the fragment molecules. The complete expression is

$$Q_{rot}(\text{int}) = \frac{Q_{st}(I_x(1),I_y(1),I_z(1))Q_{st}(I_x(2),I_y(2),I_z(2))Q_{rr}(I_{x,y}(\text{av,ext}))}{Q_{st}(I_{x,y,z}(\text{av,ext}))} \quad \text{(A-2)}$$

where Q_{st} and Q_{rr} are symmetric top and rigid rotor parition functions. $I_x(1)$, etc., refer to moments of inertia of the fragments, and $I_{x,y}(\text{av,ext})$ and $I_{x,y,z}(\text{av,ext})$ to the external moments averaged over nine fragment molecule orientations. Although eq A-1 is an approximation, it reduces to the correct expression[52,53] when applied to the case of two symmetric coaxial rotors (such as ethane, assuming free rotation).

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Conformations of Six N-Methylated Diketopiperazines in Solution

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Abstract: The conformations of six N-methylated diketopiperazines in solution are determined by a combination of NMR and CD techniques and compared to the results of X-ray crystallography. Of the six compounds, three apparently have conformations in solution substantially different from their conformations in the crystalline state. Strongly protonating solvents apparently do not change the diketopiperazine ring angle of fold. However, solvent effects may dramatically alter the side-chain rotamer populations of the aromatic substituted diketopiperazines.

Introduction

Diketopiperazines, both in the solid state and in solution, have been traditional models for studies of peptides,[1] polypeptides, and proteins.[2] One diketopiperazine is more than a model: cyclo-L-histidyl-L-prolyl (c-L-His-L-Pro) is a degradation product of thyrotropin hormone releasing hormone (THRH) and is itself biologically active.[3] Others are naturally occurring antibiotics.[4,5]

In a previous paper the X-ray structures of six N-methylated diketopiperazines, cyclobis(L-*N*-methylalanyl) [c-(L-NMeAla)$_2$], cyclo-L-methylalanyl-D-*N*-methylalanyl (c-L-NMeAla-D-NMeAla), cyclobis(L-*N*-methylvalyl) [c-(L-NMeVal)$_2$], cyclo-L-*N*-methylvalyl-D-*N*-methylvalyl (c-L-NMeVal-D-NMeVal), cyclobis(L-*N*-methylphenylalanyl) [c-(L-NMePhe)$_2$], and cyclo-L-*N*-methylphenylalanyl-D-*N*-methylphenylalanyl (c-L-NMePhe-D-NMePhe) plus cyclobis(L-valyl) [c-(L-Val$_2$], were reported.[6] The optically active alkyl-substituted compounds exhibit pseudoaxial side chains and a negative diketopiperazine ring fold, $\beta < 0°$ (Figure 1),[7] while the DL isomers assume chair conformations. The steric interaction between the side chains of the optically active isomers is less than that between the individual side chains and the carbonyl or the *N*-methyl groups on the ring. In c-(L-NMeAla)$_2$ and c-L-NMeVal-L-NMeVal, where the CD spectrum can unambiguously determine the sign of the ring fold angle β, it is of particular interest to test the conformations of the diketopiperazines in solution in comparison to the X-ray results, since in one earlier case,[7] cyclobis(L-alanyl) [c-(L-Ala)$_2$], β for the solid is positive in the crystal, while it is negative in solution. In this paper we report circular dichroism (CD) and nuclear magnetic resonance (NMR) spectra obtained from the N-methylated alkyldiketopiperazines to identify the dominant conformations in solution. From these results, we hope to elucidate the important forces determining these conformations.

It is much more difficult to decipher the optical spectroscopy of aryl-substituted diketopiperazines. The study of aryldiketopiperazines in solution by NMR has demonstrated the tendency for the side chains to "hover" over the diketopiperazine ring,[8,9] even in the face of apparent steric repulsion. In two cases, cyclo-L-phenylalanyl-L-tyrosyl (c-L-Phe-L-Tyr) and cyclobis(L-tyrosyl) [c-(L-Tyr)$_2$], it has been proposed that both aromatic chromophores share the space over a diketopiperazine simultaneously for a major fraction of the time.[10] With the problems of spectroscopic interpretation and side-chain interaction in mind and with the information about steric forces available from the alkyl-substituted compounds, we report an analysis of cyclobis-(L-*N*-methylphenylalanyl) [c-(L-NMePhe)$_2$] by CD and NMR.

Materials And Methods

Materials. The diketopiperazines c-(L-Ala)$_2$, c-L-Ala-D-Ala, c-(L-Val)$_2$, c-L-Val-D-Val, c-(L-Phe)$_2$, and c-L-Phe-D-Phe were prepared ac-

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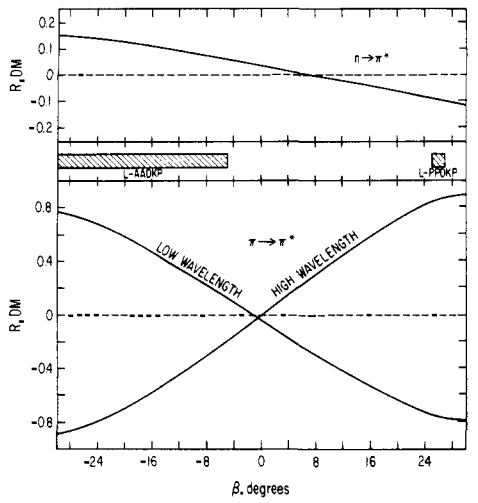

Figure 1. Quantum mechanical calculation of optical activity of alkyl-substituted LL diketopiperazine. This is taken directly from the work of Hooker et al.[7]

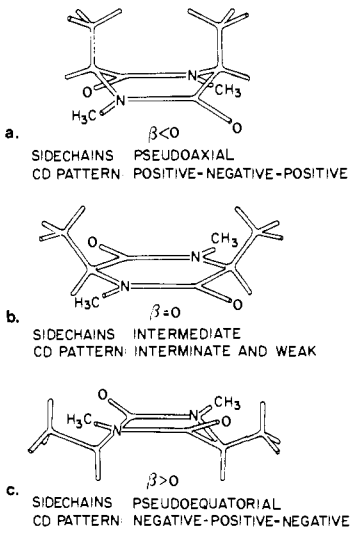

Figure 2. c-(L-NMeAla)$_2$ in three ring conformations: (a) $\beta < 0°$, (b) $\beta = 0°$, and (c) $\beta > 0°$.

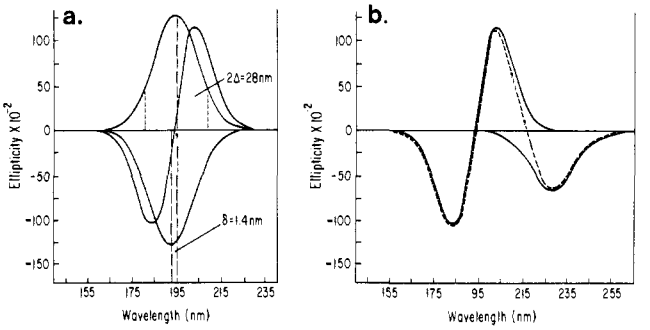

Figure 3. (a) A hypothetical CD π–π* exciton. (b) A hypothetical π–π* exciton combined with an n–π* transition.

cording to the procedure described by Nitecki.[11] N-Methylation of the cyclic dipeptides was carried out by the sodium hydride method.[12]

Cyclobis(L-*N*-methylalanyl) [c-(L-NMeAla)$_2$]. To a solution of cyclobis(L-alanyl) (290 mg, 2 mmol) in dimethylformamide (100 mL) was added methyl iodide (1.00 mL) at 0 °C. This was followed by the addition of sodium hydride (57% oil dispersion) (340 mg, 8 mmol) to the solution at 20 °C. The mixture was refluxed at 40 °C for 20 h, after which it was concentrated under reduced pressure at below room temperature. Water (in 50- and 150-mL portions), followed by chloroform (150 mL), was added to the residue extract of the product. The chloroform layer, dried over magnesium sulfate, yielded crystals on concentration under reduced pressure. The cyrstals were examined by thin-layer chromatography (silic gel F manufactured by Brinkmann). The crystalline product was purified by sublimation (100 °C (4.5 mmHg)) and was recrystallized from hot pentane in 30% yield: mp 124–125 °C; *m/e* 170; R_f value for thin-layer chromatography was 0.15 in chloroform/ethyl acetate (1:5).

Cyclo-L-*N*-methylalanyl-D-*N*-Methylalanyl (c-L-NMeAla-D-NMeAla). The conditions of methylation were the same as described above. The crude product was sublimed at 100 °C (4.5 mHg) to yield the product: mp 108–110 °C; *m/e* 170; R_f = 0.24 in chloroform/ethyl acetate (1:5).

Cyclobis(L-*N*-methylvalyl) [c-(L-NMeVal)$_2$]. Methylation was carried out as noted above. The crude product was sublimed at 85 °C (0.1–0.5 mmHg) to yield the product: mp 106–107 °C; *m/e* 226; R_f = 0.33 in chloroform/ethyl acetate (1:1).

Cyclo-L-*N*-methylvalyl-D-*N*-methylvalyl (c-L-NMeVal-D-NMeVal). The methylation was conducted as outlined above. The crude product was sublimed at 85 °C (0.1–0.5 mmHg) to provide the desired product: mp 143–144 °C; *m/e* 226; R_f = 0.55 in chloroform/ethyl acetate (1:1).

Cyclobis(L-*N*-methylphenylalanyl) [c-(L-NMePhe)$_2$]: In dimethylformamide (200 mL) cyclobis(L-phenylalanyl) (360 mg, 12 mmol) was dissolved at 40 °C. To this solution was added methyl iodide (0.61 mL, 9.9 mmol), followed by sodium hydride (57% oil dispersion) (210 mg, 5 mmol). The solution was heated at 50 °C for 3 h, before being allowed to return to room temperature. After 24 h, the solvent was removed under reduced pressure. To the residue was added benzene (200 mL). The solution was washed with water (100 mL) and then dried over magnesium sulfate. Crystals were formed on removal of the solvent. The pure product was obtained in 45% yield by recrystallization from the mixed solvent ethyl acetate/hexane (1:3): mp 150–151 °C; *m/e* 322; R_f = 0.59 in chloroform/ethyl acetate (1:1).

Cyclo-L-*N*-methylphenylalanyl-D-*N*-methylphenylalanyl (c-L-NMePhe-D-NMePhe): This product was obtained following the same procedure as outlined for cyclobis(*N*-methyl-L-phenylalanine): mp 187–189 °C; *m/e* 322; R_f = 0.35 in chloroform/ethyl acetate (1:1).

Cyclobis(L-prolyl) [c-(L-Pro)$_2$] and Cyclo-L-prolyl-D-prolyl (c-L-Pro-D-Pro) were prepared by the method of Siemion.[13]

Cyclobis(Sarcosyl) [c-(Sar)$_2$] was purchased as commercial sarcosine anhydride. The complete conversion of the NH group to an NCH_3 group was established by thin-layer chromatography, infrared, mass spectroscopy, and nuclear magnetic resonance. Since racemization leads to diastereomer formation, which could be separated by thin-layer chromatography, this technique clearly showed that no racemization occurred during our methylation procedures.

Experimental Methods. Circular dichroism was measured at 23 °C with a Cary 61 spectropolarimeter. Most measurements were carried out in cells of 0.1–0.01-cm optical path length and with the aid of computer averaging of transients.[14] Measurements in trifluoroacetic acid (TFA), however, were accomplished by using a 0.001-cm cell and cell holder especially designed for use with optically dense solvents.[15]

The CD spectra of c-(L-NMePhe)$_2$ are concentration dependent. They were consequently measured at concentrations between 0.001 and 0.015 mg/mL with the aid of the computerized CD. The concentration we used varied with solvent, but it was at most 33% of the concentration at which the spectrum no longer appeared to be concentration dependent. One CD spectrum, c-(L-NMePhe)$_2$ in chloroform/TFA, was measured in a single scan on a Cary 6001 spectropolarimeter belonging to Professor S. Beychok of Columbia University.

The NMR spectra were obtained on a Varian 220 HR generally at 0.3% (w/v) sample concentration. In some special cases, the same concentrations as those for CD spectra were used and Fourier Transform measurements averaged to obtain results.

Conformational Assignments from Spectroscopic Analyses. The ring conformations of mono- and dialkyl-LL-diketopiperazines in solution may be determined by the CD spectra, as long as the peptide bonds remain essentially planar. According to Hooker et al.,[7] each diketopiperazine CD spectrum will have three extremes above approximately 170 mm. The two extremes at shorter wavelengths are due to the π–π* exciton,[16] and the long wavelength extreme is due to the n–π* transition. The signs of these extremes fall in two patterns dictated by the angle of fold, β, of the diketopiperazine ring. The quantum mechanical calculations are

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Table I. Chemical Shifts of Alkyl-Substituted *N*-Methyldiketopiperazines[a]

molecule	solvent	$C_\alpha H$	NCH_3	$C_\beta H$	$C\gamma H$		$J_{\alpha\beta}$
c-(NMe-L-Ala)$_2$	$CDCl_3$	3.91	2.97	1.52			7.2
	TFA	4.40	3.20	1.72			7.2
c-NMe-L-Ala-NME-D-Ala	$CDCl_3$	3.98	2.99	1.57			7.0
	TFA	4.44	3.22	1.75			7.0
c-(NMe-L-Val)$_2$	$CDCl_3$	3.46	3.01	2.04	1.12	1.09	9.0
	TFA	3.94	3.23	2.23	2.23	1.23	
c-NMe-L-Val-NMe-D-Val	$CDCl_3$	3.81	2.92	2.32	1.16	1.23	2.3
	TFA	4.22	3.12	2.53	1.20	1.00	3.5

[a] Chemical shifts relative to tetramethylsilane in ppm.

summarized in Figure 1. A positive–negative–positive pattern of the CD implies that β is negative. It may be seen from Figure 2 that this conformation forces the β-carbons of the side chain into a pseudoaxial (flagpole) position. A negative–positive–negative spectrum corresponds to a positive fold angle β and pseudoequatorial (bowsprit) for the β-carbons of the side chains. This conformational assignment of Hooker et al. is contradictory to that of Blaha,[17] who attempted to correlate the optical rotatory dichroism (ORD) and ultraviolet (UV) spectra in the 200–240-nm region with NMR data.[18]

Interpretation of the spectra depends on understanding the constituent spectral bands. Figure 3a shows the CD exciton as a sum of Gaussian components. In general, exciton extrema do not match the centers of the Gaussian components. In fact, if the exciton splitting, δ, is narrow, the positions of the peaks relative to the crossover depend only on the bandwidth.[19]

Figure 3b shows the addition of an "n–π*" band to the exciton band in such a way that the hypothetical spectrum mimics the general shape of the spectrum of c-(L-Pro)$_2$.[7] It is clear that the position of the n–π* extreme depends not only on the center of the n–π* band but also on the overlap of this band with the red lobe of the π–π* exciton. As can be seen from Figure 3b, overlap causes the n–π* band to be asymmetrical. In aqueous solutions the n–π* band center is between 211 and 214 nm,[20] and there is almost always considerable overlap with the red lobe of the π–π* transition. As the solvents become less polar, the n–π* band center moves to the red,[21] even as far as 240–245 nm. Since the π–π* does not usually shift or broaden drastically as the solvents become less polar, the n–π* band becomes more symmetric. The n–π* band also appears more intense, but diminishing quadrupole–dipole interaction of the n–π* with the π–π* may work against this effect.[22]

Since commercial CD spectrophotometers do not measure to the blue of 185 nm and since it is difficult to obtain spectra below 200 nm in many solvents, interpretation of a spectrum by these rules depends on assigning the spectral extremes correctly to the n–π*, the red lobe of the π–π*, and only rarely the blue lobe of the π–π*.

The position of the n–π* band center has been documented by Nielsen and Schellman[20] for different types of peptides in various solvents. Computer averaging of spectra has also been successful in defining very weak n–π* bands in aqueous solution.[7] These experiments and the relative rigidity of the diketopiperazine ring lend confidence that solvent studies which uncover the n–π* by the red shift in nonpolar solvents do not generally alter the ring conformations.

An alternative tool for determining molecular conformation in solution is NMR spectroscopy. This technique provides insight into side-chain conformation from the $HC_\alpha C_\beta H$ coupling constants[23] (Table I).

The alanine derivatives in this study have β-carbons which presumably rotate freely for any fold of the diketopiperazine ring. However, for the sterically hindered *N*-methylvaline diketopiperazines, it is possible to ascertain a preferred side-chain conformation by applying the Karplus-type relationships,[24] although this procedure is not clearly established for cis peptides.

Nuclear magnetic resonance can also provide information about the ring fold from the chemical shift of the $C_\alpha H$ proton. As the side chains move from axial to equatorial (or pseudoaxial to pseudoequatorial, as in Figure 2a,c), the $C_\alpha H$ proton moves from an eclipsed position relative to the amide carbonyl to a staggered position. According to calculations by Narasimhan and Rogers[25] such a conformational change should result in a deshielding of the proton. These data are useful so long as the conformational change may be separated from other shielding effects, notably solvent shielding.

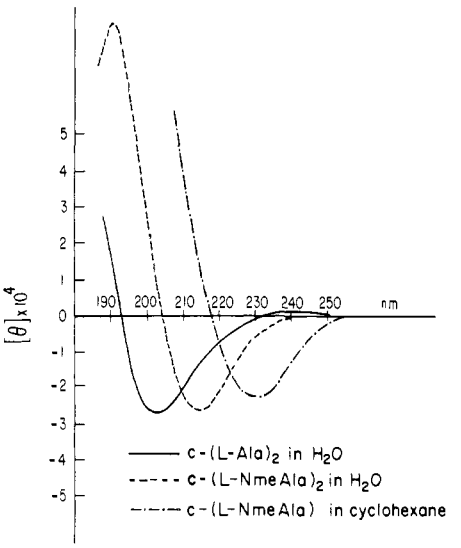

Figure 4. CD spectra of c-L-Ala in H_2O (—), c-(L-NMeAla)$_2$ in H_2O (---), and c-(L-NMeAla)$_2$ in cyclohexane (-·-).

Analysis of aryl-substituted diketopiperazines in solution is not nearly as simple as that of alkyl-substituted diketopiperazines. The CD of the LL isomers contain many more bands, because each aromatic chromophore has four electronic transitions in the far-ultraviolet region, two B transitions, the ^1L_a, and the ^1L_b.[26] Thus, a calculation of the CD spectrum of a diketopiperazine with two aromatic chromophores involves the interaction of twelve electronic transitions. Comparison with experiment is made even more difficult by the large number of side-chain conformations which must be evaluated. With the assumption that each side chain has three preferred rotational conformers, that the side chains are independent, and that the ring may have positive or negative angle of fold β, eighteen molecular conformations must be calculated and weighed correctly to evaluate the overall pattern of the CD spectrum. The problem of interpretation is enormous.

The conformational distribution of the phenylalanyl side chains of the diketopiperazines is deduced from the NMR data by the method of Pachler.[29] The rotational isomerism about the C_α–C_β bond is defined as an equilibrium among three staggered conformers A, B, and C, where the conformers are characterized by the angles of rotation of the side chains $\chi^{2,1} = 60°$, $\chi^{2,1} = 180°$, and $\chi^{2,1} = 300°$, respectively. However, Pachler's method does not uniquely identify conformers B and C. Therefore, while it is possible to derive a population proportion for the conformer with the side chain folded toward the diketopiperazine ring, in the absence of other information it is impossible to assign the other two population fractions to either B or C.

Results

Alanine Derivatives. In Figure 4 the CD spectrum of c-(L-Ala)$_2$ is compared with that of c-(L-NMeAla)$_2$. Although these spectra are superficially similar, repeated time averaging of the CD spectrum of c-(L-NMeAla)$_2$ did not show a positive band even out to 260 nm. Similar experiments easily define a small positive band for c-(L-Ala)$_2$.[7] In cyclohexane the first extreme of the c-(L-NMeAla)$_2$ spectrum (Figure 4) is at 230 nm, a value that is too far to the red to be the long wavelength lobe of the π–π* exciton. The shift of this extreme to 215 nm in water corresponds well with the predicted blue shift of an n–π* transition on going from nonpolar to aqueous solution. The c-(L-NMeAla)$_2$ spectrum therefore demonstrates the beginning of a negative–positive–

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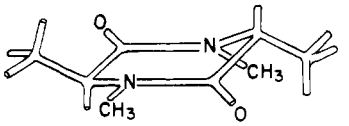
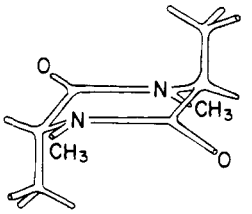


a.



b.

Figure 5. (a) c-L-NMeAla-D-NMeAla in an equatorial side-chain chair. (b) c-L-NMeAla-D-NMeAla in an axial side-chain chair.

Table II. Variations in Chemical Shifts (ppm) upon Changing Solvent from $CDCl_3$ to TFA[a]

molecule	$C_\alpha H$	NCH_3	$C_\beta H$
c-(L-NMeAla)$_2$	0.49	0.23	0.20
c-L-NMeAla-D-NMeAla)	0.46	0.22	0.18
c-(L-NMeVal)$_2$	0.48	0.22	0.19
c-L-NMeVal-D-NMeVal	0.41	0.20	0.21
c-(L-NMePhe)$_2$[b]	0.43	0.26	0.24

[a] A positive change implies TFA deshielding. [b] This TFA spectrum was obtained in $CDCl_3$/TFA (9/1, v/v) instead of pure TFA.

negative pattern which implies a positive angle of fold, β, with the side-chain methyl groups in pseudoequatorial positions (Figure 2c). This ring conformation mimics that of c-(L-Pro)[7] (Figure 2c) and is the opposite of the crystalline structure where β is negative.[6]

The X-ray analysis of c-(L-Ala)$_2$ shows the ring angle of fold, β, to be greater than zero,[29,30,31] a conformation corresponding to Figure 2c. Energy calculations carried out on this molecule indicated that it has a shallow potential well with a minimum at a negative β, a conformation corresponding to Figure 2a. Hooker et al.[7] showed that the CD spectrum is in agreement with the energy calculations. Thus, the ring folds of both c-(L-Ala)$_2$ and c-(L-NMeAla)$_2$ apparently change on going from the solid to aqueous solution.

The NMR resonance positions of the C_α and C_β protons and their coupling constants for c-(L-NMeAla)$_2$ and *c*-L-NMeAla-D-NMeAla are reported in Table I. The coupling constants, approximately 7 Hz, indicate that the side-chain methyls rotate freely and that there is little steric interaction between the side chains and the carbonyl or the *N*-methyls.

The similarity of the C_α proton deshielding in the N-methylated LL- and DL-diketopiperazines indicates that the C_α hydrogens in both cases are positioned similarly with respect to the amide carbonyl.[25] In the DL isomer this cannot be accomplished with a boat conformation but is possible with a chair. As with the optically active isomer where its side chains are pseudoequatorial, this chair possesses equatorial side chains (Figure 5a) as opposed to the axial side chains reported for the crystalline compound (Figure 5b).

The chemical shifts of these compounds in TFA and chloroform (Table I) are virtually identical for both the LL and LD isomers, indicating that the solvent deshielding effects of TFA are nearly identical for the two compounds (Table II). The CD spectrum

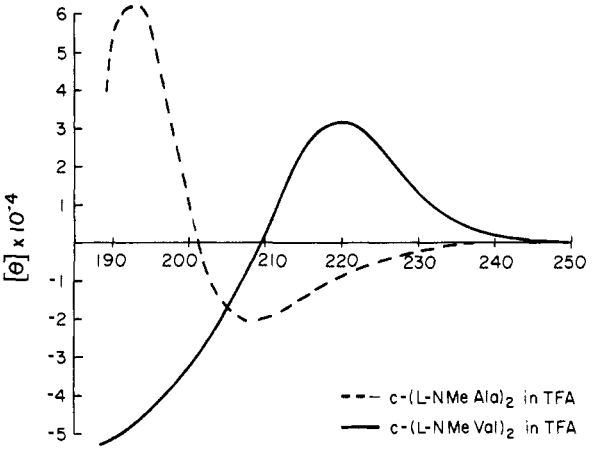


Figure 6. CD spectra of c-(L-NMeAla)$_2$ (---) and c-(L-NMeVal)$_2$ (—) in TFA taken with a 0.001-cm cell.

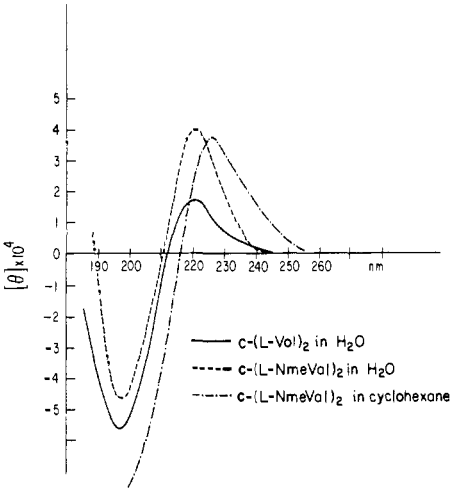


Figure 7. CD spectra of c-(L-Val)$_2$ in H_2O (—), c-(L-NMeVal)$_2$ in H_2O (---), and c-(L-NMeVal)$_2$ in cyclohexane (- - -).

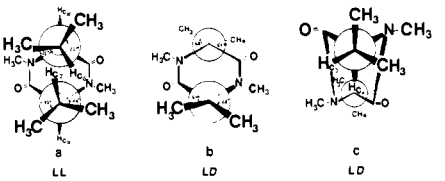


Figure 8. (a) c-(L-NMeVal)$_2$ in crystalline conformation. (b) c-L-NMeVal-D-NMeVal in crystalline conformation. (c) c-L-NMeVal-D-NMeVal in solution.

of c-(L-NMeAla)$_2$ in TFA was obtained by using a very thin cell (Figure 6). The spectrum closely resembles that taken in water, demonstrating that the diketopiperazine ring angle is maintained even in a highly acidic solvent where the ring might be expected to flatten.[9]

In solution, therefore, c-(L-NMeAla)$_2$ is always in the boat conformation with pseudoequatorial side chains and $\beta > 0°$. Since the solvent deshielding effects are so similar for the LL and LD isomers, it is probable that c-L-NMeAla-D-NMeAla in solution is always in a chair form with equatorial side chains. Both these conformations differ from the crystal conformation where the structure in Figure 5b is assumed.

Valine Derivatives. Figure 7 shows the CD spectra of the LL-valine derivatives. Both the nonmethylated and methylated compounds show the beginnings of a positive-negative-positive spectrum (Figure 7). In addition, the long wavelength part of the spectrum of c-(L-NMeVal)$_2$ in cyclohexane shows a red shift appropriate for n–π* transition. The fold angle, β, is therefore negative, and the LL-valine derivatives assume conformation a in

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Figure 2, i.e., the pseudoaxial form.

It appears that c-(L-NMeVal)$_2$ is virtually locked into the pseudoaxial side-chain conformation by the interaction of the *N*-methyls and the carbonyls with the side-chain isopropyl groups. The X-ray data show the largest ring fold of any diketopiperazine so far studied ($\beta = -41°$).[6] Even without *N*-methyl groups, c-(L-Val)$_2$ has pseudoaxial side chains in the solid state, presumably because the steric interaction of the isopropyl groups with the carbonyls is enough to force the side chains to take this position.

The double Newman projection (Figure 8a) shows the side chains of c-(L-NMeVal)$_2$ in the solid state in an exaggerated form. The *N*-methyl groups are slightly eclipsed by one of the isopropyl methyls, so that the C_β hydrogens do not come too close to each other.

In solution the strain imposed by the partially eclipsed bonds is relaxed. According to the NMR results of Mauger et al., [32] the $J_{\alpha\beta}$ coupling constants for c-Sar-L-NMeVal, c-L-NMeAla-L-NMeVal, and c-L-NMeLeu-L-NMeVal range from 4.2 to 6.0 Hz in contrast to 9.0 Hz for c-(L-NMeVal)$_2$, which increases to 9.5 Hz at 0 °C. A Karplus-type analysis (see Methods and Materials) does not provide conclusive information about the preferred rotamers of the first three compounds, indicating that the side chains have a fair amount of freedom. The splitting of 9.0 Hz for c-(L-NMeVal)$_2$ implies that the C_α hydrogen and the C_β hydrogen tend to be either eclipsed or 180° apart. Since the 0° position is obviously a very high-energy position, it seems probable that the isopropyls tend toward the position allowing the hydrogens to be trans, i.e., 180° apart.

The chemical shifts for c-L-NMeVal-D-NMeVal differ considerably from those of c-(L-NMeVal)$_2$ (Table I). Following the argument based on the calculations of Narasimhan and Rogers, the C_α hydrogens are probably in a different spatial relationship with respect to the amide carbonyls which places them at a greater distance so that the amide shielding effects are reduced. This may be accomplished by arranging the side chains in the equatorial position (Figure 5a) so that the C_α—H bond no longer eclipses the C=O. The chemical shift of 3.81 ppm for the C_α—H of c-L-NMeVal-D-NMeVal in deuteriochloroform ($CDCl_3$) is closer to the value of 3.91 ppm observed for c-(L-NMeAla)$_2$ with pseudoequatorial side chains and to 3.98 ppm for c-L-NMeAla-D-NMeAla with probable equatorial side chains than to the value of 3.46 ppm found for c-(L-NMeVal)$_2$ with its pseudoaxial side chains. Thus, if the boat form is excluded and the chair form is assumed, the evidence points to the placement of the side chains in the equatorial positions (Figure 8c). The X-ray analysis shows the side chains of this compound to be axial (Figure 8b).

An alternative explanation may proceed as follows: A symmetrical DL-diketopiperazine in the boat conformation must have severely twisted peptide bonds ($|\omega| \geq 60°$). Since in c-L-NMeVal-D-NMeVal the D and L moieties are indistinguishable, the strain of the twisted bonds must be continually traded back and forth between the D and the L halves of the molecule. The C_αH would thus have an average position intermediate between axial and equatorial, unless there were a high-potential barrier between the boat with the D C_αH equatorial and the boat with the L C_αH equatorial. No evidence for this potential barrier appears in the spectra. The C_αH chemical shift of 3.81 ppm for c-L-NMeVal-D-NMeVal is intermediate between a 3.91 ppm estimate for equatorial C_αH from c-(L-NMeAla)$_2$ and a 3.46 ppm estimate for axial C_αH from c-(L-NMeVal)$_2$, making it possible for the c-L-NMeVal-D-NMeVal to exist in boat form in solution. After consideration of both interpretations, we tentatively assign the chair form to c-L-NMeVal-D-NMeVal. The chemical shift of 3.81 ppm is much closer to 3.91 ppm than to 3.46 and in a boat form it should appear nearly half-way between the two. Interestingly, the chemical shift of 3.98 ppm for c-L-NMeAla-D-NMeAla affirms that it retains the chair form in solution. Perhaps the differences in the C_αH chemcial shifts of these two molecules may be accounted for by moderate ($|\omega| \leq 15°$) peptide bond twisting in c-L-NMeVal-D-NMeVal. Such moderate twisting appears in the X-ray structure.[6]

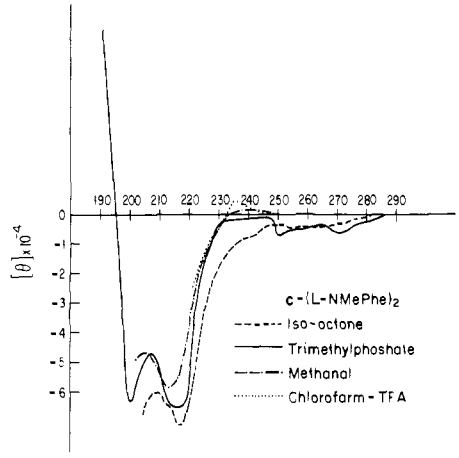

Figure 9. CD spectrum of c-(L-NMePhe) in isooctane (---), trimethylphosphate (—), methanol (-·-), and chloroform/TFA (9/1, v/v) (···).

The $J_{\alpha\beta}$ coupling constant is only 2.5 Hz at 23 °C, and it becomes slightly smaller (2.3 Hz) at −50 °C. According to Pachler,[28] such a coupling constant indicates that the isopropyl methyls are frozen with the C_α and C_β hydrogens about 90° apart and with one methyl pointing toward the ring (Figure 8b). This definite assignment for the side chains argues further against a boat conformation, where one of the side chains would always be allowed rotational freedom not reflected in the low value of $J_{\alpha\beta}$. The stability of such a conformation is supported by the minimum-energy calculation of Ramachandran and Venkatachalam,[33] who found the isopropyl methyls of c-L-Gly-L-Val in the gauche–gauche position relative to the C_α–N bond. It seems that c-L-NMeVal-D-NMeVal can achieve the conformational freedom for its side chains to take on their energetically favored spatial arrangements by assuming the chair with equatorial side chains in solution.

Examination of the change in chemical shift for both the LL and LD *N*-methylvalyl isomers on going from chloroform to TFA (Table II) reveals that the changes in chemical shift are nearly identical with one another and with those for the LL and LD isomers of *N*-methylalanine diketopiperazine (Table II). Further, as with the CD spectrum of c-(L-NMeAla)$_2$, the CD spectrum of c-(L-NMeVal)$_2$ is nearly the same in TFA as it is in water (Figure 6), signifying that the conformation is not easily altered. Apparently none of the four molecules changes conformation with introduction into strongly acidic solvents.

Phenylalanine Derivatives. Figure 9 shows the spectra of c-(L-NMePhe)$_2$ in isooctane, trimethyl phosphate, methanol, and chloroform/TFA (9/1, v/v). In isooctane and trimethylphosphate where hydrogen bonding to solvent cannot occur, the spectra are entirely negative about 200 nm. In methanol and chloroform/TFA, there are positive bands with maxima at 240 and 236 nm, respectively. These unsymmetrical bands probably represent the long wavelength end of a broad positive band centered at shorter wavelengths. A strong negative band appears at 213 nm in methanol, is shifted and slightly broadened from 213 to 217 nm in trimethylphosphate, and exhibits a shoulder at 213 nm with a 217 nm extreme in isooctane. The compound has a completely different spectrum in water (Figure 10), displaying what looks like a positive exciton band having its maximum at 222 nm and minimum at 212 nm.

Although the ^1L_a absorption band is probably mixed with both π–π^* and B transitions,[34] it is often readily identifiable in other aryl peptides as a band in the 213–230-nm region.[27,35–37] In

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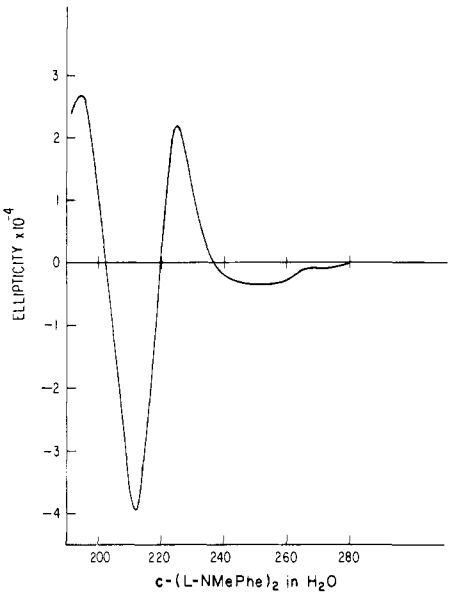

Figure 10. CD spectrum of c-(L-NMePhe)$_2$ in H_2O.

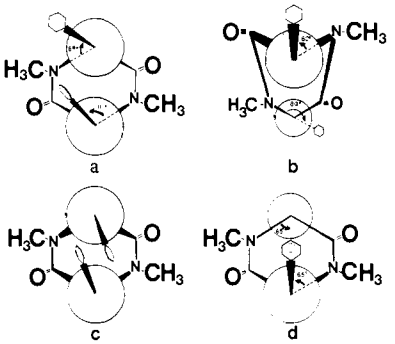

Figure 11. (a) c-(L-NMePhe)$_2$ in Me_2SO. (b) c-(L-NMePhe)$_2$ in the solid state. (c) c-(L-NMePhe)$_2$ in H_2O. (d) c-(L-NMePhe-D-NMePhe) in solid state and in Me_2SO.

tyrosine, ionization red-shifts the band considerably, while solvent changes alter its position only a few nanometers.[38] Therefore it seems that the negative band in the 213–217-nm region is due to the (predominantly) ^1L_a transition. The asymmetric positive bands in methanol and TFA/CCl_3 once again probably represent the long wavelength end of the broad quadrupole-allowed n–π* transition centered in the hydrogen-bonded region of 212–216 nm. Overlap with a narrow strong dipole-allowed ^1L_a transition removed most of this n–π* from view.

The aliphatic diketopiperazine ring does not alter conformation on transfer from hydrogen-bonding solvents to hydrophobic solvents (see above); consequently, such an alteration is not expected in the aromatic case. On transfer of c-(NMePhe)$_2$ from CCl_3 to TFA/CCl_3, the solvent shifts of the α-hydrogen and the β-hydrogen NMR lines are in the range of aliphatic solvent shifts (Table II). Thus, if possible variations in shielding from altered rotamer populations are ignored, NMR supports the contention that c-(NMe-Phe)$_2$ maintains the same diketopiperazine ring conformation in all solvents. However, the n–π* CD band should be even more visible in aprotic than in hydrogen-bonding solvents because of the n–π* red shift. Yet, there is no positive band (Figure 9).

An explanation for the lack of positive n–π* CD or for negative n–π* CD in nonpolar solvents may come from the fact that in the case of the aromatic side chain, the sign of the n–π* CD is not only determined solely by diketopiperazine ring conformation but also determined by the interactions of the n–π* quadrupole

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Table III. Coupling Constants (Hz)–Rotamer Populations[a]

molecule	solvent	$J_{\alpha\beta}$	$J_{\alpha\beta'}$	A	B	C
c-(L-NMePhe)$_2$	$CDCl_3$	6.7	4.1	0.53	0.09	0.38
				0.49	0.14	0.37
	Me_2SO	5.7	5.5	0.48	0.25	0.27
				0.45	0.26	0.28
	TFA/$CDCl_3$ (1/9)	7.6	4.0	0.44	0.08	0.47
				0.42	0.13	0.45
c-L-NMePhe-D-NMePhe	$CDCl_3$	4.3	3.2	0.88	0	0.12
				0.79	0.05	0.15

[a] Coupling constants and rotatamer populations for c-(L-NMePhe)$_2$, c-L-NMePhe-DNMePhe, and c-(L-Phe)$_2$. The top rotamer populations are calculated by using J_g and J_t values from Kopple, Wiley, and Tauke[23] and the bottom are calculated by using J_g and J_t from Pachler.[28] Conformations B and C are not distinguishable (Kopple and Ohnishi[9] call them U_I and U_{II} for "unfolded I" and "unfolded II").

Table IV. Chemical Shifts (ppm) of NMR Bands for NMePhe Model Compounds

molecule	solvent	$C_\alpha H$	NCH_3	$C_\beta H_A$	$C_\beta H_B$
LL	$CDCl_3$	4.06	2.75	2.20	2.84
	CD_3OD	4.16	2.77	2.17	2.76
	CD_3CN	4.02	2.74	2.34	2.56
	TFA/$CDCl_3$ (1/9)	4.49	3.01	2.44	2.86
LD	$CDCl_3$	3.46	2.84	2.96	3.22

with the ^1L_a dipole. Whether or not the side chains are pseudoaxial or pseudoequatorial ($\beta < 0°$ or $\beta > 0°$, Figure 11a,b), the B rotamer brings the ^1L_a dipole very close to the n–π* quadrupole, the A rotamer is somewhat more distant, and the C rotamer may be considered to interact very little. If the ^1L_a contribution to the n–π* arising from the B rotamer is strong and negative, if the contribution from the C rotamer is moderate or weak and positive, and if the populations of B and C rotamers are assigned correctly,[9] the changes in populations on going from MeSO to TFA/CCl_3 (Table III) indicate that the n–π* CD could be negative in nonpolar solvent and positive in polar solvent.

A different side-chain rotamer distribution appears in water; no other hypothesis accounts for the drastic alteration of the CD spectrum (Figure 10). The 222-nm maximum and 213-nm minimum appear to be an exciton band. In this wavelength region an exciton could only be due to coupling of the ^1L_a transitions of the two side chains, because the n–π* transitions cannot couple in an exciton manner and because the π–π* and B transitions are too far to the blue to appear in this region of the spectrum. For such strong coupling to be achieved, the rings would have to face each other at short distance, a restriction which demands the axial conformation (Figure 11c). Unfortunately, it has been difficult to obtain an NMR spectrum of the compound in D_2O, because the concentration must be very low to avoid aggregation. Given the earlier arguments that the diketopiperazine ring retains its conformation in all solvents, protic or aprotic, it seems likely that c-(NMePhe)$_2$ always has pseudoaxial side chains. This conformation agrees with the X-ray results (Figure 11a).

From the upfield position of the $C_\alpha H$ proton in the $CDCl_3$ spectrum of the LD isomer (Tables III and IV) it seems that the axial side-chain chair form shown in the crystal is also present in solution (Figure 11d). The $HC_\alpha C_\beta H$ splitting studies support this conclusion (Table III), demonstrating overwhelming populations of the folded position. There is apparently no advantage in the conformational degrees of freedom available in solution.

In solutions containing c-(L-NMePhe)$_2$ or its LD isomer the NMR spectra show that there is a well-defined tendency for the aryl side chains to fold toward the diketopiperazine ring (Table III). The axial chair form of the DL analogue (Figure 8d) is a conformation where no extraneous side chain–side chain interactions would reduce the frequency of the folded rotamer, and this rotamer is the almost exclusively populated one. If this tendency toward the folded rotamer is viewed as a steric phe-

nomenon, the reduction of the folded rotamer population in the LL pseudoequatorial isomer could be a measure of how much the side chains actually interfere with one another, even in this $\beta > 0°$ situation.

On the other hand the LL pseudoequatorial case (Figure 8b) might be thought to have infrequent side chain–side chain contact, and thus be considered the "normal" sterically controlled isomer by virtue of the fact that the phenyl group is not able to come as close to the diketopiperazine ring as it can when the side chains are axially disposed. The high population of fold rotamer in the LD axial isomer would then be due to a special ring–ring interaction.

Given the NMR-determined rotamer populations, it seems that the former explanation is more plausible, because the LL pseudoequatorial isomer of c-(L-NMePhe)$_2$ probably has significant side chain–side chain contact. If the NMR rotamer populations of both side chains are considered to be uncorrelated in $CDCl_3$ and Me_2SO, the two side chains would be nearly in contact approximately 25% of the time. Thus it is likely that the interaction between side chains forces the diketopiperazine ring into $\beta > 0°$ conformation in the first place. This view of the folded rotamer effect is also supported by calculations which arrive at an energy minimum for $\chi = 60°$ without the introduction of special interring forces,[38,39] although a simple van der Waals exclusion model could not predict the side-chain energy minimum.[40]

Conclusions

According to our interpretation of the CD and NMR data of the six N-methylated diketopiperazines studied, three have ring conformations in solution that differ significantly from the ring conformations found in their crystalline forms. The aliphatic compound c-(L-NMeVal)$_2$ and the aromatic derivatives c-(L-NMePhe)$_2$ and c-L-NMePhe-D-NMePhe maintain ring shapes close to those determined by X-ray analysis. The first is extremely sterically hindered; the second represents a case where the aromatic groups can interact with each other, with the diketopiperazine ring or with the solvent, depending on the nature of the rotamer; the third is an example of the tendency of aryl substituents to fold toward the diketopiperazine ring. The fact that some of these compounds exist in different states with opposing angles of ring fold implies that the N-methylated diketopiperazine ring may be considered somewhat flexible. This flexibility does not make the ring prone to protonation-induced shape changes. Strong proton-donating solvents TFA, H_2O, and chloroform/TFA have no apparent effect on the diketopiperazine ring structure.

Water does have a substantial effect on the distribution of aromatic side chain rotamers. Apparently, by folding both phenyl rings together over the diketopiperazine ring, c-(L-NMePhe)$_2$ achieves the minimum nonpolar surface exposure and therefore minimum solvent disruption. In less polar media, where the phenyl rings may solvate easily, there is a negligible problem with solvent disruption.

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Infrared Matrix-Isolation Studies of the Interactions and Reactions of Group 3A Metal Atoms with Water

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Abstract: It has been shown that aluminum atoms react spontaneously with H_2O at 15 K while the heavier group 3A metals form M···OH_2 and M_2···OH_2 adducts. Adduct formation causes the ν_2-bending mode of H_2O to decrease by 21.4, 16.5, and 9.6 cm^{-1} for Ga, In, and Tl and 14.4 and 10.6 cm^{-1} for Ga_2 and In_2, respectively. The divalent HAlOH molecular species is formed from reaction of the aluminum atom with water. The HGaOH and HInOH molecular species are formed by photolysis of the respective adducts while the Tl···OH_2 adduct does not react on photolysis. The monovalent molecular species MOH is readily formed by further photolysis of the divalent HMOH species. Molecular vibrational frequencies and mode assignments are given in accompanying tables. Data suggest that the diatomic Ga_2, In_2 and Tl_2 adducts with water readily rearrange when photolyzed to a hydrogen-bridged dimetal species, which in turn is converted to the M_2O molecular species by further photolysis.

I. Introduction

Our understanding of molecular beam–water reactions,[1] water-induced thin film impurities, and surface–water reactions of the group 3A metals depends in part on our knowledge of the reactivity and reaction paths of atomic and small metal clusters of the group 3A metals with water. The matrix-isolation technique along with in situ photolysis affords the opportunity of following the reaction of an atom, diatom, etc., with water from initial interaction, through intermediate products to the final products.

Previous experimental[2,3] and theoretical[4] studies have shown that matrix-isolated alkali metals will form metal–water adducts.

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