Amino acid/zwitterion equilibria II: vibrational and NMR studies of substituted thiazolidine-4-carboxylic acids

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This paper is dedicated to Dr. Richard Norman Jones

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Infrared and Raman spectra (4000–100 cm) of solid samples of seven different 2-phenyl-, *N*-benzoyl-, and 2-ethyl-2 methyl derivatives of L-cysteine and D-penicillamine have been observed and assigned. Proton and ^{13}C nuclear magnetic resonance spectra for the compounds have also been measured. Amino acid/zwitterion equilibria are discussed with reference to p*K* values and the vibrational spectra

Key words: amino acid/zwitterion equilibria, thiazolidine carboxylic acids.

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On a déterminé les spectres infra-rouge et Raman (4000–100 cm) d'échantillons solides de sept dérivés différents des 2-phényl-, *N*-benzoyl- et 2-éthyl-2-méthyl- de la L-cystéine et D-pénicillamine et on rapporte les attributions qui en découlent. On a aussi déterminé les spectres de résonance magnétique nucléaire du ^{1}H et du ^{13}C de ces composés. On discute des équilibres acide aminé/zwitterion par rapport aux valeurs de p*K* et aux spectres de vibration.

Mots clés : équilibres acide aminé/zwitterion, acides carboxyliques de thiazolidine.

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Introduction

The modes of action and adverse side effects of the drug D-penicillamine depend on several reactions, an important one being the condensation with aldehydes and ketones to form thiazolidine rings. Penicillamine might compete successfully with cysteine in vivo in this type of reaction (1). Both bind specifically to free aldehydes on collagen. Penicillamine inhibits cross-linking and depolymerizes incomplete cross-links of collagen, where these linkages are of the aldimine type. Thus, the effects of penicillamine are mainly upon skin collagen, giving rise to several side effects and some benefits (in scleroderma) as well.

The formation of thiazolidine rings is the basis for the suggested use of D-penicillamine as an alcohol detoxicant, and for treatment of scleroderma, morphea, keloid formation, and of vitreous and scleral scarring of the eye. Formation of thiazolidine rings is also the probable mode of action of D-penicillamine as vitamin B6 antagonist, and we have fully characterized the complex between pyridoxal and D-penicillamine (2).

Recently, we showed that methyl substitution at the C2 and C5 positions of thiazolidine-4-carboxylic acid affects the amino acid/zwitterion equilibrium (3). A downward shift of amino group p*K* and an upward shift of COOH group p*K* with increasing number of CH_3 groups was noted, although it was evident that other factors, such as the change in ring conformation and the degree of hydrogen bonding, played a role. The nature of the carbonyl-containing group affects the substitution at the C2 thiazolidine ring position and this in turn can affect the amino acid/zwitterion equilibrium and thus the hydrogen bonding behaviour in vivo.

A further process occurs in vivo, which may affect thiazolidine ring formation and which will affect zwitterion formation. This is substitution at the amine group. A new drug, which is used in the treatment of respiratory diseases, is *N*-acetylcysteine. This introduces a new variable, namely substitution at the N3 position. Thus we have extended our previous studies of how substitution affects these equilibria by considering a series of 2-phenyl, 2-methyl-2-ethyl-, and *N*-benzoyl-substituted thiazolidine-4-carboxylic acids.

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Materials and methods

All the starting materials were reagent grade and were used as received, after checking the NMR spectrum. D-(−)-Penicillamine free base and L-(+)-cysteine hydrochloride monohydrate were supplied by Sigma Chemical Company, St. Louis, MO. Formaldehyde, 37% v/v, was supplied by Sargent–Welsh Scientific Co., Skokie, IL. Acetaldehyde was distilled from paraldehyde, reagent grade, supplied by BDH Chemicals, Toronto, Ont. Benzaldehyde was obtained from Eastern Chemical, Hauppauge, NY, acetone from BDH Chemicals, Toronto, Ont., 2-butanone from Aldrich Chemical Company, Milwaukee, WI, and benzoyl chloride from Eastman Kodak Company, Rochester, NY. Elemental analyses were provided by Guelph Chemical Laboratories, Guelph, Ont. Melting points were determined on a Gallenkamp capillary melting point apparatus, and are not corrected. The preparation of the various substituted thiazolidine-4-carboxylic acids was carried out as indicated schematically in Table 1 and outlined in detail as follows.

2-Phenylthiazolidine-4-carboxylic acid **1**

The method of Riemschneider and Hoyle (4) was followed with modification: 1.2 g (0.01) mol of L-cysteine hydrochloride monohydrate, A, was added to a mixture of 1.08 g (0.0105 mol) of benzaldehyde in 30 mL of water at pH 8.0. The mixture was stirred at room temperature and a precipitate started appearing within 30 min. The mixture was filtered to yield 1.35 g (65%) of white solid, $C_{10}H_{11}NO_2S$, mp 154–155°C (lit. (4) mp 159–160°C).

3-Benzoyl-2-phenylthiazolidine-4-carboxylic acid **2**

The method of Clarke *et al.* (5a) was followed with modification: 0.42 g (0.002 mol) of **1** was dissolved in 20 mL of pyridine. The solution was cooled on ice and it became a light suspension. Benzoyl chloride (0.3 g, 0.0025 mol) was added dropwise to the stirred mixture, which slowly turned yellow as the solid gradually dissolved. The reaction was allowed to proceed in the refrigerator overnight. The product was recovered as follows: The mixture was evaporated under reduced pressure to leave a yellow oil and some white solid. This was dissolved in 80 mL dichloromethane, then extracted twice with 20 mL water. The combined aqueous layers were extracted once with a small

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

Reactants	Benzaldehyde	2-Butanone	Benzoyl chloride
L-Cysteine	**1**[b]	**3**	
1			**2**
3			**4**
D-Pencillamine	**5**	**6**	
6			**7**

Nomenclature: The products are secondary amino acids that are 5-membered heterocyclic rings containing a sulfur atom at position 1, a nitrogen atom at position 3, carbon atoms at 2, 4, and 5, and a COOH group attached to C4.

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

[b]**1**, 2-phenylthiazolidine-4-carboxylic acid; **2**, 3-benzoyl-2-phenylthiazolidine-4-carboxylic acid; **3**, 2-ethyl-2-methylthiazolidine-4-carboxylic acid; **4**, 3-benzoyl-2-ethyl-2-methylthiazolidine-4-carboxylic acid; **5**, 2-phenyl-5,5-dimethylthiazolidine-4-carboxylic acid; **6**, 2-ethyl-2,5,5-trimethylthiazolidine-4-carboxylic acid; **7**, 3-benzoyl-2-ethyl-2,5,5-trimethylthiazolidine-4-carboxylic acid.

volume of CH_2Cl_2. The combined organic layer was dried with anhydrous sodium sulfate, filtered, and evaporated in the rotoevaporator. The yellow solid recovered, which had only a faint pyridine odour at this stage, was dissolved in 20 mL methanol and was acidified to pH 2–3 with aqueous HCl. Water was added dropwise until the solution turned slightly cloudy. The mixture was allowed to stand at room temperature until crystals formed. These were recrystallized from hot methanol to give a pale yellow solid. Yield 0.35 g (60%), mp 128-129°C (lit. (5*a*) mp 153–154°C). Anal. calcd. for $C_{17}H_{15}NO_3S$: C 65.2, H 4.8, N 4.5, S 10.2, O 15.3; found: C 62.5, H 5.7, N 4.3, S 9.8, O 18.2. Anal. calcd. for **2**·0.7 H_2O: C 62.6, H 5.1, N 4.3, S 9.8, O 18.2.

2-Ethyl-2-methylthiazolidine-4-carboxylic acid 3

A published procedure (5*b*) was used as follows: 1.5 g (0.0125 mol) L-cysteine was added to 22 mL of 2-butanone and the mixture was refluxed for 5 h. The mixture was filtered while hot. Most of the cysteine remained undissolved and unreacted. White solid, 0.6 g, was recovered after evaporation of the filtrate, followed by recrystallization from hot 2-butanone; $C_7H_{13}NO_2S$, mp 130–132°C (lit. (5*b*) mp 132–133°C).

3-Benzoyl-2-ethyl-2-methylthiazolidine-4-carboxylic acid 4

Compound **3** (1.0 g, 0.006 mol), 30 mL of pyridine, and 0.8 g of benzoyl chloride were reacted in a flask cooled by an ice bath. Thirty minutes later a white solid started precipitating out of the pale yellow solution. The work-up procedure was identical to that reported in the preparation of **2**; 0.6 g (40%) of a white solid was obtained, mp 133–134°C. Anal. calcd. for $C_{14}H_{17}NO_3S$: C 60.2, H 6.1, N 5.0, S 11.5, O 17.2; found: C 60.5, H 6.3, N 4.8, S 11.3, O 17.2.

2-Phenyl-5,5-dimethylthiazolidine-4-carboxylic acid 5

A published procedure (5*a*) was followed: 1.0 g (0.0066 mol) of D-penicillamine was added to 1.0 mL benzaldehyde and 10 mL ethanol, and the mixture was heated for 30 min. The almost clear mixture was filtered while hot and as the filtrate cooled a white precipitate started separating. The cooled solution was filtered to obtain 1.3 g (80%) of a white solid, $C_{12}H_{15}NO_2S$, mp 141–142.5°C (lit. (5*a*) mp 145–146°C).

2-Ethyl-2,5,5-trimethylthiazolidine-4-carboxylic acid 6

To 1.5 g (0.01 mol) of D-penicillamine was added 25 mL 2-butanone and the mixture was refluxed for 4 h. The clear solution was allowed to cool but no crystallization occurred. Evaporation produced 1.5 g (70%) of white solid, recrystallized from 2-butanone, mp 117–119°C (lit. (5*c*) mp 183–183.5°C). Anal. calcd. for $C_9H_{17}NO_2S$: C 53.2, H 8.4, N 6.9, O 15.7, S 15.8; found: C 53.7, H 8.6, N 6.8, O 15.4, S 15.4.

3-Benzoyl-2-ethyl-2,5,5-trimethylthiazolidine-4-carboxylic acid 7

Compound **6** (0.20 g, 0.00125 mol) was dissolved in 15 mL pyridine and 0.15 g benzoyl chloride was added. The mixture was left in the refrigerator overnight. A yellow oil was recovered after evaporation and yielded a yellow crystalline solid after cyrstallization from methanol–water. The crystals were filtered and washed with ether to give a white solid, 0.15 g (40%), mp 182–183°C. Anal. calcd. for $C_{16}H_{21}NO_3S$: C 62.5, H 6.9, N 4.6, O 15.6, S 10.4; found: C 62.4, H 7.0, N 4.6, O 15.6, S 10.8.

Almost all of the compounds were purified by recrystallization and their melting points are close to the reported values, except for **2** and **6**. Elemental analyses were obtained for those compounds for which no mp was found in the literature, **4** and **7**. Most of these compounds had been previously prepared but very little is known about their structure and spectroscopic properties. Many of the thiazolidines known today were synthesized before 1949 from *dl*-penicillamine, so that the products obtained consisted of mixtures of stereoisomers. This might explain the melting point disparity between the published values and the values obtained here for 3-benzoyl-2-phenylthiazolidine-4-carboxylic acid **2** (128–129°C vs. 153–154°C). It is much more likely that the material described here is at least partly hydrated; the analytical figures are poor for the anhydrous material but agree fairly well with a 0.7 hydrate. An even larger disparity occurs for the melting point of **6**: 117–119°C versus 183–183.5°C published, for which no apparent explanation arises. The narrow range of observed mp's suggests the compounds are relatively pure; moreover, their proton and carbon-13 spectra were obtained and are consistent with the structure proposed, and the vibrational spectra agree with those of closely related structures.

No literature mp values could be found for 3-benzoyl-2-ethyl-2-methylthiazolidine-4-carboxylic acid **4** (mp 133–134°C) and 3-benzoyl-2-ethyl-2,5,5-trimethylthiazolidine-4-carboxylic acid **7** (mp 182–183°C), and we believe we are reporting their preparation and characterization for the first time. The elemental analyses of **4** and **7** indicate purity and stability.

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. Spectra were calibrated with polystyrene. Raman spectra were excited by means of 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 calirtated with a neon lamp; the wavelength readout scale was found not to change (±1–2 cm).

Solutions for NMR studies were prepared in D_2O and concentrations were about 100 mg (solid)/mL of solvent. External tetramethylsilane 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* values of some of the water-soluble thiazolidine-4-carboxylic acids **3**, **6**, and **7** were obtained by titration of aqueous solutions with 0.01 N NaOH and 0.01 N HCl. The pH values 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

In the *N*-benzoyl compounds and in the thiazolidine compounds derived from benzaldehyde there are peaks that result from aromatic and C—H bond stretches. These give rise to multiple absorption peaks in the 3100–3000 cm region of

the spectrum, while aliphatic C—H bond stretches occur at 3000 cm or lower. For a monosubstituted aromatic ring, carbon–carbon stretching frequencies have been assigned to bands in the regions 1608–1600, 1589–1580, 1495–1490, and 1452–1446 cm. Bands at 1240 ± 8, 1177 ± 6, 1156 ± 5, 1073 ± 4, and 1027 ± 3 cm have contributions from the bending vibrations of aromatic C—H bonds (6). We observe these bands and find that they are slightly sharper than neighbouring bands in the infrared spectra. Also we have allowed our assignments slightly wider frequency ranges than in benzene alone because of contributions from both phenyl and benzoyl groups. Medium strong bands at 751 ± 15 and 697 ± 11 cm are also characteristic of monosubstituted benzene rings. The frequency of these bands is variable because of the interaction with out-of-plane ring bending. Raman bands expected for monosubstituted benzene rings are 1620–1565, 1005–90 (very strong), 625–605, and 415–400 (very weak) cm (6).

The C—S stretching frequency has been assigned in D-penicillamine and β,β-dimethylcysteic acid to 548 cm, a very strong Raman band. In L-cysteine it has been assigned to 678 cm and it has been concluded that substituents on carbon atoms adjacent to sulfur cause a decrease in the stretching frequency of the C—S bond (7). Bands in the region of 680 and 600 cm, and 630 and 540 cm, are so assigned.

Thiazolidine ring modes have been assigned in the past as follows: 1150–1110 cm arise from CCN stretches; 380, 330, 280, and 191 cm bands result from CNC, CCS, NCS, and CSC deformations, respectively. Deformations involving sulfur usually have been found to be intense in the Raman, and showed isotopic shifts caused by the adjacent CD_3 groups in tetramethylthiazolidine-4-carboxylic acid prepared from deuterated acetone (7). The ring assignments are consistent with previous findings. The detailed spectra have been deposited.[2]

Table 2 shows selected vibrational bands that pertain to the amino acid/zwitterion equilibria, and we will discuss in detail only these features. Characteristic O—H stretching bands are present in the infrared at 3460–3400 cm for all compounds containing COOH functional groups. There are also fairly intense broad bands at about 3000 cm for some of the compounds as well as distinct bands between 2500 and 1940 cm. These broad absorptions at lower frequency are a result of moderately strong hydrogen bonds in some of these compounds. In cysteine and D-penicillamine the hydrogen bond absorptions are so strong that they mask the CH_2 and CH_3 absorptions almost completely (3).

Three types of hydrogen bonds can occur in thiazolidine-4-carboxylic acid derivatives: O—H...O, O—H...N, and N—H...O bonds. The first type can be much shorter than the other two: Hamilton and Ibers (8) have discussed correlations between the frequency of anti-symmetric stretching modes and the O...O or N...O distance in such bonds. A lowering of OH frequency correlates with a lower force constant and a longer OH bond. The O—H bond length actually increases as the O...O bond length decreases. Thus lower O—H stretching frequencies correlate with formation of stronger O—H...O hydrogen bonds. Increase of band width and intensity enhancement occur at the same time. Similar effects occur for the O—H...N and N—H...O bonds (8).

Hydrogen bonds of the type O—H...O are expected to have infrared bands between 3500 and 2600 cm for O...O distances of 2.90–2.60 Å. Hydrogen bonds between oxygen and nitrogen atoms have lower stretching frequency bands in the region of 2600–1900 cm (8).[3] All compounds in Table 2 show hydrogen bonding bands in the range 3450–3000 cm. Some compounds show splitting of bands and others have additional bands at lower wave numbers. These observations imply that there are moderate O...O hydrogen bonds, in the range of 2.88–2.65 Å (8). Hydrogen bonds of the O...N or N...O type only exist for the non-*N*-benzolyated compounds, and are characterized by two bands in the regions 2500–2350 cm and 1990–1900 cm, as observed by us for *S*-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid (7).

The two *N*-benzoyl derivatives of L-cysteine **2** and **4**, and the compounds **5**, **6**, and **7** in Table 2 are all amino acids, having C=O stretching frequencies around 1725 cm. Compounds **1**, 2-phenylthiazolidine-4-carboxylic acid, and **3**, 2-ethyl-2-methylthiazolidine-4-carboxylic acid, are zwitterions; there are no bands in the C=O region of 1740–1725 cm or in the C—O region (1215–1190 cm). COO^- stretching bands are very intense in the infrared at 1625 cm, and weaker in the Raman at 1630 and 1603 cm, for **3**, and at 1572 cm in the infrared for **1**. The latter is an unusually low frequency, but it is probaby caused by the proximity of the phenyl group, and there are three Raman bands between 1621 and 1580 cm. The symmetric vibrations of COO^- in both zwitterions are assigned to the bands at 1388–1376 cm. The amide C=O band was assigned to 1660–1620 cm, typical of disubstituted amides.

The NMR spectra

The proton NMR spectra of thiazolidine-4-carboxylic acids derived by condensation of D-penicillamine and L-cysteine with benzaldehyde and 2-butanone and their *N*-benzoyl derivatives are shown in Table 3 and the coupling constants in Table 4. Peaks at 7.42 ppm are assigned to the aromatic protons, a multiplet being observed because the protons are not equivalent. The protons on C4 have chemical shifts between 3.56 and 4.90 ppm depending on the overall structure of the molecule. The lowest chemical shift is caused by the C4 proton in 2-phenyl-5,5-dimethylthiazolidine-4-carboxylic acid, **5**, and the greatest deshielding of a C4 proton is in 3-benzoyl-2-phenylthiazolidine-4-carboxylic acid, **2**. In general the chemical shift of the C4 proton is higher in the *N*-benzoyl derivatives than in the other thiazolidine compounds. C5 protons are mostly unaffected by the presence of the benzoyl group, since they are spatially removed from the nitrogen atom, although some deshielding is noticed if there is a phenyl group on C2. Protons on C2 are markedly deshielded compared to protons on other ring carbon atoms, probably because of the presence of the phenyl ring on the same carbon atom. Deshielding is especially pronounced in the *N*-benzoyl-2-phenylthiazolidine compound, **2**, because of the proximity of the carbonyl of the amide, which attracts electron density from neighbouring atoms. The spectrum of the 2-phenylthiazolidine compound, **1**, is similar to that of the 2-methylthiazolidine compound in that both are mixtures of stereoisomers and each proton type in the molecule gives rise

[2]Tables containing the complete listing of infrared and Raman wave numbers and the approximate mode descriptions and assignments for compounds **1–7** may be purchased from: The Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, Canada K1A 0S2.

[3]I. D. Brown, personal communication.

TABLE 2. Selected vibrational bands[a]

1[b] Z[d] *RR,SR*[e]	**2** AA *RR,SR*	**3** Z,Z* *RR,SR*	**4** AA *RR,SR*	**5** AA *RS,SS*	**6** AA,Z* *RS,SS*	**7** AA *RS,SS*	Assignment[c]
2665(70) br,ir	2670(30),ir 2560(30),ir	2650,w br,ir	2505(50) br,ir			3000–2200,ir br.wing	O—H...O
		2350(55) br,ir		2490(70), br,ir	2480(70) br,ir		O—H...N
	1747(92),ir 1725,R		1733(95),ir 1727,R 1700(70),ir	1727(94),ir 1700,R	1720(95),ir 1705,R	1744(87),ir 1740,R	νC=O
1621,R		1625(100),ir 1630,R					$\nu_a CO_2$
1376,ir 1380,R		1388(93),ir 1388(25),R					$\nu_s CO_2$
	1338,ir 1212(35),ir		1326(70),ir 1215(86),ir	1328(100),ir 1222(70),ir	1324(99),ir 1232(91),ir	1318(50),ir 1213(69),ir	δ OH νC—O

[a]Infrared, ir, and Raman, R, bands, cm, relevant to the amino acid/zwitterion equilibria; intensities of some of the stronger bands in parentheses relative to strongest band = 100.
[b]Compounds **1–8** as defined in Table 1.
[c]ν = stretching frequency, a = antisymmetric, s = symmetric, δ = deformation, br = broad, w = weak.
[d]Forms present in the solid: Z, zwitterion; AA, amino acid; Z*, zwitterion in solution deduced from p*K* values.
[e]Diastereomers present.

TABLE 3. ^{1}H NMR spectra of substituted thiazolidine-4-carboxylic acids[a,b]

Compound	C_6H_5	(C-4)H	(C-5)H	(C-2)H	CH_3(C-5)	CH_3(C-2)	CH_3CH_2(C-2)
1	7.42, m, 50	3.92, t 4.22, t } 10	3.26, m, 50	5,50, s 5.68, s } 10			
2	7.30, m, 60	4.90, t, 7	3.45, m, 12	6.28, s, 6			
3		4.46, t, 7	3.36, m, 14			1.62, s, 20	1.92, dq 0.90, t } 35
4	7.35, m, 50	4.72, dd, 9	3.15, m, 25			1.83, s, 31	2.45, m, 20 0.90, t, 30
5	7.45, m, 25	3.56, s 3.67, s } 6		5.62, s 5.83, s } 6	1.63, s 1.51, s } 15 1.32, s 1.29, s } 15		
6		3.80, s 3.70, s } 16			1.45, s 1.50, s } 48 1.20, s 1.25, s } 46	1.60, s 1.57, s } 48	1.80, q, 30 1.00, t, 46
7	7.40, m, 30	4.30, s 4.38, s } 6			1.30, s 1.27, s } 20 1.60, s 1.64, s } 20	1.95, s, 20	0.93, q 1.00, t } 30

[a]Concentrated solution in DMSO-d_6.
[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 area.

to two signals. The chemical shift difference is probably very small, however, since no splitting was observed. The three-line signal of the proton at C4 was expected to be a four-line signal.

Compound **2** had some new aromatic peaks in a multiplet at 7.30 ppm, and the downfield shift of the proton at C2 was increased significantly, probably because of the proximity of the amide carbonyl group, which attracts electron density from neighbouring atoms. Even though the compound is a mixture of diastereomers, only one signal was observed for each type of proton.

The C4 proton of 2-ethyl-2-methythiazolidine-4-carboxylic acid, **3**, gives a triplet at low field and the C5 protons cause overlapping doublets of doublets. A singlet at 1.62 ppm results from the resonance of the methyl protons on C2. The ethyl

TABLE 4. Coupling constants of ^{1}H NMR spectra of thiazolidine-4-carboxylic acids

	Compound	$^2J_{H\text{-}C5,H\text{-}C5}$	$^3J_{H\text{-}C4,H\text{-}C5}$	$^2J_{H\text{-}CC2,H\text{-}CC2}$
1	2-Phenyl-	6.4	1.6	—
2	3-Benzoyl-2-phenyl-	7.8	—	—
3	2-Ethyl-2-methyl-	6.4	1.2	6.0
4	3-Benzoyl-2-ethyl-2-methyl-	6.6	2.2	7.2
5	2-Phenyl-5,5-dimethyl-	—	—	—
6	2-Ethyl-2,5,5-trimethyl-	—	—	4.4
7	3-Benzoyl-2-ethyl-2,5,5-trimethyl-	—	—	4.4

[a] *J* values in Hz; ^{1}H NMR spectra recorded at 25°C.

TABLE 5. Carbon-13 chemical shifts of substituted thiazolidine-4-carboxylic acids[a]

Compound	COOH	CO(N)	C_6H_5	C-5	C-4	C-2	CH_3(C-5)	CH_3(C-2)	CH_3CH_2(C-2)
1	173.24	—	128.76	38.30	71.11	65.88	—	—	—
	172.58		128.50	37.20	71.38	65.23	—	—	—
			127.84						
			127.53						
			127.19						
2	171.21	145.07	136.99	40.02	64.69	63.58	—	—	—
		143.89	132.95		64.28				
			129.86						
			129.36						
			128.22						
			127.32						
			126.96						
			126.68						
			125.41						
3	172.77	—	—	37.73	64.34	80.51	—	38.17	29.44
					64.18			36.29	26.59
4	171.84	138.80	132.91	32.90	67.41	76.72	—	32.90	27.22
	171.60		129.20						
			128.46						
			125.88						
5	170.79	—	128.95	61.53	74.19	69.12	29.15	—	—
	170.48	—	128.56	60.83	73.32	67.81	28.26		
			128.26				27.83		
			127.42				26.82		
			126.95						
			126.34						
6	170.64	—	—	77.22	60.20	72.69	30.77	37.70	28.69
					59.71	72.15		36.09	27.66
7	170.73	138.94	129.86	78.09	77.24	78.09	27.99	33.09	33.76
	169.64	138.69	129.22	77.91	77.01	77.01		32.78	26.71
			129.05						25.81
			128.69						
			125.70						
			125.22						

[a] Concentrated solutions in DMSO-d_6.

group shows two set of peaks: the methyl group is a triplet, and the methylene protons are nonequivalent and form part of an ABC_3 system.

The spectrum of 3-benzoyl-2-ethyl-2-methylthiazolidine-4-carboxylic acid, **4**, is essentially the same as the previous one with additional aromatic peaks. Both NMR spectra show two closely spaced signals for each type of proton, like all the other compounds in this table, because of the presence of a new asymmetric center at C2.

The 2-phenyl-5,5-dimethylthiazolidine-4-carboxylic acid, **5**, has two methyl groups, each of which gives two singlets, since they can be in one of two possible positions that are nonequivalent. Otherwise the spectrum is similar to that of the L-cysteine analogue.

TABLE 6. pK values of L-cysteine, D-penicillamine, and some thiazolidine-4-carboxylic acids

Compound	pK_1[a]	pK_2[b]	ΔG°[c]	Isoelectric point[d]
L-cysteine	1.96	8.18	−4.23	5.07
D-Penicillamine	1.8	7.9	−4.14	4.85
Thiazolidine-4-carboxylic acid	1.51	6.21	−3.19	3.86
2-Methyl-	2.8	6.0	−2.17	4.4
2,2-Dimethyl-	2.7	5.7	−2.04	4.2
5,5-Dimethyl-	2.7	5.8	−2.11	4.25
2,5,5-Trimethyl-	2.6	5.6	−2.04	4.1
2,2,5,5-Tetramethyl-	2.8	5.5	−1.83	4.15
2-Ethyl-2-methyl-	2.7	7.8	−3.50	5.25
2-Ethyl-2,5,5-trimethyl-	2.9	7.4	−3.07	5.15

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

The carbon-13 chemical shifts of the componds are shown in Table 5. Some of the peak assignments, especially those of the carbon atoms in the ring, were difficult to make. Spin-sorted spectra were obtained and compared, and it is believed the peaks are assigned correctly. Only general trends of the data listed in Table 5 will be noted here.

The carboxyl carbon atoms have very consistent chemical shifts, near 170 ppm, and thus this group is very little affected by other ring substituents. The next most deshielded carbon atom is in the amide carbonyl of the benzoylated compounds, the signal occurring at 138.7–145 ppm. The aromatic carbon atoms all have values between 125 and 137 ppm, with the two nearest the C=O being the most deshielded.

The carbon atom in the thiazolidine ring with the highest chemical shift is usually C4 except when C2 is doubly substituted. The highest-field ring carbon atom is C5, which has low chemical shift values unless there are methyl substituents directly bonded to it. Methyl groups on C5 give peaks around 30 ppm and those on C2 have slightly higher chemical shifts, 33–38 ppm. Ethyl group carbon atoms are not assigned specifically in Table 5, but the terminal methyl carbon atom gives a peak of higher field than the methylene carbon atom.

The amino acid/zwitterion equilibria

The amino acid/zwitterion equilibria are independent of the pH of solution and the presence of the cation and anion. The vibrational spectra, on the other hand, are capable of showing the presence of all three species — the cation, the neutral species, and the anion.

The pK values represent the extent of protonation of ionizable groups, and are presented in Table 6. There is a downward shift of amino group pK with increasing methyl group substitution, especially on C2. Increase of COOH group pK with methyl substitution is partly caused by inductive effects of the methyl group; also with increasing substitution the anion cannot be solvated so effectively. Thus the equilibrium $AH \rightleftarrows A^- + H^+$ is shifted to the left-hand side.

From the equation relating the Gibbs standard free energy to the equilibrium constant relationship for 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°, also shown in Table 6, vary between −4.23 and −1.83 kcal mol^{-1} for L-cysteine and *S*-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid. The isoelectric point is the pH at which the concentration of the zwitterion is at a maximum, and the concentrations of anion and cation are equal. Below the isoelectric point, the molecule is positively charged; above it, negatively. Thus at physiological pH these thiazolidine-4-carboxylic acids would all be negatively charged.

Compounds **3** and **6** have the highest values for amino group pK in this series. The carboxyl group pK values of 2.7 and 2.9 are similar to the rest of the series. The ΔG° values of −3.50 and −3.07 are closer to the value for the unsubstituted thiazolidine-4-carboxylic acid, and higher than the values for the methyl-substituted compounds.

The pK values for some compounds could not be measured because of poor solubility, but their form in the solid state can be assigned as either amino acid or zwitterion from the vibrational spectra. The compounds that exist in the amino acid form in the solid are **2**, **4**, **5**, **6**, and **7** (which includes all the *N*-benzoyl derivatives). Compounds **1** and **3** are zwitterions in the solid. Although **6** has pK values typical of the zwitterion in solution, the solid shows the characteristic C=O and C—O infrared bands of the amino acid.

The ^{13}C NMR spectra show a correlation related to these equilibria: The chemical shifts of the carboxylate carbon atoms in **1** and **3** are greater than those for the other compounds, and are close to the values for the unsubstituted thiazolidine-4-carboxylic acid. These compounds are all zwitterions in the solid.

The zwitterion forms of D-penicillamine and L-cysteine become the amino acid forms in the thiazolidine complexes because of marked changes in the pK values of the amino and carboxylic acid groups.

The amino acid/zwitterion equilibria depend both on structural variations in the ring and on the number and type of substituents on the ring (with changes at the C2 position having greater effect than those at C5). In all cases, however, the changes are large enough that they will markedly affect the hydrogen bonding behaviour in vivo, and thus this is an important feature of thiazolidine formation in biological milieu.

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