Nuclear Magnetic Resonance Studies of the Acid–Base Chemistry of Amino Acids and Peptides. II. Dependence of the Acidity of the C-Terminal Carboxyl Group on the Conformation of the C-Terminal Peptide Bond[1]

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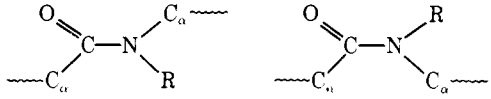
Abstract: The acid–base chemistry of selected N-substituted peptides which exist as mixtures of the cis and trans isomers with respect to the conformation of the N-substituted peptide bond has been studied by ^1H and ^{13}C nmr. Interchange between the two isomers by rotation around the peptide bond is slow on the nmr time scale. From the pH dependence of the ^1H and ^{13}C chemical shifts of the cis and trans isomers of acetylsarcosine, glycylsarcosine, and glycylglycylsarcosine and the ^{13}C chemical shifts of the cis and trans isomers of glycyl-L-proline, glycyl-L-hydroxyproline, and L-alanyl-L-proline, acid dissociation constants were determined for the N-terminal ammonium groups and the C-terminal carboxylic acid groups of the two isomers. For each peptide, the carboxylic acid group of the trans isomer is less acidic than that of the cis isomer by from 0.33 to 0.47 pK_a unit, and the proportion of peptide in the trans form markedly increases upon protonation of the carboxylate group in each of the peptides studied. These results and chemical shift data suggest intramolecular hydrogen bonding between the carbonyl oxygen of the C-terminal peptide bond and the carboxylic acid proton in the trans isomer.

It is well known that the peptide bond is planar and that, in most cases, the α-carbon atoms of the amino acid residues joined by the bond are in a trans conformation.[2] When the peptide bond is N-substituted, as in peptides containing proline and sarcosine (*N*-methylglycine), the cis form may be of comparable stability, in which case the peptide exists as a mixture of the two isomers in solution.[3] Since the rate of conformational interchange by rotation around the C–N bond of the peptide linkage is slow on the nuclear magnetic resonance (nmr) time scale,[4-11] nmr provides a convenient method for studying simultaneously the solution chemistry of the two isomers.



trans cis

In the present paper, we report the results of a study of the acid–base chemistry of the cis and trans isomers of some proline- and sarcosine-containing peptides. In each of the peptides studied, the acid dissociation constants of the two isomers are different, particularly those for the C-terminal carboxyl group. In addition, the proportion of cis isomer is strongly dependent on the state of protonation. The results are consistent with intramolecular hydrogen bonding in the fully protonated trans form.

Experimental Section

Chemicals. The peptides were obtained from Sigma Chemical Co. and if no impurities were detectable by nmr were used as received. Acetylsarcosine was prepared by the procedure of Wiley and Borum.[12] *Anal.* Calcd for $C_5H_9O_3N$: C, 45.80; H, 6.92; N, 10.68. Found: C, 45.67; H, 7.01; N, 10.35. Acetylglycylsarcosine was prepared by an analogous procedure in 12% yield. A 25% aqueous solution of tetramethylammonium hydroxide (Eastman Organic Chemicals) was filtered, neutralized with nitric acid, and evaporated to produce white crystals of tetramethylammonium (TMA) nitrate, from which a 20% aqueous stock solution of TMA nitrate was prepared. Nitric acid and potassium hydroxide were analytical grade reagents.

Nmr Measurements. ^1H nmr spectra were obtained on a Varian A60D high-resolution spectrometer at a probe temperature of 25 ± 1°. ^1H chemical shifts were measured relative to the central resonance of the TMA triplet and are reported relative to the methyl proton resonance of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Positive shifts indicate resonances less shielded than the methyl protons of TMA. The protons of TMA are 3.175 ppm less shielded than the methyl protons of DSS.

^{13}C nmr spectra were obtained either with a Bruker HFX-90 spectrometer operating at a frequency of 22.63 MHz and equipped with a Nicolet 1085 computer or on a Varian HA-100 operating at a frequency of 25.1 MHz and equipped with a Digilab FTS/NMR-3 Data System. With both instruments, the Fourier transform mode was used with proton decoupling. On the Bruker HFX-90, the ^{19}F resonance from C_6F_6 in a coaxial capillary was used for the lock; on the Varian HA-100, the deuterium resonance from C_6D_6 in a coaxial capillary served as the lock signal. ^{13}C chemical shifts are reported relative to internal dioxane, positive shifts corresponding to less shielding than in dioxane. The ^{13}C nuclei of dioxane are 67.4 ppm less shielded than those of TMS.

pH Measurements. pH measurements were made as described previously.[1] Acid dissociation constants were evaluated as concentration constants; hydrogen ion concentrations were obtained from the pH meter readings and activity coefficients calculated from the Davies equation.[13,14] p_cH is used to represent the negative logarithm of the hydrogen ion concentration and pH to represent the negative logarithm of the hydrogen ion activity.

Sample Preparation. Solutions were prepared in distilled water and appropriate amounts of the chemical-shift reference compound added (0.01 *M* TMA nitrate for pmr and 0.1 *M* dioxane for cmr). Samples used in studying dissociation of the protonated N-terminal amino group were prepared by adding sufficient solid KOH to the solution to give an initial pH of approximately 12. The first sample was then withdrawn, and subsequent samples were withdrawn at pH intervals of about 0.5 as the pH was decreased with concentrated nitric acid. Samples were taken down to a pH of approximately 5.0. Samples used in studying dissociation of the C-terminal carboxylic acid group were prepared by adding to a solution containing only peptide and chemical-shift reference compound sufficient concentrated nitric acid to give a pH of approximately 0.5. The first sample was then withdrawn, and subsequent samples were withdrawn at pH intervals of about 0.5 as the pH was increased with a concentrated solution of KOH. Samples were taken up to a pH of approximately 5.0. In this way, variations in ionic strength were minimized. Ionic strengths are reported in Table I with the acid dissociation constants. Spectra were obtained immediately after sample preparation. For glycine, glycylglycine, and in one experiment with glycylsarcosine, samples for both pmr and cmr measurements were withdrawn from the same solution. In these cases, the dioxane resonances were used as the reference in both sets of spectra.

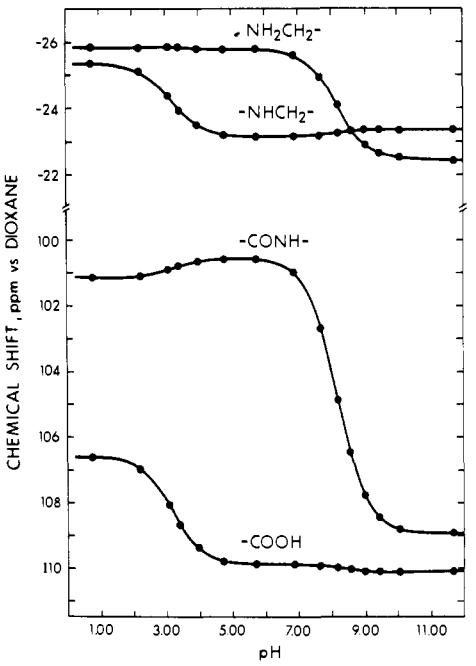


Figure 1. pH dependence of the ^{13}C chemical shifts of glycylglycine.

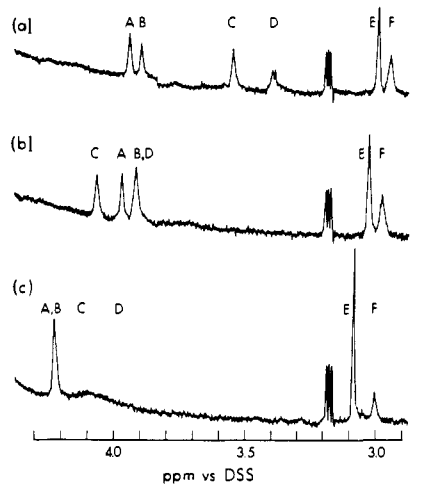
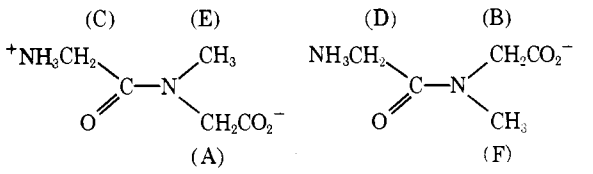


Figure 2. Nmr spectra of the carbon-bonded protons of glycylsarcosine at (a) pH 11.25, (b) pH 5.56, and (c) pH 0.66. The assignments are



Results

Measurement of Acid Dissociation Constants by Nmr. The determination of acid dissociation constants from pmr chemical-shift data is a well-documented technique.[15] To establish that cmr chemical shifts can be applied in a similar way, ^1H and ^{13}C spectra were recorded for glycine, glycylglycine, and glycylsarcosine throughout the pH range of approximately 0.5 to 12. The ^{13}C chemical shifts for Gly-Gly are shown as a function of pH in Figure 1. Acid dissociation constants were calculated using chemical-shift data in pH regions where the averaged chemical shift for a given resonance is intermediate between the shifts of that resonance for the molecule in two protonated forms. For example, the dissociation constant for the carboxylic acid group of Gly-Gly was calculated using -COOH and $-NHCH_2-$ ^{13}C chemical-shift data in the pH region 2 to 4. At a given pH, the observed chemical shift is a weighted average of the chemical shifts for the carboxylate protonated and ionized forms. A value for the acid dissociation constant was obtained from each of the data points in this region, using the equation

$$pK_a = p_cH + \log \frac{f_{protonated}}{f_{ionized}} \quad (1)$$

where $f_{protonated}$ and $f_{ionized}$ are the mole fractions of the protonated and ionized forms. The mole fractions at a given pH were calculated from the averaged chemical shift.[16] Within experimental uncertainty, the same values were obtained from pmr and cmr data: for 0.5 *M* glycine, $pK_{A,carboxyl} = 2.37$ and $pK_{A,ammonium} = 9.64$ by pmr and $pK_{A,carboxyl} = 2.39$ and $pK_{A,ammonium} = 9.67$ by cmr; for 0.5 *M* Gly-Gly, $pK_{A,carboxyl} = 3.11$ and $pK_{A,ammonium} = 8.08$ by pmr and $pK_{A,carboxyl} = 3.12$ and $pK_{A,ammonium} = 8.07$ by cmr.[17] The results for Gly-Sar are given in Table I.

Acetylsarcosine, Glycylsarcosine, and Glycylglycylsarcosine. The pmr spectra for the anion, zwitterion, and cation forms of glycylsarcosine are shown in Figure 2. Each spectrum consists of three sets of two resonances from the carbon-bonded protons of the trans and cis isomers. Figure 3 shows the chemical shifts of these resonances as a function of pH. Resonances C and D shift by approximately 0.6 ppm as the amino group is protonated and, since the other four resonances are relatively unaffected, are assigned to the glycyl methylene protons. On protonation of the carboxyl group, these two resonances shift by only about 0.05 ppm and are broadened through coupling to the ammonium protons which are exchanging slowly at this pH. Resonances A and B shift by up to 0.3 ppm and are thus assigned to the

Table I. Acid Dissociation Constants[a-d]

	Technique	Concn, *M*	pK_a, carboxyl[e] Trans	Cis	pK_a, ammonium[f] Trans	Cis
Acetylsarcosine	Pmr	0.5	3.49 (3.51)	3.09 (3.11)		
Glycylsarcosine	Pmr	0.1	2.90 (3.00)	2.57 (2.67)	8.54 (8.63)	8.49 (8.58)
	Pmr	0.5			8.55 (8.63)	8.50 (8.58)
	Cmr	0.5			8.52[g] (8.60)	
Glycylglycyl-sarcosine	Pmr	0.1	3.10 (3.20)	2.67 (2.77)	8.02 (8.11)	7.94 (8.03)
Glycyl-L-proline	Cmr	0.6	3.03 (3.05)	2.56 (2.58)	8.55 (8.61)	8.49 (8.55)
Glycyl-L-hydroxyproline	Cmr	0.5	2.88 (2.90)	*h*	8.42 (8.50)	8.32 (8.40)
L-Alanyl-L-proline	Cmr	0.5	3.22 (3.25)	*h*	8.50 (8.58)	8.24 (8.32)

[a] At 25°. [b] Values with no brakcets are concentration constants. Values within brackets are mixed activity-concentration constants; the hydrogen ion activities were obtained directly from the pH-meter readings. [c] Precision from pK_a values is ±0.02 for those obtained from pmr measurements and ±0.06 for those from cmr measurements. [d] pK_a values calculated using the resonance which shifted by the largest amount. [e] For a concentration of 0.1 *M* peptide, the ionic strength was 0.4, for 0.5 *M*, μ was 0.8, and for 0.6 *M*, μ was 0.9 in the pH range where pK_a for the carboxyl groups was measured. See Experimental Section for details on ionic strength control. [f] For a concentration of 0.1 *M* peptide, the ionic strength was 0.1, for 0.5 *M*, μ was 0.5, and for 0.6 *M*, μ was 0.6 in the pH range where pK_a for the ammonium group was measured. [g] Resonances for cis and trans isomers not sufficiently resolved for pK_a calculations for the two isomers. [h] Population of cis isomer too small to make accurate chemical-shift measurements.

Table II. Summary of Chemical Shifts of Carbon-Bonded Protons of Sarcosine-Containing Peptides[a]

		δ_{cation}		$\delta_{zwitterion}$		δ_{anion}	
		Trans	Cis	Trans	Cis	Trans	Cis
Acetylsarcosine	CH_2CO_2			4.143	4.275[b]	3.910	3.957
	NCH_3			3.130	2.940	3.064	2.910
	$COCH_3$			2.170	2.078	2.133	2.024
Glycylsarcosine	CH_2CO_2	4.217	4.217	3.965	3.912	3.942	3.892
	NCH_3	3.083	3.005	3.023	2.972	2.980	2.935
	H_2NCH_2CO	4.11[c]	3.99[c]	4.058	3.912	3.487	3.383
Glycylglycylsarcosine	CH_2CO_2	4.182	4.280	3.935	3.955	3.933	3.955
	NCH_3	3.125	2.982	3.065	2.950	3.065	2.950
	$NHCH_2CO$	4.272	*d*	4.235	4.085	4.192	4.044
	H_2NCH_2CO	3.93[c]	3.93[c]	3.907	3.907	3.405	3.405

[a] In ppm relative to DSS; ±0.002 ppm, unless otherwise indicated. [b] Chemical shifts are for neutral acetylsarcosine. [c] Estimate; resonances broadened owing to coupling to N-bonded protons; ±0.01 ppm [d] Obscured.

Table III. Fractional Concentration of the Trans Isomer[a,b]

	Cation	Zwitterion	Anion
Acetylsarcosine	0.70[c]		0.48
Glycylsarcosine	0.77	0.58	0.59
Glycylglycylsarcosine	0.75	0.61	0.62
Glycyl-L-proline	0.84	0.65	0.58
Glycyl-L-hydroxyproline	0.88	0.66	0.63
L-Alanyl-L-proline	0.89	0.65	0.53

[a] Fractional concentration of the trans isomer at 25°. [b] Uncertainties, as indicated by replicate integrations of resonance pairs in the pmr spectra of acetylsarcosine, Gly-Sar, and Gly-Gly-Sar and by the range of values from different pairs of resonances in the ^{13}C spectra of Gly-Pro, Gly-γ-Hyp, and Ala-Pro, are ±0.01. [c] For the neutral form of acetylsarcosine.

sarcosine methylene protons. The two high-field resonances are assigned to the methyl protons.

Additional support for this assignment comes from long-range proton–proton coupling through the peptide bond; coupling is larger between groups that are trans across the peptide bond.[10] Resonances D and F of anionic glycylsarcosine are a partially resolved quartet and triplet, respectively, with a coupling constant of approximately 0.6 Hz. Decoupling experiments show that these two sets of protons are coupled to each other but not appreciably to the sarcosine methylene protons of the same isomer (resonance B). Decoupling experiments also indicate that the protons giving resonances A and C are mutually coupled, with no coupling between these protons and those giving resonance E. Liberek, *et al.*,[6] have reported pmr spectra of Gly-Sar derivatives. On the basis of the pyridine-induced shifts of the two sarcosine methylene signals, they assigned the low-field resonances to the trans isomer, as here.

Integration of spectrum a shows that resonances A, C, and E have relative intensities of 2, 2, and 3, respectively, and that each constitutes 58% of the total intensity of each pair. Because of overlapping of methylene proton resonances in the zwitterionic and cationic forms, isomer populations for these protonation forms could be obtained only from the methyl resonances. As shown in Table III, the proportion of cis isomer is markedly less in the cationic form than in the other two forms.

The pmr spectra of acetylsarcosine and Gly-Gly-Sar are similar, with the exception that the sarcosine methylene resonance of the cis isomer is downfield from that of the trans isomer. The assignment for acetylsarcosine is the same as that for acetylsarcosine methyl ester in DMSO.[5] The chemical shifts of the carbon-bonded protons of acetylsarcosine, Gly-Sar and Gly-Gly-Sar in the three protonation forms are summarized in Table II, and the fractional concentrations of the trans isomer are given in Table III. The fractional concentrations for acetylsarcosine are in good agreement with those reported by Gerig.[8]

Acid-dissociation constants for the carboxylic acid group of the cis and trans isomers of acetylsarcosine, Gly-Sar, and Gly-Gly-Sar and for the ammonium group of the cis and trans isomers of Gly-Sar and Gly-Gly-Sar were obtained

Table IV. Summary of Carbon-13 Chemical Shifts[a,b]

		Acetylsarcosine		Glycylglycine			Glycylsarcosine		
		Neutral	Anion	Cation	Zwitterion	Anion	Cation	Zwitterion	Anion
N-Terminal C_α	Trans	−46.16	−45.91	−25.82	−25.77	−22.42	−26.31	−26.11	−24.61
	Cis	−46.35	−46.16				−26.50	−26.31	−24.80
N-Terminal C_β	Trans								
	Cis								
CON	Trans	108.38	107.51	101.20	100.60	108.95	101.15	100.37	107.90
	Cis	108.38	107.94				100.65	100.57	108.19
NCH_3	Trans	−29.07	−29.17				−31.21	−31.31	−31.35
	Cis	−31.79	−31.65				−31.31	−31.55	−31.35
C-Terminal C_α	Trans	−16.84	−14.85	−25.33	−23.14	−23.34	−16.74	−14.56	−14.46
	Cis	−14.27	−11.79				−16.26	−13.88	−13.88
C-Terminal C_β	Trans								
	Cis								
C-Terminal C_γ	Trans								
	Cis								
C-Terminal C_δ	Trans								
	Cis								
CO_2	Trans	106.39	109.84	106.63	109.89	110.13	106.10	109.35	109.89
	Cis	106.53	109.55				105.66	108.62	109.30

[a] In ppm relative to internal dioxane. Positive shifts correspond to less shielding than in dioxane. [b] Uncertainty ±0.05 ppm. [c] Shifts given in table for Gly-Gly-Sar CON are for N-terminal CON. Shifts for central CON are: 104.21 for the trans and cis isomers of the cationic form, 103.33 and 103.66 for the trans and cis isomers respectively, of the zwitterionic form, and 103.57 and 103.96 for trans and cis

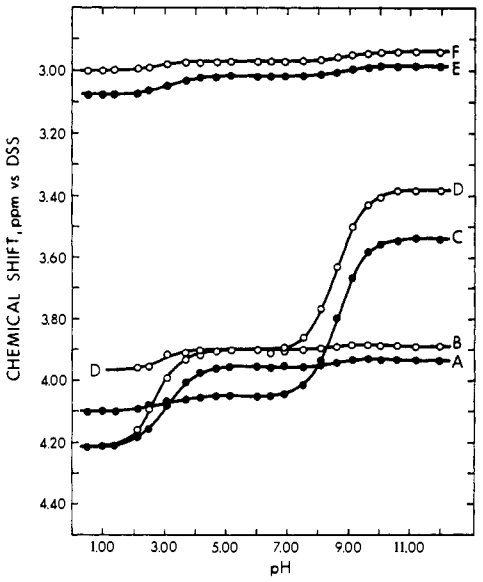


Figure 3. pH dependence of the chemical shifts for the carbon-bonded protons of the cis and trans isomers of glycylsarcosine. Assignments are given in Figure 2.

from data of the type shown in Figure 3. The results are summarized in Table I. Literature values are not available for comparison with the results in Table I, because macroscopic acid dissociation constants obtained by pH titration are a composite of those for the two isomers.

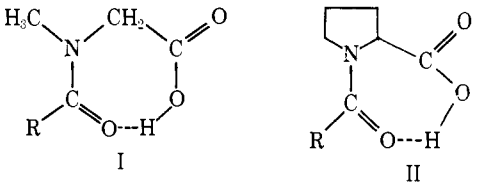
Glycylproline, Glycyl-4-hydroxyproline, and Alanylproline. The ^{13}C chemical shifts for the cis and trans isomers of the three protonation forms of each of these peptides are given in Table IV. Assignments to individual carbons and to cis and trans isomers were made on the basis of reported chemical shifts for the zwitterions of Gly-Pro and Ala-Pro,[9] reported chemical shifts for proline and, for Gly-4-Hyp, the expected shifts on substitution of a hydroxyl group in the γ position of the pyrrolidine ring.[18]

As with other proline peptides, the chemical shifts of the β and γ carbons of the proline ring are most sensitive to the conformation of the peptide bond involving the imino group of the proline residue.[9,11] The carboxyl carbon and the α carbon of the proline residue are the most sensitive to ionization of the proline carboxylic acid group, and dissociation constants for the carboxylic acid groups were evaluated from data for these carbons. The results are given in Table I. As with the sarcosine peptides, the proportion of cis isomer decreases markedly on protonation of the carboxyl group (Table III). The fractional concentrations were estimated from relative peak heights of ^{13}C resonances for cis and trans isomers. Since the two resonances being compared are in similar environments, the effects of spin-lattice relaxation and nuclear Overhauser enhancement on the resonance intensities are expected to be comparable so that their relative intensities should be a measure of relative populations. Fractional populations obtained from ^{13}C peak heights for Gly-Sar were in good agreement with those obtained by integration of pmr spectra.

Discussion

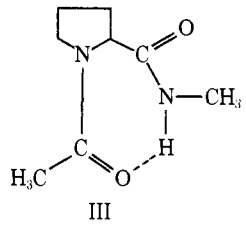
The results obtained in the present work indicate that the cis and trans isomers of these sarcosine- and proline-containing peptides have different acidities, and that the distribution between the two isomers is strongly dependent on the state of protonation. The acidity difference is particularly large for the C-terminal carboxylic acid group, the acidity of this group being greater for the cis isomer by from 0.33 to 0.47 pK_a unit. In addition, the trans isomer is the more abundant isomer when the carboxylate group is protonated, while there is an approximately equal distribution between the cis and trans isomers for the zwitterion and anion forms of the peptides.

These results suggest that there is some intramolecular hydrogen bonding between the protonated carboxylate group and the carbonyl oxygen of the C-terminal peptide bond represented schematically by I and II, which both stabilizes the protonated trans isomer and decreases its acidity; molecular models indicate such a hydrogen bond is possible only in the trans isomer. The fact that the behavior for acetylsarcosine is similar to that of Gly-Sar indicates that the interaction stabilizing the carboxylate-protonated trans



Glycylglycylsarcosine[c]			Glycyl-L-proline			Glycyl-L-hydroxyproline			L-Alanyl-L-proline		
Cation	Zwitterion	Anion	Cation	Zwitterion	Anion	Cation	Zwitterion	Anion	Cation	Zwitterion	Anion
−25.82	−25.87	−22.52	−25.95	−25.95	−23.74	−25.87	−25.87	−23.64	−18.34	−18.34	−18.99
−25.82	−25.87	−22.52	−25.95	−25.95	−23.74	−26.46	−26.45	−24.37	*d*	−18.34	−18.61
									−51.52	−51.47	−47.64
									d	−50.98	−47.64
101.10[c]	101.10[c]	109.55[c]	99.15	98.45	106.06	99.50	98.87	106.49	102.71	101.96	109.30
101.00[c]	101.00[c]	109.45[c]	*d*	98.95	106.81	*e*	99.79	107.60	*d*	102.71	110.43
−31.00	−31.11	−31.06									
−31.45	−31.31	−31.26									
−16.45	−14.37	−14.37	−7.18	−4.59	−4.75	−8.45	−6.02	−6.21	−7.07	−4.42	−4.59
−15.97	−13.64	−13.59	−8.04	−5.02	−4.96	−8.99	−6.60	−6.60	*d*	−4.69	*e*
			−37.60	−37.06	−37.06	−29.90	−29.22	−29.32	−37.82	−37.28	−37.17
			−35.82	−35.01	−35.01	−28.06	−27.18	−27.23	−35.95	−35.12	−35.12
			−42.35	−42.35	−42.29	3.25	3.35	3.35	−41.97	−41.92	−42.04
			−44.72	−44.29	−44.24	1.42	1.70	1.75	−44.88	−44.45	−44.24
			−19.96	−19.93	−20.07	−12.62	−12.52	−12.72	−19.21	−19.21	−19.37
			−19.53	−19.37	−19.48	*e*	−12.13	−12.28	*d*	−19.21	*e*
106.39	109.59	109.64	109.19	112.75	113.29	108.67	112.17	112.75	108.92	112.43	112.96
106.05	108.91	108.96	108.59	111.89	112.86	*d*	111.44	112.36	*d*	111.94	112.96

isomers of the anionic form. Shifts for the central C_α are: −25.48 and −25.63 for the trans and cis isomers of the cation, −25.38 and −25.63 for the zwitterion, and −25.58 and −25.87 for the anion. [d] Intensity of reasonance too small to detect. [c] Obscured.



III

form and decreasing its acidity does not involve the N-terminal ammonium group.

Similar intramolecular hydrogen bonding has been proposed in related systems. Infrared evidence suggests that *N*-acetylproline-*N*-methylamide (III) exists in an intramolecularly hydrogen-bonded form in nonpolar solvents.[19] Madison and Schellman[3] proposed that the carboxylate-protonated trans isomer of acetylproline is favored by a factor of 3 or 4 over the cis isomer both in nonpolar solvents and at pH 1 through similar intramolecular hydrogen bonding. Gerig proposed that intramolecular hydrogen bonding is responsible for the predominance of the trans isomer of carboxylate-protonated acetylsarcosine and Gly-Sar.[8] Although in aqueous solution intermolecular hydrogen bonding with solvent water molecules will be competing with intramolecular hydrogen bonding, it has been suggested that the amount of intramolecular hydrogen bonding is still sufficient to account for the small difference in energy[20] between the cis and trans isomers.[3,8]

Additional evidence for intramolecular hydrogen bonding in the molecules in which sarcosine is the C-terminal residue comes from the relative magnitudes of the chemical shifts of the carbon-bonded protons of the two isomers. The magnitude of the downfield shift for the methylene protons of the sarcosine residue upon protonation of the carboxylate group would be expected to be less for the trans isomer if the acidic proton was shared with the carbonyl oxygen in a hydrogen bond. This is observed to be the case; for acetylsarcosine, the shift is 0.233 ppm for the trans isomer and 0.318 ppm for the cis isomer, for Gly-Sar, 0.252 ppm and 0.305 ppm, and for Gly-Gly-Sar, 0.247 ppm and 0.325 ppm. The magnitude of the downfield shift for the NCH_3 resonance for the trans isomer upon carboxylate protonation would be expected to be larger owing to an inductive effect transmitted through the carbonyl group. For acetylsarcosine, the shift is 0.066 ppm for the trans isomer compared with 0.030 ppm for the cis isomer, for Gly-Sar, 0.060 ppm compared with 0.033 ppm, and for Gly-Gly-Sar, 0.060 ppm compared with 0.032 ppm. The relative magnitudes of the shifts for the protons on the carbon bonded to the peptide-linkage carbonyl carbon, which would be expected to shift more in the trans isomer, are not as predicted by simple inductive-effect considerations and may reflect changes in the anisotropy of the carbonyl group. The differences in the magnitudes of the shifts of the ^{13}C resonances for the cis and trans isomers upon carboxylate protonation relative to the total shifts are too small to provide evidence for intramolecular hydrogen bonding in the trans isomer, with the exception of the carbonyl carbon of the C-terminal peptide bond.

As noted by Christl and Roberts[21] in their study of the pH dependence of the ^{13}C shifts of a number of peptides which exist in the trans configuration, the carbonyl carbon of the amino acid residue directly bound to the C-terminal unit is unusual in that it becomes deshielded on protonation of the carboxylate group. This is illustrated in Figure 1 for glycylglycine. Among the possible explanations they gave for this behavior was the formation of an intramolecular hydrogen bond between the carbonyl oxygen and the carboxylate proton as depicted in I and II. Results described above provide support for such an interaction.[22] Additional effects must also be considered, however, at least in the case of acetylsarcosine and Gly-Gly-Sar where deshielding of the carbonyl carbon upon protonation of the adjacent carboxylate group was observed for both the cis and trans isomers. The lack of such deshielding for the cis isomers of the four dipeptides studied[24] suggests that the presence of a positively charged ammonium group near the carbonyl carbon may be important. Some support is obtained from the observation of deshielding of the glycyl carbonyl carbon of both isomers of *N*-acetylglycylsarcosine, whereas the glycyl carbonyl carbon of only the trans isomer of Gly-Sar is deshielded.

The similarity of the results in Tables I and II for the trans isomer of the sarcosine-containing peptides to the corresponding results for polyglycine peptides where the peptide bond is always trans suggests that the interaction that occurs in the carboxylate-protonated trans isomer of the sarcosine peptides is also present in the carboxylate-protonated polyglycine peptides. The shift of the C-terminal methylene protons on protonation of the carboxylate group of glycine peptides is 0.248 ppm for acetylglycine,[16] 0.276 ppm for Gly-Gly, 0.257 ppm for Gly-Gly-Gly, and 0.257 ppm for Gly-Gly-Gly-Gly.[25] pK_a values for the carboxylic acid groups under similar ionic strength conditions are: 3.44 for acetylglycine, 3.13 for Gly-Gly, 3.30 for Gly-Gly-Gly, and 3.31 for Gly-Gly-Gly-Gly. If this is the case, then pK_a's determined for the C-terminal carboxylate group of polypeptides are for the process

$$RCO_2H \text{ (hydrogen bonded to solvent)} + RCO_2H \text{ (intramolecularly hydrogen bonded)} \rightleftharpoons RCO_2^- + H^+$$

Where they can be measured, the pK_a's of the cis isomers may provide a better estimate of the pK_a for the reaction

$$RCO_2H \text{ (hydrogen bonded to solvent)} \rightleftharpoons RCO_2^- + H^+$$

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Elucidation of the Solution Conformation of the A Ring in Vitamin D Using Proton Coupling Constants and a Shift Reagent

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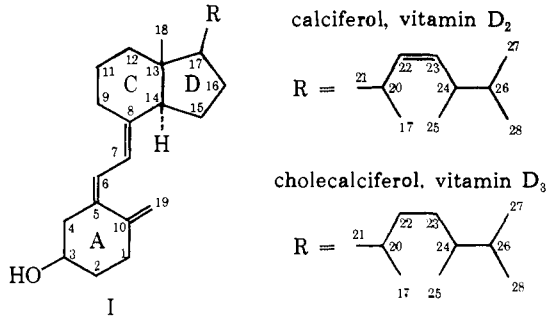
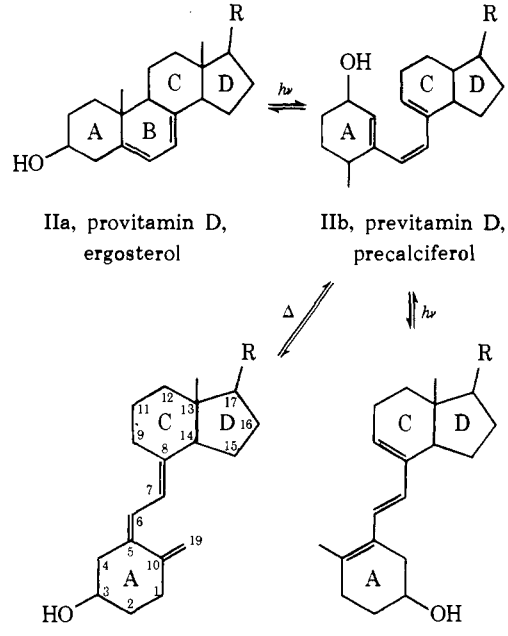
Abstract: The proton spin-spin splittings of vitamin D_2, calciferol, have been analyzed in terms of the solution conformation of the A ring. Using the known coupling constants for cyclohexanol, the observed multiplets are shown to be consistent with a dynamic equilibrium between approximately equal amounts of the α and β chair forms for the A ring. Resonances on the A ring not observable in the pure compound were resolved with the aid of the shift reagent $Eu(dpm)_3$. Analysis of the $Eu(dpm)_3$-induced dipolar shifts confirms the presence of the 1:1 mixture of conformers. The insensitivity of the proton multiplet structure to the presence of $Eu(dpm)_3$ suggests that the equilibrium is not perturbed significantly upon coordination. Conformational analysis as a function of temperature, using the induced dipolar shifts, indicates that the β chair is thermodynamically slightly more stable. The merits and problems of quantitative *vs.* qualitative use of shift reagents in solution conformational analysis of large flexible molecules are discussed.

The class of D vitamins comprises a family of fat-soluble compounds[2-5] derived photochemically from steroids, members of which have long been recognized for their antirachitic activity. Although the exact role is yet to be elucidated, the essence of vitamin D activity[2,6] is to elevate the plasma calcium and phosphate content to supersaturated levels. The most important members of this family of vitamins[4] are ergocalciferol or calciferol, also called vitamin D_2, and cholecalciferol, also known as vitamin D_3, both of which have the skeleton I. They differ only in the nature of the side chain at the 17 position. The vitamins, as indicated in I, must first be metabolized[6] to the 1,25-dihydroxy derivatives, which are thought to be the circulating active form.



I

The well-documented role of photochemical activity[2-8] in the interconversion among various forms of the D vitamins and their precursors, as found, for example, in the conversion of sterol to calciferol[6] in the skin upon uv irradiation, has led to close scrutiny of the photochemistry of this class of compounds. The currently accepted photochemical and thermal pathways for interconverting among several important intermediates are illustrated[4,5] in II for vitamin D_2.



IIa, provitamin D, ergosterol
IIb, previtamin D, precalciferol
IIc, vitamin D, calciferol
IId, tachysterol

The thermal equilibrium involving previtamin D and vitamin D is thought[4,5] to be in favor of vitamin D in spite of the instability of exocyclic double bonds in cyclohexane rings due to the influence of the strained configuration[5] of the five-membered D ring.

Because of their importance, considerable attention has been devoted toward elucidating the structure of vitamin D_2 derivatives or analogs. In tachysterol, the trans,trans,cis form, as opposed to the all-trans forms, for the A–C ring bridge was established[8] by solution NOE measurements.