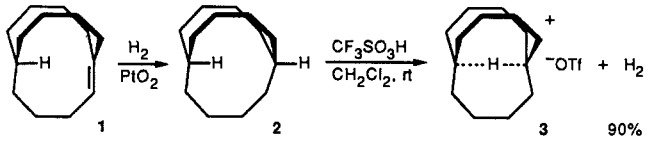
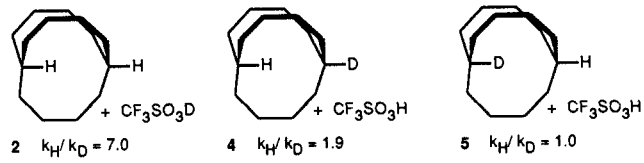
not the reactive oxidizing agent in $HSbF_6$ solutions. One particularly appealing explanation[10] is that the pentacoordinate RH_2^+ intermediate is intercepted and reduced by SbF_5 prior to the escape of H_2. Some recent work by Sorensen[11] has cast doubt on this explanation, however, leading him to state that "we...cannot really accept that the solution C-H protonation of alkanes...is an important pathway in the RH → R^+ reaction".

Clearly,[3a] the ease of carbocation formation from an alkane should depend both on the stability of the cation being formed and on the acid strength of the medium. If a stable enough carbocation is formed, it should be possible to carry out the protonolysis reaction by using weaker acids than are normally employed, thereby obviating the need for the HX/MX_n combination that appears to be the source of the trouble. We have shown in a detailed study carried out over the last four years that the hydrido-bridged *in*-bicyclo[4.4.4]-1-tetradecyl cation **3** is the most stable cycloalkyl cation known.[12] It therefore occurred to us that this cation might be formed from the corresponding alkane **2** under conditions that could allow the use of mild protic acids in the absence of an added Lewis acid. If so, it would present a unique opportunity to study a little-understood reaction.

Warming a dilute solution of **2** to 40 °C in glacial acetic acid resulted in the slow disappearance (approximately 10% completion after 1 week) of alkane starting material and the formation of cation **3**. Use of trifluoroacetic acid led to a more rapid reaction, but use of trifluoromethanesulfonic acid gave the cleanest results. When alkene **2** in a minimal volume of CH_2Cl_2 was added to CF_3SO_3H at 0 °C and gas evolution was measured,[13] more than 90% of the theoretical amount of H_2 was produced in 1 h (E_{act} = 13.5 kcal/mol) and a clean solution of cation **3** was obtained, clearly identified by its distinctive one-proton NMR absorption at δ -3.46. Quenching the solution resulted in recovery of alkene **1** in 75% isolated yield.



To gain further information about the protonolysis reaction, isotope experiments using deuterated trifluoromethanesulfonic acid, CF_3SO_3D, on unlabeled alkane **2** were carried out, and a large solvent kinetic isotope effect k_H/k_D = 7.0 at 0 °C was measured as determined by disappearance of starting material and appearance of alkene **1**.[14] Similar experiments using CF_3SO_3H on the labeled *out*-deuteriobicyclo[4.4.4]tetradecane **4**[15] gave a moderate primary isotope effect k_H/k_D = 1.9 at 0 °C. By contrast, the labeled *in*-deuterio substrate **5** showed no detectable remote isotope effect.[16] Taken together, the three results are consistent with a nonconcerted reaction whose rate-limiting step involves breakage of both the alkane C-H bond and the acid O-H bond. Were the reaction concerted, one would expect a measurable remote isotope effect in substrate **5**. Were loss of H_2 from a protonated intermediate rate-limiting, one would not expect a large solvent isotope effect on **2** but would expect some exchange of the outside bridgehead hydrogen in **2** on reaction with CF_3SO_3D.[17] No exchange of the outside bridgehead hydrogen was observed, however, when the reaction of unlabeled alkane **2** in CF_3SO_3D was allowed to go to partial completion and the recovered starting material was analyzed by mass spectroscopy.



2 k_H/k_D = 7.0 4 k_H/k_D = 1.9 5 k_H/k_D = 1.0

In summary, this work provides a clearcut example of stoichiometric hydrogen evolution in an alkane protonolysis reaction and provides good evidence that the RH → RH_2^+ → $R^+ + H_2$ pathway is a viable mechanism for carbocation formation in simple alkanes. We are unable to detect the presumed RH_2^+ intermediate, but it seems likely that a triangular structure with a closed three-center bond is energetically preferred over an alternative linear structure with an open C-H-H three-center bond.[3d] Note that the small primary isotope effect we observed is consistent with a nonlinear transition state.[18]

Acknowledgment. This work was supported by the donors of the Petroleum Research Fund, administered by the American Chemical Society, through Grant 15406-AC1 and by the National Science Foundation through Grant CHE-8615638. Thomas Lectka is the recipient of a Division of Organic Chemistry Fellowship awarded by the American Chemical Society and sponsored by the Monsanto Company.

(10) Ledford, T. H. *J. Org. Chem.* **1979**, *44*, 23-25.
(11) Kirchen, R. P.; Sorensen, T. S.; Wagstaff, K.; Walker, A. M. *Tetrahedron* **1986**, *42*, 1063-1070.
(12) McMurry, J. E.; Lectka, T.; Hodge, C. N. *J. Am. Chem. Soc.* **1989**, *111*, 8867-8872. For a preliminary communication, see: McMurry, J. E.; Hodge, C. N. *J. Am. Chem. Soc.* **1984**, *106*, 6450-6451.
(13) Gas evolution was carried out in a Clauson-Kaas microhydrogenation apparatus, which allows accurate measurement to ±0.05 mL. See: Clauson-Kaas, N.; Limborg, F. *Acta Chem. Scand.* **1947**, *1*, 884-888.
(14) For a discussion of solvent isotope effects, see: *Isotope Effects on Chemical Reactions*; Buncel, E., Lee, C. C., Eds.; Elsevier: New York, 1987.
(15) Compound **4** was prepared by protonation of alkene **1** with HCl at pH 3 in methanol solution and quenching of the cation with $NaBD_3CN$. See: Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897-2904.
(16) For a discussion of remote isotope effects, see: Sunko, D. E.; Borcic, S. In *Isotope Effects on Chemical Reactions*; Collins, C. J., Bowman, N. S., Eds.; Van Nostrand: New York, 1970; p 172.
(17) H-D exchange prior to carbocation formation is generally found in the reaction of deuterated alkanes with $HSbF_6$: Olah, G. A.; Shen, J.; Schlosberg, R. H. *J. Am. Chem. Soc.* **1970**, *92*, 3831-3832.
(18) More O'Ferrall, R. A. *J. Chem. Soc. B* **1970**, 785-790.

Isolation and Characterization of a Covalent Selenocysteine Intermediate in the Glycine Reductase System

R. A. Arkowitz and R. H. Abeles*

Graduate Department of Biochemistry
Brandeis University
Waltham, Massachusetts 02254
Received October 10, 1989

Selenocysteine occurs in the bacterial enzymes glycine reductase, formate dehydrogenase, and hydrogenase and the mammalian enzyme glutathione peroxidase.[1] The mechanistic role of this residue remains largely unknown. It has been postulated to function as a redox center, but there is little evidence supporting this suggestion.[2] We have investigated the mechanism of action of glycine reductase (eq 1) because of the unusual chemical re-

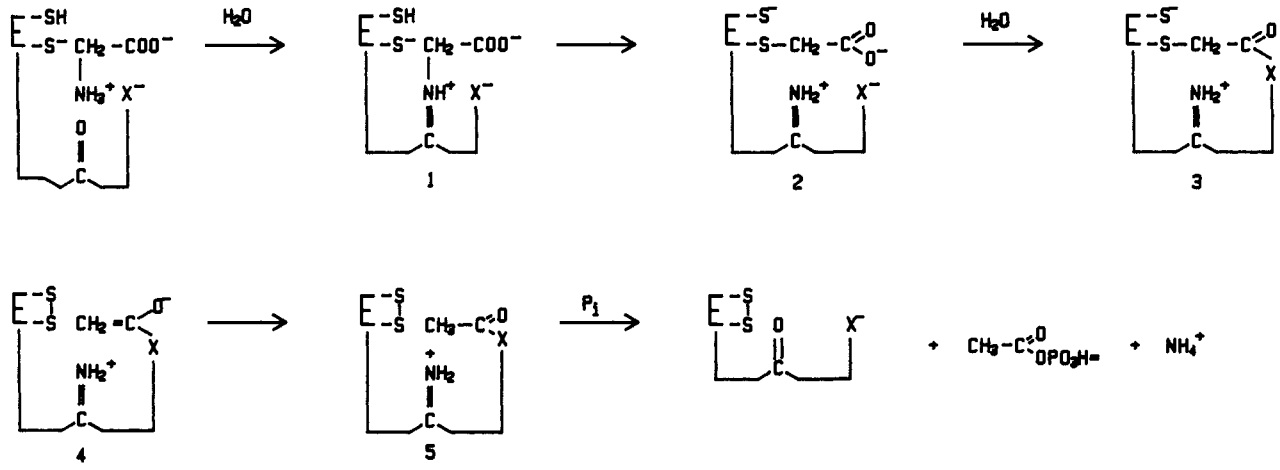
$$^+NH_3CH_2COO^- + P_i + 2e^- \rightarrow CH_3COOPO_3^{2-} + NH_4^+ \quad (1)$$

action catalyzed,[3] i.e., the reductive cleavage of a carbon-nitrogen bond. We have proposed the mechanism shown in Scheme I.[3] Clostridial glycine reductase consists of proteins A, B, and C.[5]

(1) Stadtman, T. C. *FASEB J.* **1987**, *1*, 375.
(2) (a) Pleasants, J. C.; Guo, W.; Rabenstein, D. L. *J. Am. Chem. Soc.* **1989**, *111*, 6553. (b) Sliwkowski, M. X.; Stadtman, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 368. (c) Hu, M.-L.; Tappel, A. L. *J. Inorg. Biochem.* **1987**, *30*, 239. (d) Stadtman, T. C. *Methods Enzymol.* **1984**, *107*, 576. (e) Ganther, H. E.; Kraus, R. J. *Methods Enzymol.* **1984**, *107*, 593. (f) Shamberger, R. J. *Biochemistry of Selenium*; Plenum Press: New York, NY, 1983. (g) Forstrom, J. W.; Zakowski, J. J.; Tappel, A. L. *Biochemistry* **1978**, *17*, 2639.
(3) (a) Arkowitz, R. A.; Abeles, R. H. *Biochemistry* **1989**, *28*, 4639. (b) Turner, D. C.; Stadtman, T. C. *Arch. Biochem. Biophys.* **1973**, *154*, 366.
(4) S represents a sulfur or selenium atom.
(5) (a) Tanaka, H.; Stadtman, T. C. *J. Biol. Chem.* **1979**, *254*, 447. (b) Sliwkowski, M. H.; Stadtman, T. C. *J. Biol. Chem.* **1987**, *262*, 4899.

0002-7863/90/1512-870$02.50/0

Scheme I. Proposed Mechanism of Action of Glycine Reductase[4]



Protein A contains a selenocysteine (SeCys) residue.[6] Protein B contains a covalently bound pyruvate moiety, which is necessary for activity.[3] Previously, we reported evidence consistent with the formation of an acyl enzyme intermediate,[3] located on protein C.[7] In the proposed mechanism (Scheme I) we postulated the existence of a thio- or selenoether enzyme intermediate occurring between the glycine Schiff base (**1**) and the acyl enzyme intermediate (**4**). Protein A was a likely candidate for this enzyme intermediate because it contains two cysteines and one SeCys, which are necessary for activity.[8] We report here the isolation and characterization of a covalent *Se*-(carboxymethyl)selenocysteine (SeCMSeCys) on protein A, which establishes a direct role for SeCys in the reaction catalyzed by glycine reductase.

Proteins A (0.45 mg, 31 nmol[5]) and B (1.3 mg)[9] were incubated with 50 μCi of 28 mM [^{14}C]glycine (34 000 cpm/nmol) for 5 min at room temperature, and the reaction was subsequently quenched with EtOH. After repeated washings of the protein pellet with EtOH, followed by solubilization with 6 M urea and TCA precipitation, labeled protein (230 500 cpm) was isolated. The precipitated protein was treated with 100 units of carboxypeptidase A-DFP, and the released radioactive material was analyzed for SeCMSeCys. The radioactive material chromatographed as a single spot on high-voltage paper electrophoresis, with 89% of the radioactivity comigrating with SeCMSeCys.[10] In addition, the radioactive material comigrated with SeCMSeCys on silica TLC (EtOH/H_2O, 6:4) and anion-exchange HPLC[11] and upon *o*-phthalaldehyde (OPA) derivatization on C-8 HPLC.[11]

Another experiment was carried out in which proteins A (0.4 mg) and B (1.8 mg) were treated with [^{14}C]glycine as described above. In addition, a control reaction was carried out in which protein B was inactivated with KBH_4[12] prior to addition to protein A. The reaction mixtures were subjected to gel filtration on a Bio-Rad P-60 column, which separates protein A from protein B. Radioactivity[13] was associated with protein A, and only when incubated with active protein B. HCl hydrolysis of the labeled protein A confirmed[10] the identification of SeCMSeCys as the only radioactive residue.

Aliquots of labeled protein A, prepared as described above, were incubated either alone, with protein B, or with fraction C under standard assay conditions.[14] When labeled protein A is incubated with fraction C, 91% of the protein-bound radioactive material is converted to acetate,[15] whereas no acetate is formed upon incubation of protein A alone. A small amount of product (19%) is formed when labeled protein A is incubated with protein B, probably due to the slight contamination of protein B with protein C. When fraction C is added to labeled protein A in the absence of alkaline phosphatase, Dowex1(formate) chromatography shows that acetyl phosphate is formed.[3]

Further support for a SeCMSeCys protein A intermediate was obtained by alkylation of the reduced native protein A at pH 6 (in order to minimize cysteine alkylation) with [^{14}C]iodoacetate (13 μCi/μmol). After removal of excess iodoacetate by gel filtration, the alkylated protein A was added to protein B and fraction C under standard assay conditions in the absence of alkaline phosphatase. Approximately 15% of the radioactivity associated with protein A was converted to [^{14}C]acetyl phosphate. Although this amount of turnover is quite low, it is consistent with only a small amount of the SeCys alkylation product found upon acid hydrolysis and OPA amino acid analysis.

The results reported here have established that the SeCys residue of protein A reacts with glycine to form SeCMSeCys, which forms product upon addition of fraction C. Active protein B is required for the formation of this protein A intermediate. This reaction involves a displacement of the amino (or imino) group by SeCys as proposed in Scheme I. The displacement of the substrate nitrogen by SeCys is possible because the selenolate (present at physiological pH) is a potent nucleophile.[16] For

(6) Cone, J. E.; Del Rio, R. M.; Davis, J. N.; Stadtman, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2659.

(7) Protein C alone catalyzes [^{32}P]inorganic phosphate/acetyl phosphate, phosphate exchange (unpublished data).

(8) Stadtman, T. C. *Arch. Biochem. Biophys.* **1966**, *113*, 9.

(9) Protein A was purified as described[3] (specific activity approximately 56 units/mg and ≥95% pure as judged by SDS PAGE). Protein B (specific activity approximately 19 units/mg and ≥25% pure as judged by SDS PAGE) and fraction C (specific activity approximately 37 units/mg) were purified by a modification of the described procedure.[3]

(10) After proteolysis or hydrolysis of the protein, 1 μmol of SeCMSeCys was added and the sample was chromatographed on Dowex1(formate) resin and eluted with 1 N HCl. The SeCMSeCys was then purified by high-voltage paper electrophoresis in pH 6.5 buffer, pyridine/acetic acid/H_2O (8:4:72) at 53 V/cm for 60 min. Standards were visualized by ninhydrin, strips were eluted with H_2O, and radioactivity was determined. SeCMSeCys migrated approximately 25.5 cm and *S*-(carboxymethyl)cysteine 28 cm toward the positive electrode. Specific activity of eluted SeCMSeCys calculated from recovery of SeCMSeCys (Stein, W. H.; Moore, S. *J. Biol. Chem.* **1954**, *211*, 907) compared to initial specific activity.

(11) Alltech Spherisorb SAX HPLC column eluted isocratically with 5 mM KH_2PO_4 at a flow rate of 0.8 mL/min. Both *Se*- and *S*-(carboxymethyl)cysteine eluted after 21 min. Fractions (0.5 min) were collected, and radioactivity was determined. Samples were derivatized with OPA and analyzed on an Alltech OPA-HS HPLC column equilibrated in 50 mM NaOAc, pH 5/4% THF/10% MeOH. A 20-min 10–60% MeOH gradient was developed with a 1 mL/min flow rate. SeCMSeCys eluted after 7.3 min, and *S*-(carboxymethyl)cysteine eluted after 5.9 min. Fractions (0.2 min) were collected, and radioactivity was determined.

(12) Two aliquots of 5 μmol of KBH_4 in 10 mM NaOH were added to protein B over 50 min at room temperature. The reaction was quenched with 41 μmol of sodium pyruvate and the mixture incubated for 10 min.

(13) Approximately 0.2–0.3 nmol of label/nmol of protein A was observed. This low stoichiometry is attributed to slight contamination of protein C in B and partially inactive (oxidized) protein A.

(14) Tricine (60 mM)/KOH, pH 8.1, 20 mM K_2HPO_4, 8 mM $MgCl_2$, 40 mM glycine, 40 mM DTT, and 10 units of alkaline phosphatase.

(15) The reaction mixture was incubated under an argon atmosphere at 30 °C for 30 min. Acetic acid (1 μmol) was added to the EtOH-quenched assay mixture, which was then chromatographed on Dowex50(H^+).[3] When labeled protein A and fraction C were incubated under identical conditions for 30 s, a similar amount of product was formed.

example, benzeneselenol deaminates secondary alkylamines, resulting in alkylbenzyl selenide.[17] In conclusion, we have demonstrated the first known catalytic role for selenocysteine in an enzyme.

Acknowledgment. We thank Nyla Spawn for her help in preparing the manuscript. This paper is supported by a grant from The National Science Foundation No. DMB85-05498 and a Gillette Graduate Fellowship (R.A.A.). This is publication 1698.

(16) See ref 2a. Huber, R. E.; Criddle, R. S. *Arch. Biochem. Biophys.* **1967**, *122*, 164.
(17) Krief, A.; Hevesi, L. *Organoselenium Chemistry I*; Springer-Verlag: New York, NY, 1988; pp 13-15.

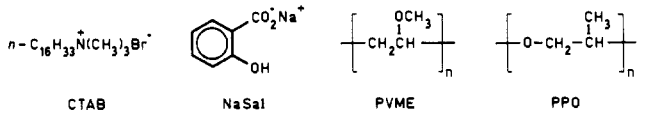
Polymer-Induced Breakdown of Rodlike Micelles. A Striking Transition of a Non-Newtonian to a Newtonian Fluid

Josephine C. Brackman and Jan B. F. N. Engberts*

Department of Organic Chemistry
University of Groningen, Nijenborgh 16
9747 AG Groningen, The Netherlands

Received October 9, 1989

Cetyltrimethylammonium salicylate (CTASal) is the archetype of a cationic surfactant that forms rodlike micelles even in dilute ($\sim 10^{-4}$ M) solutions.[1-5] At higher concentrations, the viscous, gel-like solution of CTASal shows all the peculiarities of a viscoelastic, non-Newtonian fluid.[6] Herein we report that addition of small quantities of the weakly hydrophobic but *water-soluble* poly(vinyl methyl ether) (PVME) or poly(propylene oxide) (PPO)



completely eliminates the viscoelasticity and transforms the solution into a normal Newtonian liquid of water-like viscosity.[7] This novel extension of the scope of polymer–micelle interactions[8] may have industrial importance, for example, in soap dispersion. Poly(ethylene oxide) (PEO) and poly(vinylpyrrolidone) (PVP), which are definitely more hydrophilic than PVME and PPO as well as the low molecular weight additives EtOH and *t*-BuOH, do not perturb the CTASal rods.

Although the change in the properties of the CTASal aggregates induced by PVME or PPO strikes the eye, rheological measurements[9] were performed to quantify the effect. Apparent viscosities

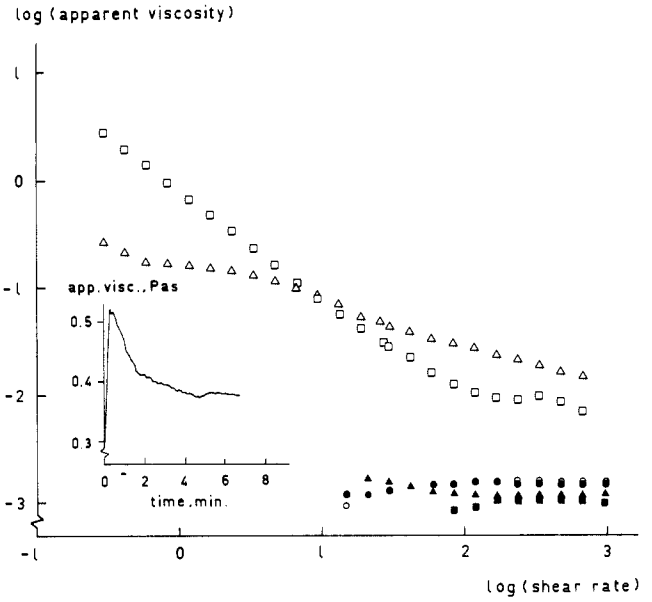


Figure 1. Double logarithmic plot of apparent viscosity vs shear rate for the following aqueous solutions of CTAB (25 mM): ■, no additives; ▲, +PEO (0.5 g dL^{-1}); ●, +PVME (0.5 g dL^{-1}); □, +NaSal (15 mM); △, +NaSal (15 mM) + PEO (0.5 g dL^{-1}); ○, +NaSal (15 mM) + PVME (0.5 g dL^{-1}). Inset: thixotropic behavior of CTAB (25 mM)/NaSal (15 mM)/PEO (0.5 g dL^{-1}). At t = 0 min, the shear rate is switched from 1.19 to 2.38 s^{-1}. Temperature = 25 °C.

Table I. The Effect of Sodium Salicylate and Several Monomeric and Polymeric Additives on the Viscosity of a Micellar CTAB Solution

[CTAB], mM	[NaSal], mM	additive[a]	viscosity, cP
25			1.08 ± 0.02
25		PVME	1.510 ± 0.0006
25		PEO	1.26 ± 0.02
25	15		2771,[b] 8.8[c]
25	15	PVME	1.630 ± 0.006
25	15	PPO	1.08 ± 0.006
25	15	PEO	274,[b] 16.9[c]
25	15	PVP	2817,[b] 15.4[c]
25	15	EtOH	3055,[b] 8.1[c]
25	15	*t*-BuOH	2213,[b] 8.9[c]

[a] [Additive] = 0.5 g dL^{-1}. [b] Shear rate = 0.2985 s^{-1}. [c] Shear rate = 477.6 s^{-1}.

as a function of shear rate (cylindrical geometry) are depicted in Figure 1. As recommended by Hoffmann et al.,[4] mixtures of equal volumes of CTAB (50 mM) and sodium salicylate (NaSal; 30 mM) were used to obtain pronounced viscoelastic and gel-like properties. These CTAB/NaSal (whether or not in the presence of PVP) and, to a lesser extent, the CTAB/NaSal/PEO solutions exhibit genuine non-Newtonian behavior (Table I), that is, the apparent viscosities vary dramatically with changing shear rate. The enormous drop in viscosity with increasing shear rate may be attributed to shear-induced aligning and/or breakdown of the rodlike micelles.[13] By contrast, the apparent viscosities of the CTAB/NaSal/PVME and CTAB/NaSal/PPO solutions and the CTAB solutions in the absence of NaSal are independent of shear

(1) Gravsholt, S. In *Rheology*; Astarita, G., Marucci, G., Nicolais, L., Eds.; Plenum Press: New York, 1980; Vol. 3, p 629.
(2) Johnson, I.; Olofsson, G. *J. Colloid Interface Sci.* **1985**, *106*, 222.
(3) Manohar, C.; Rao, U. R. K.; Valaulikar, B. S.; Iyer, R. M. *J. Chem. Soc., Chem. Commun.* **1986**, 379.
(4) Hoffmann, H.; Ebert, G. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 902.
(5) For a ^{1}H NMR study of the tumbling motions of the salicylate ions in viscoelastic solutions of CTASal, see: Anet, F. A. L. *J. Am. Chem. Soc.* **1986**, *108*, 7102.
(6) *Dynamics of Polymeric Liquids*; Bird, R. B., Curtiss, C. F., Armstrong, R. C., Hassager, O., Eds.; Wiley: New York, 1980; Vols. 1-2.
(7) Previously, it has been shown that solubilization of hydrocarbons also leads to destruction of rodlike micelles. Addition of alcohols like 1-pentanol also tends to remove the viscoelasticity, but this effect is due to shortening of the structural relaxation time of the rods. See: Hoffmann, H.; Ulbricht, W. *Tenside Surfactants Deterg.* **1987**, *24*, 1.
(8) For a review, see: Goddard, E. D. *Colloids Surf.* **1986**, *19*, 255.
(9) Brabender Rheotron rheometer, equipped with a normal F-sensor, which allows the measurement of first normal stress differences. PVME (Aldrich, mw 27 000, purified[10]) and PEO (Sigma, mw 20 000, purified[11]), PPO (Janssen, mw 1000), and PVP (Kollodion-90, BASF, purified[12]) were used.
(10) Brackman, J. C.; Engberts, J. B. F. N. *J. Colloid Interface Sci.* **1989**, *132*, 250.
(11) Witte, F. M.; Buwalda, P. L.; Engberts, J. B. F. N. *Colloid Polym. Sci.* **1987**, *265*, 42.
(12) Fadnavis, N. W.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1984**, *106*, 2636.
(13) (a) Whether the decrease of the viscosity (Figure 1; power law index n = 0) is due to a uniform structural change or a structural change only near the wall of the vessel ("effective slip at the wall")[14] has yet to be determined. Similar behavior, though in a different range of shear rates, has recently been reported; see: Strivens, T. A. *Colloid Polym. Sci.* **1989**, *267*, 269. (b) The CTAB/NaSal, CTAB/NaSal/PVP, and CTAB/NaSal/PEO solutions exhibit marked thixotropy[6] (Figure 1, inset) and rheopexy,[6] which also originates from shear-induced changes in the internal structure of the liquid. Similar effects are found for the CTAB/NaSal solutions containing EtOH or *t*-BuOH.

0002-7863/90/1512-872$02.50/0