Polydepsipeptides. 5. Experimental Conformational Analysis of Poly(L-alanyl-L-lactic acid) and Related Model Compounds

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ABSTRACT: In this paper we report an experimental conformational analysis of the depsipeptide model compounds acetyl-L-alanine methyl ester, acetyl-L-lactic acid *N*-methylamide, and acetyl-L-alanyl-L-lactic acid N-methylamide and of the sequential polydepsipeptide poly(L-alanyl-L-lactic acid). The model depsipeptides were examined in dilute organic solutions by infrared and nuclear magnetic resonance spectroscopy. Neither acetyl-L-alanine methyl ester nor acetyl-L-lactic acid *N*-methylamide assumes an intramolecularly hydrogen-bonded conformation. Acetyl-L-alanyl-L-lactic acid N-methylamide, on the other hand, in dilute chloroform or dilute carbon tetrachloride solutions, strongly favors a conformation with an intramolecular hydrogen bond between the N–*H* hydrogen atom of its *N*-methylamide group and the carbonyl oxygen atom of its acetyl group. Comparison of theoretical and experimental circular dichroism suggests that poly(L-alanyl-L-lactic acid) is partially ordered in chloroform solution with approximately 50% of its repeat units in the R_{10} helix, an ordered conformation found by our previous theoretical analysis to have a low intramolecular conformational energy.

Polydepsipeptides are copolymers of α-amino and α-hydroxy carboxylic acids with neighboring monomers linked either by an amide or an ester bond. Because of the close structural similarity of amide and ester groups, polydepsipeptides are particularly appropriate models for the conformational and optical properties of polypeptides and proteins. They also serve as models for natural depsipeptides such as valinomycin and the enniatins.

Previous papers in this series describe the synthesis of block[1] and sequential polydepsipeptides[2,3] and a theoretical conformational analysis of a sequential polydepsipeptide.[4] In this paper we present infrared and nuclear magnetic spectra of the depsipeptide model compounds acetyl-L-alanine methyl ester and acetyl-L-lactic acid *N*-methylamide. Circular dichroism, infrared, and nuclear magnetic spectra of the sequential polydepsipeptide poly(L-alanyl-L-lactic acid) are also included. The infrared spectra were recorded for dilute solutions of the amino acid and depsipeptide derivatives in carbon tetrachloride, chloroform, and dioxane, the nuclear magnetic resonance for solutions in chloroform, and the circular dichroism for solutions in chloroform and trifluoroethanol. The experimental results are interpreted in terms of the conformational properties of the depsipeptides and are compared with the previous theoretical conformational analysis.[4]

Infrared spectroscopy is especially sensitive to the presence and mode of amide hydrogen bonding. The amide NH stretching vibration occurs either near 3450 cm^{-1} in the nonhydrogen-bonded state or between 3300 and 3400 cm^{-1} when the H atom is involved in a hydrogen bond. Amide and ester carbonyl stretching frequencies shift when the oxygen atoms are hydrogen bonded. The infrared spectra presented here reveal specific intramolecular hydrogen bonding.

Conformational analysis of peptides by NMR spectroscopy relies primarily on NH proton chemical shifts, on the sensitivity of these chemical shifts to changes of temperature and solvent, and on vicinal coupling constants between NH and $C^{\alpha}H$ protons. The conformational information available from NH–$C^{\alpha}H$ coupling constants is considerably reduced for depsipeptides by the replacement of amide by ester groups. The absence of aromatic side chains in the depsipeptides studied here also eliminates the possibility of detecting specific interactions between the side chains and the backbone by aromatic ring current effects. In this report, therefore, we derive conformational information from depsipeptide chemical shifts. Confidence in this procedure follows from the good agreement obtained from all three methods of conformational analysis employed here, CD, ir, and NMR spectroscopy.

Circular dichroism (CD) is used extensively for conformational analysis of polypeptides and proteins. It is most effective for empirical correlation of conformation and spectra; the CD of a polymer in an unknown conformation is compared to standard spectra determined for similar polymers in known conformations.[5] Because of the absence of experimental standard spectra, we compare experimental polydepsipeptide CD spectra to theoretical spectra calculated according to procedures developed by Tinoco,[6] Schellman,[7] and their coworkers to account for optical activity of polypeptides.

Experimental Section

Materials and Methods. Infrared Spectroscopy. Depsipeptide infrared spectra were recorded for dilute carbon tetrachloride, deuteriochloroform, and *p*-dioxane solutions. Carbon tetrachloride was obtained from Eastman, Rochester, N.Y., as a Spectro ACS grade solvent and was dried over Linde type 4 Å molecular sieves. Deuteriochloroform, 99.8% D, was purchased from Stohler Isotope Chemicals, Rutherford, N.J. It was washed repeatedly with concentrated sulfuric acid and then with deionized water and dried by distillation onto 4 Å molecular sieves. The dioxane was an Analytical Reagent from Mallinckrodt Chemical Works, St. Louis, Mo. The infrared spectra of freshly prepared solutions, 0.01 to 0.1 mg of depsipeptide/ml, were recorded in either a 1.0 or a 5.0 cm path length cell equipped with sodium chloride windows using a Perkin-Elmer Model 180 grating spectrophotometer.

Nuclear Magnetic Resonance Spectroscopy. Deuteriochloroform (100% D, Wilmad Glass Co. Inc., Buena, N.J.), dimethyl sulfoxide-d_6 (Wilmad Glass), carbon tetrachloride (MC/B Manufacturing Chemists, Norwood, Ohio), and tetramethylsilane (Mallinckrodt Chemical Works, St. Louis, Mo.) were dried over Linde 4 Å molecular sieves. Solutions were prepared in a drybox and concentrations determined by weight. Spectra were recorded at 25 °C with either a Varian T-60 or a Varian HR-220 spectrometer equipped with a Transform Technology TT-100 Fourier transform system. Tetramethylsilane was used as an internal standard.

Circular Dichroism Spectroscopy. Chloroform (an Analytical Reagent from Mallinckrodt Chemical Works, St. Louis, Mo.) and trifluoroethanol (TFE) (Aldrich Gold Label, Aldrich Chemical Co., Milwaukee, Wis.) served as solvents for the circular dichroism (CD) measurements. They were distilled and stored over Linde type 4 Å molecular sieve. Solutions were prepared immediately before measurement; their concentrations were determined by weight (Cahn electro balance). Spectra were recorded at 25 °C in either 0.1 or 0.01 mm path length optical cells equipped with quartz windows (Helma QS). Repeated spectra were measured with a Cary Model 61 spectropolarimeter and computer averaged according to the procedure described by Radding et al.[8]

Results are expressed as mean molar ellipticity, $[\theta]$, defined as:

$$[\theta] = \frac{\theta^0}{10}\frac{M}{lC'} \text{ (deg cm}^2\text{)/dmol}$$

where θ^0 is the observed ellipticity; M is the average molecular weight of the α-amino and α-hydroxy residues, l is the optical path length in cm, and C' is the solute concentration in g/cm^3.

Calculation of Circular Dichroism. Polydepsipeptide optical activity was calculated in analogy to procedures developed by Tinoco,[6] Schellman,[7] and their co-workers. Accordingly, a polydepsipeptide is considered as an array of optically inactive amide and ester chromophores; electronic interaction between the chromophores produces the polymer's optical activity. The basis set of polymer wave functions comprises all products of wave functions for the isolated chromophores, and the polymer Hamiltonian is represented by the sum of unperturbed Hamiltonians for the isolated chromophores and the interchromophore interaction energy.

Recent studies of Schellman[9] and of Woody[10] reveal that consideration of only the n–π^* and π–π^* amide excited states is sufficient to account for most features of polypeptide optical activity. Madison and Schellman[11] also considered only the n–π^* and π–π^* excited states for both the amide and ester chromophores in their calculations of the optical activity of acetyl amino acid esters. Similarly, we have included only the amide and ester n–π^* and π–π^* excited states in our calculations of polydepsipeptide circular dichroism.

We employed the matrix formulation of Bayley, Nielsen, and Schellman.[7] The diagonal elements of the Hamiltonian matrix represent the n–π^* and π–π^* excited state energies of the constituent amide and ester chromophores. Nielsen and Schellman[12] have determined the amide excited state energies by ultraviolet spectroscopy. We show these energies in Table I. A review of the literature on ultraviolet spectroscopy of esters led Madison and Schellman[11] to suggest the values for the ester excited state energies also listed in Table I. We employed the energies of Table I in the present calculations.

The off-diagonal elements of the Hamiltonian matrix result from interactions between chromophores. We calculated these elements according to the point monopole approximation;[6] the necessary optical parameters, amide and ester transition moments, and dipole and quadrapole charges, were those of Madison and Schellman.[9,11] The interaction energy between chromophores also depends upon the polymer geometry. Polydepsipeptide geometry was determined by procedures described previously.[4] The amide and ester groups are held in their planar *trans* conformation, and bond lengths and angles are fixed at standard values. Polydepsipeptide conformation is thus defined by the set of angles, $\{\phi,\psi\}$, describing torsional rotation about the two skeletal single bonds of each α carbon atom of the chain.

Diagonalization of the Hamiltonian matrix yields polymer wave functions and electronic transition energies.[7] These are used to calculate rotational strengths, $R_K = \text{Im}\{\mu_{OK} \cdot m_{KO}\}$,[7] of each polymer transition K; μ_{OK} and m_{KO} are respectively electric- and magnetic-dipole transition moments. We consider that each transition K contributes a single Gaussian curve, whose integrated intensity is proportional to R_K, to the composite CD spectrum. The band width of each Gaussian was arbitrarily fixed at 12 nm.

Calculations were performed for chain lengths of ten structural units, five amino and five hydroxy acid residues. Chain conformations employed were the two low energy ordered polydepsipeptide structures described previously[4] and the randomly coiling chain.[4] The CD spectra of 50 random chain conformations, generated by Monte Carlo procedures, were calculated and averaged over all spectra so calculated; each spectrum was weighted in the averaging by the Boltzmann factor calculated from the energy of its associated chain conformation. We calculated polydepsipeptide conformational energy according to previously described procedures.[4]

Synthesis. Unless otherwise noted, all chemicals were Analytical Reagents from Mallinckrodt Chemical Works, St. Louis, Mo. Triethylamine and *N*-methylmorpholine were obtained from Aldrich Chemical Co., Milwaukee, Wis., distilled from ninhydrin, redistilled from sodium, and stored under nitrogen. Dimethylformamide was distilled from ninhydrin under vacuum and stored on Linde 4 Å molecular sieves. Pyridine was dried on potassium hydroxide, distilled from barium oxide, and stored on 4 Å molecular sieves. Acetic anhydride was distilled from calcium hydride onto a 4 Å molecular sieve. Methylamine (MC/B Manufacturing Chemists, Norwood, Ohio) was pased through potassium hydroxide and bubbled into dry tetrahydrofuran and the resulting solution was stored over 3 Å molecular sieves; its concentration was determined by titration with 1.0 M hydrochloric acid. Hydrochloric acid in dioxane (4 M) was purchased from Pierce Chemical Co., Rockford, Ill., and acetyl-L-alanine was supplied by Bachem Inc., Marina del Rey, Calif.

Table I
Transition Energies of Amide and Ester Groups

	Transition wavelength max, nm	
	n–π^*	π–π^*
Amide	224	184
Ester	213	167

Uncorrected melting points were obtained with a Thomas-Hoover melting point apparatus. Precoated silica gel 60 F254 plates from Merck and Co., Inc., Rahway, N.J., were employed for thin layer chromatography. Galbraith Laboratories, Inc., Knoxville, Tenn., performed the elemental analyses. Optical rotations were measured at 589 nm using a Perkin-Elmer Model 141 polarimeter.

***tert*-Butoxycarbonyl-L-alanyl-L-lactic Acid *N'*-Methylamide.** *tert*-Butoxycarbonyl-L-alanyl-L-lactic acid[3] (3 g, 11.5 mmol) was dissolved in dry THF (28 ml), *N*-methylmorpholine (1.28 ml, 11.5 mmol) was added, and the solution was cooled to −20 °C with a dry ice/2-propanol bath. Isobutyl chloroformate (1.49 ml, 11.5 mmol) was added and, after stirring 3 min, a precooled solution of methylamine in THF (1.1 M, 15 ml, 16.5 mmol) was added dropwise. The reaction mixture was stirred until temperature reached 10 °C. Solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (100 ml). After washing with saturated sodium bicarbonate, water, sodium bisulfate (1 M), and water, the ethyl acetate solution was dried on $MgSO_4$ and evaporated to dryness under reduced pressure. Residue was crystallized from ethyl acetate–hexane to give 2.3 g (73%) of the product. Recrystallization from ethyl acetate–hexane gave white needles, mp 109 °C. Thin layer chromatography (chloroform, methanol 1:1, ninhydrin after exposure to HCl vapors) showed one spot at R_f 0.79. NMR (CCl_4) δ 7.38 (d, 1 H, *NH* CH_3), 6.38 (d, 1 H, CO-*NH*-CH), 5.73 (q, 1 H, *CH*(lac)), 4.1 (quin, 1 H, α*CH*(ala)), 2.73 (d, 3 H, *CH*$_3$NH), 1.43 (m, 15 H, $(CH_3)_3$, side chains). Ir (KBr) 3250, 3330 (ν NH), 1742 (ν C-OO), 1689, 1560 cm^{-1}. (ν CO-NH urethane), 1656, 1540 cm^{-1} (ν CO-NH-CH_3). Anal. Calcd for $C_{12}H_{22}N_2O_5$: C, 52.54; H, 8.08; N, 10.21. Found: C, 52.50; H, 8.23; N, 10.07.

Alanyl-L-lactic Acid *N*-Methylamide Hydrochloride. *tert*-Butoxycarbonylalanyl-L-lactic acid *N*-methylamide (1 g, 3.6 mmol) was stirred with HCl in dioxane (4 N, 10 ml) for 0.5 h. After evaporation of solvent under reduced pressure, the residual oil was triturated twice with ether and left in ethyl acetate overnight in the cold. The precipitate was collected by filtration to give 0.7 g (91%) of product, mp 147 °C. Thin layer chromatography (chloroform, methanol 1:1) showed one ninhydrin positive spot even after treatment with HCl vapors, R_f 0.32. NMR (d_6DMSO) δ 8.83 (s, 3 H, *NH*$_3^+$), 8.31 (d, 1 H, *nh*-CH_3), 5.1 (q, 1 H, αCH(lac)), 4.13 (quin, 1 H, αCH(ala)), 2.68 (d, 3 H, *CH*$_3$-NH), 1.51 (d, 3 H, CH_3 side chain), 1.45 (d, 3 H, CH_3 side chain). Ir (KBr) 3280 (ν NH), 2495, 2042 (ν NH_3^+), 1755 (ν CO-O) 1659 (amide I), 1550 cm^{-1} (amide II). Anal. Calcd for $C_7H_5N_2O_3Cl$: C, 39.91; H, 7.18; N, 13.30; Cl, 16.83. Found: C, 38.87; H, 7.32; N, 13.41; Cl, 16.71.

Acetyl-L-alanyl-L-lactic Acid *N*-Methylamide. A solution of *N*-methylmorpholine (0.795 ml, 7.12 mmol) and glacial acetic acid (0.403 ml, 7.12 mmol) in dry tetrahydrofuran (15 ml) was cooled in dry ice/2-propanol to −20 °C and isobutyl chloroformate (0.926 ml, 7.12 mmol) was added. After stirring at −20 °C for 3 min, a precooled solution of alanyl-L-lactic acid α-methylamide hydrochloride (0.67 g, 3.18 mmol) in DMF (10 ml) was added followed by slow addition of triethylamine (0.445 ml, 3.18 mmol). Stirring was continued until the temperature reached 10 °C (approximately 1 h). The reaction mixture showed a negative ninhydrin test. The solvents were removed under reduced pressure and the residue was dissolved in water (30 ml). After adjusting the pH to 3, the water solution was extracted with ethyl acetate (5 × 50 ml). Ethyl acetate was dried (magnesium sulfate) and evaporated to dryness. The residue was crystallized from ethyl acetate–hexane to give 0.63 g (92%) of white needles, mp 110 °C. Thin layer chromatograph (chloroform, methanol 9:1) showed one spot chlorine–toluidine positive[13] and no ninhydrin positive spot, R_f 0.4. NMR ($CDCl_3$) δ 6.71 (s, 1 H, *NH*-CH_3), 6.00 (s, 1 H, CO-NHCH), 5.26 (q, 1 H, α*CH*(lac)), 4.49 (quin, 1 H, α*CH*(ala)), 2.86 (d, 3 H, *CH*$_3$CH), 2.09 (s, 3 N, *CH*$_3$CO), 1.50 (d, 6 H, side chains). Ir (KBr) 3290, 3267 (shoulder) (ν NH) 1735 (ν CO-O) 1661, 1640 (amide I), 1568 (shoulder), 1543 cm^{-1} (amide II). Anal. Calcd for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; H, 12.95. Found: C, 49.83; H, 7.51; H, 12.83.

Lactic Acid *N*-Methylamide.[27] The L-lactide (prepared by distillation of L-lactic acid benzyl ester, $[\alpha]^{25}D$ +282 (benzene *c* 0.5) lit.[14]

Table II
Infrared Absorption Maxima of Depsipeptides

Sample and solvent	Maxima, cm^{-1}		
		Carbonyl stretching	
	N–H stretching	Ester	Amide I
Acetyl-L-alanine methyl ester			
CCl_4 (0.06 mg/ml)	3420	1740	1685
$CDCl_3$ (5 mg/ml)	3430	1740	1675
Acetyl-L-lactic acid *N*-methylamide			
CCl_4 (0.01 mg/ml)	3480	1760 (1740 sh)	1690
$CDCl_3$ (0.02 mg/ml)	3450	(1755 sh) 1740	1680
Dioxane (1.5 mg/ml)	3360	1740	1680
Acetyl-L-alanyl-L-lactic acid *N*-methylamide			
CCl_4(67):$CDCl_3$(33) (0.02 mg/ml)	3450 3370	1745	1675
$CDCl_3$ (0.03 mg/ml)	3450 3370	1748	1670
Poly(L-alanyl-L-lactic acid)			
CCl_4(9):$CDCl_3$(1) (0.13 mg/ml)	3380 3290	1740	1660
$CDCl_3$ (0.012 mg/ml)	3370 3280	1740	1662
Dioxane (1.3 mg/ml)	(3380 sh) 3300	1740	1670

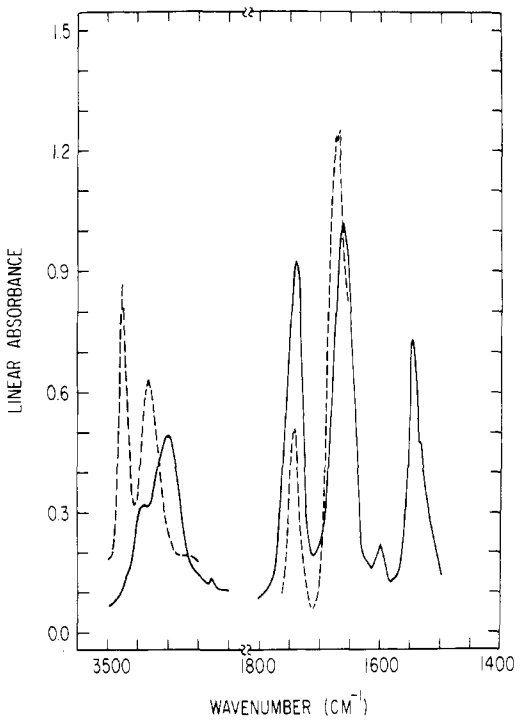

Figure 1. The infrared spectra in dilute chloroform: (—) poly(L-alanyl-L-lactic acid); (- - -) acetyl-L-alanyl-L-lactic acid *N*-methylamide.

$[\alpha]^{16}D$ +281.6 (benzene *c* 0.82)) (14.4 g, 0.1 mol) was cooled in a dry ice–methanol bath (−20 °C) and methylamine was passed through until 12.5 g were added. The reaction mixture was stirred at room temperature until the 1760-cm^{-1} band (ν CO=O) disappeared (about 24 h). Excess methyl amine was removed under reduced pressure and the oily residue crystallized in ethyl acetate–hexane. Recrystallization from ethyl acetate–hexane gave 15 g (78%) of crystals: mp 69 °C; NMR ($CDCl_3$) δ 7.04 (s, 1 M, NH), 4.8 (s, 1 M, OH), 4.18 (q, 1 H, CH), 2.63 (d, 3 H, NH*CH*$_3$), 1.4 (d, 3 H, CH-CH_3); ir (KBr) 3280–3320 (ν OH, NH), 1650 (amide I), 1550 cm^{-1} (amide II).

Acetyl-L-lactic acid *N*-Methylamide. The compound L-lactic acid *N*-methylamide (1.03 g, 10 mmol) was stirred with a mixture of pyridine (5 ml) and acetic anhydride (5 ml) for 3 h at room temperature. Solvents were removed under reduced pressure and ether was added to the residue. After evaporation, diisopropyl ether was added and the solution was refrigerated overnight. The precipitate was collected and recrystallized from diisopropyl ether to give 1.4 g (96%) of product, mp 54 °C. Thin layer chromatography (chloroform, methanol 9:1) showed one spot chlorine–toluidine positive, R_f 0.5. NMR ($CDCH_3$) δ 6.18 (s, 1 H, NH), 5.28 (q, 1 H, 2*CH*), 2.86 (d, 3 H, *CH*$_3$NH), 2.14 (s, 3 H, CH_3-CO), 1.49 (d, 3 H, *CH*$_3$CH). Anal. Calcd for $C_6H_{11}NO_3$: C, 49.65; H, 7.64; N, 9.65. Found: C, 49.78; H, 7.52; N, 9.49.

Acetyl-L-alanine Methyl Ester.[15] Acetylalanine (2.62 g, 20 mmol) and methyldiisopropylisourea[16] (3.0 ml, 1 mmol) were refluxed in dry tetrahydrofuran (20 ml) until the TLC showed complete disappearance of starting material (~12 h). After cooling, the urea was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in chloroform and passed through a MN-Kiesgel (0.08 mm) column (chloroform, methanol 9:1). The solvents were removed under reduced pressure to give 2.5 g (86%) of an oil which did not show peaks at δ 1.03 (*CH*$_3$-CH) of diisopropyl urea. Thin layer chromatography (chloroform, methanol 1:1) showed one spot chlorine–toluidine positive R_f 0.45. NMR ($CDCl_3$) δ 6.25 (s, 1 H, *NH*), 4.59 δ (q, 1 H, αCH), 3.73 (s, 3 H, O-CH_3), 2.0 (s, 3 H, C-*CH*$_3$), 1.41 (d, 3 H, CH-CH_3). Ir (CCl_4) 3420 (ν NH) 1740 (ν CO=O), 1685 cm^{-1} (ν CO-NH).

Poly(L-alanyl-L-lactic acid). The polymer sample used here was synthesized by Nissen et al.;[3] it is polymer No. AL-5 of their Table 3. Its molecular weight was determined by viscosity to be 24 000 daltons.

Results and Discussion

The Model Compounds. Infrared Spectroscopy. Examples of infrared spectra, obtained in dilute chloroform solution, are presented in Figure 1; the spectrum of acetyl-L-alanyl-L-lactic acid *N*-methylamide is drawn with the dashed line and that for poly(L-alanyl-L-lactic acid) with the solid line. Positions of the absorption maxima of the spectra in Figure 1 as well as those obtained for the remaining depsipeptides are shown in Table II.

Both acetyl-L-alanine methyl ester and acetyl-L-lactic acid *N*-methylamide exhibit only a single, sharp N–H absorption, located near 3450 cm^{-1}, in dilute carbon tetrachloride or chloroform solution. Following assignments by Mizushima et al.,[17] by Avignon et al.,[18] by Burgess and Scheraga,[19] and by Shields et al.,[20] we attribute this peak near 3450 cm^{-1} to stretching vibrations of amide N–H groups that are not hydrogen bonded. Thus neither compound forms intramolecular hydrogen bonds. Acetyl-L-alanine methyl ester and acetyl-L-lactic acid *N*-methylamide have, however, the chemical structure necessary for formation of one of the two intramolecularly hydrogen-bonded conformations assumed by the closely related compound, acetyl-L-alanine-*N*-methylamide:[19] C_5 for acetyl-L-alanine methyl ester and C_7 for acetyl-L-lactic acid *N*-methylamide. In the C_5 conformation, the acetyl N–H bonds to the L-alanine carbonyl oxygen to link five atoms in a hydrogen-bonded ring, and in the C_7 conformation

Table III
Chemical Shifts of Depsipeptide Protons in Deuteriochloroform Solution

Proton	Chemical shift, ppm[a]			
	Acetyl-L-alanine methyl ester	Acetyl-L-lactic acid *N*-methylamide	Acetyl-L-alanyl-L-lactic acid *N*-methylamide	Poly(L-alanyl-L-lactic acid)
Acetyl CH_3	1.99	2.13	2.09	
Ala N*H*	6.24		5.95	7.56
Ala $C^{\alpha}H$	4.60		4.49	4.33
Ala $C^{\beta}H_3$	1.40		1.50	1.42
Lac $C^{\alpha}H$		5.23	5.26	5.07
Lac $C^{\beta}H_3$		1.47	1.50	1.42
N*H*–CH_3		6.16	6.68	
NH–*CH_3*		2.86	2.86	
O–*CH_3*	3.73			

[a] Chemical shifts are referred to tetramethylsilane as an internal standard. Depsipeptide concentrations were all ca. 10 mg/ml.

the NH–Me group bonds to the acetyl carbonyl oxygen to form a seven-membered ring. These two conformations assume characteristic values for the angles ϕ and ψ that describe respectively torsional rotations about the N(O)–C^{α} and C^{α}–C′ skeletal bonds of the central α carbon atom: for C_5 $\phi,\psi \approx -170°,+170°$, and for C_7 $\phi,\psi \approx -80°,80°$.[21]

The conformational energy maps of both acetyl-L-alanine-*N*-methylamide[22] and acetyl-L-alanine methyl ester[4] are similar near the C_5 region. Hence it is not apparent why the C_5 conformation is adopted by the former compound and not by the latter. Reduced restriction on ψ torsional rotation for the methyl ester[4] compared to the methyl amide[22] compound may be partially responsible.

In contrast, comparison of the energy maps of acetyl-L-alanine-*N*-methylamide[22] and acetyl-L-lactic acid *N*-methylamide[4] suggests an explanation for the observed difference in stability of their C_7 conformations ($\phi,\psi \approx -80°,+80°$). The C_7 conformation occurs in a large, broad minimum in the alanine energy map,[22] while it occurs near the side of an energy barrier in the lac map.[4]

Two N–H stretching bands at 3450 and 3370 cm^{-1} were found for dilute carbon tetrachloride and chloroform solutions of acetyl-L-alanyl-L-lactic acid *N*-methylamide. We again assign the high-frequency band to stretching of nonhydrogen bonded N–H groups. The low-frequency band at 3370 cm^{-1} persists upon dilution; it assumes an asymptotic intensity in dilute (5×10^{-5} M) chloroform solution essentially equal to that of the 3450-cm^{-1} band. Thus the low-frequency band is due to N–H groups that are intramolecularly hydrogen bonded to the carbonyl oxygen of neighboring or near-neighboring residues.[17-20] Since the C_5 and C_7 hydrogen bonds are not favored by acetyl-L-alanine methyl ester or by acetyl-L-lactic acid *N*-methylamide, we believe that the intramolecular hydrogen bond of acetyl-L-alanyl-L-lactic acid *N*-methylamide is between the NH of the methyl amide group and the acetyl carbonyl oxygen to form a ten-membered ring, C_{10}. Comparison of the intensities of the free and hydrogen bonded N–H stretching bands suggests that the C_{10} conformation is strongly favored.

Stretching vibrations of the ester and amide carbonyl bonds, ca. 1740 and 1660 cm^{-1}, respectively, are less sensitive to hydrogen bonding than those of the N–H group. All ester carbonyl groups except that of acetyl-L-lactic acid *N*-methylamide exhibit a single, sharp absorption maximum near 1740 cm^{-1}. Its ester carbonyl absorption when dissolved in carbon tetrachloride comprises a maximum at 1760 cm^{-1} and a 1740-cm^{-1} shoulder; the peak and shoulder positions are reversed in chloroform. Since esters strongly favor the trans conformation, the two bands almost certainly do not represent cis and trans isomers. Laato and Isotalo[23] observed double carbonyl stretching bands for many esters with electronegative substituents. They proposed that the double bands result from different rotational isomers; the high-frequency peak is due to conformational isomers in which the electronegative substituent, such as chlorine or oxygen, is close to the carbonyl group. If this explanation is to apply to the current observation, the ester carbonyl oxygen atom of acetyl-L-lactic acid *N*-methylamide must be close to either the oxygen or nitrogen atom of the amide group. The relatively small C′OC bond angle of ca. 113°[4] at the ester oxygen atom increases the possibilities for such interaction.

The amide I bands, primarily C=O stretching vibrations, of the three model depsipeptides are not resolved; a single band for each is observed near 1670 cm^{-1} in chloroform.

Nuclear Magnetic Resonance Spectroscopy. Proton chemical shifts and their assignments for the depsipeptide model compounds and for poly(L-alanyl-L-lactic acid) are listed in Table III. The resonances of acetyl-L-alanine methyl ester and of acetyl-L-lactic acid *N*-methylamide are readily assigned. The methyl and $C^{\alpha}H$ hydrogens of acetyl-L-alanyl-L-lactic acid *N*-methylamide are assigned in analogy to the corresponding protons of acetyl-L-alanine methyl ester and of acetyl-L-lactic acid *N*-methylamide. The methyl protons of its two side chains appear as superimposable doublets at 1.50 ppm; the ala $C^{\alpha}H$ quintet and the lac $C^{\alpha}H$ quartet, at 4.49 and 5.26 ppm, respectively, are only slightly shifted from their positions in acetyl-L-alanine methyl ester or acetyl-L-lactic acid *N*-methylamide.

Broad (~20 Hz at half height), unresolved *NH* peaks of acetyl-L-alanyl-L-lactic acid *N*-methylamide occur at 5.95 and 6.68 ppm; spin decoupling experiments led to assignment of the former to the alanyl *NH* resonance and the latter to the NH–CH_3 resonance. The alanyl *NH* resonance shifts 0.29 ppm upfield from its position in acetyl-L-alanine methyl ester while the *NH*–CH_3 peak exhibits a large downfield shift of 0.52 ppm compared to the *NH* of acetyl-L-lactic acid *N*-methylamide.

A downfield shift of an *NH* resonance is expected upon incorporation in a hydrogen bond. In particular, Kopple et al.[24] noticed that the resonances of NH groups participating in intramolecular C_{10} hydrogen bonds in several proline- and glycine-containing tetrapeptides dissolved in chloroform are downfield from those of the nonbonded amide groups. Thus, the NMR data of Table III support the conclusion derived from infrared spectroscopy that the *NH*–CH_3 amide hydrogen of acetyl-L-alanyl-L-lactic acid *N*-methylamide is intramolecularly hydrogen bonded.

Thus, both ir and NMR spectroscopy indicate that acetyl-L-alanyl-L-lactic acid *N*-methylamide strongly favors a C_{10} intramolecularly hydrogen-bonded conformation in which the *NH* atom of the methylamide group is hydrogen bonded to the carbonyl oxygen atom of the acetyl group. Our previous

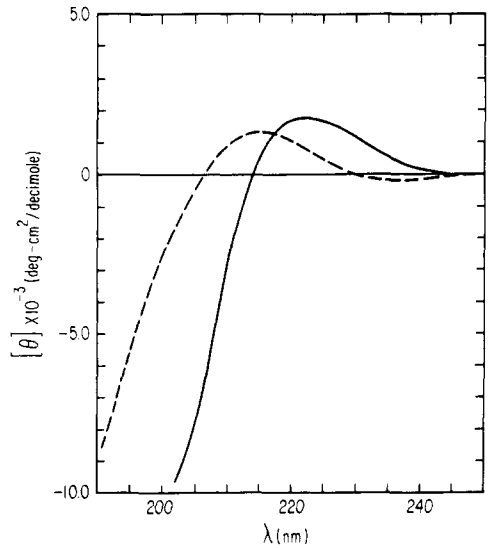

Figure 2. Circular dichroism spectra of poly(L-alanyl-L-lactic acid) expressed as mean molar ellipticity as a function of wavelength in nanometers: (—) recorded in chloroform at 6.9 mg/ml; (- - -) recorded in trifluoroethanol at 4.6 mg/ml.

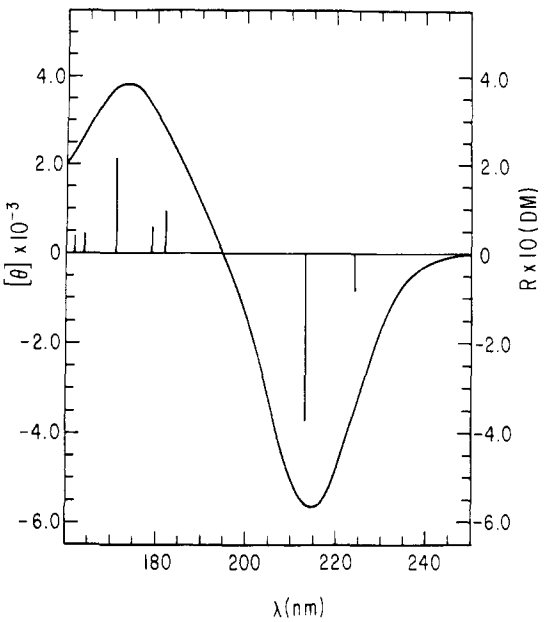

Figure 3. Theoretical circular dichroism calculated for the poly(L-alanyl-L-lactic acid) α helix. Vertical lines are positioned at optically active transitions; their lengths, as measured on the right-hand vertical axis, represent the magnitude of the rotational strength, in Debye magnetons, of the associated transition. The solid line is the composite spectrum comprising contributions from all of the optically active transitions.

energy calculations identified a low-energy C_{10} conformation available to poly(L-alanyl-L-lactic acid).[4] It is the conformation of the polymer repeat units in the R_{10} helix. The calculations also indicate (see Table V and Figure 1 of ref 4) that it is also a low-energy conformation for acetyl-L-alanyl-L-lactic acid *N*-methylamide. The R_{10} conformation is described below in connection with the conformation of poly(L-alanyl-L-lactic acid). Since the experimental results do not distinguish the R_{10} from other possible C_{10} conformations, we rely on our theoretical analysis[4] to assign tentatively the C_{10} conformation observed for acetyl-L-alanyl-L-lactic acid *N*-methylamide in chloroform and carbon tetrachloride solutions as the R_{10} conformation.

Poly(L-alanyl-L-lactic acid). Circular Dichroism Spectroscopy. Circular dichroism spectra of poly(L-alanyl-L-lactic acid) are shown in Figure 2; the solid curve was obtained in chloroform, the dashed curve was obtained in trifluoroethanol (TFE). The polymer exhibits a weak, positive CD band with a maximum near 223 nm when dissolved in chloroform. The ellipticity is negative below 215 nm. The positive band is shifted to lower wavelengths in TFE and an additional, very weak negative band with a minimum near 235 nm appears. The two bands in TFE most probably arise from the amide and ester n–π^* transitions. The CD spectra of Figure 1 do not resemble the spectra observed for polypeptides in either the random coil, α-helical, or β-sheet conformations.[9]

We proceed by comparing the experimental spectra of Figure 2 to theoretical CD spectra calculated for various polydepsipeptide conformations. Our previous theoretical conformational analysis[4] found two, low energy, ordered polydepsipeptide chain conformations. The conformation of lowest energy resembles the right-handed α helix of polypeptides; it is stabilized by hydrogen bonds between amide NH and ester carbonyl O atoms. The second conformation, the R_{10} helix, is stabilized by intramolecular hydrogen bonds between adjacent amide groups. Our previous publication[4] has a more complete definition of these depsipeptide conformations.

Circular dichroism spectra were calculated as described in the preceding section; results for the polydepsipeptide α helix are shown in Figure 3. Rotational strengths, in Debye magnetons, are indicated by vertical lines located at their theoretical transition wavelengths. The solid curve represents the composite CD spectrum.

The amide n–π^* transition is located at 223 nm, and the corresponding ester transition is located at 213 nm (Figure 3). The rotational strengths for both transitions are negative. The enhanced intensity of the ester n–π^* rotational strength (it is approximately four times larger than that for the amide) results primarily from its strong coupling to the energetically similar amide π–π^* transition by interaction between the quadrapolar charge distribution of the former transition with the dipolar charge distribution of the latter. Schellman[7] has recently called attention to the importance of this type of interaction, designated by him as μ–m coupling, in determining the optical activity of peptides. The π–π^* rotational strengths are all positive.

The resultant CD spectrum exhibits a long wavelength minimum at 215 nm, a crossover at 200 nm, and a maximum at 175 nm. It is similar in shape to the CD of polypeptides in the right-handed α-helix conformation.[9] It is clearly very different, however, from the polydepsipeptide CD spectra reported here. Apparently, poly(L-alanyl-L-lactic acid) does not assume the right-handed α-helical conformation to any important extent in chloroform or TFE solutions.

Theoretical optical activity of the R_{10} helix is shown in Figure 4. The amide and ester rotational strengths for the n–π^* transitions are both positive, while all the intense π–π^* rotational strengths are negative. The composite spectrum, drawn with the solid line in Figure 4, has a maximum at 215 nm, a crossover near 200 nm, and a minimum at 186 nm. The dashed spectrum was calculated without including μ–m interactions between the ester n–π^* and amide π–π^* transitions; the importance of such μ–m coupling is thus vividly illustrated by comparison of the dashed and solid spectra of Figure 4. The dotted spectrum was calculated without μ–m coupling between amide groups. It is readily apparent that this μ–m coupling is of negligible importance in determining polydepsipeptide CD in the R_{10} helix. The relative importance of these two μ–m interactions results mostly from: (i) the closer proximity of neighboring amide and ester groups in the chain sequence compared to that for succeeding amide groups; and (ii) the smaller difference between the ester n–π^* and amide

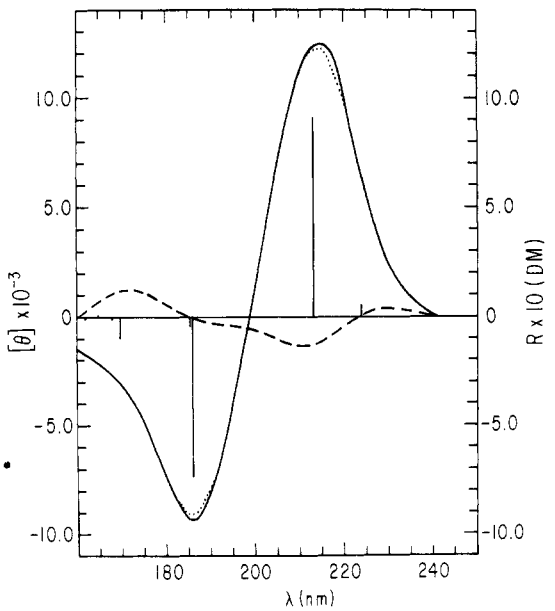

Figure 4. Theoretical circular dichroism calculated for the R_{10} helix of poly(L-alanyl-L-lactic acid). The vertical lines and solid curve have the same meanings as those of Figure 2. The dotted curve was calculated without including μ–m coupling between amide groups and the dashed curve without μ–m coupling between the ester n–π^* and amide π–π^* transitions. See text for description of μ–m coupling.

π–π^* transition energies compared to that for the amide n–π^* and π–π^* transitions.

A comparison of the experimental spectra of Figure 2 and the theoretical spectrum of Figure 4 reveals a rough, qualitative agreement; both exhibit a maximum near 215 nm and negative ellipticity at shorter wavelengths. However, the maximum molar ellipticities of the experimental spectra of Figure 2 are much smaller than those for the calculated spectrum. The experimental spectra may reflect polydepsipeptide conformations in which only a relatively small fraction of the units adopt the R_{10} conformation, with the remaining units assuming disordered conformations.

The CD spectrum calculated for the randomly coiling polydepsipeptide conformation is drawn with the dashed line of Figure 5. The solid line is the spectrum calculated for a polydepsipeptide with 50% of its units in the R_{10} helix and the remainder randomly coiling. The theoretical CD of the partially ordered polydepsipeptide exhibits a weak minimum between 230 and 255 nm similar to that observed for poly(L-alanyl-L-lactic acid) in TFE. The minimum at 235 nm reflects a negative contribution from the amide n–π^* transition. The molar ellipticity of the maximum at 215 nm is considerably reduced by replacement of R_{10} by disordered conformations. It is apparent from examination of the spectra of Figures 2, 4, and 5 that good agreement between the experimental and theoretical CD spectra could be obtained by variation of the random content from the single value of 50% used here. However, we believe that the limited quantitative accuracy of the CD calculations would make the random content obtained by such a procedure unreliable.

Infrared Spectroscopy. The infrared spectrum of poly(L-alanyl-L-lactic acid) in dilute chloroform solution is shown in Figure 1; its infrared absorption maxima in carbon tetrachloride, chloroform, and dioxane solutions are listed in Table II.

Poly(L-alanyl-L-lactic acid) exhibits two N–H stretching bands in carbon tetrachloride:chloroform mixtures and in chloroform solutions; one is at 3370 cm^{-1} and the other at 3280 cm^{-1}. The former band occurs at the same frequency as the hydrogen bonded N–H of acetyl-L-alanyl-L-lactic acid *N*-methylamide and most probably reflects C_{10} hydrogen bonding in the polymer. The following observations aid assignment of the absorption near 3300 cm^{-1}: (i) the spectra reported by Shields et al.[20] of oligopeptides in chloroform solution exhibit a broad absorption at 3300 cm^{-1} attributed to intermolecular hydrogen bonding, and (ii) the N–H groups of polypeptides in the α helix, the ω helix, and the cross-β conformations absorb near 3300 cm^{-1}.[25] Thus we believe that depsipeptide absorption near 3300 cm^{-1} is due to N–H groups participating in intermolecularlike hydrogen bonds either between different molecules or between remote chain segments of the same polymer that are brought into close spatial proximity by the conformation of the intervening chain section. In both cases the absence of conformational restrictions allows ideal hydrogen bonds to form.

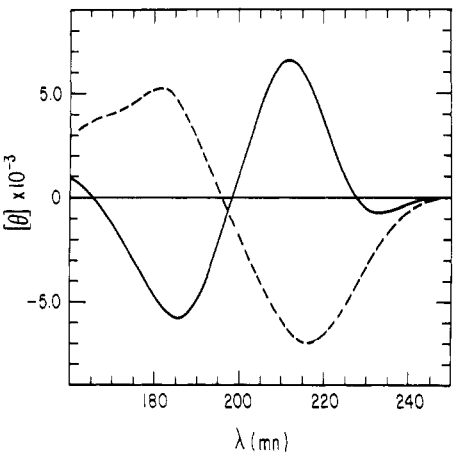

Figure 5. Theoretical circular dichroism spectrum for poly(L-alanyl-L-lactic acid): (—) 50% random coil and R_{10} helix; (- - -) the random coiling conformation.

The relative integrated intensities of the 3370 and 3280 cm^{-1} bands, ~1 to 3, suggest that an enhanced local concentration of amide and ester groups resulting from the conformational characteristics of the disordered polydepsipeptide chain encourages nonspecific hydrogen bonding compared to C_{10} hydrogen bonding in dilute chloroform solution.

Poly(L-alanyl-L-lactic acid) exhibits a single ester carbonyl stretching vibration at 1740 cm^{-1} in all three solvents employed here. The polymer amide I band also occurs as a single peak at 1660 cm^{-1} in carbon tetrachloride:chloroform, at 1662 cm^{-1} in chloroform, and at 1670 cm^{-1} in dioxane. Thus the polymer does not have the split amide I band that is characteristic of polypeptides in the β sheet and cross-β conformations.[25]

Nuclear Magnetic Resonance Spectroscopy. Proton chemical shifts for poly(L-alanyl-L-lactic acid) in chloroform are listed in Table III.

The side chain methyl groups of poly(L-alanyl-L-lactic acid) appear as two overlapping doublets centered near 1.42 ppm; its $C^{\alpha}H$ resonances are similar to those of acetyl-L-alanyl-L-lactic acid *N*-methylamide. A single, unresolved *NH* peak occurs at 7.56 ppm. Observation of a single resonance for the ala $C^{\alpha}H$, for the lac $C^{\alpha}H$ and for the amide NH indicates: (i) the polydepsipeptide sample is free from contamination by low molecular weight oligomers, and (ii) the amide and ester groups adopt exclusively their planar trans conformation. The polymer NH peak is shifted downfield from corresponding resonances of the model compounds. Substituent effects from incorporation of the amide groups in a polymer interior are at least partly responsible for the downfield shift. In addition, nonspecific, intramolecular hydrogen bonding, noted from ir spectroscopy, may also contribute to the shift.

Conformation of Poly(L-alanyl-L-lactic acid). Its cir-

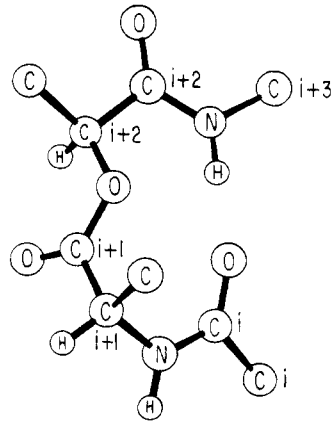

Figure 6. Schematic diagram of the depsipeptide R_{10} conformation.

cular dichroism clearly indicates that the poly(L-alanyl-L-lactic acid) α helix, found by energy calculations to be the conformation of lowest intramolecular energy,[4] is not stable in chloroform solution at room temperature. This contrasts with the very stable α helix formed by poly(L-alanine) in chloroform.[26] Apparently comprehension of depsipeptide thermodynamic stability will require consideration of entropy and solute:solvent interactions in addition to the intramolecular interactions included in our theoretical conformational analysis.

Comparison of the experimental CD spectra of Figure 2 with the theoretical spectra of Figure 4 suggests that poly(L-alanyl-L-lactic acid) is partially ordered in chloroform and trifluoroethanol solutions with approximately half of its residues in the R_{10} helical conformation. A segment of the R_{10} helix is illustrated in Figure 6. The carbonyl oxygen of a hydroxy acid unit i is hydrogen bonded to the N–*H* atom of amino acid unit $i + 3$. The amide groups lie approximately in the plane of Figure 6 while the ester carbonyl oxygen atom is below the plane. Torsional angles are $\phi,\psi = 51°,-94°$ for the amino acid unit and $\phi,\psi = -144°,30°$ for the hydroxy acid unit.

The ir spectra of poly(L-alanyl-L-lactic acid) reveal both specific and nonspecific intramolecular hydrogen bonding in carbon tetrachloride and chloroform solutions. Comparison with the results obtained for the model compounds suggests that the specific hydrogen bonding observed for the polymer forms primarily a C_{10} conformation. Our previous energy calculations and the CD results presented here support assignment of the C_{10} observed by infrared as that of the R_{10} helix.

It is interesting to compare the depsipeptide R_{10} conformation with peptide C_{10} conformations. Two C_{10} conformations, β bend type I and type II, are available to peptide chains.[28] Both conformations have the same pattern of hydrogen bonding as occurs in the R_{10} depsipeptide conformation of Figure 6. The peptide β bends I and II are distinguished by the orientation of the C=O carbonyl bond of residue $i + 1$; this bond is directed below the plane of Figure 6 for type I and above the plane for type II. A hydroxy acid cannot occur at position $i + 2$ in a peptide type II β bend because of the resulting steric repulsion between $C{=}O_{i+1}$ and C_{i+2} caused by the relatively small bond angle at the ester oxygen atom.[4] The steric requirements of a type I bend, on the other hand, do not exclude a hydroxy acid at $i + 2$.

The depsipeptide R_{10} conformation differs from the peptide type I β bend mainly in the orientation of the amide group that links residues i and $i + 1$ (Figure 6). Examination of a molecular model reveals that reduced steric interactions of the C=O of the ith residue with the ester oxygen of the R_{10} conformation compared to those with the NH_{i+2} of the peptide β bend type I allow formation of a more linear hydrogen bond in the former conformation than is possible in the latter. A bend conformation is therefore favored by a hydroxy acid unit in position $i + 2$.

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