2*R*,4*S*-2-(2′-Methyl-3′-hydroxy-5′-hydroxymethylenepyridine-C4′)-5,5-dimethylthiazolidine-4-carboxylic acid, the product of the reaction of D-penicillamine and vitamin B6

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We have studied the reaction of both D- and L-penicillamine with pyridoxal hydrochloride and examined the products by single crystal X-ray diffraction. The structure of the title compound, **A**, formed by the reaction of D-penicillamine and pyridoxal was determined. Crystals are orthorhombic, $P2_12_12_1$, with cell dimensions $a = 9.507(5)$, $b = 19.185(5)$, $c = 7.766(2)$ Å and $Z = 4$. The structure was solved by standard methods and refined to $R = 0.088$, $R_w = 0.079$ for 2433 independent reflections. **A** exists as a zwitterion, and bond lengths and angles are normal. In the solid state, **A** and the corresponding product obtained from L-penicillamine, **B**, have identical geometrical structure but are of opposite chirality; that is, D-penicillamine produces the 2*R*,4*S* diastereomer and L-penicillamine produces the 2*S*,4*R* diastereomer (with no *S*,*S* and *R*,*R* components). In solution, however, NMR spectra show the presence of both pairs of diastereomers (2*R*,4*S* and 2*S*,4*S*; 2*S*,4*R* and 2*R*,4*R*). In neutral or alkaline solution there appears to be a rapid epimerization at the thiazolidine carbon atom attached to the pyridoxal moiety. Features of the mass, ^{1}H NMR, vibrational, and electronic spectra are also discussed.

Key words: D-penicillamine, vitamin B6, pyridoxal hydrochloride, therapeutic uses, bischemical reactions, structures.

R. FAGGIANI, H. E. HOWARD-LOCK, C. J. L. LOCK et R. ORGIAS. Can. J. Chem. **69**, 1 (1991).

On a étudié la réaction des D- et L-pénicillamines avec le chlorhydrate du pyridoxal et on a examiné la nature des produits obtenus par diffraction des rayons-X par un cristal unique. On a déterminé la structure du composé **A** mentionné dans le titre et formé par la réaction de la D-pénicillamine avec le pyridoxal. Les cristaux sont orthorhombiques, $P2_12_12_1$, avec $a = 9,507(5)$, $b = 19,185(5)$ et $c = 7,766(2)$ Å et $Z = 4$. On a résolu la structure par les méthodes standards et on l'a affinée jusqu'à des valeurs de $R = 0,088$ et $R_w = 0,079$ pour 2433 réflexions indépendantes. Le composé **A** existe sous le forme d'un zwitterion; ses angles et les longueurs de ses liaisons sont normaux. À l'état solide, les composés **A** et **B** possèdent des structures géométriques identiques, mais de chiralités opposées; c'est-à-dire que la D-pénicillamine conduit au diastéréoisomère 2*R*,4*S* alors que la L-pénicillamine conduit au diastéréoisomère 2*S*,4*R* (sans contamination par les composantes *S*,*S* ou *R*,*R*). Toutefois, en solution, les spectres RMN montrent qu'il existe deux paires de diastéréoisomères (2*R*,4*S* et 2*S*,4*S*; 2*S*,4*R* et 2*R*,4*R*). Il semble que, en solutions neutres ou alcalines, il se produit une épimérisation rapide de l'atome de carbone de la thiazolidine qui est attaché à la portion pyridoxal. On discute aussi des caractéristiques spectrales (masse, RMN du ^{1}H, vibrationnels et électroniques) de ces produits.

Mots clés : D-pénicillamine, vitamine B6, chlorhydrate du pyridoxal, usage thérapeutique, réactions bischimiques, structures.

[Traduit par la revue]

Introduction

D-(−)-Penicillamine is a synthetic amino acid, which has been used in the treatment of rheumatoid arthritis since 1973 when its efficacy as an anti-arthritic drug was firmly established. In addition, D-(−)-penicillamine has an older, well-established use in the treatment of patients suffering from Wilson's disease, as well as uses in the treatment of heavy metal poisoning, cystinuria, multiple sclerosis, and chronic aggressive hepatitis (1).

Only the stereochemically pure D-isomer of penicillamine is employed as a therapeutic agent because it has been shown that the administration of the racemate or the L-isomer causes greater toxicity. Penicillamine causes inhibition of the kynurenine pathway and pyridoxine metabolism (2), the reduction of the activities of liver transaminases and liver cysteine desulfhydrase (3, 4), the inhibition of collagen fibre crosslinking (5), and the inhibition in bone marrow of DNA, RNA, and protein synthesis (6). In all but the last of the toxic effects cited above, penicillamine is acting on an ubiquitous coenzyme called pyridoxal-5-phosphate. This fact is borne out by studies that have shown that the administration of penicillamine creates a need for supplements of vitamin B_6, a pyridoxal-5-phosphate precursor (2, 7).

The principal derivatives of vitamin B_6 are pyridoxal, pyridoxal-5-phosphate, pyridoxamine, and pyridoxine, all of which are interconvertible in vivo (2). The postulated targets of penicillamine are the aldehydes, pyridoxal and pyridoxal-5-phosphate. The aldehydic function present in these structures has been shown to be the site through which the coenzyme binds to different apoenzymes to form active holoenzymes. This binding takes the form of a Schiff's base linkage (8).

Penicillamine, being an aminothiol, reacts with aldehydes to form five-membered ring thiazolidine-4-carboxylic acids, which are stable under physiological conditions (9). More significantly, competition experiments have shown that aminothiols can react with Schiff's bases to form thiazolidines (10). This result implies that penicillamine can interact directly with enzyme bound pyridoxal, and such a reaction is believed to occur when penicillamine is administered as a drug.

Previous work has provided substantial evidence that the thiazolidine products of the reactions are formed in solution (9), but the preparation of the reaction products in the solid state has been dealt with only fleetingly. Heyl *et al.* (11) reported the preparation of a thiazolidine product from pyridoxal and penicillamine but their characterization of the product was minimal, and was confined to an elemental analysis and a melting point.

We became interested in the factors that control the absolute

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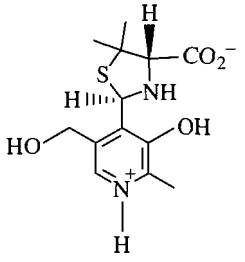
stereochemistry at the C2 position of the thiazolidine ring. In this study, we have attempted to add to the present understanding of the chiral nature of the penicillamine – vitamine B_6 interaction by preparing and characterizing the thiazolidine products (**A** and **B**) arising from the separate reactions of D-(−)- and L-(+)-penicillamine, respectively, with pyridoxal.



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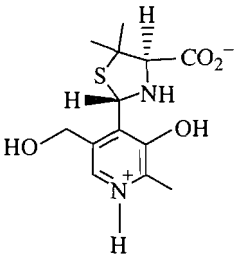

B

The reaction products 2-(2′-methyl-3′-hydroxy-5′-hydroxymethylenepyridine-C4′)-5,5-dimethyl-4-thiazolidine carboxylic acid (**A** = 2*R*,4*S*; **B** = 2*S*,4*R*) were prepared in a crystalline form to allow study of their configurations by single crystal X-ray diffraction. Although the crystal structures of both **A** and **B** were determined, they are enantiomers with no significant differences in their structures; hence only data for **A** are reported here. Spectroscopic characterization (NMR, vibrational, electronic, mass) of the reaction products was also undertaken.

Experiments

Pyridoxal hydrochloride, L-(+)-penicillamine (free base), and D-(−)-penicillamine (free base) were of reagent grade as supplied by Sigma Chemical Co., St. Louis, MO. All other reagents were of standard laboratory grade.

*2-(2′-Methyl-3′-hydroxy-5′-hydroxymethylenepyridine-C4′)-5,5-dimethyl-4-carboxylic acid (**A** and **B**)*

Pyridoxal hydrochloride (0.5000 g, 2.5 mmol) was dissolved in 50 mL of water and the pH adjusted to 8.0 by the dropwise addition of dilute NH_4OH. The pH was monitored with a pH meter (Corning model 120). To this solution was added 0.3665 g (2.5 mmol) of D- or L-penicillamine (to give the products **A** and **B**, respectively). The mixture was stirred at room temperature with a magnetic stirrer until the gradual disappearance of the characteristic yellow colour of pyridoxal signalled that the reaction was complete (about1 h). The solution was filtered by suction to remove any unreacted solids, and was stored undisturbed at room temperature in the dark. Some crystallization was evident after 24 h but the solution was allowed to sit for 4 days before being filtered. The pale yellow crystals were collected by suction on a fritted glass filter and washed successively with water, ethanol, and ether. Yield: 0.5661 g (77.4%) (**A**), 0.5968 g (81.5%) (**B**).

Analysis (by Guelph Chemical Laboratories Ltd., Guelph, Ontario) for **B**: calcd. for $C_{13}H_{18}N_2O_4S$: C 52.5, H 6.0, N 9.4, S 10.7; found: C 52.5, H 6.2, N 9.4, S 9.9. For **A**, $[\alpha]_D^{27}$ +19.4° (*c* 1, 0.1 N HCl); for **B**, $[\alpha]_D^{27}$ −19.7° (*c* 1, 0.1 N HCl). **A** and **B** were insoluble in saturated hydrocarbon solvents, chlorinated methanes, and ether. They were slightly soluble in methanol and ethanol and were soluble in dimethyl sulfoxide on warming. Solubility in water depended on pH; they were insoluble in neutral water but soluble in acidic or basic solutions.

*2-Phenyl-5,5-dimethylthiazolidine-4-carboxylic acid (**C** and **D**)*

Benzaldehyde (0.05 mL, 0.5 mmol) was mixed with 0.0733 g (0.5 mmol) of either D- or L-penicillamine (to give the products **C** and **D**, respectively) in 10 mL of water at pH 8.0 (pH adjusted with dilute NH_4OH). The solution yielded a white precipitate after 2 h of stirring. The precipitate was collected by suction and washed with ethanol and ether. Yield: 0.0490 g (41%) (**C**), 0.0341 g (29%) (**D**).

Spectral measurements

Infrared specrtra were recorded over the range 4000–200 cm^{-1} on a Perkin Elmer model 283 spectrophotometer with samples in the form of KBr pellets (at a concentration of ~1–10% by weight). The spectra were calibrated with polystyrene. Infrared spectra were also recorded in the range 500–100 cm^{-1} on a Nicolet 7199 FT-IR spectrometer with samples prepared as Nujol mulls between polyethylene plates. These spectra were automatically corrected for background noise. Raman spectra were obtained by excitation of solid samples with λ 5145 Å radiation from an Innova 90 series argon ion laser operating at an output power of 0–2 W. The spectra were recorded on a Spex 14018 double monochromator. The spectrometer was calibrated against neon and indene, and the wavenumber readout was accurate to ±2 cm^{-1}. Ultraviolet–visible spectra were recorded on a Hewlett Packard 8451 Å diode array spectrophotometer. The samples were contained in quartz cuvettes with path lengths of 1 cm; sample concentrations were 10^{-5}–10^{-4} molar. All spectra were of water solutions and run against an overlaid water reference. Sample solutions of varied pH were prepared by the addition of 0.1 N HCl or 0.1 N KOH to standard solutions. The optical activities of aqueous solutions were measured with use of the sodium D line (589 nm) and a Perkin Elmer 241 MC polarimeter, the sample chamber temperature being kept at 27°C. Samples were prepared in 0.1 N HCl solution at concentrations of 10 g L^{-1} and were measured against an overlaid reference solution. Mass spectral data were obtained with a VG micromass 7070F spectrometer equipped with a Digital PDP-8 computer. The electron impact method of ionization was used, with an electron potential of 70 eV, direct probe introduction, an ion source temperature of 220°C, and an emission current of 100 μA. The ^1H NMR spectra were recorded at 90 MHz on a Varian EM 390 spectrometer. Samples were prepared in DMSO-d_6 and D_2O solutions at concentrations of approximately 20 g L^{-1} of solvent. Tetramethylsilane and trimethylsilylpropionic acid were used as internal standards.

Collection of the X-ray diffraction data

The X-ray studies were carried out on single crystals sealed in Lindeman capillary tubes, in order to protect against decomposition. Precession photographs of the crystals indicated that they were orthorhombic. The unit cell parameters were obtained from least-squares fit of χ, φ, and 2θ for 15 reflections in the range $19.1° < 2\theta < 31.8°$, measured on a Syntex $P2_1$ diffractometer having graphite monochromated MoKα radiation (λ = 0.71069 Å). Crystal data and other parameters related to data collection are summarized in Table 1. The density of the crystals was measured by suspension in a dichloromethane–bromoform mixture. Intensities were also measured with use of the Syntex $P2_1$ diffractometer and a coupled θ(crystal)–2θ(counter) scan. The methods of selection of scan rates and initial data treatment have been described (12, 13). Corrections have been made for Lorentz and polarization effects but not for absorption. This will make a maximum error in F_o of 1.0%.

Solution of the structure

Phases were determined by direct methods with use of 230 reflections with $|E| > 1.789$ and 12 sets of starting phases. All non-hydrogen atoms were found in the resulting *E* map. Subsequent refinement and electron density difference syntheses revealed all the hydrogen atoms except one. Attempts to refine hydrogen atoms led to divergent functions, so the hydrogen atoms were placed at the positions given in the difference synthesis with temperature factors of about twice those of the atoms to which they were attached, and were not refined in further cycles. Temperature factors for sulfur and oxygen atoms and adjacent carbon atoms were made anisotropic. The significance of the additional parameters was tested at each stage (14). Full-matrix least-squares refinement, which minimized $\Sigma w(|F_o|-|F_c|)^2$, was continued until the maximum shift/error was <0.1. No corrections were needed for secondary extinction.

TABLE 1. Crystal data

Formula	$C_{13}H_{18}N_2O_4S$
Formula weight	298.36
Crystal shape, size (mm)	Acicular, 0.10 × 0.11 × 0.35
Systematic absences	$h00$, $h = 2n + 1$, $0k0$, $k = 2n + 1$, $00l$, $l = 2n + 1$
Space group	$P2_12_12_1$ (No. 19)
Unit cell (Å)	a = 9.507(5), b = 19.185(5), c = 7.766(2)
Volume (Å^3)	1416.5(9)
Z	4
ρ_{calc}, ρ_{obs} (g cm^{-3})	1.399, 1.40(1)
Temperature (°C)	22
Linear absorption coeff (cm^{-1})	2.44
Reflections collected, max 2θ	h, k, $\pm l$, 55°
Standard reflections (esd)	3 0 2 (0.016), 2 2 −1 (0.015)
No. of reflections collected	5354
R_{merg}	0.0284
No. of unique reflections	2510
No. with $I > 0$, used	2433
Final R,[a] R_w[a]	0.0876, 0.0787
Final shift/error, max, ave.	0.013, 0.002
Final difference map, max, min (e Å^{-3})	1.10, −0.73
Weighting scheme	$w = (\sigma_F^2 + 0.00090F_o^2)^{-1}$
Error in an observation of unit wt., S[b]	1.422

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = \{\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2\}^{1/2}$.
[b] $S = [\Sigma w(|F_o| - |F_c|)^2/(m-n)]^{1/2}$.

Scattering curves were taken from Cromer and Waber (15) and anomalous dispersion corrections were taken from Cromer (16) and applied to the curves for S, N, O, and C. The atom parameters are listed in Table 2.[3,4]

Discussion

The structure of **A** is shown in Fig. 1 and bond distances and angles are given in Table 3. As can be seen, the configuration at the chiral C2 carbon atom is *R* while that at C4 is *S*. The conformation is such that the hydrogen atoms at C2 and C4 are *trans*. The thiazolidine ring resembles an open envelope with C5 lying 0.682(5) Å out of the rough plane of the other four atoms. This is different from both (*S*)-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid (21) and (*R*)-thiazolidine-4-carboxylic acid (22). The small N3,C4,C1,O1 torsion angle (11.9(4)°) is in agreement with those in related structures we and others have examined (21–25) and again appears to be caused by non-bonding steric interactions rather than intramolecular hydrogen bonding. Although the N3...O1 distance is short, the N3—H3...O2 angle (118°) lies well outside the N...O/N—H...O relationship established by Brown (26). The pyridine ring (max. dev. C2′, 0.007(6) Å) and the exocyclic atoms (largest dev. C51′, 0.035(9); O5′, 0.066(9); C2, 0.053(8) Å) are very close to planar. The dihedral angle of 60.7(2)° between the pyridine ring and the thiazolidine ring is determined by the strong internal hydrogen bond between O3′—H3′ and N3. Since N3 is roughly coplanar with the pyridine ring, the thiazolidine ring may be regarded as bent out of the pyridine ring plane about a C2—N3 hinge.

TABLE 2. Atomic positional parameters (×10^4) and temperature factors (Å^2 × 10^3).

Atom	x	y	z	U_{eq}[a]
S1	281(2)	1024(1)	2522(2)	40
C2	1004(5)	1009(2)	4722(6)	26
N3	603(5)	1681(2)	5524(5)	28
C4	−298(5)	2115(2)	4408(6)	28
C5	104(5)	1975(2)	2509(7)	30
C1	−124(5)	2890(2)	4917(6)	31
O1	858(4)	3043(2)	5901(5)	40
O2	−1023(5)	3303(2)	4304(5)	51
C51	1489(7)	2320(3)	1991(8)	49
C52	−1102(8)	2157(3)	1266(8)	55
N1′	−369(4)	−779(2)	7499(6)	32
C2′	−838(5)	−160(2)	8020(6)	28
C3′	−433(5)	436(2)	7113(5)	22
C4′	476(5)	372(2)	5706(6)	23
C5′	948(5)	−298(2)	5230(6)	27
C6′	505(6)	−856(2)	6166(6)	32
C21′	−1805(6)	−135(3)	9532(7)	43
O3′	−940(4)	1041(2)	7667(5)	42
C51′	1964(7)	−405(3)	3736(8)	47
O5′	2304(5)	−1101(2)	3460(6)	51

[a] $U_{eq} = 1/3\ (U_{11} + U_{22} + U_{33})$.

[3]Tables of anisotropic temperature factors, hydrogen atom positions, bond lengths and angles involving hydrogen atoms, best planes, dihedral and torsional angles, moduli of F_o and F_c for **A**, and all vibrational bands in the region 4000–100 cm^{-1} for D-penicillamine, **A** (or **B**), and **C** (or **D**) may be purchased from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont., Canada K1A 0S2.

Tables of hydrogen atom positions and bond lengths and angles involving hydrogen atoms have also been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

[4]All calculations were carried out on a CYBER 170/815 computer. Initial data treatment was performed with programs from the XRAY76 package (17). The structure was solved by means of SHELX (18). The least-squares planes calculations used NRC-22 (19) and diagrams were prepared with the use of SNOOPI (20).

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TABLE 3. Interatomic distances (Å) and angles (°)

S1—C2	1.842(5)	C2–N3	1.481(6)	N3—C4	1.476(6)
C4—C5	1.547(7)	C5—S1	1.833(4)	C2—C4′	1.526(6)
C4—C1	1.548(7)	C1—O1	1.241(6)	C1—O2	1.260(6)
C5—C51	1.528(8)	C5—C52	1.539(8)	N1′—C2′	1.332(6)
C2′—C3′	1.396(6)	C3′—C4′	1.399(6)	C4′—C5′	1.411(6)
C5′—C6′	1.360(7)	C6′—N1′	1.336(7)	C2′—C21′	1.492(7)
C3′—O3′	1.330(6)	C5′—O51′	1.524(8)	C51′—O5′	1.390(6)
C5—S1—C2	93.1(2)	S1—C2—N3	106.3(3)	C2—N3—C4	113.2(4)
N3—C4—C5	108.6(4)	C4—C5—S1	101.0(3)	S1—C2—C4′	110.7(3)
C4′—C2—N3	113.7(4)	N3—C4—C1	109.3(4)	C1—C4—C5	112.5(4)
C4—C1—O1	117.7(4)	C4—C1—O2	115.9(4)	O1—C1—O2	126.4(5)
C4—C5—C51	112.9(4)	C4—C5—C52	112.0(4)	S1—C5—C51	110.7(4)
S1—C5—C52	107.3(3)	C6′—N1′—C2′	122.9(4)	N1′—C2′—C3′	118.9(4)
C2′—C3′—C4′	119.6(4)	C3′—C4′—C5′	118.7(4)	C4′—C5′—C6′	118.6(4)
C5′—C6′—N1′	121.3(4)	N1′—C3′—C31′	118.3(4)	C3′—C2′—C21′	122.8(4)
C2′—C3′—O3′	116.8(4)	C4′—C3′—O3′	123.6(4)	C3′—C4′—C2	121.7(4)
C3′—C4′—C2	119.6(4)	C4′—C5′—C51′	121.6(4)	C6′—C5′—C51′	119.8(4)
C5′—C51′—O5′	113.2(4)	C51—C5—C52	112.1(5)		

Possible hydrogen bonds

O3′...N3	2.535(5)	O3′—H3′	0.69[c]	H3′...N3	1.89	O3′—H3′...N3	156
N1′...O1[a]	2.620(5)	N1′—H1′	0.96	H1′...O1	1.67	N1′—H1′...O1	169
O5′...O2[b]	2.719(6)						

[a,b]Atoms are related to those in Table 2 by the relationships *a*; -*x*, *y*-1/2, 3/2-*z*; *b*; -*x*, y-1/2, 1/2-*z*.
[c]No errors are given since the hydrogen atom parameters were not refined.

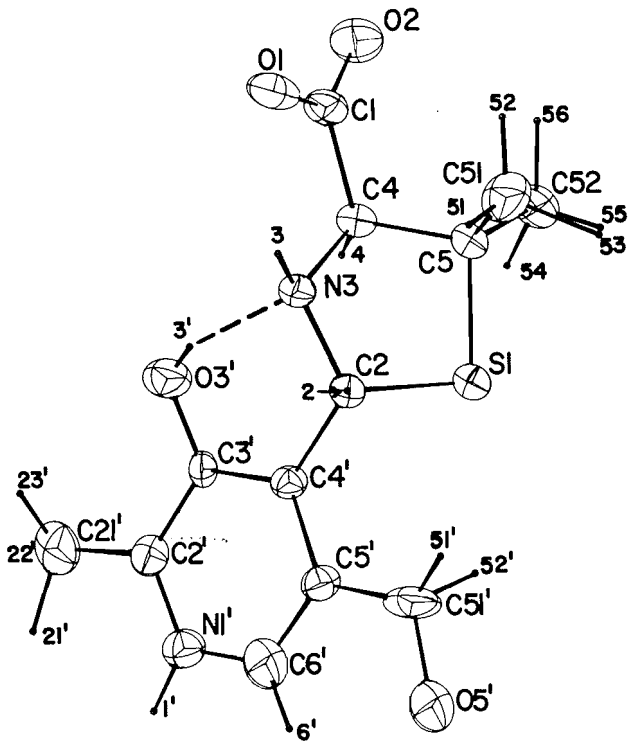

FIG. 1. The molecule of **A** showing the atom numbering. The view is down the H2—C2 bond. Hydrogen atoms are indicated by affixes only in smaller print.

The bond lengths and angles within the thiazolidine ring are consistent with values found for (*S*)-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid, except for the C2—N3—C4 angle, which is significantly larger (113.2(4)° vs. 108.6(4)°, 107.9(4)°) (21) and closer to the value found for protonated amines as in bis((*S*)-5,5-dimethylthiazolidine-4-carboxylic acid)hydrogen chloride hydrate (112.2°, 109.7°) (27). The structural results show no protonation of the nitrogen atom, although there is a very strong internal hydrogen bond to it (N3...O3′, 2.535(5) Å). In the difference map, however, a small peak appeared on the same N3...O3′ axis, about 1 Å from the nitrogen atom, but this disappeared when attempts were made to refine it with partial occupancy. It may be that, in the crystal, there is a small amount of the $O^-...NH_2^+$ tautomer, as well as the major OH...NH tautomer, which could not be detected by the X-ray refinement procedure. The distances and angles within the carboxylate ion differ from those found in both the above structures and are close to those found in (*R*)-thiazolidine-4-carboxylic acid, which exists as the zwitterion tautomer (22). The bond lengths and angles in the pyridoxal ring agree well with average values for a number of N-protonated pyridoxyl rings (28–34). In particular the C6′—N1′—C2′ angle is significantly larger (122.9(4)° vs. 111.3°) than a comparable angle in a non-protonated pyridoxyl ring (35), an effect first noted by Singh (36).

The packing is shown in Fig. 2 and is dominated by the strong hydrogen bonds between adjacent molecules related by the 2_1 axes along the *b* direction. N1′...O1 (2.620(5) Å) is between molecules related by the axis at $x = 0$, $z = 3/4$, whereas O5′...O2 (2.70(5) Å) is between molecules related by the $x = 0$, $z = 1/4$ axis. The resultant bonding of one molecule to four others provides a hydrogen bonding net in the *c* direction as well. Interactions in the *a* direction are van der Waals.

The ^{1}H NMR chemical shifts and assignments for **A** and **B** are presented in Table 4 together with those of **C** and **D** so that analogies can be drawn between the spectra. Both sets of spectra can be readily interpreted if it is assumed that pairs of diastereomers are present in solution for each compound (**A** has *S*,*S* and 2*R*,4*S* and **B** has *R*,*R* and 2*S*,4*R*). This is shown by the splitting of all peaks except that for H6′. We assume that the lack of visible splitting of the H6′ proton peak is caused by limita-

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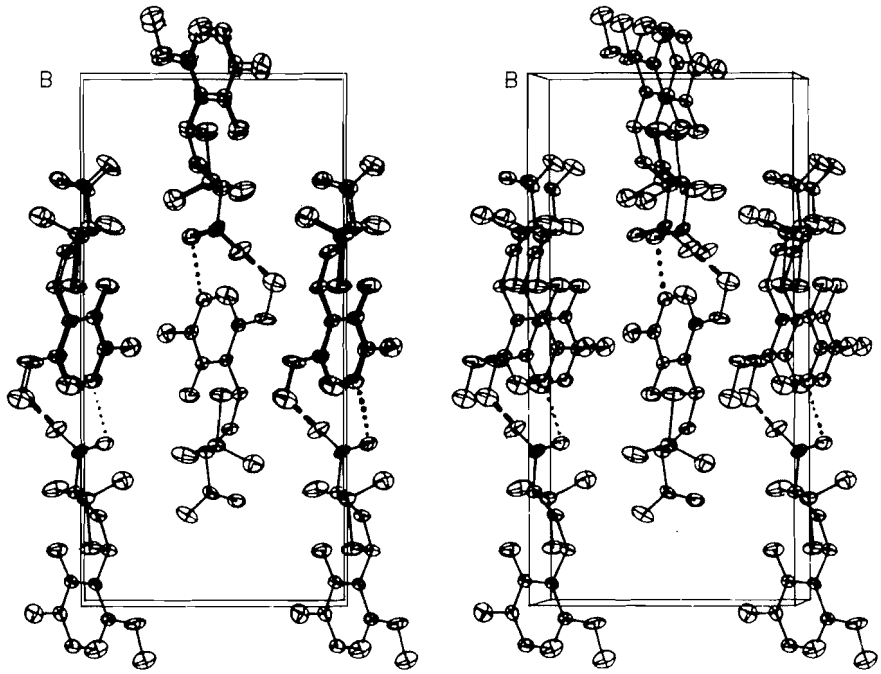

FIG. 2. The packing **A** within the unit cell. *a* and *b* are parallel to the bottom and side of the page and the view is down *c*. Hydrogen bonds are given by dashed (O5′---O2) or dotted (N1′...O1) lines.

TABLE 4. ^{1}H NMR chemical shifts of various thiazolidine-4-carboxylic acids

Compound[a]	%	Diastereomer	α-CH_3 H-7	β-CH_3 H-8	H2i′ *i* = 1–3	H2	H5i′ *i* = 1,2	H4	H6′	Aromatic
A	22	*S,S*	1.38	1.58	2.30	3.88	4.45	6.33	7.82	—
	78	2*R*,4*S*	1.43	1.63	2.35	3.75	4.45	5.93	7.82	—
B	20	*R,R*	1.38	1.59	2.30	3.90	4.45	6.33	7.84	—
	80	2*S*,4*R*	1.44	1.63	2.35	3.76	4.45	5.95	7.84	—
C	30	*S,S*	1.33	1.58	—	3.63	—	5.90	—	7.4 m
	70	2*R*,4*S*	1.40	1.70	—	3.72	—	5.67	—	7.4 m
D	33	*R,R*	1.33	1.56	—	3.60	—	5.88	—	7.4 m
	67	2*S*,4*R*	1.38	1.68	—	3.67	—	5.65	—	7.4 m

m = multiplet

[a]**A** and **B** are solutions of 2*R*,4*S* and 2*S*,4*R* 2-(2′methyl-3′-hydroxy-5′-hydroxymethylenepyridine-C4′)-5,5-dimethylthiazolidine-4-carboxylic acid. **C** and **D** are solutions of the 2-phenyl-5,5-dimethylthiazolidine-4-carboxylic acids derived from *S*-penicillamine and *R*-penicillamine respectively.

tions in resolution of the NMR instrument or chance coincidence. Comparison of the spectra of **C** and **D** with those of **A** and **B**, together with consideration of the intensities, shows that the peak at $\delta \approx 2.3$ is caused by the methyl group of the substituted pyridine ring. The resonance at $\delta \approx 7.82$ is assigned to the hydrogen atom at the C6′ position of the substituted pyridine ring and the resonance at $\delta \approx 4.45$ to the CH_2 protons at the 5′ position. The proton of the C4 position of the thiazolidine ring can be assigned the resonances at ≈5.92 and ≈6.3. The assignment is consistent with assignments of similar compounds examined by other workers (37). The C51 and C52 methyl group protons are assigned by analogy to penicillin (38).

The assignment of the resonances to *S,R* and *S,S* (or *R,S* and *R,R*) is based on the work of McMillan and Stoodley (37). Talking about the protons at C2 and C4 in similar compounds, they state: "In particular, if both stereoisomers at C2 are available, the isomer in which the C4 proton appears at lower field possesses the *trans* configuration." Thus the "*trans*" diastereomer, which is the one of which the structure was determined, is the major component in solution and this is more stable. The apparent contradiction, that only one diastereomer was found in the solid state, whereas the ^{1}H NMR spectra showed that in solution both diastereomers were present (that is, a solution of **A** contained both 2*R*,4*S* and *S,S* diastereomers with the corresponding pair for **B**), can be explained in two ways:

(a) The products **A** and **B** were obtained from a mixture of diastereomeric crystals but the crystals chosen for study just happened to be 2*R*,4*S* and 2*S*,4*R*.

(b) The products **A** and **B** in the solid state are single diastereomers that undergo epimerization in solution to give a mixture of diastereomers.

Possibility (a) was tested by performing a powder diffraction study on the product **B**. The measured diffraction pattern agreed with that calculated from the single crystal parameters. It was concluded therefore that possibility (b) holds: rapid epimerization of the thiazolidine products **A** and **B** occurs in solution probably by means of a Schiff's base intermediate (39). The

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TABLE 5. Selected vibrational bands[a]

D-Penicillamine[b]	Pyridoxal·HCl[b]	A[b]	Assignment[c]
		2480, IR	O—H...N bonding
2008, IR		2080, IR	
	1638, IR	1650, IR	ν C=N
	1630, R		
1616, IR		1610, IR	ν_a CO_2^-
1597, IR			
1558, IR			δ_a NH_3^+
	1552, IR	1540, IR	δ NH, δ NH^+
1524, IR			δ_s NH_3^+
1518, R			
1400, R		1400, IR	ν_s CO_2^-
1397, IR		1395, R	

[a]Infrared bands (IR) and Raman shifts (R), in cm^{-1}.
[b]Compounds as defined in the Experiments. D-Penicillamine in single enantiomer, zwitterionic, non single crystalline form; **A** in crystalline form.
[c]Notations: ν = stretching frequency, a = antisymmetric, s = symmetric, δ = deformation.

TABLE 6. Electronic spectra of **A** and pyridoxal as a function of pH

A			Pyridoxal		
pH	λ_{max}[a]	Absorbance (M^{-1} cm^{-1})	pH	λ_{max}	Absorbance (M^{-1} cm^{-1})
3.0	245sh[b]	~2500	1.1		
	295sh	~2500		286	8524
	326	5330			
7.0	245sh	~2500	6.2	250	5574
	295sh	~2500			
	326	4887		314	8463
10.0	245sh	~4000	12.5	236	8437
	318	5249		298	5654
				392	1870

[a]λ_{max}, wavelength in nanometers of band maximum.
[b]sh = shoulder.

diastereomer found in the solid state is that which is least soluble and will be determined not only by molecular stability but also by intermolecular interactions.

The infrared and Raman spectra of **A** and **B** were identical (as were those of **C** and **D**). The frequencies and assignments of the major vibrational bands of the starting materials D-penicillamine and pyridoxal hydrochloride, and of **A**, are listed comparatively in Table 5.[3] The two strong bands between 2500 and 1900 cm^{-1} are typical of medium strong O—H...N bonding in the solid state. That the compounds **A** and **B** exist as zwitterions in the solid state is shown by the presence of strong bands at ca. 1620 and 1400 cm^{-1}, typical of ν_a and ν_s of the CO_2^- group, and the absence of a strong νC=O absorption at 1720 cm^{-1}. Assignments are based on previous work by ourselves (23) and others (40, 41).

The electronic spectra were of interest because the reaction between penicillamine and pyridoxal was monitored by the appearance of a peak at 326 nm, which is characteristic of thiazolidines of pyridoxal (42). The ultraviolet–visible spectra of **A** were examined at three pH values: 3.2, 7, and 10.2. The spectral results are given in Table 6. The spectra of the starting material, pyridoxal hydrochloride, were also obtained at several pH values. For pyridoxal at pH 1.5, where the pyridine nitrogen

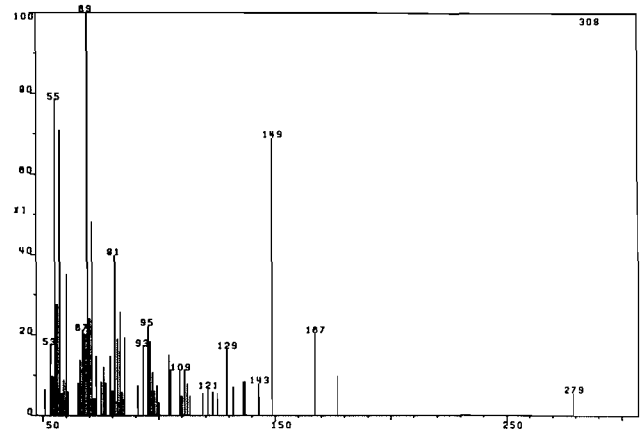

FIG. 3. The mass spectrum of **A**.

atom is protonated, only one absorption maximum at 286 nm is evident, while at pH 12.5, where the phenolic group is deprotonated, three maxima are present. Pyridoxal forms bright yellow solutions at pH values greater than 7.5 because of the presence of the phenoxide-like chromophore.

Compound **A** also has a pH dependent UV spectrum. Formation of the thiazolidine reduces the extent of the delocalization of the phenoxide-like chromophore, and hence the absorption maxima occur at lower wavelengths and the characteristic yellow colour observed for pyridoxal solutions is not present.

A titration of **B** against 0.01 N HCl shows the presence of three titratable protons at pK values of 4.5, 7.5, and 10.1. By comparison with ^{13}C NMR studies done by other workers (43), we have made the following assignments: pK_a 4.5, proton of pyridinium nitrogen; pK_a 7.5, proton of N3 of thiazolidine ring; pK_a 10.1, phenolic hydrogen. These assignments can also be seen to support the pH dependent ultraviolet data.

The mass spectrum is shown in Fig. 3. No parent peak, P^+, is seen; the highest peak at m/e = 279 corresponds to $(P-OH_3)^+$, but the fragment cannot be identified. The principal features below m/e = 200 are very similar to the corresponding spectra for pyridoxal phosphate and pyridoxal, for which the fragmentation patterns have been fully discussed (44, 45). There is little evidence of fragments from the thiazolidine ring through the normal 1,4 cleavage. Peaks at m/e = 69 and 55 have been observed previously for decompositions of thiazolidines derived from D-penicillamine (21). In that case the m/e = 69 peak was derived from a 1,4 fragmentation, whereas in this case it can only be derived from a 2,5 fragmentation. The m/e = 55 peak can also arise from the 2,5 fragmentation, even though the first fragment (m/e = 114) is not observed.

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