A strong steric interaction of the groups within the carbonyl ylide should lead to twisting of the structure away from the planar conformation, which is presumably the most stable conformer when steric effects are absent. Indeed a twisted structure has been detected by X-ray crystallography in the case of a substituted thiocarbonyl ylide.[25]

The structures of carbonyl ylides have been the subject to numerous theoretical investigations.[26] In one of the most recent[4] the effects of twisting on the relative energies of the molecular orbitals was studied in detail. It was concluded that twisting reduced the energy gap between the highest occupied molecular orbital and the lowest unoccupied molecular orbital. This suggests that a red shift in the absorption spectrum of carbonyl ylides should be detected with increasing twisting induced by steric crowding. Such an effect was observed in this work since the absorption spectrum of the carbonyl ylide obtained with cycloheptanone was substantially red shifted with respect to that formed from cyclobutanone.

Other Carbenes. It is important to note that the reactions discussed above are unlikely to be a unique property of fluorenylidene. Indeed, diphenyl carbene has been observed to react with benzophenone at −196 °C in a *tert*-butyl alcohol glass to give the corresponding carbonyl ylide which on warm up yields tetraphenyloxirane.[27] Moreover, there seem to be other interesting pathways which lead to the formation of carbonyl ylides. For example, ylides have been detected in flash photolysis experiments on the cyclization of aryl vinyl ethers to dihydrofurans.[28]

(25) Arduengo, A. J.; Burgess, E. M. *J. Am. Chem. Soc.* **1976**, *98*, 5021–5023.
(26) For a review of the literature see ref 4.
(27) Tomioka, H.; Miwa, T.; Suzuki, S.; Izawa, Y. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 753–756.

Summary

In this work we have demonstrated that carbonyl ylides could be generated via the addition of fluorenylidene to ketones. The rate constant for the formation of the acetone ylide was 1×10^7 M^{-1} s^{-1} and its decay yields the corresponding oxirane. The decay kinetics were unaffected by the concentration of the ketone; hence the reverse reaction of further reaction with the ketone did not compete with ring closure. The activation energy for the ring closure reaction which presumably requires some C–O bond rotation was 10.96 kcal/mol^{-1} with an *A* factor of $10^{13.26}$ s^{-1}. The carbonyl ylides reported here could be efficiently quenched by electron-withdrawing olefins and oxygen but not by methanol. This work is being extended to studies of the reactions of other carbenes with ketones.

Acknowledgment. Thanks are due to Mr. S. E. Sugamori for his technical assistance. We are also grateful to Professor G. B. Schuster for the communication of unpublished results and to Professors A. M. Trozzolo, G. W. Griffin, and N. J. Turro for their helpful comments.

Registry No. 1, 81277-93-2; **2**, 746-47-4; **3**, 3300-03-6; **4**, 1530-12-7; **5**, 2071-44-5; **6** (R = Me), 81277-94-3; DAF, 832-80-4; acetone, 67-64-1; acetone-d_6, 666-52-4; methyl ethyl ketone, 78-93-3; cyclobutanone, 1191-95-3; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; cycloheptanone, 502-42-1; methyl *tert*-butyl ketone, 75-97-8; fluorenylidene, 2762-16-5; 9-fluorenyl radical, 2299-68-5.

(28) Wolff, T. *J. Org. Chem.* **1981**, *46*, 978–983.

Novel Conformational Distributions of Methylproline Peptides

Norma G. Delaney† and Vincent Madison*

Contribution from the Department of Medicinal Chemistry, University of Illinois at the Medical Center, Chicago, Illinois 60680. *Received February 1, 1982*

Abstract: Steric interactions of strategically placed methyl substituents can produce novel conformational effects. Herein, we have determined the main features of the conformational distributions in solution of *N*-acetyl, *N'*-methylamide derivatives of 2-methylproline, *anti*-3-methylproline, and *syn*-3-methylproline (anti, the methyl group is on the opposite side of the proline ring from the carboxamide; syn, on the same side). For the 3-methylproline peptides, there is about 25% cis peptide bond isomer (acetyl methyl cis to C^α) in water and 15–20% cis in chloroform as observed for other proline peptides. For Ac-2-MeProNHMe, there is no cis isomer detected in any solvent. The cis isomer is destabilized by steric interactions between the 2-methyl and the acetyl methyl groups. The conformational states of Ac-*anti*-3-MeProNHMe are nearly identical with those of AcProNHMe. The C_7 conformer dominates in nonpolar solvents; there is a mixture of C_7, α_R, and P_{II} in acetonitrile; and P_{II} dominates in water. For Ac-*syn*-3-MeProNHMe, C_7 is destabilized by steric interactions between the methyl group and the proline carbonyl oxygen. There is little C_7 conformer in chloroform. Although there is a substantial population of C_7 in carbon tetrachloride, the intramolecular hydrogen bond is weaker than usual. In contrast, for Ac-2-MeProNHMe, the hydrogen bond in the C_7 conformer is unusually strong. Significant amounts of this intramolecularly hydrogen-bonded conformer seem to be retained in aqueous solution. The C_7 conformer may be stabilized both by steric effects and by shielding of the peptide hydrogen bond from solvent.

Local interactions between an amino acid side chain and the adjacent peptide groups are a dominant conformational determinant.[1] However, the conformational preferences of the common amino acids are not absolute; a number of conformers are possible for each. In polymers and proteins, long-range cooperative effects stabilize unique conformers. Since such effects are not possible for oligopeptides, they often populate mixtures of conformers in solution.

Naturally occurring oligopeptides often contain constraints that restrict their conformational freedom.[2] Restrictions may be introduced by means of amino acids with cyclic side chains and/or by methyl substitution in the backbone or the side chain. Of

Table I. Nomenclature and Approximate Dihedral Angles for Proline Peptide Conformers

conformer	ϕ, ψ, deg	polymer and protein structure
P_{II}	−80, 150	polyproline II
C_7	−80, 80	γ turn
α_R	−80, −50	right-handed α helix

* Address correspondence to this author at the Department of Physical Chemistry, Hoffman-LaRoche Inc., Nutley, New Jersey 07110.
† Present address: Department of Organic Chemistry, Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08540.

(1) Anfinsen, C. B.; Scheraga, H. A. *Adv. Protein Chem.* **1975**, *29*, 205–300.

0002-7863/82/1504-6635$01.25/0

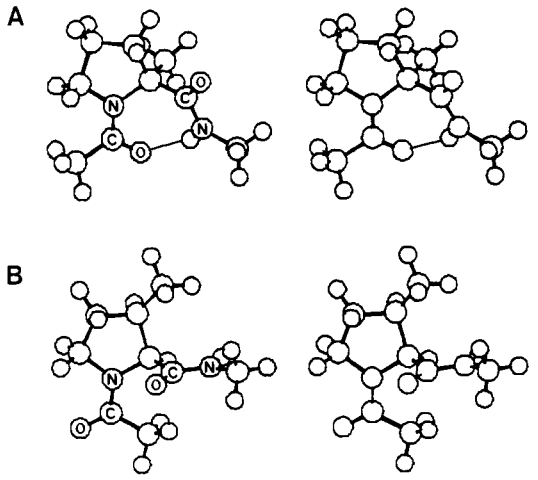

Figure 1. Stereo views of *N*-acetyl, *N'*-methylamide derivatives of two methylprolines. (A) C_7 conformer of Ac-L-2-MeProNHMe. The 3→1 hydrogen bond is indicated by a light line. The trans isomer of the acetyl–proline peptide bond is a prerequisite for this conformer. The five-membered ring exhibits form-A puckering. (B) cis isomer of acetyl–proline peptide bond for Ac-L-*syn*-3-MeProNHMe (syn, the methyl group and the carboxamide are on the same side of the proline ring; anti, they are on opposite sides). In this conformer, ψ = 150°. Form B ring-puckering is shown.

course, similar constraints have been incorporated into synthetic peptides.

We have reported conformational data on the model peptide *N*-acetyl-L-proline *N'*-methylamide (AcProNHMe).[3] From this report and some more recent data,[4] the following conclusions can be drawn: (1) the intramolecularly hydrogen-bonded C_7 conformer predominates in nonpolar solvents, (2) there are roughly equal amounts of three conformers in acetonitrile, and (3) the P_{II} conformer predominates in dimethyl sulfoxide and water. (See Table I for the definition of conformers.) This conformational information allows us to infer the importance of nonspecific peptide–solvent interactions in acetonitrile and of specific peptide–solvent binding in water and dimethyl sulfoxide.

Herein, we investigate novel conformational distributions of proline peptides that are constrained by methyl substituents in the side chain. The methyl substituents alter the conformational distributions by steric interactions with the peptide groups and, perhaps, by altering solvation of the peptide groups. Conformational results will be reported for *N*-acetyl, *N'*-methylamide derivatives of three methylprolines: 2-MePro, *syn*-3-MePro, and *anti*-3-MePro (see Figure 1 for the definition of the substituent and peptide-bond isomer nomenclatures). The steric bulk of the 2-methyl substituent may destabilize the P_{II} region and stabilize the intramolecular hydrogen bond in the C_7 conformer by shielding it from solvent. The syn 3-methyl group should destabilize the C_7 conformer due to steric interactions with the carbonyl oxygen. As the anti 3-methyl group is not expected to alter backbone conformations, Ac-*anti*-3-MeProNHMe indicates which effects are simply due to the presence of a methyl substituent. Conformational influences of methylprolines in oligopeptides can be inferred from the results obtained. Our results will also aid in the assessment of the relative importance of intramolecular peptide interactions vs. peptide–solvent interactions in stabilizing particular conformers.

Experimental Section

Synthetic Methods. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at ambient temperature (25 ± 3 °C) at concentrations of 10–20 mg/mL. Analyses were performed by Micro-Tech Laboratories, Skokie, IL, and unless noted otherwise agreed within ±0.4% of the calculated values.

Table II. Analytical Data for AcMeProNHMe

peptide	recryst solvent	mp, °C	$[\alpha]_D$,[a] deg	analyses
2-MePro	EtOAc–pet. ether[b]	127–130.5		C, H, N
L-2-MePro	EtOAc–pet. ether	159.5–161	−58.1	C, H, N
anti-3-MePro		oil		*c*
L-*anti*-3-MePro	Et_2O–pet. ether	62.5–67.5	−49.2	C, H, N
syn-3-MePro	EtOAc	112–114.5		C, H, N
D-*syn*-3-MePro	EtOAc–pet. ether	96.5–101	−9.92	C, H, N

[a] Determined in methanol. [b] Petroleum ether. [c] Anal. Calcd for $C_9H_{16}N_2O_2$: C, 58.67; H, 8.75; N, 15.20. Found: C, 56.24; H, 8.89; N, 14.17.

Racemic 2-MePro was prepared according to Ellington and Honigberg[5] and resolved by the procedure of Overberger and Jon.[6] The stereospecific synthesis of Kollonitsch et al.[7] was utilized for the preparation of L-*anti*-3-MePro. *N*-Ts-*syn*-3-MePro was prepared by the method of Meinwald and Ottenheym;[8] its resolution and conversion to D-*syn*-3-MePro are described below. *N*-Ts-*anti*-3-MeProOEt, a side product in the synthesis of *N*-Ts-*syn*-3-MePro, was separated by using the preferential saponification procedure of Mauger et al.[9] Racemic *syn*-3-MePro and *anti*-3-MePro were obtained by the detosylation procedure described below.

N-Acetyl *N'*-methylamide derivatives of the methylprolines were prepared by the general procedure described previously[10] and were judged homogeneous by thin-layer chromatographic analysis, as well as by their proton and carbon-13 NMR spectra. Physical constants are given in Table II.

(+)-*N*-Ts-D-*syn*-3-MePro. A solution of 11.42 g (35.2 mmol) of quinine in 175 mL of warm acetone was added to a warm solution of 10.0 g (35.2 mmol) of racemic *N*-Ts-*syn*-3-MePro in 100 mL of acetone, and the resulting solution was concentrated (with heating) to 200 mL. Upon standing at room temperature overnight, 10.09 g of a powdery white solid separated. Two recrystallizations from acetone yielded 6.32 g (59%) of a salt: mp 187–188.5 °C; $[\alpha]_D$ −43.5° (methanol).

This salt was suspended in 1 N NH_4OH (50 mL) at 5 °C, and 25 mL of chloroform was added. After the solution was stirred for 1 h at 10–15 °C, the mixture was transferred to a separatory funnel and the aqueous layer was separated. The chloroform layer was extracted with 1 N NH_4OH (3 × 5 mL), and the combined aqueous extracts were backwashed with chloroform and then acidified (concentrated HCl). The resulting white precipitate was extracted into ethyl acetate, and the ethyl acetate solution was washed with water, dried (anhydrous Na_2SO_4), and evaporated to give 2.58 g (89%) of a white crystalline solid, mp 155–158 °C. One recrystallization from ethyl acetate afforded the desired product as chunky white needles: mp 160–162 °C (softening at 156 °C); $[\alpha]_D$ +72.2° (methanol). Anal. ($C_{13}H_{17}NO_4S$) C, H, N.

(−)-*N*-Ts-L-*syn*-3-MePro. The filtrate from the initial crystallization of *N*-Ts-D-*syn*-3-MePro quinine salt was concentrated to approximately 100 mL, and 100 mL of ether was added. After the solution stood at room temperature overnight, 9.48 g of colorless crystals were collected. Two recrystallizations from acetone–ether (2:1) furnished 6.01 g (56%) of colorless crystals: mp 167.5–168.5 °C, $[\alpha]_D$ −158.5° (methanol).

Liberation of the free acid as described above gave 2.39 g of a white crystalline solid, mp 154–160 °C. One recrystallization from ethyl acetate yielded chunky white needles: mp 159–162 °C; $[\alpha]_D$ −71.6° (methanol). Anal. ($C_{13}H_{17}NO_4S$) C, H, N.

D-*syn*-3-MePro. *N*-Ts-D-*syn*-3-MePro (2.5 g, 8.82 mmol) and phenol (1.7 g, 18.1 mmol) were dissolved in 15 mL of 34% HBr in glacial acetic acid solution and allowed to stand tightly stoppered at room temperature.[11] After 24 h, the solvent was evaporated. The residue was taken up in ether and evaporated several times to remove excess HBr. The syrupy residue was added dropwise with vigorous stirring to 300 mL of anhydrous ether, and the slightly red-tinged precipitate that resulted was

(2) (a) Iitaka, Y. In "Bioactive Peptides Produced by Microorganisms"; Umezawa, H., Takita, T., Shiba, T., Eds.; Kodansha Ltd.: Tokyo, 1978; pp 153–182. (b) Bycroft, B. W.; Wels, C. M. In "Amino Acids, Peptides and Proteins"; Sheppard, R. C., Ed.; Burlington House: London, 1976; Vol. 8, pp 310–337.
(3) Madison, V.; Kopple, K. D. *J. Am. Chem. Soc.* **1980**, *102*, 4855–4863.
(4) Kopple, K. D., unpublished nuclear Overhauser enhancement data.
(5) Ellington, J. J.; Honigberg, I. L. *J. Org. Chem.* **1974**, *39*, 104–106.
(6) Overberger, C. G.; Jon, Y. S. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 1413–1421.
(7) Kollonitsch, J.; Scott, A. N.; Doldouras, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3624–3626.
(8) Meinwald, J.; Ottenheym, H. C. J. *Tetrahedron* **1971**, *27*, 3307–3315.
(9) Mauger, A. B.; Irreverre, F.; Witkop, B. *J. Am. Chem. Soc.* **1966**, *88*, 2019–2024.
(10) Delaney, N. G.; Madison, V. *Int. J. Pept. Protein Res.* **1982**, *19*, 543–548.
(11) Weisblat, D. I.; Magerlein, B. J.; Myers, D. R. *J. Am. Chem. Soc.* **1953**, *75*, 3630–3632.

Table III. Fraction of Cis Peptide-Bond Isomer for Ac-X-NHMe

peptide (X)	% cis[a] chloroform[b]	water[c]
2-Me	0	0
syn-3-Me	22	27
anti-3-Me	15	23
Pro	15	24

[a] Estimated from average ratio of peak areas for each proline ring carbon, error ±4%. [b] Concentrations 0.05–0.10 M. [c] Concentrations 0.10–0.20 M.

collected yielding 1.78 g of D-*syn*-3-MePro·HBr.

A concentrated aqueous solution of the salt was applied to a column of Dowex MSC-1 (H^+ form). The column was washed with water until the effluent was no longer acidic and then slowly eluted with 1N NH_4OH. Evaporation of the NH_4OH effluent yielded 1.06 g (93%) of D-*syn*-3-MePro as a white crystalline solid. Recrystallization from ethanol–ether yielded material with the following characteristics: mp 221–224 °C, $[\alpha]_D$ +68.4 (water); [lit.[7] mp 227–230 °C, $[\alpha]_D$ +69.8° (water)].

Spectroscopic Methods. Proton (60 MHz) and most carbon-13 (15 MHz) nuclear magnetic resonance (NMR) spectra were measured on a Varian T-60 spectrometer equipped with a Nicolet pulse-transform accessory. Additional carbon-13 spectra (45 MHz) were obtained on a Bruker CXP-180 spectrometer. Carbon-13 resonances of the methylproline peptides were assigned by analogy to proline peptides and through the use of off-resonance decoupling. The multiplicities of the partially coupled peaks were observed, and the decoupling frequencies for the carbon resonances were correlated with the frequencies of the proton resonances of the peptides. Circular dichroism (CD) spectra were measured at 25 °C on a Jasco J-40A spectrometer, which is interfaced to a Data General Nova 2 computer for data acquisition and signal averaging. CD spectra were obtained for Ac-D-*syn*-3-MeProNHMe. The reported values were obtained by changing the sign of the molar ellipticity so that all spectra are for L enantiomers. Fourier-transform infrared spectra were measured at 20 °C on a Nicolet Model 7199 spectrometer.

Spectrophotometric grade solvents were used. The chloroform used for CD measurements was stabilized with 0.03% hydrocarbon. The deuteriochloroform had no added stabilizer. Neither of these solvents contained ethanol. The carbon-13 NMR measurements reported in Table V were made in deuteriochloroform, but those of Figure 2 in ethanol-stabilized chloroform.

Theoretical Methods. Intramolecular conformational energies were computed by the consistent force field (CFF) method of Lifson and co-workers[12] using parameters that they have reported.[13] In this method the covalent skeleton is allowed complete flexibility within the constraints of the force field. We computed energies at 10° increments in ϕ and ψ by means of an artificial potential that constrains these dihedral angles. Full 360° rotation was considered for ψ. The ϕ values included the range of ±30–40° from the minimum for each of the two forms of ring-puckering.[14] Populations in each of the major conformational regions were computed from the CFF energies by using the Boltzmann distribution. Partition functions were computed as the sum of the Boltzmann terms. CFF energies were computed with dielectric constants of unity and infinity; these will be referred to as total energy and steric energy, respectively.

Results and Discussion

Carbon-13 NMR. Two resonances are observed for each carbon (except two of the methyls) for Ac-*anti*-3-MeProNHMe (Figure 2). By analogy of the chemical shift pattern to that of Ac-ProNHMe,[3,15] the minor resonances can be assigned to the cis peptide-bond isomer and the major ones to the trans isomer. For the acetyl, methylamide derivatives of proline, *syn*-3-methylproline, and *anti*-3-methylproline, there is about 25% cis in water and 15–20% cis in chloroform (Table III). Percentages in other solvents fall within these ranges. Ac-2-MeProNHMe is exceptional; no cis peptide isomer is detected in any solvent.

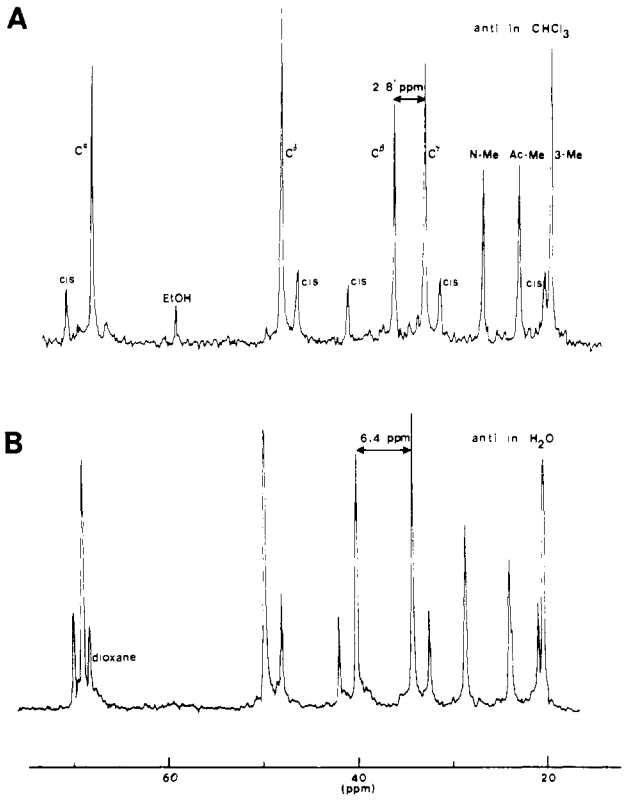

Figure 2. Carbon-13 NMR spectra at 15 MHz for Ac-*anti*-3-MeProNHMe. Assignments, resonances for cis peptide-bond isomers, and the chemical shift separation between C^β and C^γ ($\Delta\delta_{\beta\gamma}$) are indicated. (A) 0.25 M peptide in chloroform containing 0.75% ethanol as a stabilizer. (B) 0.65 M peptide in water containing 1% dioxane as an internal reference.

Table IV. Carbon-13 Chemical Shifts of Ac-X-NHMe in Water

carbon	peptide isomer	chemical shift, ppm[a] Pro	2-MePro	*anti*-3-MePro	*syn*-3-MePro
ring-Me	trans		21.24	18.01	14.66
	cis			18.57	
Ac-Me	cis	21.99			
	trans	22.26	23.40	22.15	22.03
C^γ	cis	23.28		30.94	31.00
	trans	24.90	23.73	32.77	32.61
N-Me	cis & trans	26.71	27.19	26.80	26.48
C^β	trans	30.73	40.34	39.15	36.36
	cis	32.35		40.92	38.08
C^δ	cis	47.88		46.92	47.32
	trans	49.50	50.36	48.74	48.78
C^α	trans	61.21	67.84	68.21	64.68
	cis	62.77		69.60	66.44
C=O			172.85	173.80	173.84
			177.60	175.20	

[a] Referenced to internal *p*-dioxane assumed to be at 67.41 ppm.

Chemical shifts for the methylproline peptides in aqueous solution are given in Table IV. Values for the *N*-methyl and acetyl-methyl resonances are nearly constant for the four peptides. For Ac-2-MeProNHMe, C^α and C^β experience large downfield shifts relative to the corresponding resonances of AcProNHMe, while C^γ has a small upfield shift. For the 3-methylproline peptides, C^α, C^β, and C^γ all undergo substantial downfield shifts in both cis and trans peptide isomers. The changes in chemical shifts for the methylproline peptides are in accord with generalizations that have been made for the effect of methyl substitution in amino acid side chains.[16] There are smaller chemical shift

(12) (a) Warshel, A.; Lifson, S. *J. Chem. Phys.* **1970**, *53*, 582–594. (b) Warshel, A.; Levitt, M.; Lifson, S. *J. Mol. Spectrosc.* **1970**, *33*, 84–99.
(13) (a) Hagler, A. T.; Huler, E.; Lifson, S. *J. Am. Chem. Soc.* **1974**, *96*, 5319–5327. (b) Hagler, A. T.; Lifson, S. *Ibid.* **1974**, *96*, 5327–5335. (c) Hagler, A. T.; Leiserowitz, L.; Tuval, M. *Ibid.* **1976**, *98*, 4600–4612.
(14) Madison, V. *Biopolymers* **1977**, *16*, 2671–2692.
(15) Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379–2383.
(16) Deslauriers, R.; Smith, I. C. P. In "Topics in Carbon-13 NMR Spectroscopy"; Levy, G., Ed.; Wiley-Interscience: New York, 1976; pp 1–80.

Table V. $^{13}C^{\beta}$-$^{13}C^{\gamma}$ Chemical Shift Difference for Ac-X-NHMe in Various Solvents

	$\Delta\delta_{\beta\gamma}$, ppm[a]						
	2-MePro	*syn*-3-MePro		*anti*-3-MePro		Pro[b]	
solvent	trans	trans	cis	trans	cis	trans	cis
$CHCl_3$	15.37	3.88	6.85	2.42	9.49	2.01	9.06
dioxane	16.05	3.90	6.91	3.97	9.61	3.13	9.03
CH_3CN	16.62	3.42	7.01	5.42	9.77	4.72	9.08
CH_3OH	16.69	3.83	7.37	6.02	10.09	5.52	9.28
H_2O	16.61	3.75	7.08	6.38	9.98	5.81	9.05

[a] Concentrations 0.05–0.10 M in chloroform and ca. 0.5 M in other solvents. [b] From ref 3.

differences between *syn*- and *anti*-Ac-3-MeProNHMe. The C^{α}, C^{β}, and 3-methyl resonances are about 3 ppm upfield for Ac-*syn*-3-MeProNHMe relative to the anti isomer.

The chemical shift difference between C^{β} and C^{γ}, $\Delta\delta_{\beta\gamma}$, has been shown to be correlated with the ψ angle in proline peptides.[3,17] This effect is primarily due to an upfield shift of C^{β} when it is eclipsed by the carbonyl oxygen in the C_7 conformer. $\Delta\delta_{\beta\gamma}$ increases by about 4 ppm for AcProNHMe and Ac-*anti*-3-MeProNHMe when this eclipsing is relieved by conformational changes. The C_7 conformer is precluded when the peptide bond preceding proline is cis. $\Delta\delta_{\beta\gamma}$ undergoes little variation with solvent for this fraction of the population (Table V, Figure 2). In the absence of the C_7 conformer, $\Delta\delta_{\beta\gamma}$ is about 3 ppm smaller for trans than cis peptide-bond isomers in AcProNHMe and Ac-*anti*-3-MeProNHMe.

Since C^{β} and C^{γ} are shifted downfield by comparable amounts due to the methyl substituent, $\Delta\delta_{\beta\gamma}$ observed for Ac-*anti*-3-MeProNHMe are comparable to those for AcProNHMe for both cis and trans peptide isomers in all solvents. Small values in chloroform indicate the C_7 conformer. Increases in more polar solvents indicate progressive depopulation of C_7 (Table V, Figure 2). For Ac-*syn*-3-MeProNHMe, $\Delta\delta_{\beta\gamma}$ varies little with solvent for either cis or trans isomers. Because C^{β} is about 3 ppm upfield in this peptide compared to the anti isomer, $\Delta\delta_{\beta\gamma}$ values for cis isomers in all solvents and the value for the trans isomer in water are about 3 ppm smaller for Ac-*syn*-3-MeProNHMe than the corresponding values for the anti isomer. We conclude that there is little change in $\Delta\delta_{\beta\gamma}$ due to conformational effects for trans Ac-*syn*-3-MeProNHMe in any of the solvents of Table V and hence little C_7 conformer.

For Ac-2-MeProNHMe, $\Delta\delta_{\beta\gamma}$ is much larger due to chemical shifts caused by the methyl substituent. The variation in going from chloroform to water is only 1.2 ppm compared to 3.8 ppm for AcProNHMe and 4.0 ppm for Ac-*anti*-3-MeProNHMe. We infer that there is less conformational variation with solvent for Ac-2-MeProNHMe. In this case there are no values from the cis isomer for comparison, so that these data are insufficient to define the conformation of Ac-2-MeProNHMe.

In chloroform solutions, our measurements covered the peptide concentration range from 0.05 to 1.0 M. Although the NMR parameters showed some concentration dependence, the values at the lowest concentrations are close to estimated asymptotic limits. There is little concentration dependence in the other solvents. The reported parameters should apply to peptide monomers.

Circular Dichroism. The CD spectra of Ac-2-MeProNHMe and Ac-*anti*-3-MeProNHMe have large negative n–π^* bands near 230 nm in chloroform and cyclohexane (Figure 3, Table VI). This spectral feature is indicative of the C_7 conformer.[3,18] For Ac-*syn*-3-MeProNHMe in cyclohexane, the n–π^* band is only about one-third that of the anti diastereomer. In chloroform, the band for the syn peptide is even smaller. For the three peptides, the CD spectra in carbon tetrachloride are slightly red-shifted and increased in magnitude relative to those in cyclohexane.

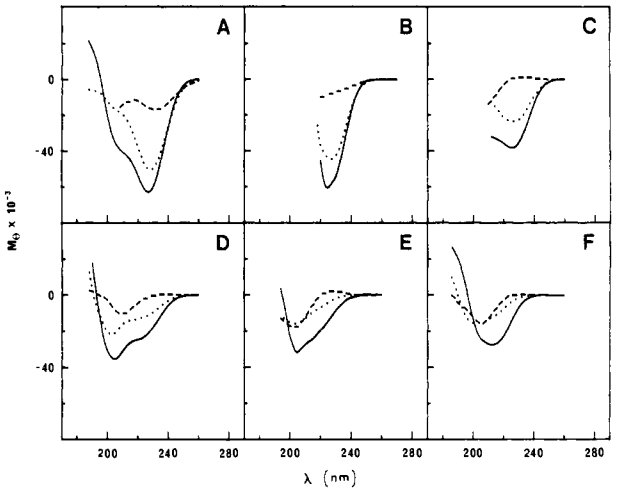

Figure 3. CD spectra of methylproline peptides in various solvents. Ac-L-2-MeProNHMe (solid line), Ac-L-*anti*-3-MeProNHMe (dotted line), and Ac-L-*syn*-3-MeProNHMe (dashed line) in (A) cyclohexane, (B) chloroform, (C) *p*-dioxane, (D) acetonitrile, (E) methanol, and (F) water.

Table VI. CD for Ac-X-NHMe in Various Solvents

	M_θ (225 nm) × 10^{-3}			
solvent	*syn*-3-MePro	*anti*-3-MePro	Pro[a]	2-MePro
cyclohexane	−15	−50		−62
chloroform	−9	−44	−48	−60
dioxane	0	−24	−31	−38
acetonitrile	−2	−12	−17	−23
methanol	+2	−4	−4	−14
water	0	−4	−4	−15

[a] From ref 3.

As the polarity of the solvent is increased, the magnitude of the n–π^* band decreases for Ac-*anti*-3-MeProNHMe. These changes exactly parallel those for AcProNHMe (Table VI). For Ac-*syn*-3-MeProNHMe, the n–π^* CD band is rapidly diminished in going from cyclohexane to chloroform to dioxane. In water, the CD spectra of *syn*- and *anti*-Ac-3-MeProNHMe are similar to each other (Figure 3) and to that of AcProNHMe (Table VI). This suggests that the P_{II} conformer predominates in aqueous solution for the 3-methylproline peptides as has been established for AcProNHMe.[3,4]

The n–π^* CD bands of Ac-2-MeProNHMe are more negative than those of the other peptides in all solvents. This is consistent with an altered conformation or a larger population for the hydrogen-bonded C_7 conformer. The unique CD spectrum in water, especially the negative ellipticity at 215 nm, may indicate retention of a significant fraction C_7.

With one exception, observed molar ellipticities did not change upon 10–100-fold dilution down to concentrations of 2×10^{-5} M in chloroform and 2×10^{-6} M in cyclohexane. This indicates that peptide aggregates do not make significant contributions to the CD spectra. The exceptional case, Ac-*syn*-3-MeProNHMe in carbon tetrachloride, showed substantial changes in molar ellipticities at concentrations above 5×10^{-4} M. These data could be fitted assuming an equilibrium between monomer and dimer (Figure 4). Least-squares analysis yielded an association constant of 360 M^{-1} and molar ellipticities of −8800° for the monomer and −60° for the dimer at 248 nm. From this association constant and additional data, ellipticities of −23 000° for the monomer and +9200° for the dimer at 236 nm were obtained. For comparison, −51 000° is observed for Ac-*anti*-3-MeProNHMe at 236 nm in carbon tetrachloride.

Infrared Spectra. For Ac-*anti*-3-MeProNHMe and Ac-ProNHMe in dilute carbon tetrachloride solutions, 95% of the

(17) (a) Siemion, I. Z.; Wieland, Th.; Pook, K. H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 702–703. (b) Deber, C. M.; Madison, V.; Blout, E. R. *Acc. Chem. Res.* **1976**, *9*, 106–113. (c) Gierasch, L. M.; Deber, C. M.; Madison, V.; Niu, C.-H.; Blout, E. R. *Biochemistry* **1981**, *20*, 4730–4738.

(18) Madison, V. *Biopolymers* **1973**, *12*, 1837–1852.

Table VII. Infrared Bands for NH Stretch of Ac-X-NHMe

peptide (X)	solvent	concn, M	ν_{free}, cm^{-1}	ν_{bound}, cm^{-1}	% free[a]	% bound
2-MePro	CCl_4	3.6×10^{-5}	3484	3294	5	95
	$CDCl_3$	3.6×10^{-4}	3482, 3465	3300	20	80
	CH_3CN	1.4×10^{-2}	3417	3300	70	30
Pro	CCl_4	3.9×10^{-5}	3454	3327	5	95
	$CDCl_3$	3.2×10^{-4}	3454	3333	15	85
	CH_3CN	1.4×10^{-2}	3401	3330	65	35
anti-3-MePro	CCl_4	3.6×10^{-5}	3460, 3448	3327	10	90
	$CDCl_3$	4.1×10^{-4}	3454	3333	25	75
syn-3-MePro	CCl_4	3.8×10^{-5}	3462	3360	40	60
	$CDCl_3$	3.6×10^{-4}	3456	3360	85	15
	CH_3CN	1.8×10^{-2}	3401		95	5

[a] From relative areas of absorption bands. Estimated error ±5%.

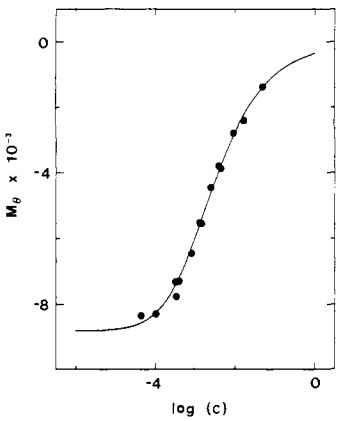

Figure 4. Molar ellipticity at 248 nm for Ac-L-*syn*-3-MeProNHMe in carbon tetrachloride vs. the logarithm of the molar concentration. The circles are experimental points. The line is drawn from parameters giving the best least-squares fit to the data assuming an equilibrium between monomer and dimer.

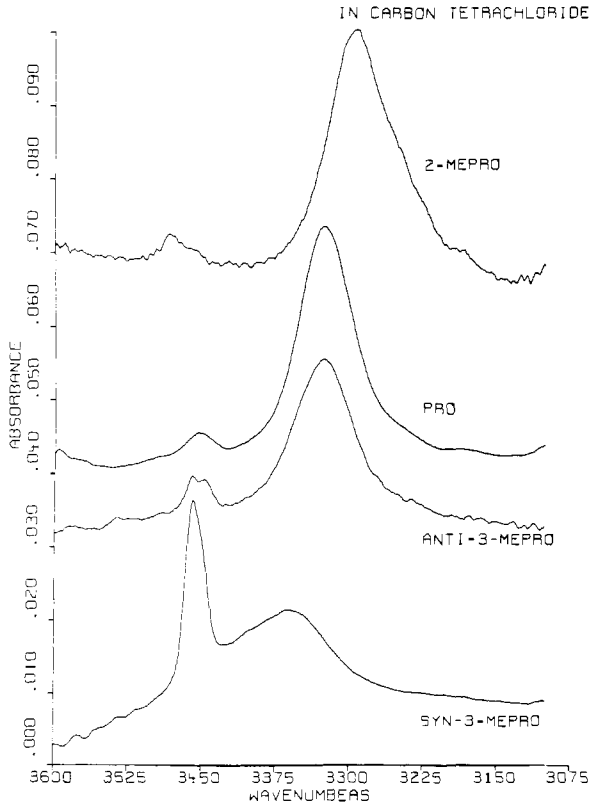

Figure 5. NH stretching region of infrared spectra for Ac-X-NHMe in carbon tetrachloride with a 10-cm cell with peptide concentrations ca. 4×10^{-5} M. The spectra have been offset vertically but are all on the same scale. For Ac-*syn*-3-MeProNHMe, the area from 3230 to 3430 cm^{-1} was assigned to the hydrogen-bonded NH and that from 3430 to 3550 cm^{-1} to the free NH.

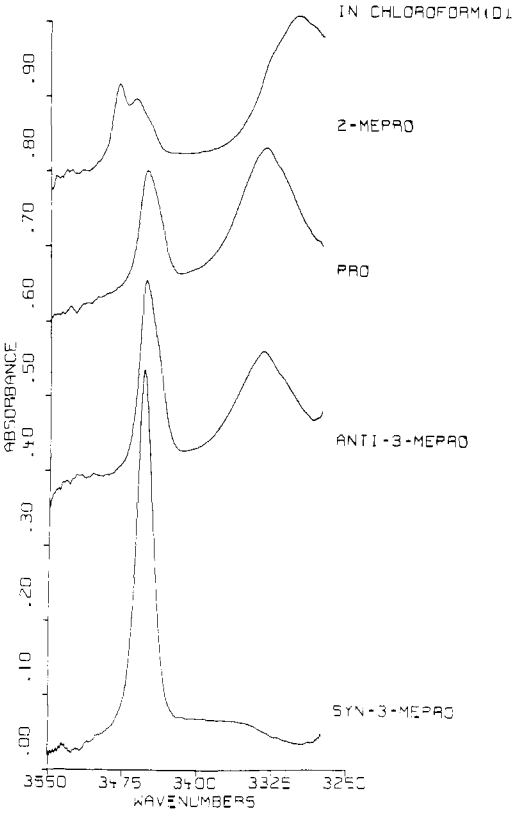

Figure 6. NH stretching region of infrared spectra for Ac-X-NHMe in chloroform (D) with a 10-cm cell with peptide concentrations ca. 4×10^{-4} M. The spectra have been offset vertically but are all on the same scale. The spectra are truncated at 3275 cm^{-1} due to high solvent absorbance. The area of the hydrogen-bonded NH band for Ac-2-MeProNHMe was estimated by doubling the area from 3300 to 3400 cm^{-1}.

integrated intensity of the absorption from the NH stretch is in a broad band at 3327 cm^{-1} (Figure 5, Table VII). The remaining 5% of the intensity is in narrower bands near 3455 cm^{-1}. In pioneering work on AcProNHMe, Tsuboi et al.[19] established that the major, broad band is due to the NH in the intramolecularly hydrogen-bonded C_7 conformer while the higher frequency band arises from non-hydrogen-bonded conformers. For Ac-2-MeProNHMe, the C_7 conformer also dominates. For this peptide, the hydrogen-bonded NH band occurs at lower frequency (3294 cm^{-1}), indicating a stronger hydrogen bond. The free NH occurs at higher frequency (3484 cm^{-1}). In contrast, for Ac-*syn*-3-MeProNHMe, the population of the C_7 conformer is reduced to 60%. The high frequency (3360 cm^{-1}) of the hydrogen-bonded NH indicates a weak hydrogen bond. The free NH is at 3462 cm^{-1}, similar to the frequencies observed for Ac-*anti*-3-MeProNHMe and AcProNHMe.

(19) Tsuboi, M.; Shimanouchi, T.; Mizushima, S. *J. Am. Chem. Soc.* **1959**, *81*, 1406–1411.

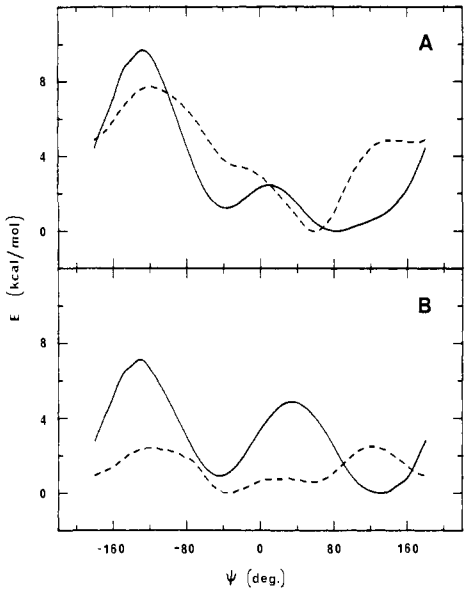

Figure 7. Intrapeptide potential energies computed by the CFF method for the trans peptide-bond isomers of Ac-L-2-MeProNHMe (dashed line) and Ac-L-*anti*-3-MeProNHMe (solid line). For each ψ, the plotted energy is the minimum with respect to all other degrees of freedom, including ϕ and the type of ring-puckering. (A) Total energy ($\epsilon = 1$). (B) Steric energy ($\epsilon = \infty$).

The infrared spectra of the peptides in chloroform are similar to those in carbon tetrachloride except that the population of C_7 conformer is reduced and the frequency of the hydrogen-bonded NH band increases by six wavenumbers (Figure 6, Table VII). For Ac-2-MeProNHMe, AcProNHMe, and Ac-*anti*-3-MeProNHMe, about 80% is in the C_7 conformer. However, for Ac-*syn*-3-MeProNHMe, only 15% remains in this hydrogen-bonded conformer.

In acetonitrile solutions, the population of C_7 conformer is further reduced to about 30% for Ac-2-MeProNHMe and AcProNHMe (Table VII). For Ac-*syn*-3-MeProNHMe, the amount of hydrogen-bonded NH is comparable to the experimental error (ca. 5%). The free NH band is near 3400 cm^{-1} and much broader than in the less polar solvents. This is apparently due to interactions of the NH with acetonitrile.

Conformational Energies. Intramolecular potential energies, computed by the CFF method, show the same dependence on backbone conformation for Ac-*anti*-3-MeProNHMe as for AcProNHMe. The total energy for Ac-2-MeProNHMe has a deeper well at C_7 and the minimum is shifted about 20° to smaller ψ (Figure 7). For this peptide, both the P_{II} and α_R regions have been destabilized. Considering only the steric energy, the P_{II} region becomes favored for Ac-*anti*-3-MeProNHMe. The steric energy for Ac-2-MeProNHMe is virtually constant between $\psi = -40°$ and +60°; there is little barrier separating the α_R and C_7 regions.

Populations in various conformers were computed from the Boltzmann distribution. For AcProNHMe, Ac-*anti*-3-MeProNHMe, and Ac-*syn*-3-MeProNHMe, about 75% of the population in the C_7 conformer is predicted from the total energy; about 70% in the P_{II} conformer is predicted from the steric energy (Table VIII). For Ac-2-MeProNHMe, 96% in the C_7 conformer is predicted from the total energy. From the steric energy, 37% in the α_R conformer is predicted with another 30% in the adjoining region $\psi = -20$ to +30°.

There are two major ways in which the five-membered proline ring is puckered. These have been designated form A (χ_1 ca. −30°) and form B (χ_1 ca. +30°).[20] Form A is predicted to dominate for Ac-*anti*-3-MeProNHMe and form B for Ac-*syn*-3-MeProNHMe. These predictions have been verified from the vicinal proton coupling constants in chloroform, $^3J_{HC^\alpha C^\beta H} = 3.2$ Hz for the anti diastereomer and 7.0 Hz for syn, in conjunction with a Karplus-like relationship.[21] For the other two peptides, the two forms are predicted to be more equally populated and the computed populations change with the potential function used (Table VIII).

Table VIII. Computed Conformational Distributions for Ac-X-NHMe

	% population[a]									
	P_{II}		C_7		α_R		A pucker		partition function	
peptide (X)	totl[b]	st[c]	totl	st	totl	st	totl	st	totl	st
Pro	13	*73*	*77*	18	7	8	63	84	28	16
2-MePro	0	10	*96*	17	0	*37*	13	49	7	24
anti 3-MePro	16	*71*	*76*	17	5	11	82	82	15	13
syn-3-MePro	24	*69*	*71*	25	4	5	0	2	12	12

[a] Populations given for backbone conformers within ±30° of the following ψ values, 150° for P_{II}, 80° for C_7, and −60° for α_R. For 2-MePro, the center of the C_7 region shifts to 60°. [b] From total intramolecular potential energy ($\epsilon = 1$). [c] From steric energy ($\epsilon = \infty$).

The magnitude of the partition function is indicative of the number of conformational states that contribute significantly to the Boltzmann average. For the two 3-methylproline peptides, the magnitude is little changed between the two potential energies even though the majority of the population is shifted from C_7 for the total energy to P_{II} for the steric energy. For AcProNHMe and Ac-2-MeProNHMe, the partition function increases when the population of the two forms of ring-puckering become more nearly equal, regardless of the backbone conformation (Table VIII). We infer that the configurational entropies of the C_7 and P_{II} conformers should be similar but that population of both forms of ring-puckering could make a substantial entropic contribution.

Conclusions

There is qualitative agreement of the spectroscopic methods on the populations of conformers in a particular solvent and the extent to which the populations change with solvent. From this correlation of the methods, we conclude that relative values of $\Delta\delta_{\beta\gamma}$ reflect conformational changes for both the methylproline and proline peptides.

The conformational states of Ac-*anti*-3-MeProNHMe are nearly identical with those of AcProNHMe. C_7 dominates in nonpolar solvents; there is a mixture of C_7, α_R, and P_{II} in acetonitrile; P_{II} dominates in water. For Ac-*syn*-3-MeProNHMe, the C_7 conformer is destabilized. Even in the least polar solvents, cyclohexane and carbon tetrachloride, there is no more than 60% of the population in C_7. The high infrared frequency and small magnitude of the n–π^* CD band indicate that the intramolecular hydrogen bond is weakened. Steric interactions would increase the ψ angle, thus lengthening the hydrogen bond. For Ac-*syn*-3-MeProNHMe, the C_7 conformer is depopulated upon slight increases in solvent polarity. In aqueous solution, this peptide also seems to populate the P_{II} conformer preferentially.

The low infrared frequency indicates a strengthened hydrogen bond in the C_7 conformer for Ac-2-MeProNHMe. Infrared spectra indicate that the population of C_7 conformer is comparable for Ac-2-MeProNHMe, Ac-*anti*-3-MeProNHMe, and AcProNHMe in nonpolar solvents. The magnitude of the n–π^* CD band is largest for Ac-2-MeProNHMe, indicating a shorter hydrogen bond. Steric interactions seem to be responsible for this shortening. The unique CD spectrum of Ac-2-MeProNHMe in aqueous solution may indicate that significant amounts of C_7 are retained.

The amounts of C_7 conformer predicted from total conformational energies are in good accord with experiments in all nonpolar solvents for Ac-2-MeProNHMe and Ac-*anti*-3-MeProNHMe, but only in cyclohexane and carbon tetrachloride

(20) Ramachandran, G. N.; Lakshminarayan, A. V.; Balasubramanian, R.; Tegoni, G. *Biochim. Biophys. Acta* **1970**, *221*, 165–181.

(21) Kopple, K. D.; Wiley, G. R.; Tauke, R. *Biopolymers* **1973**, *12*, 627–636.

for Ac-*syn*-3-MeProNHMe. The predicted population is greater than that observed in chloroform for the latter peptide. This overprediction could be due to underestimation of steric interactions. Perhaps for Ac-*syn*-3-MeProNHMe, peptide–solvent interactions shift the conformational distribution in chloroform relative to that in carbon tetrachloride or cyclohexane. Peptides can interact strongly with such solvents. For example, the solvation energy of *N*-butylacetamide in carbon tetrachloride is −14 kcal/mol.[22] The solvation enthalpy of Ac-*anti*-3-MeProNHMe is −4 kcal/mol in chloroform relative to carbon tetrachloride.[23] The ability of polar solvents to attenuate intramolecular electrostatic interactions can be qualitatively considered by comparing the total and steric energies. The predictions from the steric energy are in qualitative agreement with observations in aqueous solution. Nevertheless, consideration of the conformational distributions in the full range of solvents shows that polar solvents have effects in addition to attenuation of intrapeptide electrostatic interactions. Polar, aprotic acetonitrile stabilizes the α_R conformer by dipolar interactions of the peptide with bulk solvent. Water, which is more polar, does not simply increase the population of α_R via stronger dipolar interactions with the peptide. Rather, the P_{II} conformer is populated due to the preferential binding of water to the peptide in this conformation.

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Registry No. AcMeProNHMe, 82320-42-1; Ac-L-2-MeProNHMe, 83365-32-6; Ac-*anti*-3-MePrNHMe, 83365-33-7; Ac-L-*anti*-3-MePrNHMe, 83434-50-8; Ac-*syn*-3-MeProNHMe, 82320-41-0; Ac-D-*syn*-3-MeProNHMe, 83434-51-9; racemic *N*-Ts-*syn*-3-MePro, 33443-67-3; *N*-Ts-D-*syn*-3-MePro·quinine, 83434-80-4; (+)-*N*-Ts-D-*syn*-3-MePro, 83434-52-0; (−)-*N*-Ts-L-*syn*-3-MePro, 83434-53-1; D-*syn*-3-MePro·HBr, 83434-81-5; D-*syn*-3-MePro, 10512-88-6.

(22) (a) Konicek, J.; Wadso, I. *Acta Chem. Scand.* **1971**, *25*, 1541–1551. (b) Ojelund, G.; Skold, R.; Wadso, I. *J. Chem. Thermodyn.* **1976**, *8*, 45–54.
(23) Madison, V.; Delaney, N. G. *Biopolymers*, in press.

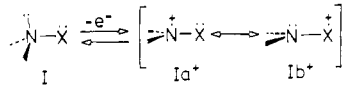
One-Electron Oxidation of Trialkylsulfenamides

Stephen F. Nelsen,* Daniel J. Steffek, Glen T. Cunkle, and Peter M. Gannett

Contribution from the S. M. McElvain Laboratories of Organic Chemistry, Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706. *Received March 29, 1982*

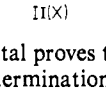
Abstract: Trialkylsulfenamides give long-lived enough radical cations for their cyclic voltammograms to be electrochemically reversible at fast enough scan rates. Radical cation lifetimes are increased by α branching in the nitrogen substituent, decreased by replacing a methyl sulfur substituent by *tert*-butyl, and decreased when pyridine is added and at higher sulfenamide concentration. For the five compounds for which hydrazine analogue (S replaced by NMe) data are available, $(CH_2)_4NSMe$ (**1**), $(CH_2)_5NSMe$ (**2**), *i*-Pr_2NSMe (**3**), 9-SMe-9-ABN (**4**), and Me_2NS-*t*-Bu (**5**), $E^{\circ\prime}$ values are 0.72, 0.81, 0.77, 0.69, and 0.95 V vs. SCE (acetonitrile), respectively, 0.45–0.58 eV more positive than $E^{\circ\prime}$ for the analogous hydrazine. The IP_1 values of **1**–**5** are 8.47, 8.42, 7.71, 7.82, and 8.34, respectively, 0.06–0.45 eV greater than for the analogous hydrazines. The nitrogen inversion barrier $\Delta G^{\ddagger}$ (T_c) for **4** is 8.7_0 (−84 °C) kcal/mol and that of 9-Et-9-ABN is 7.1_3 (−118 °C). $\mathbf{1}^{+\cdot}$ has ESR splittings of 21.7 (2 H), 18.2 (2 H), 13.8 (N), and 8.5 (3 H) G and that of $\mathbf{4}^{+\cdot}$ 14.1 (N) and 8.3 (3 H) G. The significance of these data is discussed.

Electron loss from nonconjugated amino nitrogen compounds has unusual properties because a large geometry change takes place during the electron transfer. Tetraalkylhydrazines (I, X = NR_2) have received much study.[1] Neutral hydrazines prefer



conformations with the lone-pair axes approximately perpendicular and the nitrogens approximately tetrahedral and prove to have a nitrogen inversion barrier that is very sensitive to the NN rotation angle.

Hydrazine radical cations prefer to have planar nitrogens (but their bending force constants are low) and have a two-atom, three-electron π bond (shown as two resonance forms, $Ia^{+\cdot}$ and $Ib^{+\cdot}$ in eq 1). The equilibrium constant for electron transfer (relative values conveniently determined by measuring the formal potential, $E^{\circ\prime}$ (I, $I^{+\cdot}$), by cyclic voltammetry, CV) does not depend principally upon ionization potential but on the difference in strain energy between I and $I^{+\cdot}$. The rate of electron transfer is sensitive to NN rotation angle, and the electron transfer shows non-Brønsted behavior in that a plot of $\Delta G^{\ddagger}$ vs. ΔG° for electron loss has a slope greater than one.[1c] A study of electron loss from other nonconjugated amino nitrogen compounds is plagued by short radical cation lifetimes. Loss of a proton from a carbon attached to nitrogen is so rapid that $E^{\circ\prime}$ usually cannot be measured by CV. A solution to this kinetic problem has been to use "Bredt's rule protected" R_2N groups such as the 9-azabicyclo[3.3.1]nonyl group II(X).[2] Holding the α-hydrogens in the nodal plane of



II(X)

the cation's nitrogen p orbital proves to provide powerful kinetic stabilization, allowing determination of $E^{\circ\prime}$ for X groups as noncation stabilizing as *tert*-butyl and as thermodynamically cation destabilizing as Cl and NMe_3^+. Disappointingly however, II(O-CH_3) proved to give a cation radical so short-lived that no re-

(1) (a) Nelsen, S. F. *Isr. J. Chem.* **1979**, *18*, 45. (b) Nelsen, S. F. *Acc. Chem. Res.* **1981**, *14*, 131. (c) Nelsen, S. F., Kinlen, P. J.; Evans, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 7045.

(2) Nelsen, S. F.; Kessel, C. R.; Brien, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 702.

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