Amino-acid zwitterion equilibria: vibrational and nuclear magnetic resonance studies of methyl-substituted thiazolidine-4-carboxylic acids

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This paper is dedicated to Professor Arthur N. Bourns

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Infrared and Raman spectra (4000–100 cm^{-1}) of solid samples of six different methyl substituted thiazolidine products of D-penicillamine and L-cysteine hydrochloride have been observed and assigned. Infrared spectra in D_2O solutions have been obtained for comparison in order to study the amino-acid zwitterion equilibria. Proton and ^{13}C nmr spectra for the compounds have also been measured.

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On a déterminé et interprété les spectres infrarouges et Raman (4000–100 cm^{-1}) d'échantillons solides de six produits différents de la D-pénicillamine et du chlorhydrate de la L-cystéine avec des thiazolidines substituées par des groupements méthyles. Dans le but d'examiner l'équilibre acide aminé zwitterion, on a mesuré les spectres infrarouges dans le D_2O. On a aussi mesuré les spectres rmn du ^1H et du ^{13}C de ces composés.

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Introduction

The modes of therapeutic action and adverse side effects of the drug D-penicillamine depend on several biochemical reactions, an important one being the ability to form thiazolidine rings with aldehydes and ketones. Thiazolidine-4-carboxylic acid has also been found to act on the cell membrane of tumour cells, possibly causing a reverse transformation to normal cells through restoration of contact inhibition (1). Therefore, we have been investigating thiazolidine-4-carboxylic acids, and in particular measuring their physical, chemical, and spectroscopic properties, as part of our overall program of studying reactions having biological significance or potential medical applications.

The essential amino acids normally exist in the zwitterion form both in the solid state and in aqueous solutions. Recently, we have shown by vibrational spectroscopy and single crystal X-ray diffraction that (*S*)-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid exists in the amino acid form in the solid state and to a minor extent in aqueous solution (2). The unsubstituted thiazolidine-4-carboxylic acid, however, exists in the zwitterion form (2–4). In this work, we attempt to establish by vibrational spectroscopic studies whether methyl substitution at the C2 and C5 positions of the thiazolidine ring affects the amino-acid zwitterion equilibrium. In addition, we have characterized the various species by ^1H and ^{13}C nmr spectroscopy.

Materials and methods

The D-(−)-penicillamine (free base) and L-(+)-cysteine hydrochloride were of reagent grade, as supplied by Sigma Chemical Co., St. Louis, MS. Formaldehyde, 37%, reagent grade was supplied by Sargent Welch Scientific Co., Skokie, IL. Acetaldehyde was distilled from paraldehyde, reagent grade, supplied by BDH; acetone, reagent grade, was supplied by BDH Chemicals, Toronto, Ont. The preparation of the several thiazolidine-4-carboxylic acids was carried out as indicated schematically in Table 1 and outlined in detail as follows:

Thiazolidine-4-carboxylic acid, I

The method of Ratner and Clarke (5) was followed with modification. L-Cysteine hydrochloride hydrate, **A**, 0.5 g (0.003 mol) was dissolved in 2 mL H_2O to which 0.4 ml (0.4 g or 0.003 mol) of 37% (w/w) formaldehyde was added; the mixture was left to react for 15 h at room temperature. Then 0.5 mL pyridine was added. In 30 min, a white solid started separating slowly. Ethanol, 1 mL, was added and the mixture was placed in the refrigerator. Prismatic crystals were separated from the pyridine ethanol mixture by filtration. The compound was recrystallized from ~10 mL hot H_2O to give 0.3 g (0.002 mol, 70%) of a white, crystalline product, mp 185–187°C (dec.), lit. 184–185°C (dec.) (5).

2-Methylthiazolidine-4-carboxylic acid, II

The method of Riemschneider and Hoyle (6) was followed with modification. To 1.4 mL H_2O was added 2.0 g **A** (0.01 mol) 1 mL, glacial acetic acid, followed by 20 mL EtOH. The solution was placed on ice and after 30 min the solid hydroacetate precipitated. This was filtered out, dissolved in water, and the solution placed on ice. Freshly prepared acetaldehyde, 0.5 g (0.013 mol), was added. The clear mixture was allowed to stand in the refrigerator 48 h; then 0.5 mL pyridine was added. A few days later a thick white precipitate was filtered out and air-dried. Weight 1.0 g (yield 60%); mp 153–155°C (dec.), lit. 161–163°C (6).

2,2-Dimethylthiazolidine-4-carboxylic acid, III

The method of Sheehan and Yang (7) was followed with modification. **A**, 0.7 g (0.0057 mol), was added to 180 mL freshly distilled acetone in a 250 mL round bottom flask. The mixture was refluxed for 7 h and then allowed to cool. The undissolved material (0.5 g unreacted cysteine) was removed by filtration. Acetone was removed by distillation and the remaining 10 mL solution was allowed to stand in the refrigerator. Within a few days a cluster of long thin crystals formed in the acetone solution. These were recrystallized from hot acetone to give 0.1 g product, (62% based on the 0.2 g L-cysteine which reacted, 11% based on the initial 0.7 g); mp 163–165°C, lit. 163–165°C (7).

5,5-Dimethylthiazolidine-4-carboxylic acid, IV

The method of Nagasawa *et al.* (8) was followed with modification for **IV** and **V**. D-Penicillamine, **B**, 0.50 mg (0.0034 mol), was mixed with 10 mL EtOH and 2.5 mL H_2O, resulting in a white suspension. Formaldehyde, (37% w/w), 0.5 mL (0.004 mol), was added with stirring and within 30 min the mixture was clear. In 1 h the mixture turned into a white jelly-like solid suspension. This was left at room temperature for approximately 15 h, and then a few drops of pyridine were added. There was no apparent change in the reaction mixture. The white suspension was filtered off and dried in a desiccator for several days. Yield 0.285 g product (0.0018 mol, 53%); mp 194–196°C, lit. 196–197°C (dec.) (8).

2,5,5-Trimethylthiazolidine-4-carboxylic acid, V

B, 1.55 g (0.01 mol), was dissolved in 15 mL H_2O and the mixture was filtered to remove a small amount of undissolved material. The solution was cooled in an ice bath and 1.2 mL (0.02 mol) acetaldehyde was added with stirring; the flask was then sealed with parafilm. In

TABLE 1. Nomenclature and preparation scheme for the thiazolidine-4-carboxylic acids[a]

Reactants	Formaldehyde	Acetaldehyde	Acetone
A, L-cysteine	**I**, thiazolidine-4-COOH	**II**, 2-monomethyl-	**III**, 2-dimethyl-
B, D-penicillamine	**IV**, 5,5-dimethyl-	**V**, 2,5,5-trimethyl-	**VI**, 2,2,5,5-tetramethyl-

Nomenclature: The products are secondary amino acids which are 5-membered heterocyclic rings containing sulfur at atom position 1, nitrogen at position 3, carbons at 2, 4, and 5, and the COOH group attached to the carbon at 4.

[a]Reflux overnight at room temperature or gentle heat (~80°C).

~1½ h a white precipitate started to form and the cold bath was removed. After 3 h stirring at room temperature the mixture became clear. The solvent was evaporated in a Buchi rotoevaporator and the white residue was recrystallized from hot ethyl acetate to give 0.721 g of product (0.004 mol, 40%); mp 161–162°C, lit. 165.5–167.5°C (8).

2,2,5,5-Tetramethylthiazolidine-4-carboxylic acid, VI

The method of Howard-Lock *et al.* (2) was followed: **B**, 3.0 g (0.02 mol), was dissolved in 20 mL H_2O and excess acetone (40 mL) was added. The mixture was stirred for 15 h at room temperature. The solvent was evaporated under N_2 to near dryness, and the resulting solid was filtered off and recrystallized from hot acetone. Yield: 2.70 g product (0.014 mol, 70%).

Spectral measurements

Infrared spectra were recorded on both Nicolet 7199 FT-IR and Perkin-Elmer Model 283 spectrophotometers. The samples were ground with KBr at a concentration of approximately 1% by weight and then pressed into pellets. To confirm that certain peaks were real, and not manifestations of the pelleting procedure, the spectra of certain samples were also run in Nujol and again in hexachlorobutadiene. D_2O solution spectra were run with AgCl windows. Spectra were calibrated with polystyrene. Raman spectra were excited by means of the λ5145 Å radiation from a Spectra-Physics Model 164-02 argon ion laser and recorded on a Spex 14018 double monochromator. Solid samples were contained in glass melting point tubes, and solutions in nmr tubes. The spectrometer was calibrated regularly against an indene standard and had previously been calibrated with a neon lamp; the wavelength readout scale was found not to change (± 1–2 cm^{-1}).

Solutions for nmr studies were prepared in D_2O and concentrations were about 100 mg (solid) mL^{-1} of solvent. Internal TSP (trimethylsilylpropionic acid) was used as the reference. Proton nmr spectra were recorded on a Varian T60 spectrometer, and ^{13}C nmr spectra were recorded on a Bruker WP-80 spectrometer operating at 20.115 MHz.

The p*K*'s of the various thiazolidine-4-carboxylic acids were obtained by titration of an aqueous solution with 0.01 *N* NaOH and 0.01 *N* HCl. The pH's were measured with a Corning Model 130 pH meter which was standardized with Scientific Products potassium hydrogen phthalate pH 4.00 buffer, BDH pH 7.00 buffer, and Scientific Products boric acid/potassium hydroxide pH 10.00 buffer. The pH measurements are reliable to ±0.03.

Results and discussion

The vibrational spectra

The thiazolidine-4-carboxylic acids are secondary α-amino acids which are five-membered heterocyclic molecules having no symmetry elements. There is very little published work relating to the vibrational spectra of thiazolidine-4-carboxylic acids. Some isolated C—S bond frequencies are listed by Freeman (9) for various cyclic sulfides. A study of the vibrational spectra of thiazolidine and ND thiazolidine has been reported, followed by a similar study of three monomethyl thiazolidines (substituted at the C(2), C(4), and C(5) positions (10). These studies did not include work on the 4-carboxylic acids. The infrared spectra of some chelates of thiazolidine acids, including the spectra of free ligands 2-methyl-, 2,2-dimethyl-, 2,2,5,5-tetramethyl- and 2-benzylthiazolidine-4-carboxylic acids have been reported, for the range 4000–400 cm^{-1} (11). Our spectra of these ligands cover a greater cm^{-1} range, and they differ in many details. Detailed spectroscopic studies of D-(−)-penicillamine and its deuterated derivatives, in solid solution, both acid and zwitterion forms (12) and of (*S*)-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid and several deuterated species (2) have been reported recently by us.

The spectra of the thiazolidines show many similarities to those of D-penicillamine and its deuterated derivatives, both ref. 12 and this work, and to those for L-cysteine, the main differences being (*i*) the absence of νS—H and δS—H bands; (*ii*) the replacement of bands of the NH_3^+ group by those of the NH group; and (*iii*) the presence of the three unique ring deformations δCSC, δCNC, and δNCS, which are not present in D-penicillamine or L-cysteine.[1]

We discuss here only those features of the spectra which pertain to the CO_2^- (or COOH) and NH_2^+ (or NH) groups; that is, the features relevant to the amino-acid zwitterion equilibria (see Table 2). Strong, broad bands in the infrared spectrum at about 2500 and 1950 cm^{-1}, compounds **IV**, **V** and **VI**, (slightly higher for compounds **I**, **II**, **III**) are typical of fairly strong O—H···N bonding. These bands appear only in the infrared, and not in the Raman spectra for compounds **IV–VI**. They are present in all samples, whether prepared as KBr discs or Nujol or hexachlorobutadiene mulls (and thus are not spurious bands from moisture in KBr). These bands are less consistent in the L-cysteine based compounds, with compounds **I** and **II** showing a single broad band at 2330 and 2630 cm^{-1} and **III** showing bands at 2528 cm^{-1} (ir, Raman) and 2075 cm^{-1} (ir alone).

Bands attributed to the COOH group: The strong infrared bands at 1744–1715, 1330, and 1190–1215 cm^{-1} are assigned as νC=O, δOH, and νC—O, respectively, in **III**, **V**, and **VI**, having mainly the amino-acid form. These assignments are consistent with the findings of the X-ray work for (*S*)-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid, a compound which exists in the solid in the strongly hydrogen-bonded acid form (2). Bands attributable to the CO_2^- and NH_2^+ groups help identify compounds in the zwitterion form, **I** and **IV**. The specific assignments are in the regions 1620–1555 and 1425–1393 cm^{-1} for $\nu_a CO_2^-$ and $\nu_s CO_2^-$, and 1550–1585 cm^{-1} for δNH_2^+. The spectra of **II** show bands for both forms.

Nuclear magnetic resonance spectra

The ^1H nmr spectra and assignments of the methyl-substituted thiazolidine-4-carboxylic acids are given in Table 3. The

[1]Tables containing the complete listing of infrared and Raman frequencies and the approximate mode descriptions and assignments for compounds **A**, **B**, **I–VI** (6 pages) may be purchased from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont., Canada K1A 0S7.

TABLE 2. Selected vibrational bands[a]

I[b] Z[d] *R*[e]	II AA and Z *RR, SR*	III AA *R*	IV Z *S*	V AA, A* *RS, SS*	VI AA, Z* *S*	Assignment[c]
2330, ir	2630, ir	2528, ir	2435, ir	2450, ir	2470, ir	O—H...N
2320, R		2075, ir				
—	1732, ir	1745, ir⎫	—	1732, ir⎫	1728, ir	ν C=O
		1740, R⎭		1730, R⎭		
1628, ir⎫	1610, ir	—		1634, ir*		
1630, R⎭			1598, ir⎫		1612, ir*	$\nu_a CO_2^-$
1552, ir	1536, ir	—	1598, ir⎭			δNH_2^+
1380, ir⎫	1388, ir	—	1385, ir	1400, R*	—	$\nu_s CO_2^-$
1388, R⎭						
1340, ir	—	1335, ir	—	1322, ir	1329, ir	δ OH
—	1218, ir	1200, ir	—	1227, ir	1216, ir	ν C—O

[a]Infrared and Raman bands, cm^{-1}, relevant to the amino acid and zwitterion equilibria.
[b]Compounds **I**–**VI** as defined in Table 1.
[c]ν = stretch frequency, a = antisymmetric, s = symmetric, δ = deformation.
[d]Forms present in the solid state: Z, zwitterion; AA, amino acid; Z*, zwitterion detected by ir bands in D_2O solution.
[e]Enantiomers and diastereomers present.

TABLE 3. ^1H nmr spectra of methyl-substituted thiazolidine-4-carboxylic acids[a,b]

Compound	H(C-2)	H(C-4)	H(C-5)	CH_3(C-2)	CH_3(C-5)
Thiazolidine	4.35, s, 10	4.40, dt, 5	3.32, m, 10	—	—
2-Methyl-	4.60, q, 6	5.05, t, 6	3.50, m, 12	1.73, d, 18	—
				1.76, d	
2,2-Dimethyl-	—	4.77, t, 3	3.45, d, 8	1.70, s, 18	
			3.50, d		
5,5-Dimethyl-	4.45, s 12	3.95, s, 7	—	—	1.42, s, 21
	4.50, s				1.68, s, 21
2,5,5-Trimethyl-	4.90, q, 5	3.94, s, 5	—	1.58, d, 14	1.37, s, 28
		4.02, s			1.63, s
2,2,5,5-Tetramethyl-	—	4.2, s, 44	—	1.76, s, 16	1.43, s, 18
				1.94, s, 18	1.68, s, 17

[a]Solutions in D_2O, TSP used as internal standard.
[b]The chemical shift in ppm is followed by peak multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and by the integrated peak area.

non-methylated compound has the simplest spectrum with the C2 hydrogens giving rise to a singlet which indicated both protons have the same average chemical environment. The downfield signal is the hydrogen at C4, which is next to an electron-withdrawing carboxyl group having a deshielding effect; the signal is split into a triplet by the two neighbouring protons on C5. The signal of the C5 protons is upfield; these protons have slightly different chemical environments because of their proximity to the carboxyl group; each proton gives rise to a doublet as a result of splitting by the vicinal proton on C4.

2-Methylthiazolidine-4-carboxylic acid, **II**, is a mixture of two stereoisomers: C4 is a fixed chiral center, derived from L-cysteine in this case and with the same *R* conformation, but there is a new chiral carbon, C2, which can be *R* or *S*. The lowest field triplet is the C4 hydrogen. C2-H is split into a quartet by the methyl group attached to the same carbon. The C5 proton signal, of chemical shift 3.5 ppm, is a multiplet because it results from two overlapping doublets of doublets. The doublet of doublets of each proton arises from it being split by the C4 proton and by the geminal proton. The methyl group on C2 gives a doublet and there are two such signals because the methyl group can be on the same side of the ring as the carboxyl group or on the opposite side, because both *R* and *S* isomers are present.

The two methyl groups of the 2,2-dimethyl compound, **III**, give only one singlet in the spectrum, and therefore, they have equivalent chemical environments. They are situated far enough away not to be affected by the carboxyl group, but the two C5 protons, both doublets of doublets, give rise to two distinct signals, split by the C4 proton and in turn by each of the C5 protons, resulting in a quartet of peaks of equal intensity. For the C4 proton, a triplet is observed.

For the 5,5-dimethyl- compound, **IV**, it is somewhat surprising that there are two different C2 proton signals in the spectrum. The two methyl groups appear as two singlets, because there is no plane of symmetry in the molecule.

2,5,5-Trimethylthiazolidine-4-carboxylic acid, compound **V**, also has two chiral centers, and can exist as two diastereomers (*S*,*R* or *R*,*R*) in any proportion. Two sets of resonance signals for each type of proton in the molecule are expected; only one signal was observed for the C2 proton and another one for the methyl group, but the presence of the two diastereomers is shown by the signals for C4.

2,2,5,5-Tetramethylthiazolidine-4-carboxylic acid, compound **VI**, exhibits the expected nmr spectrum. It consists of five singlets, one for each methyl group and one for the single proton bonded to C4. The methyls on C2 are the downfield singlets because they are more deshielded by S and N, both of which are

TABLE 4. Carbon-13 chemical shifts of methyl-substituted thiazolidine-4-carboxylic acids[a]

Compound	C-2	C-4	C-5	C(OOH)	CH_3(C-2)	CH_3(C-5)
Thiazolidine	50.00	65.30	34.29	173.14	—	—
2-Methyl-	62.04	64.84	33.99	172.89	19.80	—
	62.57	65.60	34.20		18.85	
2,2-Dimethyl-	63.52	55.63	32.81	171.36	25.04	—
					27.85	
5,5-Dimethyl-	53.84	73.08	46.87	170.16	—	26.45
						28.26
2,5,5-Trimethyl-	58.38	73.29	54.18	170.86	28.49	26.79
	58.83	74.51	54.84		30.94	28.14
2,2,5,5-Tetramethyl-	73.46	73.46	61.62	171.31	32.24	28.52
					34.36	29.61

[a]Solutions in D_2O.

TABLE 5. pK values of L-cysteine, D-penicillamine and related thiazolidine-4-carboxylic acids

Compound	pK_1[a]	pK_2[b]	$\Delta G°$	Isoelectric point[c]
L-Cysteine	1.96[d]	8.18[d]	−4.23[e]	5.07
D-Pencillamine	1.8	7.9	−4.14	4.85
Thiazolidine-4-carboxylic acid	1.51[d]	6.21[d]	−3.19	3.86
2-Methyl-4-carboxylic acid	2.8	6.0	−2.17	4.4
2,2-Dimethyl-4-carboxylic acid	2.7	5.7	−2.04	4.2
5,5-Dimethyl-4-carboxylic acid	2.7	5.8	−2.11	4.25
2,5,5-Trimethyl-4-carboxylic acid	2.6	5.6	−2.04	4.1
2,2,5,5-Tetramethyl-4-carboxylic acid	2.8	5.5	−1.83	4.35

[a]pK_1 is pK of COOH group.
[b]pK_2 is pK of amino group.
[c](1/2) (pK_1 + pK_2).
[d]Ratner and Clarke, ref. 5.
[e]Calculated in kcal mol^{-1}, from $\Delta G = -RT \ln (K_2/K_1)^{1/2}$.

electron withdrawing. This assignment was confirmed previously by deuteration of the C2 methyl groups (2).

All the C5 methyl groups in Table 2 have very consistent chemical shifts, generally to high field of the C2 methyl groups.

NOE experiments have been carried out with derivatives of penicillin to study thiazolidine ring conformation (13), and have shown that the signals of pro-*R* methyl hydrogens are always at higher field than the hydrogens of pro-*S* methyl groups. By analogy, the 1.68 ppm peak in the spectrum of the 2,2,5,5-tetramethyl compound **VI**, spectrum is assigned to the pro-*R* methyl protons of C5 and the 1.43 singlet to the pro-*S* methyl protons. A study of *erythro* and *threo* sterochemistry of five-membered rings by proton nmr lists also the spectra of tetramethylthiazolidine-4-carboxylic acid, and the assignments agree with those above (14).

Table 4 lists the carbon-13 shifts observed.

The carboxyl carbon is highly deshielded by the two oxygens, and its signal is well downfield, always 170–174 ppm. C4 is expected to be the next most deshielded carbon. As previously discussed with respect to the ^{1}H nmr spectra, the proton on C4 is also the most deshielded because of the proximity to the carboxyl group. C5 is more deshielded than C2 as illustrated by compound **I**, with C2 assigned a chemical shift of 50, and C5, 34.29. This trend continues down the table, although peaks are quite close.

The carbon-13 chemical shifts of compounds **II** and **I** are very similar. C4 and C5 are deshielded to nearly the same extent. The greatest change was observed with C2, with the CH_3 group on C2 causing increased chemical shift, as expected.

In compound **III**, the value of C2 increased only slightly, compared to the same carbon in the 2-methyl compound. C4 has decreased, which cannot be easily explained because the two methyl groups on C2 appear to be too far away from C4 to cause an alteration in the chemical shift unless the conformation of the ring was changed. The carboxyl carbon has its chemical shift lowered by >1 ppm. The methyl carbon on C2 was shifted downfield considerably compared with 2-methylthiazolidine.

For compound **IV**, an increase in the chemical shift of C5 was observed, compared to the values for compounds **I–III** derived from L-cysteine. There is also a large increase in the value for C4; it appears that these methyl groups are close enough to C4 to have an effect similar to that observed for the C5 carbon to which the methyls are directly bound. The chemical shift of C2 has reverted to nearly the same value as in the non-methylated thiazolidine compounds. The C5 methyl groups always occur to slightly higher field than the C2 methyl groups, presumably because of the γ-shielding of the COOH group. This is a general trend which becomes more apparent as more methyl groups are introduced into the molecule.

With compound **V**, C2 again showed an increase in chemical shift relative to compound **IV**. This molecule, **V**, like the 2-methyl compound, **II**, contains two chiral centers, the one at C4 being always *S*, and it can occur in *R*,*S* and *S*,*S* configurations. The compounds are probably about 50:50 mixtures of the two diastereomers, judging from the heights of the duplicated proton nmr signals. The increased chemical shift of C2 relative to that for the compound **IV** reflects the presence of a new methyl group on that carbon. C4 also increased

slightly, which is consistent with the trend previously noted of increasing chemical shifts with every new methyl group added to the thiazolidine ring. The chemical shift of C5 has increased by almost 8 ppm; the presence of one more methyl group on another carbon in the molecule is not a sufficient reason to explain such a large effect, unless the stereochemistry of the ring has changed in such a way as to bring the new methyl spatially close the C5 atom so that the nuclear shielding is altered.

In the compound **VI**, C2 again has a higher value than in the previous compound which only had one C2 methyl group. It should be noted that since there is peak overlap in this compound, either of 61.62 or 76.46 could be assigned to C2 and it is arguable which of the assignments is correct. The chemical shift of C2 is more likely to be 73.46 since it is expected to be higher than that of C5, because of the higher deshielding effects of the N and S atoms around C2 in the ring. For this compound, the methyl group chemical shifts have been assigned previously to C2 and C5 methyl carbons by deuteration of the C2 methyls (2). The 61.62 ppm peak assigned to C5 is of higher chemical shift than expected from the values in ppm assigned to C5 of the other thiazolidine molecules listed in Table 3. Previosly published spectra of *N*-formyl-2,2,5,5-tetramethylthiazolidine compounds (15) helped in the assignment of peaks, as well as the recording of "spin-sort" spectra in which the peaks arising from carbons with an odd number of protons show up below the baseline while carbons with an even number of protons give rise to peaks above the baseline. The methyl group carbons deserve a reference. In thiazolidines that have both C2 and C5 methyl substituents, the methyls on C2 have higher chemical shifts than the C5 methyls, probably because of the proximity of the electronegative N and S. In substituted penicillins the relative shifts were C2 > C5, pro *S* CH_3(C5) > pro *R* (16). If 2,2,5,5-tetramethylthiazolidine-4-carboxylic acid can be considered analogous, the assignments have the same order.

The amino-acid zwitterion equilibria

The p*K* values represent the extent of protonation of ionizable groups, and are presented in Table 5. There is a downward shift of amino group p*K* with increasing CH_3 substitution, especially C2. Increase of COOH group p*K* with methyl substitution is partly caused by inductive effects of the methyl group. Also the anion cannot be solvated so effectively, so that the equilibrium $AH \rightleftarrows A^- + H^+$ is shifted to the left hand side.

From the usual relationship of the Gibbs standard free energy to the equilibrium constant associated with a given reaction in solution, we have calculated the free energy of conversion from the amino acid to the zwitterion form. The values of ΔG^0, also shown in Table 5, vary between -4.2 and -1.8 kcal mol^{-1} for L-cysteine and tetramethylthiazolidine-4-carboxylic acid. If the compounds were tabulated in order of ΔG°, the 2,2-dimethyl-compound, **III**, would be placed below the 5,5-dimethyl compound, **IV**.

Compounds which exist in the amino-acid form in the solid include 2,5,5-trimethyl- and 2,2,5,5-tetramethylthiazolidine-4-carboxylic acid, **V** and **VI**, both derived from D-penicillamine, and the 2,2-dimethyl species, **III**, derived from L-cysteine. When **V** is crystallized from D_2O, howver, it occurs as the zwitterion in the solid state. In D_2O solutions, zwitterion forms are present for all three compounds, although compound **VI** still has a significant concentration of the acid form, as shown by the infrared spectra.

We have shown that methyl substitution at the C2 and C5 positions of the thiazolidine ring does indeed affect the amino-acid zwitterion equilibrium. It was originally assumed that the acidity mght be proportional to the number of CH_3 groups. The shift in equilibrium did not follow this simple relationship, however, since from the infrared spectra the 5,5-dimethyl compound, **IV**, showed no amino acid form in the solid, while the 2-methyl and 2,2-methyl compounds, **II** and **III**, showed evidence for both amino acid and zwitterion forms in the solid. It seems likely that both the number and position of the methyl groups affect the ring conformation and the hydrogen bonding patterns in ways which result in stabilization of either the amino acid or the zwitterion.

The 2-methyl and 2,5,5-trimethyl compounds, **II** and **V**, do have one other feature in common; namely, they can exist as two diastereomers, *RR*, *SR* and *RS*, *SS* respectively. The vibrational spectra of these two compounds show many extra bands, a fact which is consistent with both diastereomers being present. There is, however, no evidence that this factor alone is sufficient to explain the change in the amino acid zwitterion equilibria, although it would mean different entropy values.

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