RSSR$^-$ and RSSR with a $k_{(RSSR^-+RSSR)}$ = (2.0 ± 0.1) × 10^7 dm^3 mol^{-1} s^{-1}. The products of this reaction are still unknown but they do not appear to absorb at 410 nm. In all our experiments conditions were chosen such that the reaction of RSSR$^-$ with RSSR does not compete with reactions 1 or 2.

It is shown in Table I that the rate constants of reactions 1 and 2 decrease with increasing mole fraction of the nonaqueous components. In principle, the reverse reactions of 1 and 2 may retard the observed overall reaction rate. However, for both reactions the decay of the RSSR$^-$ absorbance followed a first-order rate law over at least 4 half-lives for all binary solvents studied.

An attempt was made to correlate the rate constants for both reactions with solvent property parameters such as the dielectric constant, viscosity, and heat of mixing. The values of rate constants for reaction 1 are correlated in Figure 1a with the static dielectric constants of the matrix in order to see the effects of electrostatic interactions of RSSR$^-$ radicals with solvent molecules on the dissociation rate. Figure 1b presents the dependence of $k_1/(k_1)_w$ ratio (subscript w refers to water) on the reciprocal values of solution viscosity.
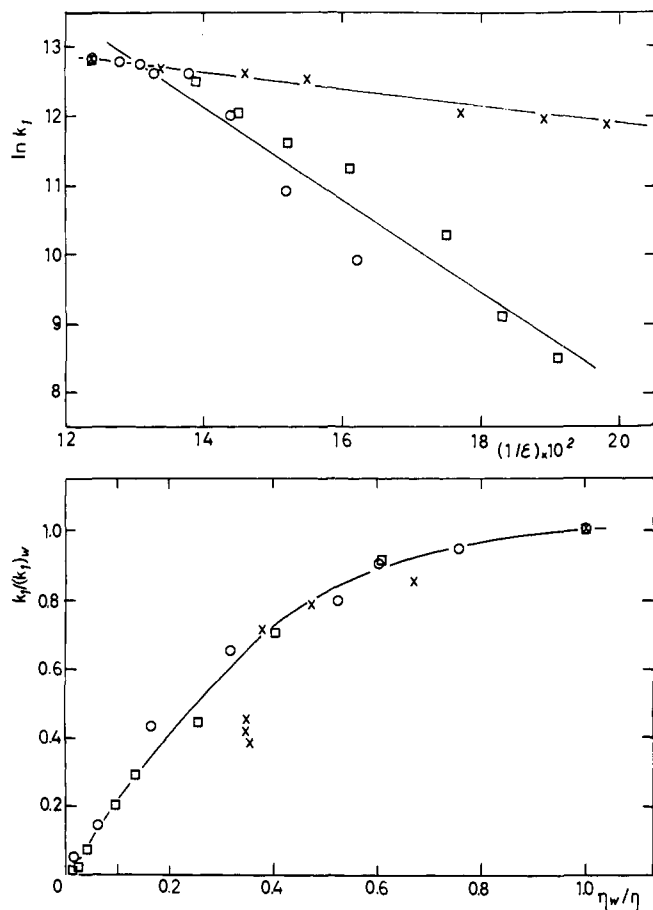
Figure 1. Rate of reaction 1 ($RSSR^- \rightarrow RS + RS^-$) in different aqueous binary systems: (a) dielectric constant influence and (b) viscosity influence. ($\times$) water–ethanol; ($\square$) water–glycerol; ($\bigcirc$) water–sucrose system.

The rate constants of reaction 2 as a function of heat of mixing parameters of water–glycerol solutions are presented in Figure 2a. The partial molal excess functions $-(\partial \Delta H^M / \partial n_2)/X_2$ (n_2 = number of moles and X_2 = mole fraction of water) were used for presentation of the results and compared with sets of data for the rate constants of different reactions, determined in water–glycerol solutions for which heat of mixing plots show regularity.[16] The plot of ln k_2 against reciprocal dielectric constants of the matrix, presented in Figure 2b, shows nearly linear dependence for all the solutions studied.

Discussion

Analysis of experimental results shows extreme sensitivity of the $RSSR^-$ radical anions dissociation, reaction 1, to the solvent environmental properties. Electrostatic interactions of $RSSR^-$ radicals with solvent molecules have no influence (Figure 1a) on the dissociation rate since the rate constants only slightly decrease with increasing ethanol content in water–ethanol solution, while the dielectric constants decrease markedly and viscosity increases only slightly (Table I).

The reaction rate decreases with increasing viscosity (Figure 1b) presumably because of the increasing difficulty of separation of RS and RS^- products. Solvent cage effects can be important for free-radical reaction in the solution.[17] Thus reaction 1 can be considered as the process of conversion of an intimate pair, [RS $\cdots$ RS^-], held in close proximity by a wall of solvent molecules into completely separate RS and RS^- products.
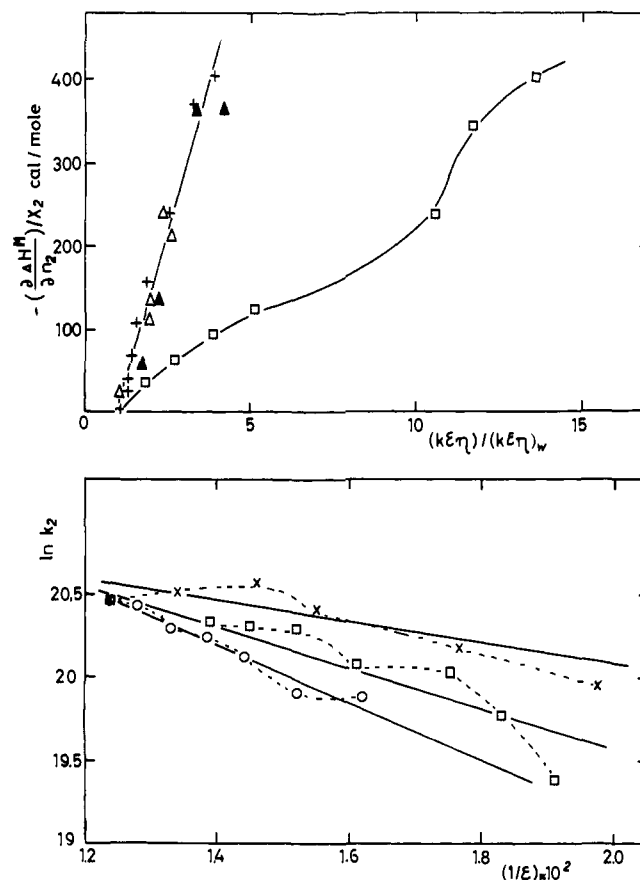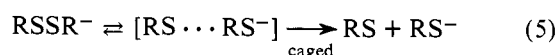
$$RSSR^- \rightleftarrows \underset{\text{caged}}{[RS \cdots RS^-]} \longrightarrow RS + RS^- \qquad (5)$$

Figure 2. Rate of reaction 2 ($RSSR^- + O_2 \rightarrow RSSR + O_2^-$) in different aqueous binary ssytems. (a) Heat of mixing parameter plot for several reactions in water–glycerol system; ($\triangle$) neutralization of bromocresol green ($H^+ + BG^{2-} \rightarrow (HBG)^-$), ref 13; ($+$) self-diffusion coefficients of glycerol, ref 14; ($\blacktriangle$) electron transfer reaction ($CH_3C_6H_5CO^- + (C_6H_5)_2CO \rightarrow CH_3C_6H_5CO + (C_6H_5)_2CO^-$), ref 2a; ($\square$) reaction 2. Heat of mixing parameters in calories per mole calculated from ref 15. Representations: n_2, number of moles, and X_2, mole fraction of water. (b) Effect of dielectric properties of matrix: ($\times$) water–ethanol; ($\square$) water–glycerol; ($\bigcirc$) water–sucrose system.

In more viscous media the escape of products from the solvent cage can be severely hindered and becomes a rate-determining step. Increase of the activation energy of reaction 1 for higher glycerol and sucrose content (Table I) supports the idea of a solvent cage effect on the dissociation process. The increase of activation energy in more viscous media shows that, besides the $RSSR^-$ radicals' characteristic property, the strength of interaction with solvent molecules is also a factor determining activation energy.

We compared reaction 2 with data available for several reactions previously studied in water–glycerol solutions. The diffusion-controlled reaction of the neutralization of bromocresol green,[13] the electron transfer reaction between acetophenone anion radical and benzophenone,[1] and self-diffusion coefficients data,[14] presented in Figure 2a as a function of heat of mixing parameters, all lie on the same straight line. For reaction 2 the plot is curved and located below the straight line. The reaction 2 is activation controlled. The relative positions of the curves in Figure 2a can help to distinguish between activation and diffusion-controlled reactions since in nonideal solvent systems there is no linear correlation between rate constants and the reciprocal value of matrix viscosity.

Electron transfer reaction 2 shows that the rate constants decrease with increasing content of the nonaqueous component for all the systems studied (Table I). This effect is determined by the change of dielectric properties of solutions (Figure 2b). Viscosity has no special influence on the rate of reaction 2.

Reaction 2 is controlled by an activation process so that a change of dielectric properties affects reorganization of the solvent around both the reactant and the activation complex. The reaction is enhanced in polar media. Results in Figure 2b show that the slopes, $\partial \ln k_2 / \partial(1/\epsilon)$, depend on the nature of the nonaqueous component. The slopes of the straight lines are -60, -120, and -170 for water–ethanol, water–glycerol, and water–sucrose solutions, respectively. This indicates existence of specific solvation and interaction of reacting species with the solvent shell at the initial and transient states.

References and Notes

(1) (a) Boris Kidrič Institute of Nuclear Sciences; (b) California Institute of Technology.
(2) (a) Part 1, O. I. Mićić and B. Čerček, *J. Phys. Chem.*, **78**, 285 (1974); (b) M. J. Blandamer and J. Burgess, *Chem. Soc. Rev.*, **4**, 55 (1975).
(3) (a) J. Khaladji, *Ann. Chim. (Paris)*, **13**, 555 (1958); (b) L. Eldjarn, *Acta Chem. Scand.*, **5**, 677 (1951).
(4) M. Z. Hoffman and E. Hayon, *J. Am. Chem. Soc.*, **94**, 7950 (1972).
(5) G. E. Adams, G. S. McNaughton, and B. D. Michael in "The Chemistry of Ionization and Excitation", G. R. A. Johnson and G. Scholes, Ed., Taylor and Francis, London, 1976, p 281.
(6) M. Bonifačić, K. Schäfer, H. Möckel, and K.-D. Asmus, *J. Phys. Chem.*, **79**, 1496 (1975).
(7) (a) V. M. Marković, D. Nikolić, and O. I. Mićić, *Int. J. Radiat. Phys. Chem.*, **6**, 224 (1974); (b) O. I. Mićić and M. T. Nenadović, *J. Phys. Chem.*, **80**, 940 (1976).
(8) J. R. McPhee, *Biochem. J.*, **64**, 22 (1956).
(9) J. Timmermans, "Physico-Chemical Constants of Binary Systems", Vol. 4, Interscience, New York, N.Y., 1960.
(10) J. S. Moore and A. F. Norris, *Int. J. Radiat. Biol.*, **29**, 489 (1976).
(11) J. V. Davies, W. Griffiths, and G. O. Phillips in "Pulse Radiolysis", M. Ebert, J. P. Keen, A. J. Swallow, and J. H. Baxendale, Ed., Academic Press, New York, N.Y., 1965, p 181.
(12) M. Simić, P. Neta, and E. Hayon, *J. Phys. Chem.*, **73**, 3794 (1969).
(13) P. Warrick, Jr., J. J. Auborn, and E. M. Eyring, *J. Phys. Chem.*, **76**, 1184 (1972).
(14) Y. Nishijima and G. Oster, *Bull. Chem. Soc. Jpn.*, **33**, 1649 (1960).
(15) "International Critical Tables", Vol. 5, McGraw-Hill, New York, N.Y., 1929, p 157.
(16) P. A. Carapellucci, *J. Am. Chem. Soc.*, **98**, 3016 (1976).
(17) (a) C. Walling, "Free Radicals in Solution", Wiley, New York, N.Y., 1957, p 76; (b) R. M. Noyes, *Prog. React. Kinet.*, **1**, 152. (1961).

Conformational Studies of Tentoxin by Nuclear Magnetic Resonance Spectroscopy. Evidence for a New Conformation for a Cyclic Tetrapeptide

Daniel H. Rich* and Pradip K. Bhatnagar[1]

Contribution from the School of Pharmacy, University of Wisconsin—Madison, Madison, Wisconsin 53706. Received August 11, 1977

Abstract: The conformations of the phytotoxic cyclic tetrapeptide tentoxin, *cyclo*-(L-MeAla^1-L-Leu^2-MePhe[(Z)Δ]^3-Gly^4) and the analogues, L-Pro^1-tentoxin and *N*-L-[*methyl*-^{13}C]Ala^1-tentoxin, have been studied by ^{1}H and ^{13}C NMR in chloroform, and by ultraviolet and circular dichroism spectroscopy in methanol. The data from these studies indicate that the three compounds have essentially the same conformation and that the conformation previously proposed for tentoxin is incorrect. A new conformation is proposed for tentoxin with the following torsion angles: ϕ_1, $-80°$, ψ_1, $-10°$; ϕ_2, $-120°$, ψ_2, $+70°$; ϕ_3, $-90°$, ψ_3, $-20°$; ϕ_4, $-120°$, ψ_4, $+70°$. The new ring conformation differs from the centrosymmetric conformation previously proposed for tentoxin by the sign reversal of the torsion angles ψ_4,ϕ_1 while ϕ_2,ψ_2, ϕ_3,ψ_3, and all ω torsional angles are unchanged. The conformation proposed for these compounds is a new conformation for the 12-membered cyclic tetrapeptide ring system.

Tentoxin (**1**) is a phytotoxic cyclic tetrapeptide produced by the plant pathogen *Alternaria tenuis*.[2] When applied to germinating seedlings, tentoxin causes chlorosis in some plant species but has little apparent effect on others.[3] This selective toxicity has been linked to the presence in susceptible species of a high affinity ($k_{assoc} = 2 \times 10^8$ M) tentoxin binding site[4] on chloroplast coupling factor 1 (CF$_1$), a key protein involved in chloroplast synthesis of ATP.[5] Resistant species usually contain a form of CF$_1$ which does not bind tentoxin tightly.

The configuration and sequence[6] of the amino acids in tentoxin and the configuration of the double bond in the *N*-methyldehydrophenylalanyl residue (MePhe[(Z)Δ])[7] are well established and have been confirmed by total synthesis.[8,9] In addition, a conformation was proposed[6] for tentoxin (Figure 1a), by analogy to that observed by Dale and Titlestad for several model cyclic tetrapeptides (Figure 1b),[10] based on the close similarity in the ^{1}H NMR chemical-shift and coupling constant data for the two systems.[6c] However, whereas the Dale–Titlestad conformation (Figure 1b) has been confirmed by x-ray crystallography,[11] no substantiating evidence for the proposed tentoxin conformation has been reported.

We first came to question the proposed tentoxin conformation[6] as a result of synthesizing the analogue D-MeAla^1-tentoxin (**2**) which differs from **1** only in the stereochemistry

of the methyl group at C-11 (see Figures 1a and 1b for the numbering used here). Examination of molecular models of tentoxin in conformation **1a** revealed that the alanine β-carbon was about 2.3 Å from the glycine α-carbon at C-8. This carbon–carbon distance is very close and should be very hindered.[12] It was expected that the alanine β-carbon would be in an unhindered, "pseudoequatorial" position in the D-MeAla analogue **2** and therefore would be no less stable than tentoxin **1**. In fact, when synthesized, D-MeAla^1-tentoxin was found to exist in several conformations, two of which could be isolated,[9] and this occurrence of multiple conformations was inconsistent with conformation **1a**.

As a result we decided to test the proposed tentoxin conformation by synthesizing the analogue, L-Pro^1-tentoxin (**3**), in which L-proline replaces L-*N*-methylalanine. The five-membered ring of proline limits the C$^\delta$–N–C$^\alpha$–C$^\beta$ vicinal bond angle to values less than ± 0–20° whereas, in the proposed conformation for tentoxin (Figure 1a), the C–N–C$^\alpha$–C$^\beta$ vicinal bond angle is about 130°. Thus, L-Pro^1-tentoxin (**3**) cannot exist in a conformation directly analogous to that proposed for tentoxin. If **3** were synthesized and found to have similar conformational and biological properties to tentoxin **1**, then the proposed conformation shown in Figure 1a could not be correct.