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$$ROO\cdot + HBr \longrightarrow ROOH + Br\cdot$$

Spectroscopic and structural information are not available for the compound ROOBr for the calculation of $\Delta V_{triplet}$. However, the low bond dissociation energy of HOBr suggests that the value for the peroxy bromide would be very low, and thus the transfer reactions will manifest a very low $\Delta V^{\ddagger}$.
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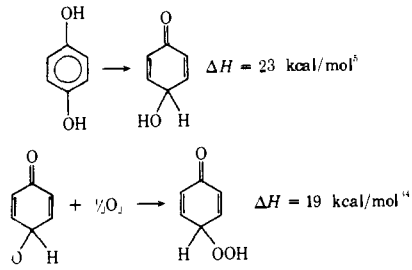

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Charge Relay at the Peptide Bond. A Proton Magnetic Resonance Study of Solvation Effects on the Amide Electron Density Distribution[1a]

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Abstract: The proton magnetic resonance (^{1}H NMR) manifestations of amide solvation in polypeptides have been studied using alumichrome and alumichrome C as model compounds. The extreme structural rigidity of the alumichromes allowed the investigation to center on the chemical shifts of the amides as conformational drifts are precluded. The data have been analyzed primarily in terms of two main events: (a) H bonding of the amide nitrogen to basic solvents (Me_2SO, DMF, and pyridine), and (b) protonation of the amide carbonyl by Brønsted acids (chloroform, TFE, and TFA). The two solvent types cause a low-field shift of the amide proton resonance which in case (a) arises from a direct effect, while in case (b), consistent with an earlier suggestion of Schwyzer and Ludescher, the deshielding would result from the extreme electronic lability of the peptide link which permits an electron density flow to the carbonyl. The solvent-induced chemical shift is shown to depend on the extent of exposure of the pertinent hydrogen and oxygen atoms so that the magnitude and direction of the effect reflect conformational features of the molecule at the amide sites. The distinct amide spectra for the two alumichromes, which differ only in a single L-alanyl for glycyl substitution at site 2, can thus be rationalized from their structures. The study proves that the temperature coefficient of the proton resonance frequency affords an excellent criterion to determine the extent of exposure of the NH group, whatever the solvent. The chemical shifts in amphoteric water can be explained by combining the effects observed for the nucleophilic and acidic solvents. However, as judged by the amides' NH ^{1}H NMR, water behaves more like a proton acceptor than as a donor. The extent of the NH resonance frequency shifts indicates synergistic effects between intramolecular H bonding and C=O protonation. On the basis of the amide solvation effects, the apparent pK_a for $DCCl_3$ as a base is estimated to be about −6.

In 1969 Schwyzer and Ludescher[2] tentatively distinguished "internal" from "external" amides in cyclic peptides on the basis of the shifts induced on their proton magnetic resonance (^{1}H NMR)[3] by solvent perturbation. The authors rationalized that carbonyl protonation in an acidic solvent (TFA) and amide-to-solvent H bonding in a basic

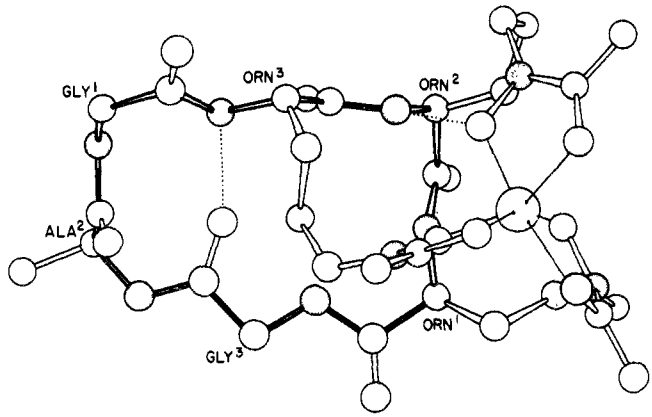


Figure 1. The structure of ferrichrome C.[10] The amino acid residues are labeled in accordance with the convention used by Zalkin et al.[8a] for ferrichrome A and follow that used in previous papers in this series.[1),33] The figure is a slightly modified version of the crystallographic models for ferrichrome A and ferrichrysin.[8b] Sites 1, 2, and 3 are occupied by Gly^1, Ala^2, and Gly^3, and sites 4, 5, and 6 are occupied by Orn^1, Orn^2, and Orn^3. In ferrichrome the primary sequence differs in that the residue at site 2 (Ala^2) is substituted by a glycyl residue. The dotted lines represent the intramolecular H bonds suggested by the X-ray data[8] and confirmed by the ^1H NMR studies.[10,11]

solvent (DMSO) should decrease the proton shielding at the peptide link, the extent of the interaction depending on the degree of exposure of the amide C=O and NH groups. In spite of the wide use of solvent perturbation in this type of study,[4] little attention has been paid to the proposal, probably because of a likely simultaneity between solvation effects on the amides and solvent-driven conformational shifts. The argumentation has usually been based on the dielectric or H-bond acceptor character of the solvent rather than on electron density shifts along the peptide bond. Indeed, such an analysis has been ignored in two recent studies[5] specifically dealing with amide solvation effects. Furthermore, since the interaction is temperature dependent, not always in a predictable manner, this further complicates the interpretation in terms of static chemical shifts and has led some authors[6] to question the validity of chemical shift temperature dependence studies.

We have in the past extensively studied the conformational state of the ferrichromes in solution.[7] The ferrichromes are ferric cyclohexapeptides which chelate the metal with high affinity ($K \sim 10^{30}$). The metal can be replaced to form stable Al^{3+} and Ga^{3+} complexes. ^1H NMR studies of these diamagnetic analogs of the ferric complex showed that glycyl- and L-seryl-containing ferrichromes exhibit conformations that are identical, for all practical purposes, with that found in crystalline ferrichrome A and ferrichrysin as revealed by X-ray studies.[8] The ^1H NMR studies have been extended to alanyl-containing ferrichromes (sake colorant A[11] and ferrichrome C[12]) which have shown that, independent of the primary composition, the conformations of the chelated homologous peptides are essentially identical (Figure 1). This is a result of the structural constraints imposed by the complexation center, which rigidly determine the overall configuration of the polypeptide backbone.[7]

Structurally rigid peptides should be especially suited to help understand the ^1H NMR manifestations of the amide solvation effects. This explains why diketopiperazines have been previously considered useful model compounds by investigators looking into the problem.[2,5a] In this regard, the suitability of the ferrichromes for this kind of study immediately comes to mind. Furthermore, while the two amides in diketopiperazines are equivalent, with both the carbonyl and the amino proton exposed in a cis configuration (absent in most natural oligopeptides), the six ferrichrome amides are trans and differ in the extents of proton and carbonyl exposure and in the degree of intramolecular H bonding.

Table I. Solvent Properties

Solvent	ϵ_{20}[a]	ϵ_{25}[a]	pK_{a_1}[b]	pK_{a_2}[b]
TFA	8.42[c]		~−10.6[k]	0.2[d]
TFE	27.68[e]	26.67[e]	~−8.2[k]	12.4[f]
Chloroform	4.81[m]	4.90[g]	~−6.0[l]	24.0[h]
Water	80.37[i]	78.54[i]	≳−1.8[f]	15.3[d]
Me_2SO	48.9[j]	46.4[m]	0[f]	
DMF		36.7[m,n]	−0.01[m]	
Pyridine		12.3[m]	5.19[m]	

[a] Dielectric constants ϵ_{20} and ϵ_{25} are at 20 and 25°, respectively. [b] The pH for half-protonation of the neutral bases is given under pK_{a_1} while pK_{a_2} corresponds to the pH of half-deprotonation of the Brønsted acid. These pK_a values (measured at about 25°) are meant only to be representative as the acid–base character is solvent-dependent. In particular, pK_a values below ~−2 and above ~15 are quite approximate. [c] Various tables in J. J. Lagowski, Ed., "The Chemistry of Nonaqueous Solvents", Vol. III, Academic Press, New York, N.Y., 1970, p 366. [d] F. G. Bordwell, "Organic Chemistry", Macmillan, New York, N.Y., 1963, pp 866–873. [e] J. Murto and E. L. Heino, *Suom. Kemistil. B*, 39, 263 (1966). [f] E. M. Arnett, *Prog. Phys. Org. Chem.*, 1, 223 (1963). [g] L. Scheflan and M. B. Jacobs, "The Handbook of Solvents", Van Nostrand, New York, N.Y., 1953. [h] Z. Margolin and F. A. Long, *J. Am. Chem. Soc.*, 95, 2757 (1973). [i] "Handbook of Chemistry and Physics", 54th ed, CRC Press, Cleveland, Ohio, 1973–1974, p E-54. [j] I. Mellan, "Industrial Solvents Handbook", Noyes Data Corp., Park Ridge, N.J., 1970. [k] Estimated as described in the text. [l] "Effective" value suggested by this work (see text and Figure 5). [m] See footnote *c*, p 2. [n] See footnote *c*, Vol. II, p 194.

In recording the amide proton resonance frequencies (and their temperature coefficients) of the glycyl- and L-seryl-containing alumichromes in both water and Me_2SO-d_6, we found that while the conformation is unaffected by the solvent change the chemical shifts are extremely sensitive to the perturbation.[11] Since then, we have noted that alumichrome maintains its conformation even in TFA, a solvent widely used in NMR studies but that is known to denature polypeptides to a random coil configuration. This unusual stability, together with our casual observation that ferrichrome C is soluble in relatively nonpolar (chloroform) as well as polar (water) solvents, prompted us to attempt a study of the amide solvation effects by using alumichrome and alumichrome C as model compounds (Figure 1). These two peptides further afford the convenience of lacking residues with side chains capable of forming H bonds, such as, e.g., seryl $-CH_2OH$, which could complicate the analysis of the data.

Carbonyl protonation[13] by the action of a moderately strong acid such as TFA provides a classic example of solvent-to-amide proton transfer.[15] The nucleophilic character of the amide group should also be exhibited when dissolved in weaker Brønsted acids. The H-bond donor character of chloroform in the presence of suitable H acceptors has been investigated widely.[16] Moreover, there are studies showing that fluoro alcohols are good H-bond donor solvents.[18] In particular, Strassmair et al.[19] have demonstrated binding of TFE to the peptide carbonyl group in poly-L-proline. Chloroform (pK_a = 24), TFE (pK_a = 12.4), and TFA (pK_a = 0.2) afford thus a convenient set of solvents to test the effect of protonation on the amide ^1H NMR as they cover a wide acidity range and have relatively low dielectric constants (Table I). Furthermore, these solvents should exhibit weak H-bond acceptor properties especially considering that the basic properties of halogen atoms bonded to carbon are significantly repressed.[20] In general, H bonding induces a chemical shift to low field, consistent with a decreased elec-

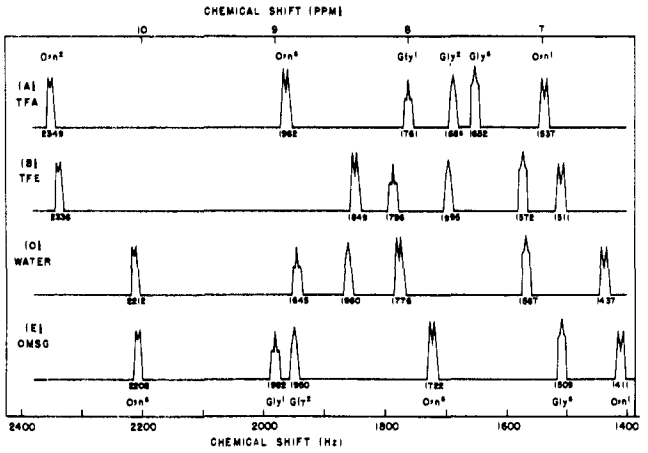


Figure 2. Schematic ^1H NMR spectra for the amide region of alumichrome at 25°, in different solvents, referred to internal Me_4Si. The data for water (D) and Me_2SO (E) have been taken from a previous paper.[11a] The numbers below each peak indicate the exact resonance frequency (in hertz) as interpolated from the linear least-squares fit of the chemical shift vs. temperature plots.

tron density at the nuclear locus.[21] Hence, amide H bonding to suitable acceptor solvents, such as pyridine (pK_{a_1} = 5.2) and DMF or Me_2SO (both with $pK_{a_1} \simeq 0$), should result in a relative low-field position of the (exposed) amide proton resonance.

Because of both its relevance as a biological solvent and its more frequent use as an NMR solvent to observe low-field lines, the interaction of water with amides has also been studied. In principle, water can behave both as a proton acceptor and a proton donor. Furthermore, because of its high polarity (ϵ_{20} = 80.1), it might tend to destabilize intramolecular H bonds[23] and cause unfolding (amide exposure) of conformationally loose peptides.[11a,24]

In this study we center our attention on the effects on the amide resonances of Me_2SO-d_6, pyridine-d_5, chloroform-d, H_2O, TFE, and TFA. These solvents cover a wide acidity-basicity range and are commonly used in spectroscopic studies on polypeptides. The observations here reported should serve as a guide to estimate the amide ^1H NMR effects of other solvents from their known acidity, basicity, and dielectric properties.

Experimental Section

Alumichrome belonged to the batch used in previous experiments.[11a] Ferrichrome C was extracted from the culture medium of *Cryptococcus melibiosum*[12] as described elsewhere.[10] The preparation of the metal-free peptide and the formation of the Al^{3+} chelate (alumichrome C) have also been reported.[10] Spectral quality TFE, TFA, and the deuterated NMR solvents were obtained from commercial sources. Water was quartz double-distilled and TFA was redistilled at 70–71°; the other solvents were used without further purification.

The spectra were taken with a Varian HR-220 spectrometer which operates at 220 MHz. The amide region was recorded on a 1000-Hz spectral width scale and the frequency calibrated by sideband modulation of the internal reference peak. In the case of aqueous solutions, the internal standard was *tert*-butyl alcohol and the chemical shifts were referred to Me_4Si by adding 246.2 Hz to the TBA-referred frequencies; 246.2 Hz is the relative shift of the methyl groups of TBA when referred to Me_4Si in Me_2SO-d_6 and within ±2.2 Hz was found invariant over the temperature range studied. This procedure allows all the amide chemical shifts to be compared by reference to internal TMS and is equivalent to comparing the amide chemical shifts in water to those in Me_2SO-d_6 referred both to TBA, and then comparing the chemical shifts in Me_2SO to those in the other solvents with Me_4Si as standard.[11b] The spectrometer probe temperature was determined with either ethylene glycol or methanol and based on the temperature calibration chart supplied by Varian.

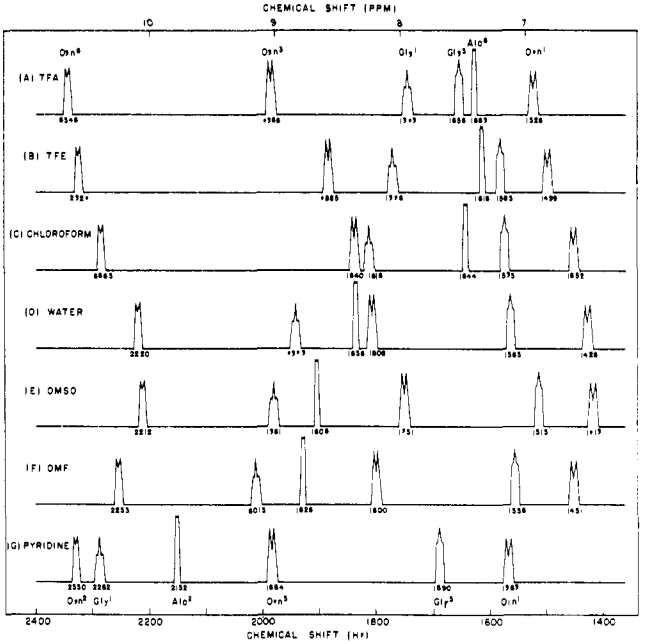


Figure 3. Schematic ^1H NMR spectra for the amide region of alumichrome C, at 25°, in different solvents and referred to internal Me_4Si. The numbers below each peak indicate the exact resonance frequency (in hertz) as interpolated from the linear least-squares fit of the chemical shift vs. temperature plots.

Results and Discussion

(a) Acidity–Basicity Effects. A scheme showing the amide ^1H NMR spectra at 25°, for alumichrome solutions in TFA, TFE, water, and Me_2SO, is shown in Figure 2. Similar data for alumichrome C dissolved in these solvents as well as in chloroform, DMF, and pyridine, are given in Figure 3. It is apparent that there are two groups of amides with respect to their response to the solvent basicity change. The resonance arising from the Gly^1 and Gly^2 in alumichrome and from Gly^1 and Ala^2 in alumichrome C (residues at sites 1 and 2 in both peptides) exhibit a consistent monotonic move from high-field positions in TFA and TFE to low-field positions in Me_2SO. These amides are exposed and the shift is consistent with a strengthening of the H-bond acceptor quality of the solvent. In contrast to this move, the other four resonances (Gly^3 and three ornithyl amides) shift from low to high field in the transitions from TFA to TFE to $HCCl_3$ to H_2O and achieve a maximal high-field position in Me_2SO, a nonprotic nucleophile. These latter four residues are characterized by having internal NH's while their peptide-linked carbonyls are exposed. The trend from TFA to Me_2SO is hence consistent with an increased diamagnetic shielding of the amide proton as the solvent acidity is decreased. The data further indicate that the Orn^3 NH resonance is the one that senses the acidity variations the most as it moves, in alumichrome, from 1962 Hz in TFA to 1722 Hz in Me_2SO ($\Delta\delta$ = −1.10 ppm) in contrast to the Orn^1, Orn^2, and Gly^3 resonances that shift −0.27, −0.64, and −0.65 ppm, respectively. In alumichrome C the shifts of the corresponding amides are of the same magnitudes.

The external amides differ in that while the site 1 NH has its peptide linked C=O exposed, hence responsive to acidity changes, the site 2 NH-linked C=O is internal (Figure 1). On going from chloroform to DMF ($pK_{a_1} \sim 0$), the Ala^2 amide significantly shifts from 1644 to 1929 Hz, $\Delta\delta$ = +1.3 ppm (Figure 3). For the Gly^1 NH the shift is from 1814 (chloroform) to 2013 Hz (DMF), $\Delta\delta$ = +0.91 ppm. Since on going from chloroform to DMF the solvent

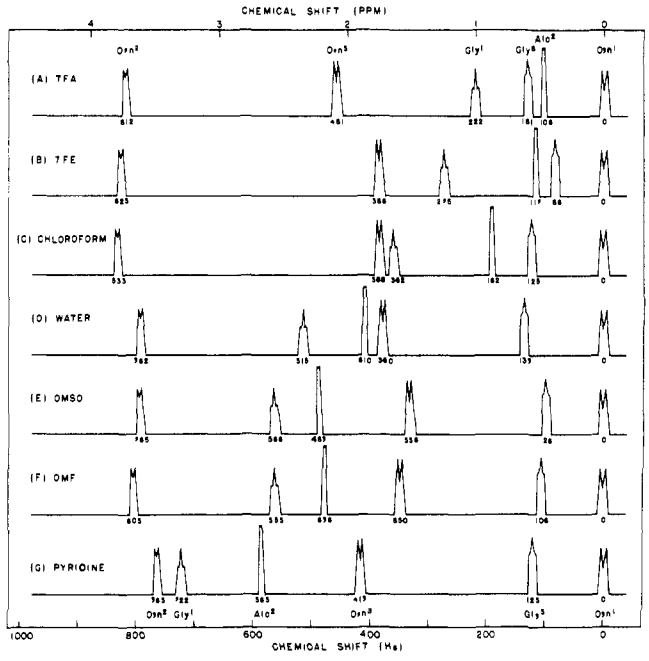


Figure 4. Schematic ^{1}H NMR spectra for the amide region of alumichrome C, at 25°, in different solvents, referred to the Orn^1 peak. The data are the same as in Figure 3, merely shifted to bring the Orn^1 peaks into registry.

acidity decreases, the loss of any carbonyl protonation effect at the (Ala^2)C=ONH(Gly^1) link subtracts from the deshielding caused by the increase in H-bond strength, thus accounting for the 0.4-ppm smaller response of Gly^1 relative to Ala^2 upon the solvent change.

Me_2SO is a solvent of basicity similar to DMF (Table I). The trend for the chloroform to Me_2SO transition parallels that for the chloroform to DMF change, an almost exact correspondence for the chemical shifts in Me_2SO being obtainable if the resonances in DMF are shifted by −0.1 to −0.2 ppm, as if DMF were somewhat more nucleophilic than Me_2SO. Explanations for the slightly different effects of the two solvents should be sought in their particular dielectric properties (Me_2SO is more polar than DMF), steric ability for H-bond pairing, and diamagnetic anisotropic effects of the H-acceptor groups.

On going from Me_2SO (or DMF) to pyridine (pK_{a_1} = 5.2), a further deshielding ought to be expected for the exposed Gly^1 or Ala^2 NH. Gly^1 shifts from 1981 (Me_2SO) to 2289 Hz (pyridine), $\Delta\delta$ = +1.39 ppm, and Ala^2 moves from 1904 (Me_2SO) to 2152 Hz (pyridine), $\Delta\delta$ = +1.13 ppm (Figure 3). However, these shifts should not be attributed only to a strengthening of the amide to solvent H bond, since the pyridine-N:HN pairing will result in an extra deshielding from the ring current at the H-bond proton site.[17] Furthermore, the aromatic cycle itself provides a nucleophile capable of pairing with electronically deficient atoms.[25] It has been observed, for example, that the α-proton resonances of cyclo(tri-L-prolyl) shift upon addition of benzene to a solution of the peptide in CD_2Cl_2.[26] Some resonance shifts are observed in the aliphatic region of the alumichrome C spectrum on going from Me_2SO to pyridine. More relevant to our discussion, the solvent change shifts the unexposed NH's of Gly^3, Orn^1, Orn^2, and Orn^3 by +0.80, +0.68, +0.54, and +1.06 ppm, respectively.

Both Gly^3 and Orn^1 have internal amides and exhibit similar response to the various solvent transitions (Figures 2 and 3). The lower field position of the Gly^3 resonance suggests its amide dipole may be somewhat stabilized by H bonding to the Orn^3 C=O and that this H bond may be about as stable as that of the site 2 NH toward TFA. A relative estimate of the intramolecular H-bond strengths can be obtained by referring the amide shifts to Gly^1, whose NH has its peptide-linked C=O exposed, as do the other three intramolecularly H-bonded amides. The fact that in TFA Gly^1 resonates at higher frequency (1747 Hz) than Gly^3 (1656 Hz) indicates that the intramolecular H bonding of Gly^3 is weaker than the H bonding of Gly^1 to the solvent, i.e., it is very weak. Similarly, the close equivalence between Orn^3 and Gly^1 in chloroform implies that the Orn^3-NH···O=C-Gly^3 H bond is slightly more stable than that of an amide to chloroform.[27] Finally, the close equivalence between Orn^2 and Gly^1 in pyridine indicates that the intramolecular H bond of Orn^2 is somewhat stronger than that of an amide to pyridine. The above estimate of the relative H-bond strengths is in full agreement with the prediction based on the X-ray crystallographic distances between the H donor (N) and H acceptor (O) atoms.[8]

Figure 4 more directly illustrates the effects of solvent basicity on the alumichrome C resonances. The data shown in Figure 3 are replotted by referring the chemical shifts to the Orn^1 (NH buried) amide, thus correcting for the consequences of C=O protonation. It is interesting to note that while in Figures 2 and 3 the site 2 NH exhibits a relative insensitivity to the acidity decrease on going from TFA to TFE to $DCCl_3$ (as would be expected from the internal location of its peptide-linked C=O), Figure 4 reveals this amide to be responsive to the basicity variations among these solvents.

It is possible to estimate approximate pK_{a_1} values for TFE and TFA on the basis of their acidities (pK_{a_2}) relative, e.g., to ethanol ($pK_{a_1} \sim -3.1$, $pK_{a_2} \sim 17.5$)[20] and acetic acid ($pK_{a_1} \sim -6.1$, $pK_{a_2} = 4.75$),[20] respectively. Thus, on going from TFE to ethanol, $\Delta pK_{a_2} \sim 17.5 - 12.4 = 5.1$ so that $pK_{a_1} \sim -3.1 - 5.1 = -8.2$ may be approximated for TFE. Similarly, on going from TFA to acetic acid, $\Delta pK_{a_2} \sim 4.7 - 0.2 = 4.5$ so that $pK_{a_1} \sim -6.1 - 4.5 = -10.6$ may be estimated for TFA. A pK_{a_1} estimate of this kind is not feasible for chloroform; however, on the basis of the acidities of the corresponding acids, a relative basicity scale may be assumed ad hoc: pK_{a_1}(chloroform) > pK_{a_1}(TFE) > pK_{a_1}(TFA). That is, as the proton donor pressure (pK_{a_2}) of the acid decreases, its basicity increases, and the observable trend in the (exposed) NH chemical shift results from a subtraction of the deshielding lost because of a weaker carbonyl protonation (as exhibited, e.g., by Orn^1) from the deshielding gained from the increase in solvent basicity (H-bond acceptor capacity). Since the ^{1}H NMR of the external Gly^1 NH moves from 1747 (TFA) to 1774 (TFE) to 1814 Hz (chloroform), net $\Delta\delta$ = 0.30 ppm (Figure 3), this indicates that the solvent basicity effects dominate the deshielding lost by the acidity drop as, on the contrary, the resonance move would have been of the opposite sign. This is not surprising considering (a) that the amide hydrogen is directly involved in the H bond to a base while the effects from carbonyl protonation are indirect, and (b) the fact that amides are better acids than they are bases. The extent of the alumichrome C Gly^1 NH shift on going from TFA to TFE (Δf = +27 Hz) is similar to that for the same residue in alumichrome (Δf = +25 Hz) and indicates that steric hindrance of the Ala^2 side chain is not an important factor in this regard. Similarly, going from TFE to chloroform, the alumichrome C Ala^2 resonance moves from 1616 to 1644 Hz, the +28-Hz shift being indicative of more important effects caused by a basicity gain than by an acidity loss.

A plot of the Gly^1 NH chemical shift referred to the Orn^1 amide resonance vs. the solvent pK_{a_1} is given in Figure 5. A linear dependence on pK_{a_1} is indicated for the H-bond

deshielding, the two protons becoming magnetically more equivalent as the basicity decreases. On the basis of the line shown, an apparent $pK_{a_1} \sim -6$ is suggested for chloroform as an H acceptor from an amide. The 1-ppm shift in TFA may be ascribed to a finite nucleophilic character for this solvent. Our results are then consistent with the data of Klotz and collaborators[15] showing that TFA significantly protonates peptides. Yet, by having some H-acceptor properties, TFA can also adequately solvate an exposed NH thus explaining, as proposed by Bovey,[28] its effectiveness in causing helix-coil transition in polypeptides.

(b) Amide Hydration and Synergistic Effects of the H Bond. Water is both a weak base ($pK_{a_1} \lesssim -1.8$) and a weak acid ($pK_{a_2} = 15.7$). Figures 2-4 suggest that, in spite of its pK_a values, water should be classified among the basic rather than the acidic solvents in regard to its interaction with polypeptide amides.

Amide hydration will be discussed by reference to the effects of TFE and Me_2SO. On going from the more acidic to the more basic solvents, Ala^2 shifts from 1616 (TFE) to 1838 (H_2O) ($\Delta\delta = +1.02$ ppm) to 1904 Hz (Me_2SO) ($\Delta\delta = +0.29$ ppm), while, in comparison, Gly^1 shifts from 1774 (TFE) to 1943 (H_2O) ($\Delta\delta = +0.77$ ppm) to 2013 Hz (Me_2SO) ($\Delta\delta = +0.18$ ppm). Given that the site 2 and site 1 NH's differ in the extent of exposure of the linked C=O, the relative magnitude of the resonance moves of these two amides shows that their spectrum is more sensitive to the basicity rather than to the acidity changes. The H-donor character of the aqueous solution should, however, account for the lesser effect of the solvent transitions on Gly^1 relative to Ala^2 and it may provide a rationalization for the shifts observed for the internal amides. For example, the buried Orn^1 NH whose peptide linked C=O is exposed moves from 1499 (TFE) to 1428 (H_2O) ($\Delta\delta = -0.32$ ppm) to 1417 Hz (DMSO) ($\Delta\delta = -0.05$ ppm), consistent with the acidity drop in each transition. The Orn^1 resonance move ought to be compared with that of the similarly situated Orn^2 NH as the latter, in contrast, strongly H bonds to its side chain (Figure 1). Thus, the Orn^2 resonance moves from 2324 (TFE) to 2220 (H_2O) ($\Delta\delta = -0.47$ ppm) to 2212 Hz (Me_2SO) ($\Delta\delta = -0.04$ ppm), consistent with a cooperative effect on the H-bond deshielding.

The Gly^3 NH shifts from 1583 (TFE) to 1565 (H_2O) ($\Delta\delta = -0.08$ ppm) to 1515 Hz (Me_2SO) ($\Delta\delta = -0.23$ ppm). The Orn^3 NH, in turn, moves from 1885 (TFE) to 1808 (H_2O) ($\Delta\delta = -0.35$ ppm) to 1751 Hz (Me_2SO) ($\Delta\delta = -0.26$ ppm). While the monotonic decrease in their chemical shifts suggests that protonation of the peptide linked (external) carbonyl is controlling the proton shielding, the stronger effect of the acidity decrease on the intramolecularly H-bonded Orn^3 resonance should be noted.

On going from chloroform to DMF, the buried Orn^1 resonance barely moves (Figure 3). In the same solvent transition Gly^3 moves from 1575 ($DCCl_3$) to 1556 Hz (DMF) ($\Delta\delta = -0.08$ ppm), Orn^3 moves from 1840 ($DCCl_3$) to 1800 Hz (DMF) ($\Delta\delta = -0.18$ ppm), and Orn^2 moves from 2285 ($DCCl_3$) to 2253 Hz (DMF) ($\Delta\delta = -0.14$ ppm). For the latter two intramolecularly H-bonded amides, the carbonyl protonation effect again appears to be more important. The solvent-induced resonance shift is hence somewhat dependent on whether or not the intramolecular amide is H bonded. This indicates that in determining the extent of ^1H NMR deshielding a synergism exists between H bonding and the solvent stabilization of the local peptide dipole.

Relative to Orn^1, Orn^2 appears to be more responsive to the solvent basicity increase, becoming consistently more shielded on going from TFA to pyridine (Figure 4). The relatively higher sensitivity of the short Orn^2 H bond to the acidity of the solvent would thus result from the fact that the C=O end of the peptide link should be more negatively polarized in Orn^2 than in Orn^1 because of the further stabilization of the positive NH charge by the hydroxamate NO^- group. A similar synergism would explain the Orn^3 shift (Figure 4). For this amide, however, the H bond-paired carbonyl (Gly^3 C=O, Figure 1) has its peptide-linked NH exposed so that, as the solvent basicity is increased, a further cooperativity may result. This would explain the relative low-field move of this amide on going from Me_2SO to DMF to pyridine (Figure 4).

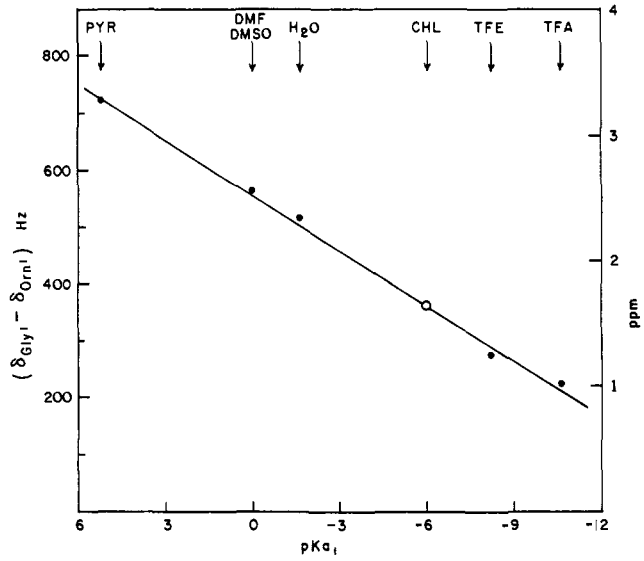


Figure 5. Solvent basicity dependence of the Gly^1 NH chemical shift referred to the Orn^1 NH resonance frequency. Basicities (pK_{a_1}) for the TFE and TFA have been estimated as described in the text. The basicity of chloroform is not known; this plot suggests a pK_{a_1} value of about −6.

The Ala^2 amide differs from the corresponding Gly^1 group in that its C=O is internally H bonded by the Orn^3 NH (Figure 1). The strength of this H bond should depend on the acidity of the Orn^3 amide which, in turn, is affected by the Orn^2 C=O protonation by acids. Hence, a more important weight for the acidic relative to the basic properties of the solvent might be expected for Ala^2 than for Gly^1. Indeed, on going from TFA to TFE the Ala^2 NH ^1H NMR moves from 1629 to 1616 Hz, $\Delta\delta = -13$ Hz (Figure 3). Although the shift is small, it is opposite to that exhibited by the Gly^1 NH in the same solvent transition. A similar trend is exemplified by the 7-Hz shift observed for the Gly^2 NH in alumichrome compared to 25 Hz for Gly^1 in the same peptide. Thus the exposed site 2 NH again suggests the kind of synergistic effect already encountered, only that here the external solvent control of the intramolecular H-bond strength appears to be transmitted, through the participating carbonyls, to a rather removed NH.

(c) Temperature and Dielectric Effects. The temperature dependence of the ^1H NMR chemical shift has been recognized as a useful criterion for distinguishing "exposed" (solvent interacting) from "internal" (buried or intramolecularly H bonded) amides, as the former usually exhibit a larger temperature coefficient than do the latter.[29] In the case of the alumichromes dissolved in water or in Me_2SO, the criterion was found to apply rigorously, lending strong support to the method soon after it was proposed.[11] The temperature coefficients of the amide NH chemical shifts are included in Tables II and III and exemplified in Figure 6 for alumichrome C dissolved in TFA and in pyridine.

The temperature coefficients for the internal and external amide protons of gramicidin S in TFE have been re-

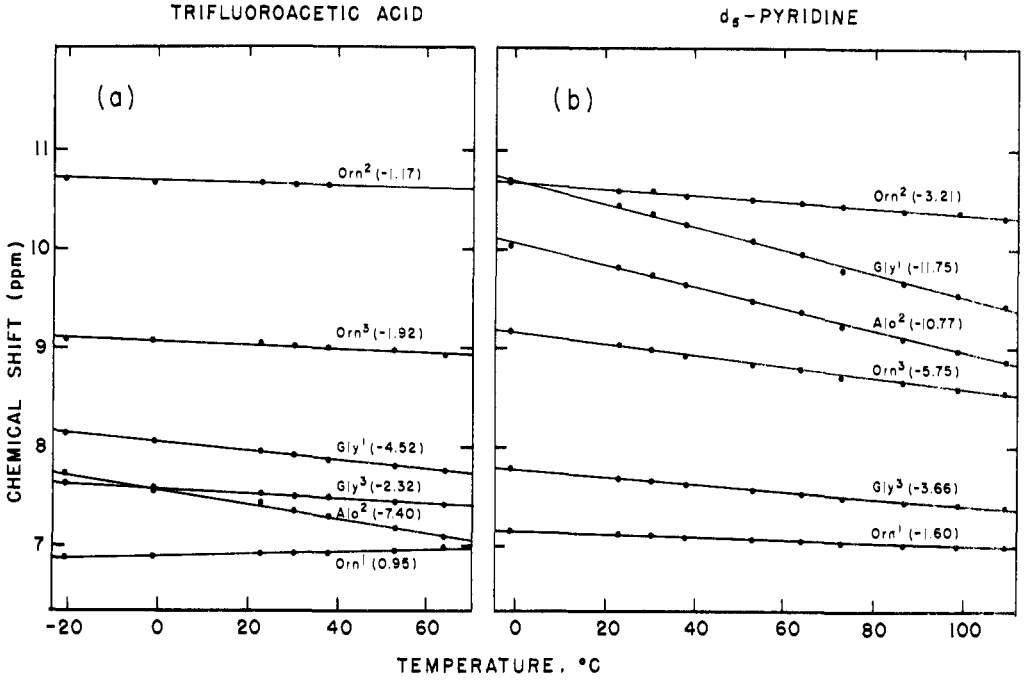


Figure 6. The temperature dependence of the chemical shifts of the amide NH protons of alumichrome C in TFA and in pyridine. The numbers in parentheses are 10^3 times the slope of the corresponding lines expressed in the graph units, i.e., $-1.17 = -1.17 \times 10^{-3}$ ppm/°C. The chemical shifts are referred to internal Me_4Si. The slopes for similar plots in other solvents are given in Table III.

Table II. Temperature Coefficients [(ppm/°C) × 10^3] for the Chemical Shifts of the Alumichrome Amide Resonances in Various Solvents

	Gly¹	Gly²	Gly³	Orn¹	Orn²	Orn³
TFA	−4.59	−6.35	−2.49	1.23	−2.75	−3.15
TFE	−5.40	−8.07	−1.74	0.43	−2.57	−3.38
H_2O	−6.81	−5.12	−2.67	1.35	−1.81	−2.92
Me_2SO	−6.38	−5.12	−0.97	0.83	−1.90	−1.87

ported to be very similar.[35] In contrast, our data for alumichrome (Table II) and alumichrome C (Table III) show that in this solvent the exposed sites 1 and 2 protons exhibit slopes that are significantly larger than those of any of the other internal amides, proving that TFE is not an exception to the above criterion. Indeed, in spite of the fact that the slopes vary from one solvent to another, the six amides show similar trends relative to each other. Thus, the exposed Gly¹ always exhibits a stronger temperature dependence than does the intramolecularly H-bonded Orn². Similarly, the Gly¹ and Ala² lines in alumichrome C (or Gly¹ and Gly² in alumichrome) are those with the highest slope value, being quite parallel in the basic solvents and less so in the acidic ones where protonation of the site 2 carbonyl directly affects the Gly¹ NH. By the same token, both Gly³ and Orn³, which structure the distorted β sheet, show reduced slopes. The Orn² NH, intramolecular and strongly H bonded, exhibits the weakest, negative coefficient while the buried Orn¹ amide is the only NH to exhibit small positive slopes in all solvents except pyridine.

On going from Me_2SO to DMF to pyridine, the shift of the internal NH resonances parallels those of the external NH's, although to a somewhat lesser extent (Figures 2 and 3). This is surprising as the internal amides, in contrast to the exposed sites 1 and 2 NH's, should not sense the solvent basicity changes since their direct H bonding to the solvent is highly impaired. It is likely that, as the dielectric constant decreases (as on going from Me_2SO to DMF to pyridine, Table I) the amide–solvent interaction increases for the overall peptide unit. The effect may be somewhat magnified in DMF, and even more so in pyridine, due to magnetic an-

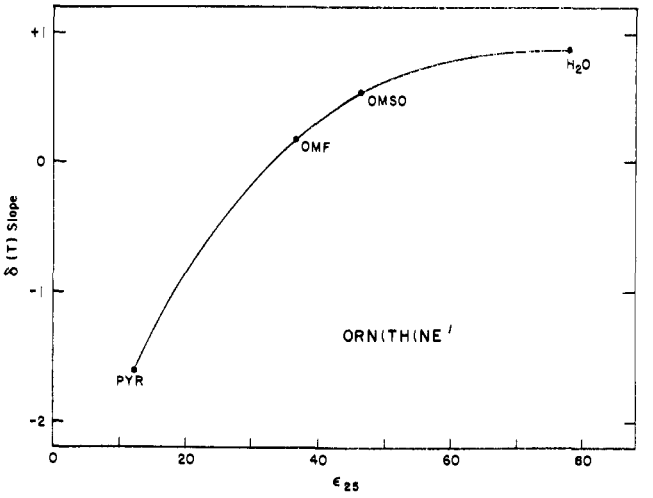


Figure 7. Temperature coefficient of the buried Orn¹ NH chemical shift (in units of ppm/°C × 10^{-3}) vs. the solvent dielectric constant.

Table III. Temperature Coefficients [(ppm/°C) × 10^3] for the Chemical Shifts of the Alumichrome C Amide Resonances in Various Solvents

	Gly¹	Ala²	Gly³	Orn¹	Orn²	Orn³
TFA	−4.52	−7.40	−2.32	0.95	−1.17	−1.92
TFE	−5.33	−8.35	−1.72	0.90	−1.61	−2.87
Chloroform	−5.22	−8.57	−2.65	1.36	−1.89	−3.91
Water	−6.49	−6.23	−2.36	0.88	−1.77	−2.65
Me_2SO	−5.44	−4.67	−1.06	0.55	−1.66	−1.70
DMF	−6.94	−6.25	−1.79	0.18	−2.34	−2.79
Pyridine	−11.75	−10.77	−3.66	−1.60	−3.21	−5.75

isotropic shifts arising from the unsaturated nucleophiles. This would explain the anomalous slope of the Orn¹ NH in pyridine; the temperature coefficient of this amide monotonically follows the dielectric constant of the solvents as if the thermally activated process changed from H-bond formation to H-bond breakage upon decrease of solvent polarity (Figure 7). For the acidic solvents the correlation does

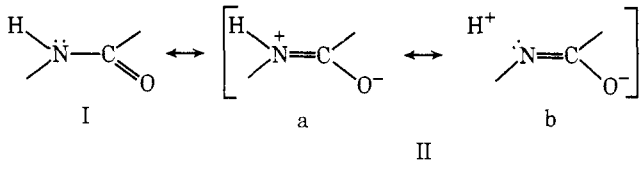
not necessarily hold as they act from the outside, namely, on the (peptide bonded) Orn[2] carbonyl.

In DMF the Orn[1] slope is 0 ppm/°C up to 64°, at which temperature it increases to $+0.179 \times 10^{-3}$ ppm/°C. We have observed a similar curvature for one of the amides of deferriferrichrysin in Me_2SO,[24] which indicates that even if no conformational transition is involved, the temperature dependence of the amide chemical shift is not always rigorously linear.

Conclusions

A wealth of experimental evidence shows that the polypeptide backbone is protonated by organic acids.[30] Moreover, O-protonation of amides would be favored over N-protonation,[14a,31] a contention which has received further support from theoretical ab initio SCF studies,[32] especially if amide planarity is enforced.[33] Thus upon protonation or H bonding, tautomers II (Scheme I), which are responsible

Scheme I



for amide planarity, ought to be stabilized. This effect has, indeed, been indicated by experiments.[31b,34]

Formation of an H bond is usually accompanied by a change in the dipole moment of the interacting species.[17] According to the charge-transfer theory, the proton-donor group tends to acquire excess electronic density directly from the basic electron-donor complement.[35] In the case of the amide this would decrease the negative charge at the carbonyl, which, in turn, can be relieved by an electron density shift from the nitrogen atom lone-pair orbital. The net effect would be to reduce somewhat the electronic density at the amide hydrogen atom. Upon H bonding, a more polar charge distribution (emphasizing resonance structures II over I) has been indicated by ab initio SCF calculations on polyglycine, as on going from fully extended to α-helical conformations.[36] Similar conclusions have been reached in the case of *N*-methylacetamide on the basis of infrared spectroscopic data for the molecule in the gaseous state and the structural parameters of the crystalline species.[37] According to the authors, the evidence suggested that "*when H bonds are formed, a redistribution of the electronic density takes place, mostly within the -HNCO- group*".[37] Thus, the H–N bond force constant (k_{NH}) drops from 7.25 mdyn/Å in the gas to 6.36 mdyn/Å in the crystal, consistent with an electronic rearrangement at this bond. Furthermore, ab initio MO calculations by Johansson and Kollman[38] have shown an enhanced H-bond acid strength for formamide relative to ammonia as a result of the a ⇄ b resonance in tautomer II. In any event, as shown by the alumichromes, the direction of the electron density shift from the NH to the carbonyl results in a decreased magnetic shielding for the amide proton and hence results in shift to lower fields of its ^{1}H NMR.

The rationalization given above for the peptide resonance shifts explains the upfield move observed in diketopiperazine (NH and C=O exposed) and in the exposed NH's (C=O buried) of the ornithyl and leucyl residues of gramicidin S, as well as the downfield shift of its internal valyl and leucyl NH's (C=O external) on going from methanol to TFE.[5a] Similar, though more exaggerated, shifts had been reported earlier by Schwyzer and Ludescher[2] for the gramicidin S resonances on going from methanol to TFA. Analogous downfield and upfield shifts were observed by Kopple and Schamper[5b] for the internal and external amides of cyclic peptides, respectively, on going from Me_2SO to hexafluoro-2-propanol. The present study thus provides a basic guideline for the interpretation of the pattern of amide resonance shifts upon solvent perturbation and should help to establish the convenience and limitations of the method in conformational studies by ^{1}H NMR spectroscopy.

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