hydration shell might be a subpicosecond event.[25] Just because the stabilization of the H_3O^+ in the hydration complex is of paramount importance in the dissociation event, any perturbation at this step might be crucial for the rest of the reaction to occur.

Figure 3a demonstrates that the effect of salts on the rate of proton dissociation is not a simple function of the salt concentration. The rate measured in equimolar concentrations of NaCl, LiBr, or $MgCl_2$ varied markedly. Other concentration parameters of the solution such as molality or mole fraction were of no further advantage. On the other hand, the function which reflects the properties of the solvent in the solution, the activity of H_2O, was found suitable: Experimental results obtained with the three electrolytes fit a single linear function

$$\log k' = \log k_0 - n \log a(H_2O) \quad (3)$$

where k' and k_0 are the proton transfer rates in salt solutions and in pure water, respectively.

Equation 3 is compatible with a reaction mechanism where the excited molecule transfers a proton to a hydration complex of n water molecules. Such a presentation is a gross oversimplification as n becomes a stoichiometic factor which states that no reaction will take place with the species $(H_2O)_{n-1}$ or $(H_2O)_{n+1}$. Thus, a less stringent explanation should be looked for.

Searcy and Fenn[10] and Kebarle[11] measured the clustering of water molecules around free protons in the gas phase. Clusters with varying size were observed and the respective enthalpy of formation was calculated. The difference in enthalpy of hydration of a proton vs. the cluster number n, designated as $-\Delta H^\circ_{n,n+1}$, shows a remarkable decrease while increasing the cluster number n. The hydration enthalpy difference between a monomer H_3O^+ and a dimer is $-\Delta H^\circ_{1,2}$ = 32 kcal/mol, while $\Delta H^\circ_{2,3}$ = 22 kcal/mol. These values are comparable with the results obtained by quantum-mechanical calculations.[12,13] The hydration enthalpy $-\Delta H^\circ_{n,n+1}$ is reaching a limiting value of about 10 kcal/mol when the cluster number is about 10. By analogy with these results, the enthalpy of proton hydration in solution will also increase with the size of the hydration complex. Yet, in liquid water one exception should be made: In order to increase the size of the complex by one water molecule, a water molecule must first be removed from the bulk, with energy investment of 10 kcal/mol (heat of evaporation of water). Therefore, the hydration complex of a proton will not exceed the state where the energy gain of further hydration will be comparable with the heat of evaporation. Using the results of Kebarle[11] and Searcy and Fenn,[10] we estimate that the hydrating complex in dilute electrolyte solution ($a(H_2O)$ = 1) will be of 10 water molecules, or less. The above conclusion bears directly on our observation.

In concentrated salt solutions, the vapor pressure is lower than that of pure water and hence it exhibits reduced water activity. This phenomenon is explained by the fact that a considerable fraction of the water molecules are associated with the hydration of the salt ions. The binding energy of these water molecules (which form the first and second hydration shells), to the center ion, is larger than 10 kcal/mol. Therefore, they are less likely than the free water molecules to participate in the process of the hydration of the initially formed H_3O^+. In order to obtain a proton hydrate greater than H_3O^+, the thermodynamically stable complex must be formed within the ion-pair lifetime. The depletion from the solution of water molecules available for this reaction will lower the probability of the successful dissociation. As demonstrated in Figure 5, indeed this function decreases with the activity of the water in the solution.

Finally, we wish to demonstrate the applicability of this technique for estimation of $a(H_2O)$, in contrast to the usual techniques based upon colligative properties or emf measurements of electrochemical cells. The values calculated from measurements using different proton emitters are practically identical (Figure 6) and are comparable with values calculated by vapor pressure studies (Figure 5). The applicability of this method for estimation of the equivalent water activity in the microspace of an active site of a protein has already been demonstrated.[17]

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Registry No. H_2O, 7732-18-5; 2-naphthol-6-sulfonic acid, 93-01-6; 2-naphthol-3,6-disulfonic acid, 148-75-4; 2-naphthol-6,8-disulfonic acid, 118-32-1; 8-hydroxyl-1,3,6-pyrenetrisulfonic acid, 27928-00-3; 2-naphthol, 135-19-3.

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Intramolecular Hydrogen Bonding in Gramicidin S. 2. Ornithine

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Abstract: The conformation of the ornithine side chains in gramicidin S (GrS) in solution was investigated by ^1H and ^{15}N NMR spectroscopy at 11.7 T. Rotational averaging of the chemical shifts of the Orn $C^\delta H_2$ protons was incomplete, the degree to which the apparent motility of the side chain is limited varying inversely with the ability of the solvent to compete for hydrogen-bonding (H-bonding) donor or acceptor sites. Methylation of GrS to give [2,2′-N^δ-trimethylornithyl]GrS resulted in an upfield shift of 3.5 ppm in the ^{15}N resonance of Pro in MeOH and abolished the correlation of the Orn $C^\delta H_2$ splitting with solvent basicity. The data are consistent with the presence of intramolecular Orn $N^\delta H_3^+$--O=C D-Phe H bonds, each with formation constant ~1.1 in MeOH at 23 °C, and exerting a substantial charge relay effect on the Pro ^{15}N chemical shift. Thermodynamic analysis of the Orn $C^\delta H_2$ proton splitting yielded estimates of $-\Delta H^\circ$ = 2.3 ± 0.4 kcal mol^{-1} and $-\Delta S^\circ$ = 7.5 ± 1.0 cal deg^{-1} mol^{-1} for the transition of each residue from the inter- to the intramolecularly H-bonded configuration in MeOH and +10.1 ppm for the total charge relay shifted at Pro ^{15}N. Proton exchange kinetics and NOE measurements indicate that the H bonds are formed in the $i \rightarrow i + 2$ sense. Estimates for the Orn side chain torsional angles in the intramolecularly H-bonded configuration are given, and the possible origin of the Orn $C^\delta H_2$ chemical shift inequivalence is discussed. The possible functional role of the H bonds is considered.

The cyclic decapeptide antibiotic gramicidin S (GrS; *cyclo*-[Val[1]-Orn[2]-Leu[3]-D-Phe[4]-Pro[5]]$_2$, Figure 1a) contains, as do a number of other biologically active peptides, cationic amino acid side chains which are essential for activity.[1] While considerable

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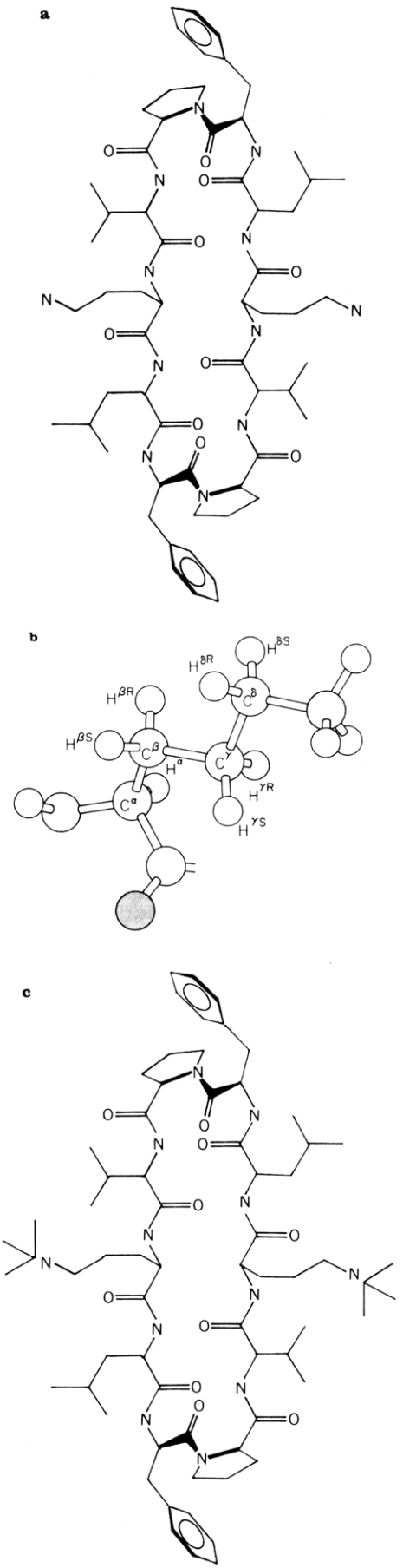

Figure 1. (a) Diagrammatic representation of the structure of gramicidin S. (b) Stereochemical labels for the protons of the ornithine side chain. (c) Diagrammatic representation of the structure for [2,2'-N^{δ}-trimethylornithyl]gramicidin S.

progress has been made toward elucidating the solution structure, particularly of the peptide backbone[2-5] and the side chains of D-Phe[6] and Pro,[7] the conformation and possible molecular interactions of Orn (Figure 1b) are uncertain. Because of the central role postulated for Orn in mechanisms proposed for the interaction of GrS with bacteria[1] and model membranes,[8] we have undertaken a study of the conformation of these residues in solution.

GrS possesses C_2 symmetry in solution on the NMR timescale.[2] The backbone is relatively rigid[9] and in all solvents thus far investigated[2-5] assumes an antiparallel β-pleated structure stabilized by two pairs of transannular hydrogen bonds (H bonds) of moderate strength.[10] Magnetization transfer experiments[6] indicate that the D-Phe-Pro sequences form type II' β turns,[11] a structural feature which also persists in different solvents.[6] Structure–activity correlations of GrS analogues have demonstrated the necessity of the rigid backbone,[12] a D-amino acid at position 4,[13] hydrophobic residues at positions 1 and 3, and cationic residues at positions 2[1] for full potency. The high activity of *enantio*-GrS argues against the existence of a specific macromolecular receptor.[8]

Earlier attempts to define the tertiary structure of Orn have been deterred by the flexibility of the aminopropyl side chain. The presence of rapid rotational isomerism in solution renders estimation of dihedral angles or interatomic distances by conventional NMR methods extremely difficult without making major assumptions concerning the rotational potential,[14] and when isomerism obtains along a flexible chain of several segments' length, the problem becomes all but intractable. The most comprehensive NMR study of the solution structure of GrS published to date[15] does not treat the side-chain dihedral angles beyond χ^1. Pachler analysis[16] of the $H^{\alpha}H^{\beta}$ vicinal coupling constants of Orn indicated a predominance (~70%) of one of the classical staggered rotamers, corresponding either to $\chi_2{}^1 = 180°$ or to $\chi_2{}^1 = -60°$,[16] but without stereoselective ^2H labeling it was not possible to distinguish between them.

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Several lines of evidence have suggested that, interestingly, there exists a preferred conformation for the distal portion of the Orn side chain. Levels of potency of GrS analogues were found to be related to the ability of spin-labeled polar side chains to achieve spatial proximity.[8] ^{13}C spin–lattice relaxation times in MeOH and Me_2SO[9] showed an appreciable decrease in apparent rotational correlation time only between C^{β} and C^{γ}; this may be contrasted, for example, to the highly mobile Lys side chain in lysine vasopressin.[17] Finally, a significant chemical shift difference between the Orn $C^{\delta}H_2$ protons was observed in MeOH[15] which can only arise from incomplete averaging about χ_2^3.

The possibility of intramolecular H bonds involving Orn $N^{\delta}H_2$ was discussed in the semiempirical calculation of Dygert et al.[18] The lowest energy conformation contained a pair of Orn $N^{\delta}H_2$--O=C D-Phe H bonds formed in the $i \rightarrow i-3$ sense, while other low energy conformations lacked the bonds. The minimization was not exhaustive for the side chains; an additional difficulty with the structures proposed by Dygert et al. is that the transannular H bonds involving Val NH and Leu C=O, later demonstrated experimentally,[10] were not predicted. The crystalline urea complex of GrS contained a single $i \rightarrow i+2$ Orn $N^{\delta}H_3^+$--O=C D-Phe H bond per molecule.[19] Here, backbone distortion induced by the urea diminished the symmetry.[19]

Whether the terminal amino groups of Orn are actually intramolecularly H bonded in solution remains to be experimentally verified. Steric factors[20] or the formation of relatively long-lived complexes with the solvent could equally well explain the apparent decrease in motility of the side chain. Selecting from these alternatives is difficult because the necessary experimental criteria are not readily available. Proton exchange rates are useful for identifying potential amide H bond donors in peptides, but model compound data are not available for amines. IR spectrophotometry should also be less helpful here than with amides, because of both the impracticality of isotopically isolating[10] the amine vibrations and the likelihood that the vibrational bands will be broadened by coupling of the NH oscillators and interactions with the solvent.[21] The study of solvent-induced NMR chemical shift changes in amide group resonances can identify solvent-shielded amide groups in peptides as long as the conformational perturbations caused by the solvents are minor. On the other hand, when the stability of an intramolecular H bond itself varies with the solvent, the variation of these chemical shifts with solvent may equal or exceed those of fully exposed amides.[22]

In view of these difficulties, we elected to begin not by attempting a direct demonstration of H bonding, but rather by investigating the unusual rotational isomerism in the Orn side chains as evidenced by the chemical shift inequivalence of the Orn $C^{\delta}H_2$ protons (ΔO^{δ}_{obsd}). It is found that ΔO^{δ}_{obsd} varies inversely with increasing solvent acidity or basicity, as expected in the case of solvent-labile side chain to backbone H bonds. This correlation is abolished when GrS is exhaustively methylated to give [2,2'-N^{δ}-trimethylornithyl]GrS (Me_6GrS; Figure 1c), a derivative in which Orn remains ionized but cannot function as an H-bond donor. Concomitantly, permethylation induces a substantial upfield shift in the Pro ^{15}N resonance in MeOH, identifying D-Phe C=O as the likely acceptor group in an intramolecular H bond to Orn $N^{\delta}H_3^+$. The thermodynamics and spatial orientation of the H bonds and the side-chain torsional angles of Orn in the intramolecularly H-bonded configuration are then investigated by NMR-based methods.

Table I. Proton NMR Chemical Shifts of GrS[a]

residue	resonance	solvent				
		Me_2SO	H_2O	MeOH	F_3EtOH	AcOH
Val	NH	7.22	7.62	7.73	7.86	7.59
	α	4.42	4.13	4.17	3.98	4.18
	β	2.08	2.16	2.26	2.37	2.26
Orn	NH	8.68	8.61	8.70	7.66	8.36
	α	4.77	4.97	4.97	5.11	5.10
	β_d	1.75	1.96	2.05	2.18	1.99
	β_u	1.60	1.66	1.64	1.65	1.75
	γ_d	1.65	1.70	1.79	1.92	1.90
	γ_u	1.65	1.70	1.79	1.81	1.84
	δ_d	2.85	3.01	3.05	3.16	3.10
	δ_u	2.75	2.99	2.91	2.94	3.10
Leu	NH	8.34	8.81	8.80	8.92	9.03
	α	4.58	4.64	4.66	4.71	4.78
	β_d	1.34	1.48	1.55	1.59	1.59
	β_u	1.34	1.41	1.41	1.55	1.51
	γ	1.41	1.38	1.50	1.51	1.51
D-Phe	NH	9.11	8.99	8.90	7.82	8.50
	α	4.36	4.67	4.50	4.53	4.53
	β_d	2.99	3.12	3.10	3.10	3.14
	β_u	2.88	2.97	2.96	3.01	3.08
Pro	α	4.32	4.40	4.35	4.29	4.66
	β_d	1.96	1.94	2.00	1.98	2.08
	β_u	1.49	1.85	1.69	1.89	1.73
	γ_d	1.52	1.69	1.71	1.80	1.70
	γ_u	1.52	1.64	1.59	1.63	1.61
	δ_d	3.60	3.68	3.73	3.77	3.76
	δ_u	2.48	2.59	2.48	2.48	2.46

[a] In ppm downfield of Me_4Si at 22 °C in the deuterated solvents; *t*-BuOH was present in the aqueous sample, and the shifts were corrected to Me_4Si.

Experimental Methods

Materials. GrS dihydrochloride (Sigma) was dissolved in H_2O–dioxane (5:2 v/v), filtered, and lyophilized prior to use. It migrated as a single spot on TLC in several solvent systems. Other chemicals were reagent grade and used without further purification.

[2,2'-N^{δ}-Trimethylornithyl]GrS Chloride (Me_6GrS). The title compound was synthesized according to a modification of the method of Granados and Bello.[23] GrS (Sigma, 3.29×10^{-4} mol) was dissolved in 10 mL of 50% aqueous EtOH containing 0.010 M $Na_2B_4O_7(H_2O)_{10}$. The solution was adjusted to glass electrode reading 10.0 on a pH stat with solvent A (50 mL of 10 N NaOH + 50 mL of EtOH, with sufficient water to obtain a single phase). Me_2SO_4 (0.0296 mol) was added and the solution maintained at an apparent pH of 10.0 with additional alkali. The initial and final delivery rates of solvent A were 35 and 5 μL min^{-1}, respectively. The solution was then diluted fourfold with 40% EtOH and chromatographed on Sephadex G-10 in 40% aqueous EtOH containing 0.020 M NH_4Cl. The protein-containing fractions were rechromatographed on Sephadex G-15 in 40% aqueous EtOH containing 1×10^{-3} M HCl. Removal of solvents yielded a white powder which on TLC migrated as a single ninhydrin-negative spot in several solvent systems. A ^1H NMR spectrum at 500.13 MHz in Me_2SO-d_6 is consistent with a single species with the structure of the title compound, containing a singlet at δ 3.15 (18 protons) assigned to Orn $N^{\delta}Me_3$, and devoid of Orn amino protons; yield, 325 mg (76%).

NMR Spectroscopy. All spectra were obtained on a Bruker WM-500 multinuclear NMR spectrometer employing an Oxford Instruments 11.7-T superconducting magnet and an Aspect 2000 computer. ^1H spectra at 500.13 MHz were obtained on 0.02 M solutions of peptide in the indicated solvent, with the exception of ^2H_2O, in which the concentration was 5×10^{-3} M. For variable-temperature runs the thermocouple was calibrated against MeOH. NOE difference spectra were acquired by alternating on- and off-resonance single-frequency CW irradiation, both with and without truncation. For ensured selectivity the decoupler power was set well below saturating levels, and the NH protons exchanged with ^2H to circumvent the problem of magnetization transfer within the peptide backbone at low temperatures.

Natural abundance ^{15}N spectra at 50.68 MHz were obtained on MeOH solutions of GrS and Me_6GrS containing 10% MeOH-d_4 for internal locking. The recycle time was 3.41 s, including a 3-s relaxation delay between pulses. Chemical shifts were measured without NOE to avoid nulling the Pro resonance.[24] There was no detectable concentration

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Table II. Proton NMR Chemical Shifts of Me_6GrS[a]

residue	resonance	solvent				
		Me_2SO	H_2O	MeOH	F_3EtOH	AcOH
Val	NH	~7.2	7.61	7.54	7.81	7.66
	α	4.40	4.04	4.10	4.01	4.28
	β	2.05	2.18	2.25	2.36	2.25
Orn	NH	8.74	8.52	8.46	7.42	8.33
	α	4.88	4.81	4.95	5.08	5.18
	β_d	1.70	1.91	1.87	1.90	1.92
	β_u	1.37	1.73	1.64	1.73	1.74
	γ_d	1.70	1.78	1.80	1.88	1.90
	γ_u	1.60	1.78	1.80	1.88	1.82
	δ_d	3.67	3.35	3.57	3.38	3.66
	δ_u	3.31	3.35	3.33	3.25	3.53
Leu	NH	8.32	8.50	8.62	8.81	8.96
	α	4.62	4.54	4.54	4.63	4.78
	β_d	1.34	1.46	1.55	1.65	1.59
	β_u	1.34	1.40	1.40	1.50	1.46
	γ	1.42	1.35	1.55	1.56	1.50
D-Phe	NH	9.17	8.99	8.75	7.61	8.64
	α	4.34	4.56	4.37	4.53	4.56
	β_d	2.95	3.03	3.06	3.05	3.12
	β_u	2.80	2.97	2.87	2.89	3.05
Pro	α	4.23	4.38	4.21	4.35	4.52
	β_d	1.91	1.89	1.98	1.99	2.04
	β_u	1.51	1.85	1.68	1.82	1.80
	γ_d	1.55	1.67	1.68	1.74	1.72
	γ_u	1.55	1.67	1.63	1.61	1.62
	δ_d	3.55	3.61	3.66	3.70	3.74
	δ_u	2.49	2.60	2.43	2.43	2.48

[a] Chemical shift referencing as in Table I.

effect on the shifts for solutions up to 0.15 M. Peak assignments in Me_6GrS were confirmed by single-frequency ^1H decoupling. The chemical shift reference was external Me_2NCHO, which was assigned[25] a shift of 82.2 ppm downfield from the NH_4^+ resonance of 5 M $^{15}NH_4NO_3$ in 2 M HNO_3.

Results and Discussion

NMR Spectra of GrS and Me_6GrS at 11.7 T. The ^1H NMR shifts of GrS and Me_6GrS, displayed in Tables I and II, were obtained by standard decoupling techniques. For either compound, a number of solvent dependencies are evident, particularly in the amide NH resonances which are strongly influenced by the capacity of the solvent to donate or accept protons.[26] The pleated backbone conformation of GrS is little affected by the medium,[2-5] and the shift variations in the nonexchangeable resonances are thus predominantly the result of solvent screening interactions not related to H bonding[27] and induced tertiary structural effects. Individual absolute shifts are not readily analyzable in terms of specific contributions and are not treated in detail at present.

Quaternization of Orn eliminates the possibility of H bonding by the amino groups without introducing substantial or long-range conformational perturbations. This is supported by the following observations: (a) With the exception of Orn, the root-mean-square (rms) deviation of the methylene and methyne ^1H shifts in Me_6GrS from GrS is small and demonstrates no significant solvent dependence. The rms deviations are, in 10^{-3} ppm (values for Orn α, β, and γ protons in parentheses): Me_2SO, 40 (120); water, 56 (96); MeOH, 60 (80); F_3EtOH, 57 (136); AcOH, 51 (49). (b) With the exception of Orn, the ^{13}C shifts of the proton-bearing C atoms of Me_6GrS in MeOH are identical within 1 ppm of the corresponding resonances in GrS.[9] Orn C^δ moves downfield by 20.2 ppm, Orn C^γ moves upfield by 4.6 ppm, and a singlet at 53.0 ppm (downfield of Me_4Si), assigned to Orn NMe_3, appears in the derivative. (c) The amide hydrogen exchange (HX) rates of Leu and Val NH are substantially slowed in Me_6GrS (Table III), as they are in GrS,[28,29] indicating that the two pairs of internal interamide H bonds remain intact. (d) Vicinal coupling constants $^3J_{N\alpha}$ and $^3J_{\alpha\beta}$ measured in MeOH do not differ appreciably between GrS and its derivative. These observations lead us to conclude that the conformational perturbation introduced by methylation is minor, limited primarily to the Orn side chain, and not disparately large in any particular solvent.

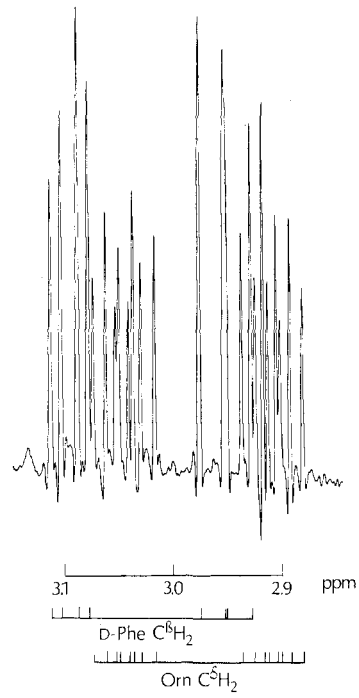

Figure 2. 500.13 MHz ^1H NMR spectrum of the D-Phe $C^\beta H_2$ and Orn $C^\delta H_2$ resonances in GrS in MeOH-d_4 at 295 K, with resolution enhancement.

Solvent Dependence of ΔO^δ_{obsd}. If the nonvanishing chemical shift difference between the Orn $C^\delta H_2$ protons (Figure 2) arises from the existence of intramolecularly H-bonded conformers in which the motility of the Orn side chain is hindered, in rapid exchange with a fully solvated state in which the intramolecular motion is unrestricted, we can deduce the form of solvent dependence expected for ΔO^δ_{obsd}. Because NMR detects the time-averaged chemical shifts, $\Delta O^\delta_{obsd} = \sum_i f_b(i)\Delta O^\delta_b(i)$, where $f_b(i)$ and $\Delta O^\delta_b(i)$ refer to the probability of occupancy and the $C^\delta H_2$ chemical shift inequivalence of the ith hypothetical bound state involving the side chain of Orn, respectively. We take $\Delta O^\delta = 0$ in the fully solvated state (occupancy $= 1 - \sum_i f_b(i)$). ΔO^δ_{obsd} is affected by solvent both through changes in the formation constants of the H-bonded conformers and by conformational and solvent influences on the absolute shifts. For the purposes of comparison between solvents and to allow computation of thermodynamic quantities (vide infra), two simplifications are made: first, the intramolecularly H-bonded states are described by their ensemble average, e.g., $\overline{\Delta O^\delta_b} \equiv \sum_i f_b(i)\Delta O^\delta_b(i)/\sum_i f_b(i) = \sum_i f_b(i)\Delta O^\delta_b(i)/F_b$, and thus $\Delta O^\delta_{obsd} = \overline{\Delta O_b}^\delta$; and second, $\overline{\Delta O}^\delta_b$ is taken (to first order only) to be independent of solvent, so that ΔO^δ_{obsd} is influenced chiefly by variation in F_b. The *apparent* free energy change per H bond for the transition from the solvated to the intramolecularly H-bonded state (eq 1), as determined by the

$$RNH_2^+\text{-H--SH} + \text{SH--O=CNHR'R''} \rightarrow RNH_2^+\text{-H--O=CNHR'R''} + \text{SH--SH} \quad (1)$$

partially averaged chemical shift difference, is then $\Delta G^0_b = RT$

(24) Hawkes, G. E.; Randall, E. W.; Bradley, C. H. *Nature (London)* **1975**, *257*, 767–772. Hawkes, G. E.; Litchman, W. M.; Randall, E. W. *J. Magn. Reson.* **1975**, *19*, 255–258.
(25) Levy, G. C.; Lichter, R. L. "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy"; Wiley-Interscience: New York, 1979; p 32.
(26) Llinas, M.; Klein, M. P. *J. Am. Chem. Soc.* **1975**, *97*, 4731–4737.
(27) Buckingham, A. D.; Schaefer, T.; Schneider, W. G. *J. Chem. Phys.* **1960**, *32*, 1227–1233.
(28) Philson, S. B.; Bothner-By, A. A. *Pept. Struct. Biol. Funct., Proc. Am. Pept. Symp. 6th* **1979**, 209–212.
(29) Fischman, S. J.; Wittbold, W. M.; Wyssbrod, H. R., Mount Sinai Medical Center, personal communication, 1981.

Table III. Proton Exchange Parameters for GrS and Me_6GrS[a]

	GrS[b]				Me_6GrS			
residue	k_D	$k_{OD} \times 10^{10}$	R'	pD'	k_D	$k_{OD} \times 10^{10}$	R'	pD'
Val	1.34	0.0453	0.154	3.24	1.49	0.0290	0.130	3.36
Orn	10.5	1.13	2.15	2.99	11.7	0.573	1.62	3.16
Leu	0.907	0.256	0.301	2.78	0.836	0.215	0.265	2.80
D-Phe	7.30	7.13	4.51	2.51	3.98	2.95	2.14	2.57

[a] In D_2O at 21 °C. The rate law is $R = k_D[D^+] + k_{OD}[OD^-]$, where k_D and k_{OD} are the pseudo-first-order rate constants for specific acid and specific base catalyzed exchange, respectively, in L mol^{-1} min^{-1}, and R is the macroscopically observed exchange rate. $R' = 2(k_D k_{OD} K_{D_2O})^{1/2}$ in $(10^2$ min$)^{-1}$ is the calculated minimum value of R with respect to pD; pD' is the pD at which $R = R'$. [b] Reference 29, corrected to 21 °C.

Table IV. Solvent Dependence of ΔO^{δ}_{obsd}

solvent[c]	pK_a	pK_b	$h(K)$[b]	ΔO^{δ}_{obsd} (GrS)[a]	ΔO^{δ}_{obsd} (Me_6GrS)[a]
pyridine		5.2	7.3	1[d]	
AcOH	4.75	−6.1	5.4	1[d]	63
Me_2SO	31	0	2.1	48	183
H_2O	15.7	−1.7	0.4	10	1[d]
MeOH	16	−2.2	−0.1	68	109
F_3EtOH	12.4	−8.2	−2.2	107	65

[a] In Hz at 22 °C. [b] See text for definition. [c] Dissociation constants are representative values from the following: ref 26 and references therein. Noller, C. R. "Chemistry of Organic Compounds"; W. B. Saunders: Philadelphia, 1965. Gordon, A. J.; Ford, R. A. "The Chemists' Companion"; Wiley-Interscience: New York, 1972; Chapter 1, and references therein. [d] No $C^{\delta}H_2$ splitting was detected, and a value of 1 Hz was assigned for computational purposes.

Table V. ^{15}N NMR Chemical Shifts of GrS and Me_6GrS[a]

residue	GrS	Me_6GrS
Val	97.3	96.2
Orn N′	105.0	104.2
Orn N^{δ}	10.8	27.2
Leu	106.7	106.8
D-Phe	106.2	105.9
Pro	115.5	112.0

[a] In ppm downfield of $^{15}NH_4NO_3$ in 2 M HNO_3 at 25 °C in MeOH.

$\ln(\overline{\Delta O^{\delta}}_b/\Delta O^{\delta}_{obsd} - 1) \equiv RTg(\Delta O^{\delta})$.

The more acidic (than RNH_3^+) or the more basic (than O=CNHR′R″) the solvent, the more effectively it will compete for the acceptor or donor moieties in the peptide. In the case of intramolecular H bonding, a direct relationship should then exist between the apparent free energy change for eq 1 and solvent–peptide p*K differences*, and it should be possible to establish a correlation between $g(\Delta O^{\delta})$ and some function $h(K)$ of the p*K*'s.[30] This must take into account both the donor and acceptor roles of the solvent ($pK_{ap} - pK_{as}$ and $pK_{bp} - pK_{bs}$, where p denotes peptide, s solvent, and $pK_{ap} = pK_a(RNH_3^+) \sim 10.2$, $pK_{bp} = pK_b(O{=}CNHR'R'') \sim -2.1$)[31] and should not be dominated by contributions from conjugate species (such as $MeC(OH)_2^+$ in AcOH) which are in effect invisible in the H-bonding transition. A simple function which meets these requirements is $h(K) = \log(K_{as}/K_{ap} + K_{bp}/K_{bs})$, which scales as the free energies of deprotonation and is linear in the free energies for the set of monofunctional solvents used.[32]

Measurements of ΔO^{δ}_{obsd}, with the corresponding solvent p*K*'s, are listed in Table IV. For GrS, ΔO^{δ}_{obsd} is largest in F_3EtOH, which is substantially weaker as an acid than Orn $N^{\delta}H_3^+$ and as a base than O=CNHR′R″, while no chemical shift difference could be resolved in AcOH ($pK_{as} = 4.75$) or pyridine ($pK_{bs} = 5.2$). For the remaining solvents, intermediate values of ΔO^{δ}_{obsd} are measured. For Me_6GrS, ΔO^{δ}_{obsd} increases with solvent in the order F_3EtOH < MeOH < Me_2SO, which is the reverse of the trend observed for GrS, and a 63-Hz difference is detected in AcOH. When values for $\overline{\Delta O^{\delta}}_b$ of 120 Hz for GrS (determined in MeOH, vide infra) and 184 Hz for Me_6GrS (maximum ΔO^{δ}_{obsd} plus 1 Hz) are employed, regression of $g(\overline{\Delta O^{\delta}})$ against $h(K)$ yields correlation coefficients $\rho = 0.908$ (GrS) and −0.181 (Me_6GrS). The values chosen for $\overline{\Delta O^{\delta}}_b$ are not critical in the calculation of ρ.[33] It is concluded that, in the case of GrS, variation in ΔO^{δ}_{obsd} is indeed correlated with this elementary measure of the capacity of solvents to disrupt solute–solute H bonds ($P < 0.01$ for the two-tailed test), while for Me_6GrS, in which the putative H-bond donor site is blocked without the introduction of any solvent-specific conformational shifts, no correlation exists. The ^{1}H NMR data are thus consistent with the presence of Orn side chain to backbone H bonds in GrS. The Orn $C^{\delta}H_2$ chemical shift inequivalences in the quarternary derivative, in contrast, derive from the bulkiness of the $RNMe_3^+$ groups and solvent-mediated dipolar interactions.

Acceptor Site. ^{15}N NMR chemical shifts in amides are sensitive to H bonding.[34] The effect is most pronounced when the carbonyl group interacts with proton donors, in which case a decrease in electron density on the adjacent nitrogen[35] is accompanied by a low-field shift of the corresponding ^{14}N or ^{15}N resonance.[35,36] Downfield shifts as large as 10.5 ppm have been reported[37] in model peptides on transfer from Me_2SO to F_3AcOH, and shifts of 2.8–7.5 ppm were observed on transfer from Me_2SO to F_3EtOH of alumichrome[38] and GrS.[4,5] ^{15}N resonances adjacent to solvent-shielded amide C=O groups, such as D-Phe and Orn in GrS,[5] exhibit little or no dependence on solvent acidity.

When the amide ^{15}N shifts of GrS and Me_6GrS obtained in MeOH at 25 °C are compared (Table V), it is evident that the Pro resonance moves well upfield (3.5 ppm) on methylation, while the remaining shifts are virtually unchanged. The Pro ^{15}N chemical shift in Me_6GrS, 112.0 ppm, is identical with that predicted for Pro in GrS in MeOH on the basis of primary structure alone.[5] This result is precisely what would be expected were the principal effect of Orn N^{δ} quaternization to be the disruption of an Orn $N^{\delta}H_3^+$--O=C D-Phe H bond. Substitution

(30) Other solvatochromatic parameters which have been developed for predicting reaction free energy dependencies on solvent acidity and basicity are reviewed in Kamlet et al. (Kamlet, M. J.; Abboud, J. L. M.; Taft, R. W. *Prog. Phys. Org. Chem.* **1981**, *13*, 485–630). Standard solvatochromatic methods have not yielded reliable parameter estimates for protic solvents such as water, F_3EtOH, MeOH, or AcOH, and provide no measure of the acidity of conjugate acids of primary amines.

(31) Homer, R. B.; Johnson, C. D. "The Chemistry of Amides"; Zabicky, J., Ed.; Wiley-Interscience: London, 1970; Chapter 3. Mahler, H. R.; Cordes, E. H. "Biological Chemistry"; Harper and Row: New York, 1971; p 95.

(32) In the case of solvent-labile amide–amide H bonds, solvents such as water and MeOH might behave in a bifunctional manner and the dependence on $h(K)$ would necessarily be more complex.

(33) If $\overline{\Delta O^{\delta}}_b = 250$ Hz is assumed, ρ(GrS) = 0.906 and ρ(Me_6GrS) = −0.146.

(34) Reference 25; Chapter 6.

(35) Saito, H.; Tanaka, Y.; Nukada, K. *J. Am. Chem. Soc.* **1971**, *93*, 1077–1081.

(36) Kamlet, M. J.; Dickinson, C.; Taft, R. W. *J. Chem. Soc. Perkin Trans. 2* **1981**, 353–355. Marchal, J. P.; Canet, D. *Org. Magn. Reson.* **1981**, *15*, 244–246. Burgar, M. I.; St. Amour, T. E.; Fiat, D. *J. Phys. Chem.* **1981**, *85*, 502–510.

(37) Gattegno, D.; Hawkes, G. E.; Randall, E. W. *J. Chem. Soc. Perkin Trans. 2* **1976**, 1527–1531.

(38) Llinas, M.; Horsley, W. J.; Klein, M. P. *J. Am. Chem. Soc.* **1976**, *98*, 7554–7558.

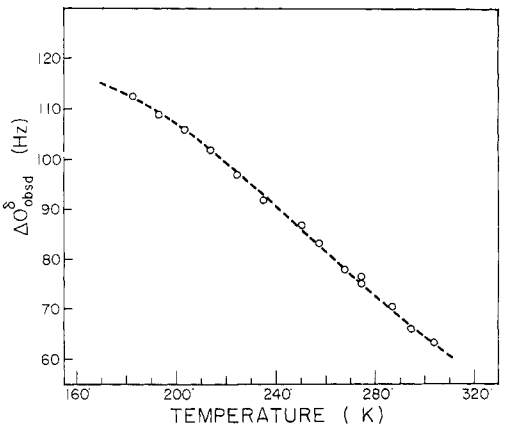

Figure 3. Measured values (circles) of ΔO^{δ}_{obsd} in GrS in MeOH-d_4. The dashed line is a plot of $\Delta O^{\delta}_{obsd} = \overline{\Delta O^{\delta}}_b/(1 + \exp(\Delta H^\circ/RT - \Delta S^\circ/R))$ for $\overline{\Delta O^{\delta}}_b$ = 120 Hz, ΔH° = −2.3 kcal mol^{-1}, and ΔS° = −7.5 cal deg^{-1} mol^{-1}.

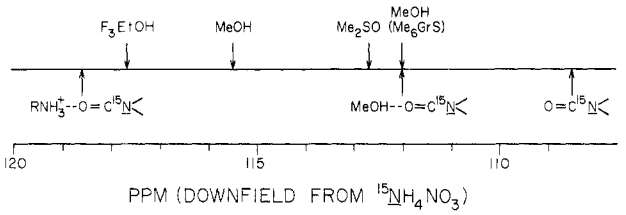

Figure 4. Pro ^{15}N shifts for several associational states of D-Phe C=O. Markers above and below the line indicate observed and computed shifts, respectively. The observed shifts in F_3EtOH and Me_2SO and the computed shifts of the MeOH and non-H-bonded complexes are from ref 5.

of a weaker acid (MeOH) for a stronger one (Orn $N^{\delta}H_3^+$) as the H bond donor to D-Phe C=O results in a relatively higher degree of diamagnetic shielding of the Pro N atom and an upfield shift of the corresponding resonance in Me_6GrS relative to GrS.

Examination of molecular models indicates that when GrS assumes the generally accepted β-pleated sheet–β-II′ turn backbone conformation, Orn $N^{\delta}H_3^+$--O=C D-Phe H bonds can be reversibly formed in either the $i \rightarrow i + 2$ or $i \rightarrow i - 3$ sense with minimal perturbation of the remaining structure. It is then entirely plausible that quaternization of Orn N^{δ} should eliminate the charge relay effect at Pro N produced by intramolecular H bonding without introducing other chemical shift anomalies. The displacement of the Pro ^{15}N resonance in Me_6GrS and the ^{1}H NMR chemical shift inequivalence of the Orn $C^{\delta}H_2$ protons thus emerge as complementary manifestations of the same intramolecular interaction.

Thermodynamics and Limiting Shifts. To investigate the thermodynamics of the transition given in eq 1 subject to the assumptions noted above, the temperature dependence of ΔO^{δ}_{obsd} was measured for GrS between −91 and 30 °C in MeOH (Figure 3). The limiting chemical shift inequivalence $\overline{\Delta O^{\delta}}_b$ and the standard enthalpy and entropy change on intramolecular H-bond formation were fitted according to the relation $\Delta O^{\delta}_{obsd} = \overline{\Delta O^{\delta}}_b/(1 + \exp(\Delta H^\circ/RT - \Delta S^\circ/R))$, yielding values of $-\Delta H^\circ$ = 2.3 ± 0.4 kcal mol^{-1}, $-\Delta S^\circ$ = 7.5 ± 1.0 cal deg^{-1} mol^{-1}, and $\overline{\Delta O^{\delta}}_b$ = 120 Hz. From these, a standard free energy change of −0.08 kcal mol^{-1} at 25 °C is calculated, corresponding to a simple formation constant $K_b = F_b/(1 - F_b)$ of 1.1. The thermodynamic quantities contain contributions both from the donor/acceptor couples (eq 1) and from changes in the rotamer populations about single bonds in the Orn side chain.

From the calculated thermodynamic parameters, the averaged Pro ^{15}N shift in the Orn $N^{\delta}H_3^+$--O=C D-Phe complex can be estimated. The observed shift $\delta_{obsd} = F_b'\bar{\delta}_{intra}{}^+ (1 - F_b')\delta_{solv}$, where the primes indicate that the ensemble average is taken with respect to the ^{15}N shift. Making the approximation $F_b' = F_b = K_b/(1 + K_b)$ and using δ_{solv} = 112.0 ppm (as indicated by the spectrum of Me_6GrS and supported by model compound studies[5]) and δ_{obsd} = 115.5 ppm in MeOH, we calculate $\bar{\delta}_{intra}$ = 118.6 ppm. Observed and calculated Pro ^{15}N shifts for a variety of possible donors to D-Phe C=O are diagrammed in Figure 4. In the absence of any H bonding to D-Phe C=O, a shift of 108.5 ppm was predicted,[5] and a shift of 112.7 ppm observed in Me_2SO.[5] If the Orn $N^{\delta}H_3^+$ interaction accounts entirely for the downfield shift and $\bar{\delta}_{intra}$-(Me_2SO) = $\bar{\delta}_{intra}$(MeOH), a limiting $\overline{\Delta O^{\delta}}_b$ of 115 Hz is projected for GrS in Me_2SO from ΔO^{δ}_{obsd} = 48 Hz (Table IV), in reasonable agreement with the esimate of $\overline{\Delta O^{\delta}}_b$ for MeOH. The calculated magnitude of the Pro ^{15}N shift change from the non-H-bonded to the fully intramolecularly H-bonded state, 10.1 ppm, is comparable to the largest solvent-induced amide ^{15}N shifts recorded.[37]

Spatial Orientation of the Orn Side Chains. It is possible to orient the Orn $N^{\delta}H_3^+$--O=C D-Phe H bonds in either the $i \rightarrow i + 2$ or the $i \rightarrow i - 3$ sense. Since the preceding work could not differentiate between the two alternatives, additional experiments were performed in which it was possible to identify specific regions of the peptide backbone in close proximity to the Orn side chain. The evidence obtained supports the $i \rightarrow i + 2$ orientation observed for the single H-bonded Orn in the crystalline urea complex[19] rather than the $i \rightarrow i - 3$ orientation proposed earlier.[18]

Comparison of the HX kinetics of GrS and Me_6GrS in ^2H_2O (Table III, Figure 5) shows that Val and Leu do not differ significantly, while the HX profile of Orn exhibits a small shift which

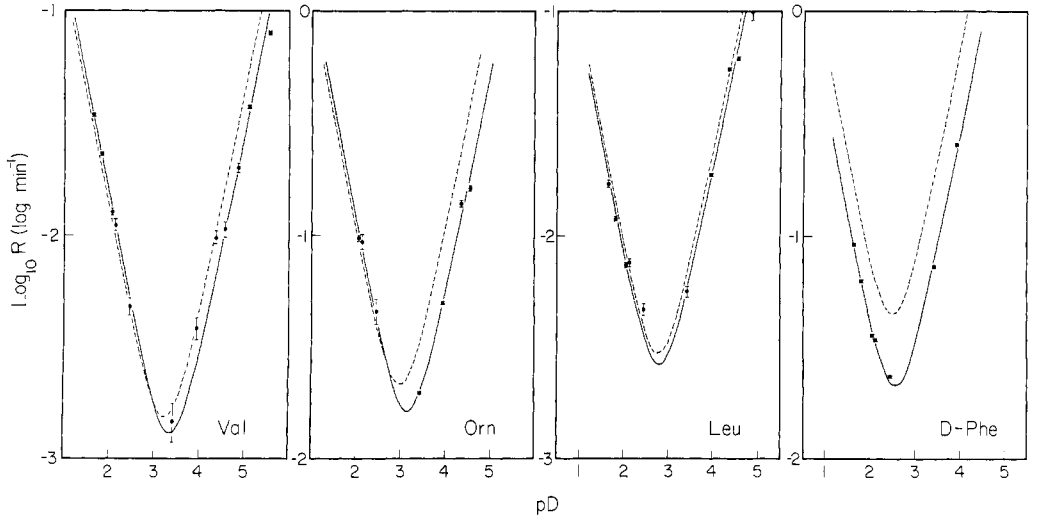

Figure 5. Observed proton exchange kinetics for Me_6GrS in D_2O; experimental values are shown with standard errors. The exchange profiles (solid lines) were fitted by a weighted nonlinear least-squares routine. Exchange profiles measured previously in D_2O for GrS[29] are also shown (dashed lines).

Table VI. Ornithine ^1H–^1H NMR Coupling Constants in GrS[a]

solvent	temp, °C	ΔO^{δ}_{obsd}	$^3J_{\alpha\beta_d}$	$^3J_{\alpha\beta_u}$	$^2J_{\beta_d\beta_u}$	$^3J_{\beta_d\gamma}$	$^3J_{\gamma_d\delta_d}$ ($=^3J_{\gamma_u\delta_u}$)	$^3J_{\gamma_d\delta_u}$ ($=^3J_{\gamma_u\delta_d}$)	$^2J_{\delta_d\delta_u}$
Me_2SO	23	48	8.5	6.0			9.0	6.8	−12.3
MeOH	22	68	10.3	5.2	−12.5	6.5	9.8	5.8	−12.5
MeOH	−40	94	11.0[b]	4.2[b]			10.9	5.3	
F_3EtOH	22	109	11.5	4.2	−14.0	5.5	11.5	4.9	−12.5

[a] All splittings in Hz at 500.13 MHz. The sign of the geminal coupling constants is assumed. The chemical shift difference between the $C^{\gamma}H$ protons was unmeasurably small in Me_2SO and MeOH and in these solvents the labeling of these protons is arbitrary. [b] Reference 16, at −44 °C.

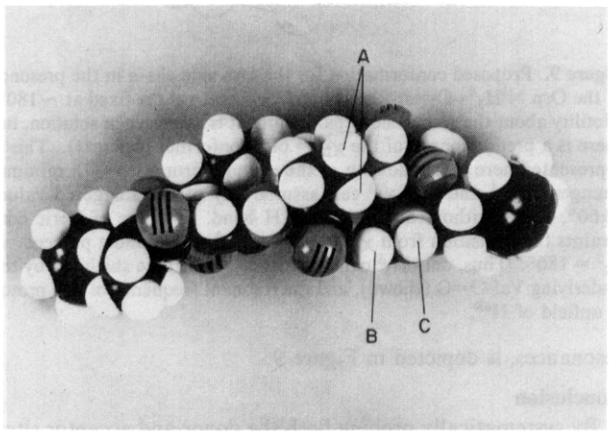

Figure 6. CPK model of GrS, viewed perpendicular to the C_2 axis, with the Orn $N^{\delta}H_3^+$--O=C D-Phe H bonds oriented in the $i \rightarrow i + 2$ sense. The side chains of valine and leucine are omitted. The torsional angles illustrated in Figure 9 are assumed for ornithine residues and the backbone contains that twist observed in the crystal.[19] The proximity of Orn $C^{\gamma}H_2$ (A), Leu $C^{\alpha}H$ (B), D-Phe NH (C), and the terminal amino group of ornithine is evident.

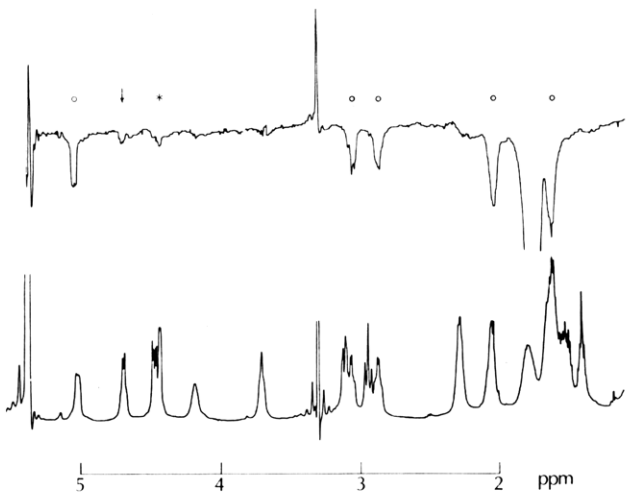

Figure 7. (Upper trace) NOE difference spectrum of GrS (per-N-deuterated) in MeOH at 226.5 K: Irradiation of Orn $C^{\gamma}H_2$ at subsaturating levels for 1 s; acquisition time = 1.64 s. Intraornithyl enhancements are indicated by circles and the Orn $C^{\gamma}H_2$–Leu $C^{\alpha}H$ NOE is identified by an arrow. A small NOE at Pro $C^{\alpha}H$ (asterisk) is the result of decoupler spillover into the nearby Pro $C^{\beta}H_2$ resonance. (Lower trace) Spectrum of GrS for comparison of chemical shifts; vertical scale much reduced.

is most likely the consequence of side-chain modification.[39] However, exchange at D-Phe is slowed more than twofold in Me_6GrS. D-Phe NH is too far from the site of modification to manifest an altered primary structure effect on HX.[39] The existence of a preferred conformation in which the quaternary ammonium group shields D-Phe NH from the solvent could conceivably retard HX, but there is no evidence to support such an interaction and the high degree of mobility of the Orn side chains of Me_6GrS in ^2H_2O indicated by $\Delta O^{\gamma}_{obsd} = \Delta O^{\delta}_{obsd} = 0$ argues against it. The most plausible explanation is that in the unmodified peptide the terminus of the Orn side chain is, on average, sufficiently close to D-Phe NH that a general catalytic effect is exerted on HX which accelerates it twofold.[40] In the intramolecularly H-bonded configuration Orn $N^{\delta}H_3^+$ is close to D-Phe NH (3–4 Å) only when the bonds form in the $i \rightarrow i + 2$ sense (Figure 6).

Additional support for the $i \rightarrow i + 2$ orientation derives from the observation of a specific nuclear Overhauser enhancement (NOE) at Leu H^{α} when the Orn H^{γ} resonance is irradiated in MeOH below −25 °C (Figure 7). Working at low temperatures facilitates detection of the NOE by increasing its maximum magnitude, controlled by the solvent viscosity,[41] and by increasing the population of the intramolecularly H-bonded conformers. Cross-relaxation effects become especially significant outside the extreme narrowing limit, however,[41] and we have not attempted to calculate internuclear distances from NOE data. Rather, it is the qualitative pattern of NOE's observed upon Orn side-chain irradiation which is considered here; only in the $i \rightarrow i + 2$ case is the Orn H^{γ}–Leu H^{α} enhancement expected ($r_{HH} \leq 4$ Å, Figure 6), while in the $i \rightarrow i - 3$ case, either no NOE or an Orn–Val H^{α} NOE would be observed.

Conformation of the Orn Side Chain. Any account of the solution conformation of the Orn side chain must take into consideration that there is residual motional freedom even in the intramolecularly H-bonded configuration, particularly about χ_2[2].

Measured ^1H–^1H coupling constants in several solvents are given in Table VI. It is evident that as the fractional population of the H-bonded conformers decreases, as indicated by ΔO^{δ}_{obsd}, the ^3J's approach the fully averaged value of 6–7 Hz.[42] This is in accord with the treatment developed above in which variation in ΔO^{δ}_{obsd} was attributed to the admixture of a formally solvent-H-bonded state in which rotation about the side-chain single bonds is unrestricted. The ^3J's in F_3EtOH, in which Orn $N^{\delta}H_3^+$--O=C D-Phe H bonding is maximally favored, will therefore be presumed sufficiently close to the limiting case to provide reasonable estimates of the torsional angles in the intramolecularly H-bonded conformation in general.

The $H^{\alpha}H^{\beta}$ coupling constants have been subjected to Pachler analysis[16] and a statistical weight of 0.67 calculated for $\chi_2^1 = -60°$ or 180° in MeOH. Since the H bond forms in the $i \rightarrow i + 2$ sense, $\chi_2^1 = 180°$ is the proper choice (the form of averaging of ^3J cannot actually be specified by NMR) and the H^{β_d} resonance is assigned to $H^{\beta S}$ (d and u denote the downfield and upfield resonances of a geminal pair, *R* and *S* the stereochemical position; see Figure

(39) Molday, R. S.; Englander, S. W.; Kallen, R. G. *Biochemistry* **1972**, *11*, 150–158.

(40) Some evidence exists for such effects exerted by nitrogenous bases on amide HX kinetics, but it is generally difficult to achieve sufficiently high activities of potentially catalytic agents in pure water. NH_2OH has been shown to accelerate both acid- and base-catalyzed HX in AcNHMe in D_2O: Klotz, I. M.; Frank, B. H. *J. Am. Chem. Soc.* **1965**, *87*, 2721–2728.

(41) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971; Chapter 2.

(42) As an approximation, the Karplus relationship $^3J(\theta) = 9.4 \cos\theta - 1.4 \cos\theta + 1.6$ for the $NCCHCH_2C$ fragment is employed: Bystrov, V. F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41–81. In view of the uncertainty associated with substituent electronegativities and orientational effects, ^3J_t and ^3J_g may be in error by ~10%. This should not influence the conclusions concerning preferred rotamers about χ_2^1 and χ_2^3.

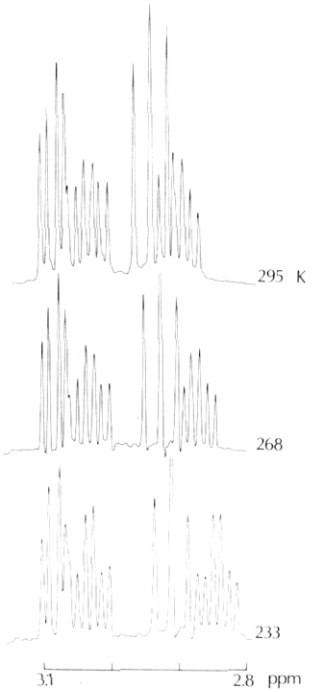

Figure 8. Temperature variation of the Orn $C^{\delta}H_2$ and D-Phe $C^{\beta}H_2$ proton resonances in GrS in MeOH-d_4, depicting the upfield shift of the Orn H^{δ_u} with decreasing temperature.

1b). In F_3EtOH, values of $^3J_{\alpha\beta_d} = 4.2$ Hz and $^3J_{\alpha\beta_u} = 11.5$ Hz indicate that $\chi_2^1 = 180°$ is more heavily weighted than in MeOH.

Extensive rotational averaging of the $H^{\beta}H^{\gamma}$ coupling constants is expected in light of the twofold increase in ^{13}C NT_1 on proceeding from C^{β} to C^{γ},[9] and this is observed in MeOH. However, in F_3EtOH the relatively high stability of the Orn $N^{\delta}H_3^+$--O=C D-Phe H bonds is associated with the existence of preferred conformers even about the $C^{\beta}C^{\gamma}$ bond, as indicated by the chemical shift inequivalence of the Orn $C^{\gamma}H_2$ protons. The $^3J_{\beta_d\gamma}$'s in F_3EtOH, both smaller than the fully averaged value, suggest a predominance[43] of $\chi_2^2 \sim +60°$, in which $H^{\beta S}$ is gauche to both H^{γ} protons. It was not possible to characterize the H^{β_u} or the H^{γ} multiplets.

The large difference between the measured $H^{\gamma}H^{\delta}$ coupling constants for each H^{δ} resonance in F_3EtOH demonstrates, as does the large ΔO^{δ}_{obsd}, that there is considerable constraint on the motility of the terminal side-chain segment. That $^3J_{\gamma_d\delta_d} = {}^3J_{\gamma_u\delta_u}$ and $^3J_{\gamma_d\delta_u} = {}^3J_{\gamma_u\delta_d}$ can only be explained by a major contribution from $\chi_2^3 = 180°$ in the H-bonded configuration, regardless of the precise magnitude of the coefficients used in the Karplus equation.[42]

The origin of the chemical shift difference between the $C^{\delta}H_2$ protons has not been explicitly treated in the discussions of its temperature and solvent variation, but it is possible, by modeling the environment of these protons, to relate the phenomenon to the preferred torsional angles of the side chain. When χ_2^1 and $\chi_2^3 \sim 180°$ and χ_2^2 takes any value near +60°, one of the $C^{\delta}H_2$ protons ($H^{\delta S}$) becomes closely apposed to the C=O group of Val in the internally H-bonded network and should thus be expected, upon formation of the Orn $N^{\delta}H_3^+$--O=C D-Phe bond, to manifest increased diamagnetic shielding (Figure 9). Comparison of the absolute shifts of the H^{δ} resonances in MeOH at several temperatures (Figure 8) reveals that the increase in ΔO^{δ}_{obsd} is almost entirely attributable to an upfield shift of H^{δ_u}. We therefore assign the H^{δ_u} resonance to $H^{\delta S}$, and since, as indicated by the $^3J_{\gamma\delta}$'s, H^{δ_u} and H^{γ_d} are gauche, we assign the H^{γ_d} resonance in F_3EtOH to $H^{\gamma R}$. A proposed conformation for the Orn side chain in the H-bonded state, with stereospecific assignment of the ^1H NMR resonances, is depicted in Figure 9.

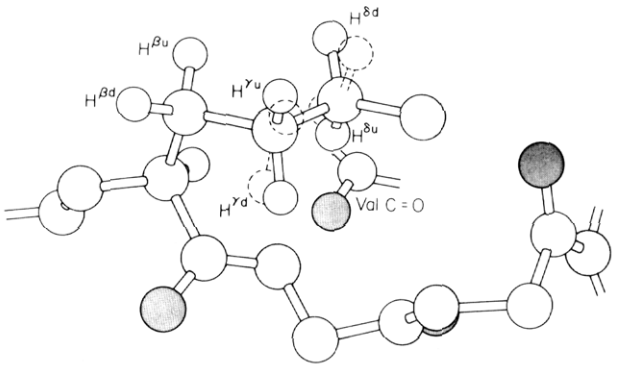

Figure 9. Proposed conformation for the Orn side chain in the presence of the Orn $N^{\delta}H_3^+$--O= D-Phe H bond. χ_2^1 and χ_2^3 are fixed at ~180°. Motility about the second side-chain segment is extensive in solution, but there is a predominance of the $\chi_2^2 = 60°$ conformer (see text). This is represented here by shadowing of the γ and δ protons. With minimal changes in χ_2^1 and χ_2^3, χ_2^2 can assume any of the staggered values (±60°, 180°) without disrupting the H bond. Because of steric constraints the transition from $\chi_2^2 = -60°$ to $\chi_2^2 = +60$ must proceed via $\chi_2^2 = 180°$. Thus, only H_{δ}^S can experience anisotropic shielding by the underlying Val C=O (shown), and the resonant frequency of this proton is upfield of $H^{\delta R}$.

Conclusion

By systematically probing both the donor and acceptor sites, we have identified a pair of side chain to backbone H bonds in GrS not previously known to exist in solution. These H bonds provided constraints on the torsional angles that might be accessible to the side chains of the two Orn residues and greatly facilitated the delineation of the conformation of the Orn side chains in solution by high-resolution NMR methods even in the presence of residual rotational averaging.

Studies of the solution structure of GrS have revealed the extraordinary prevalence of intramolecular H bonding in this small peptide. Two pairs of symmetry-related interamide H bonds, the first between Leu NH and Val C=O and the second between Val NH and Leu C=O, were proposed in the initial conformational studies[44] and demonstrated experimentally by difference IR spectrophotometry.[10] The spectroscopic data thus far obtained[2-5] strongly suggest that they are not disrupted by variation of the solvent. Elimination of these internal bonds by alteration of the primary structure yields a peptide with dramatically altered chiroptical properties,[12,45] NMR spectra,[45] and reduced or absent antibiotic activity.[8] There is, in addition, a pair of solvent-labile H bonds between Orn $N^{\delta}H_3^+$ and D-Phe C=O. In contrast to the transannular bonds, stability is strongly solvent dependent, and the backbone conformation shows little if any change when the bonds are eliminated by covalent modification.

The question of the biological importance of the Orn $N^{\delta}H_3$--O=C D-Phe bonds cannot presently be resolved. It is difficult to modify the peptide covalently in such a manner as to eliminate these H bonds completely without introducing extraneous steric effects or altering the partition coefficients of the amino acid side chains at position 2. The data suggest a relatively small fraction of intramolecularly H-bonded conformers in water (Table IV), but it is not essential that the H bonds exist to a major extent in aqueous solution in order for them to be functionally relevant. It is possible, for example, that the peptide readily assumes the H-bonded conformation upon transfer to a less polar environment; there is considerable evidence that GrS is membrane active.[1,8] Alternatively, the antibiotic action of GrS may involve the formation of a complex in solution with a polyvalent anion, such as an organic polyphosphate. In this case, Orn $N^{\delta}H_3$--O=C D-Phe

(43) Simple rotamer analysis (Pachler, K. G. R. *Spectrochim. Acta* **1964**, *20*, 581), using $^3J_t = 12.4$ and $^3J_g = 3.25$ Hz, gives p (180°) = p (−60°) = 0.25, p (+60°) = 0.50 for χ_2^2.

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H bonding might facilitate binding by ensuring that a critical intercationic distance is maintained which is complementary to the architecture of the anionic species. The possibility of anion binding, which is raised by the study of the solution conformation of the Orn residues, is currently under investigation.

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Structural Characterizations of Salts of $HCr(CO)_5^-$ and $(\mu\text{-}H)_2BH_2Cr(CO)_4^-$ and Studies of Their Interconversions

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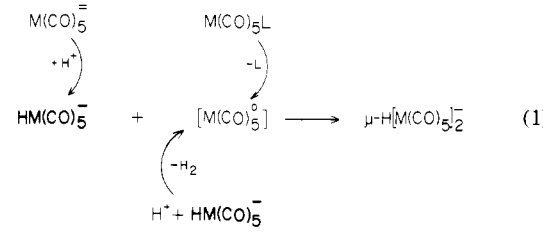
Abstract: At 0 °C, BH_3 reacted with $HCr(CO)_5^-$ in THF to abstract hydride, presumably producing coordinatively unsaturated $Cr(CO)_5^0$, which immediately aggregated with remaining $HCr(CO)_5^-$ to yield the very stable $(\mu\text{-}H)[Cr_2(CO)_{10}]^-$. At room temperature two bridging hydride products were obtained. In addition to the binuclear bridging hydride, a second product, $(\mu\text{-}H)_2BH_2Cr(CO)_4^-$, resulted from CO loss either prior to or following Cr–H---BH_3 adduct formation. The borohydride complex could be reconverted to $HCr(CO)_5^-$ on addition of CO; however, $(\mu\text{-}H)[Cr_2(CO)_{10}]^-$ was also formed in the process. Salts of both title anions were characterized by solution spectroscopic probes as well as X-ray structural analysis. Crystals of $[Ph_4P][HCr(CO)_5]$ were found to belong to the tetragonal space group $P4/n$, with a = 13.234 (2) Å, b = 13.234 (2) Å, c = 7.472 (2) Å, and Z = 2. $R(F)$ = 3.9% for 1796 reflections with $I > 3\sigma(I)$. Deep red crystals of $[PPN][(\mu\text{-}H)_2BH_2Cr(CO)_4]$ belong to the triclinic space group $P\bar{1}$, with a = 11.708 (3) Å, b = 14.572 (6) Å, c = 11.454 (3) Å, α = 101.98 (3)°, β = 91.69 (2)°, γ = 77.34 (3)°, and Z = 2. $R(F)$ = 6.6% for 2880 reflections with $I > 3\sigma(I)$. Most notably, $HCr(CO)_5^-$ showed bending of the crystallographically identical equatorial CO groups toward the hydride ligand ($\angle(CO)_{ax}$–Cr–$(CO)_{eq}$ = 95.4 (1)°), as has been exhibited by all mononuclear hydridocarbonyl complexes whose structures are known. Analysis of the ν(CO) infrared spectrum indicated that this pseudooctahedral structure persisted in solution. The hydride ligand induced only a very small trans effect on the Cr–C bond length with $Cr\text{–}C_{trans}$ = 1.852 (4) Å and $Cr\text{–}C_{cis}$ = 1.865 (3) Å. The hydride ligand was located 1.66 (5) Å from Cr. In contrast the Cr–C bonds of $(\mu\text{-}H)_2BH_2Cr(CO)_4^-$ showed considerable asymmetry with $Cr\text{–}C_{eq}$(trans to H) = 1.81 (1) and 1.82 (1) Å and $Cr\text{–}C_{ax}$(cis to H) = 1.87 (1) and 1.85 (1) Å. In addition, the axial CO groups bend away from the $[(\mu\text{-}H)_2BCr]$ planar unit, $\angle(CO)_{ax}$–Cr–$(CO)_{ax}$ = 175.6 (4)°, whereas the equatorial CO groups expand into the space made available by the small requirement of the $(\mu\text{-}H)_2B$ bidentate ligand, $\angle(CO)_{eq}$–Cr–$(CO)_{eq}$ = 94.8 (4)°. Carbon-13 NMR spectroscopy showed the CO groups of $(\mu\text{-}H)_2BH_2Cr(CO)_4^-$ to be stereochemically rigid at +30 °C whereas ^1H NMR spectroscopy showed rapid interchange of bridging and terminal hydrogens, even at −80 °C.

Introduction

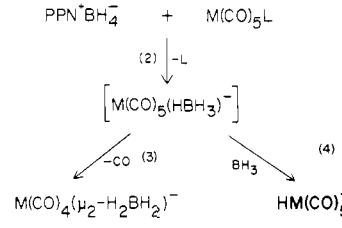
Recent progress in the syntheses of soluble salts of $HM(CO)_5^-$ (M = Cr, Mo, W)[2] has allowed for the development of the chemistry of these highly reactive metal carbonyl hydrides.[3] Although they were reported by Behrens et al., some 20 years ago,[4] these simple metal carbonyl hydrides were unavailable in soluble form for solution characterization and the molybdenum derivative was unknown. The synthetic advancements made lately were based on (1) circumventing reactions that are of an aggregative acid/base type, i.e., the interaction of the metal hydride with a transition-metal Lewis acid, $[M(CO)_5^0]$ (Scheme I), and (2) the successful utilization of hydride from an inexpensive light main-group metal hydride source (Scheme II). As also shown in Scheme II a competing reaction that involves CO loss may occur and is prominent for M = Mo.

Scheme I



(1)

Scheme II



Concurrent developments that emphasized the need for understanding the chemical and spectral characteristics of $HM(CO)_5^-$ provided yet other routes to the hydrides.[3,5–7] The re-

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