of 4 to 6, depending on $\Delta\phi$. The s^2 for these same conformations differ to a considerably lesser extent. Consequently the value of $g_{\gamma 2}$, but not g,[10] for 3-ethylpentane is sensitive to the distinction between σ and τ.

Comparison with Previous Work. Previous investigations of isotactic vinyl polymers have shown that the computed values for the characteristic ratio,[14,15] strain birefringence coefficient Γ_2,[16] and optical anisotropy[17] decrease as $\Delta\phi$ increases. The present work shows that an increase in $\Delta\phi$ at the trifunctional branch point in an alkane containing three articulated branches also brings about a decrease in $\langle\gamma^2\rangle$. Good agreement between experimental[18] and calculated[17] optical anisotropies for 2,4-dimethylpentane has been obtained when $\Delta\phi = 10°$. The characteristic ratio of isotactic vinyl polymers[14] and the optical rotation for poly[(*S*)-methylhept-1-ene][19,20] become larger as the value assigned to τ decreases. The present work shows that $\langle\gamma^2\rangle$ for an alkane consisting of three articulated branches emanating from a common atom also increases as the value assigned to τ decreases.

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Vacuum Ultraviolet Circular Dichroism of β-Forming Alkyl Oligopeptides

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Abstract: The vacuum ultraviolet circular dichroism, to 140 nm, is reported for films of oligopeptides having the general formula BOC(L-X)$_n$OMe, where X = Ala, Val, Nva and n = 2-7. Substantial amounts of β conformation are indicated at the trimer in the alanine series, the hexamer in the norvaline series, and the heptamer in the valine series. The alanine and valine heptamer conformations are assigned to the antiparallel and parallel sheets, respectively. The norvaline heptamer conformation is assigned to a mixture of parallel and antiparallel sheets, in approximately equal amounts. The assignments are in agreement with published infrared data for these peptides.

The formation of secondary structure in peptides is directed by chemical structure, i.e., the nature of the side chains and the chain length, and by a large number of environmental factors. In recent years work has been carried out in our laboratories on the synthesis and conformational analysis of monodisperse linear homo-oligopeptides,[1b,2] and on the extension of circular dichroism measurements on polypeptides into the vacuum ultraviolet spectral region.[1a,3] In this work we report the vacuum ultraviolet circular dichroism (VUCD)[4] to 140 nm of films of oligopeptides having the general formula BOC(L-X)$_n$OMe, where X = Ala, Val, Nva and n = 2-7.

Palumbo et al.[2] have reported solid state infrared absorption data for the same oligopeptide series. Shifts in the positions of the amide I and V bands indicate that the higher members of all three series are in the β conformation in the solid state. Absorption was found in the deblocked heptamers, HCl·H(L-X)$_7$OMe, of alanine and norvaline at 1694 and 1692 cm^{-1}, respectively, but none was found in the deblocked valine heptamer. It was concluded that the β conformation which develops in the alanine and norvaline heptamers definitely contains antiparallel chains whereas parallel chains are predominant in the case of valine.

Theoret et al.[5] measured the solid state infrared absorption of the zwitterionic alanine dimer through hexamer. Absorption was found in the tetramer through hexamer at 1697 cm^{-1} indicating the presence of the antiparallel β conformation in the solid state of those zwitterionic forms; bands were found in the dimer and trimer at 1685 and 1692 cm^{-1}, respectively. The zwitterionic alanine dimer through hexamer as also studied in the solid state by Sutton and Koenig[6] with Raman spectroscopy. The antiparallel β conformation was indicated in the tetramer through hexamer by the appearance of a band at 1663 cm^{-1}; in the dimer and trimer the band was found at 1680 and 1659 cm^{-1}, respectively. Fujie et al.[7] reported infrared data and x-ray diffraction patterns for the alanine dimer, trimer, tetramer, and nonamer. The tetramer x-ray diffraction pattern is similar to that of the nonamer, but the dimer and trimer patterns are qualitatively different. Komoto et al.[8] observed development of the β conformation in the early stages of solid state polymerization of L-valine *N*-carboxyanhydride.

Of particular importance in the present work is the question of the consistency of infrared absorption and circular dichroism as analytical tools for the detection of the β conformation in solid state oligopeptides and, especially, in their ability to distinguish between the parallel and antiparallel sheets.

Experimental Section

Synthesis. The details of the synthesis and the chemical and optical characterizations of BOC(L-X)$_n$OMe, where X = Val, Nva, Ala and n = 2-7, are reported in ref 9-11.

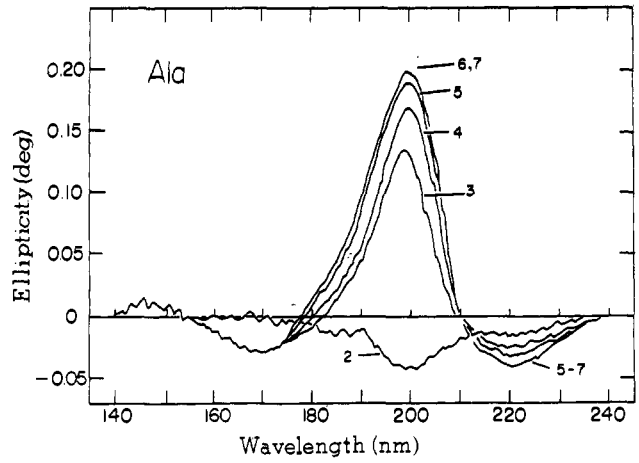

Figure 1. Circular dichroism of BOC(L-Ala)$_n$OMe, n = 2-7.

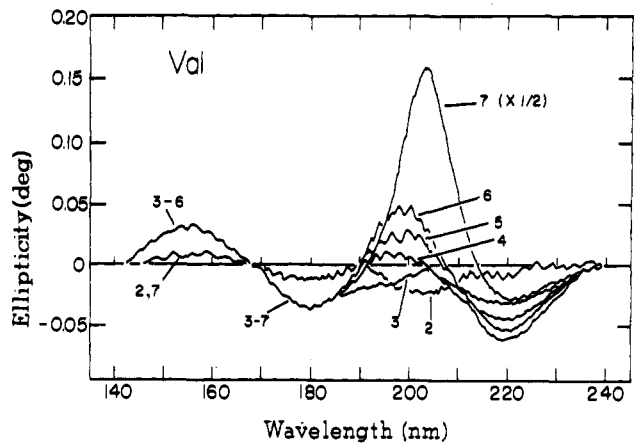

Figure 2. Circular dichroism of BOC(L-Val)$_n$OMe, n = 2-7.

Circular Dichroism. Trifluoroethanol solutions (2 mg/ml) were allowed to stand for 48 h. Films were cast on 1 mm thick CaF_2 disks by evaporation of the solution to dryness in a nitrogen-filled glove bag at 23 °C. The spectrometer[3] was operated with a spectral width of 1.6 nm, a time constant of 10 s, and a scan rate of 2 nm/min. Absorption spectra obtained on a Cary 14 spectrometer showed no evidence of scattering in the nonabsorbing spectral region. Rotation of the films about the optical axis had no effect on the signals reported here, and there was no indication of flattening of the CD bands. Films which did show a dependence of the signal on orientation were recast until they showed no such dependence. It was particularly difficult to obtain unoriented norvaline hexamers.

Except for the dimers, there was no change in the spectra after the films had been in the vacuum chamber for several hours, and there was furthermore no difference in the spectra after storage in a desiccator for several weeks. We found that the dimers were slowly pumped off the CaF_2 disks with a half-life of about 5 h; however, the first two scans, which were taken within 30 min of each other, were identical. In the case of the alanine dimer, the circular dichroism changed after storage in a desiccator overnight, or in the vacuum chamber for 1 h. The general appearance of the film also changed, from a clear film resembling all the other films we studied, to a partially cloudy and striated film. The CD which we measured with newly prepared films is the one we report here, since (see below) it corresponds to the amorphous material. The CD which develops at low humidity (not shown) contains a single broad negative band near 220 nm, which we take to be a scattering artifact and/or the result of a microcrystalline structure taken up by the alanine dimer, but not by the other dimers.

The instrument was not operating near the limits of its sensitivity; spectra were obtained at gains corresponding to a full scale (10 in.) pen deflection of 1.0 or 0.5° ellipticity.

We cannot at the present time measure absorption and circular dichroism simultaneously, so that absorption measurements could not be made without moving the films. The possibility of film thickness inhomogeneities under these conditions makes it impossible to determine precisely how much material was in the light beam during the CD measurements. We did however scale our data to an approximately uniform absorbance of unity by calibrating the photomultiplier gain at the wavelength of maximum absorption. Such a procedure corresponds to calibrating the dynode voltage on commercial instruments. This estimate agreed with the absorption measured on a Cary 14 absorption spectrometer assuming a uniform film thickness. Our results are therefore reported in Figures 1-4 in terms of total ellipticity, θ, in units of degree, scaled to (approximately) uniform maximum absorbance.

Results and Discussion

Alanine. Figure 1 shows the film CD for the series BOC(L-Ala)$_n$OMe, n = 2-7 from 140-240 nm. The bands at 200 and 220 nm can be taken as indicating the presence of the β conformation.[12,13] Figure 1 shows that the β conformation predominates in the solid state for all alanine oligomers except the dimer. The dimer spectrum above 190 nm resembles the contribution to CD from an internal Ala-Ala peptide chromophore within a randomly coiled chain.[14] Palumbo et al.[2] found infrared absorption in KBr pellets of BOC(L-Ala)$_2$OMe at 1655 (amide I) and 626 cm^{-1} (amide V) which is characteristic of disordered chains. On the other hand ordered structures were found in x-ray studies of L-alanyl-L-alanyl hydrochloride[15] and the zwitterionic dimer.[16] As mentioned above, the CD of our dimer changes in a low humidity which may indicate the development of one of the ordered structures found in the x-ray studies.

At 200 nm the CD of the trimer and tetramer indicate large but not complete conversion to the β conformation. The CD of the pentamer through heptamer are virtually identical near 200 nm, as are the positions of the amide I (1634 cm^{-1}) and amide V (703 cm^{-1}) bands.[2] Both techniques lead to the conclusion that development of the β conformation in the solid state is essentially complete with the pentamer. The CD positive maximum is at 199 nm.

At 185 nm the dimer shows a negative band, but all higher members of the series show positive dichroism in the form of shoulders in the large 199-nm band. The crossover which appears at 182 nm in the trimer shifts to 178 nm in the fully developed sheet.

Valine. Figure 2 shows the film CD for the series BOC(L-Val)$_n$OMe, n = 2-7 from 140 to 240 nm. The dimer spectrum above 190 nm resembles the contribution to CD of an internal Val-Val peptide chromophore within a randomly coiled chain.[14] The infrared bands seen in the dimer[2] at 1651 (amide I) and 630 cm^{-1} (amide V) are also characteristic of disordered chains. Near 200 nm the CD becomes progressively more positive within the series, indicating the development of the β conformation. The largest change in CD occurs between the hexamer and heptamer, with a very large increase in the intensity of the positive band and a shift in position to 203 nm. The development of the β conformation within this series is also reflected in the position of the first CD crossover, which shifts from 203 nm in the tetramer to 214 nm in the heptamer. This shift is probably brought about by the shift in position of the large positive band.

In the valine oligomers there is no shoulder on the high energy side of the positive band. There is, however, a negative band at 180 nm in the dimer, which becomes somewhat more intense in the higher members of the series. The crossover in the heptamer is at 191.5 nm.

Because of the uncertainty (see above) in scaling the measured CD to a uniform absorbance, it is worth noting that the ratio of the intensity of the 200-nm band in the valine heptamer to that in the alanine heptamer is 1.6 in this work, which is very close to the value of that same ratio measured in trifluoroethanol solutions.[9,17]

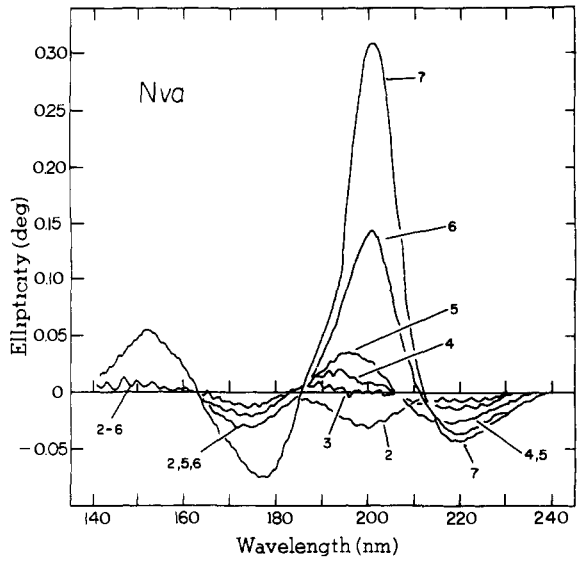

Figure 3. Circular dichroism of BOC(L-Nva)$_n$OMe, n = 2-7.

Norvaline. Figure 3 shows the film CD for the series BOC(L-Nva)$_n$OMe, n = 2-7, from 140 to 240 nm. Increasing positive dichroism near 200 nm within the series indicates the development of the β conformation. The dimer spectrum above 190 nm resembles the contribution to CD of an internal Nva-Nva peptide chromophore within a randomly coiled chain,[14] and infrared bands characteristic of disordered chains are found with KBr pellets of BOC(L-Nva)$_2$OMe at 1659 (amide I) and 618 cm^{-1} (amide V).[2] The CD of the trimer has a maximum near 189 nm; the tetramer has a maximum near 192 nm and a shoulder near 200 nm; the pentamer shows a single broad band near 195 nm; and the hexamer and heptamer show a shoulder near 190 nm and a positive band near 200 nm. These results can only indicate that there are two bands in this region, one near 190 nm and another near 200 nm.

Growth of the positive band near 200 nm in the trimer, tetramer, and pentamer indicates the onset of development of β structure. A large CD change occurs at the hexamer, with the positive band approximately quadrupling in intensity. Another large change occurs between the hexamer and heptamer; the positive band is located at 201 nm in the heptamer.

At 190 nm the dimer shows a weak shoulder of negative dichroism; the trimer shows a very weak positive band. The positive dichroism at 190 nm becomes progressively larger in the tetramer and pentamer, and is clearly apparent as a shoulder in the positive band of the hexamer and heptamer. There is a crossover in the CD of all members of the series above the dimer, located at 186 nm.

Chain Length Dependence of β Formation. Figure 1 shows that the β conformation is well developed in the alanine trimer, and its development in the pentamer is essentially complete. The change in CD at 200 nm as a function of chain length behaves very much like the change in the position of the amide I band;[2] graphical representations of those two parameters are virtually coincident. Figure 2 shows that the β conformation is completely developed in the valine series only with the heptamer. According to Figure 3 the norvaline hexamer forms a well-developed sheet.

The requirement for β formation of longer valine chains, compared with alanine or norvaline, shows up in the infrared data as a lack of convergence of the position of the amide V band even at the heptamer of valine, whereas convergence is reached at the hexamer of norvaline and the pentamer of alanine.[2] With respect to the convergence of the amide V band position, therefore, the infrared data reflect the same conclusion as obtained from the 200-nm CD band intensity; β formation sets in at shorter chain lengths of alanine, compared with valine, and norvaline is intermediate in this respect. Bulkiness of the side chain and β branching apparently both determine these differences, since the behavior of norvaline is not identical with that of valine oligomers. Postulating that bulkiness and β branching both contribute to the inhibition of β formation, relative to alanine oligomers, would rationalize the apparent difference between the norvaline and valine series.

Conformational Assignments. Figures 1-3 show that the β conformation develops in all three oligopeptide series in films cast from trifluoroethanol. It is of interest to determine whether the differences in the CD of the three heptamers can be understood in terms of differences in interstrand orientation, as has been done using infrared absorption data.[2]

The appearance of an infrared band near 1690 cm^{-1} in peptide β sheets has come to be taken as diagnostic of the antiparallel interstrand orientation.[18,19] This band represents the component of the amide I band polarized parallel to the chain direction (y). In the parallel pleated sheet that component is too near the x-polarized band to be easily resolved from it. Palumbo et al.[2] found an infrared band near 1690 cm^{-1} in KBr pellets of the deblocked alanine and norvaline heptamers and concluded that the antiparallel conformation is present, at least in part, in those structures. The absence of such a band in the deblocked valine heptamers led them to suggest that the parallel sheet characterizes that structure.

The amide π-π^* band also splits into components in the β conformation, since the same group theoretical principles apply equally well to electronic and vibrational excitations. Pysh[20] and others[21-23] have calculated the splitting patterns expected for parallel and antiparallel sheets of various sizes. In both sheets the low-energy component is predicted to show strong positive CD (which is confirmed by experimental data), but that component should appear at wavelengths 3-5 nm higher in a parallel sheet than in an antiparallel sheet, all other factors being equal. In addition, the positive CD band (y component) should be closer to the absorption maximum (x component) in the antiparallel sheet than in the parallel sheet. These predicted differences in the two types of sheet are observed experimentally in the alanine and valine CD spectra (Figures 1 and 2), suggesting that they take up the antiparallel and parallel sheets, respectively. The differences are small, however, and probably cannot be used with great reliability.

Larger differences are expected in the vacuum ultraviolet region of the spectrum, according to the calculations.[20-23] The perpendicular (x) component of the amide band should show weak positive CD in the antiparallel sheet and negative CD in the parallel sheet; i.e., the distinction involves a change in the sign of CD. The experimental CD of the alanine heptamer shows a pronounced shoulder on the high-energy side of the strong positive band. The shoulder appears to be located near 190 nm but the positive (x) component giving rise to it could well be located near 192-193 nm which is the position of the absorption maximum. The CD in the valine heptamer is negative at 190 nm, the crossover being at 192 nm. These features are compatible with the negative (x) component being located near 194-195 nm, since the strongly positive nonconservative nature of the CD in this region indicates that polarizability contributions to CD are very large and seriously modify the exciton-only spectrum.

This difference in the sign of the CD near 190 nm, which we attribute here to the perpendicular x component, results in a very large difference between the spectra in the positions of the crossover in this region, that crossover being at 178 nm in alanine and 192 nm in valine. If the assignments of the alanine and valine heptamers to the antiparallel and parallel sheets, respectively, is correct, the location of this crossover may provide the most sensitive CD criterion for distinguishing between the two sheets.

The negative z component CD band predicted[20-23] to arise from exciton interactions in the antiparallel sheet is either canceled by polarizability contributions to that component's CD, or makes up part of the negative CD band actually observed near 175 nm.

According to all CD criteria, the observed CD of the norvaline heptamer is intermediate between those of the alanine and valine heptamers. We find its CD positive band at 201 nm (compare 199 nm for alanine and 203 nm for valine). That band is 7 nm from its absorption maximum (compare 6 nm for alanine and 8.5 nm for valine). Most significantly, there is a CD crossover at 186 nm (compare 178 nm for alanine and 192 nm for valine). On the basis of this consistent behavior, and in spite of the small differences involved in the first two criteria, we believe it very likely that the norvaline heptamer conformation contains both parallel and antiparallel chains. That is, with respect to any given norvaline chain, the probabilities that a neighboring chain is oriented parallel, or antiparallel, to it are both large, and in this case approximately equal.

We can use the total CD spectrum from 140 to 240 nm to show further evidence that this is so. Figure 4a shows the spectrum of the alanine heptamer; Figure 4b shows the spectrum of the valine heptamer; and Figure 4c shows a composite spectrum made up of a simple linear combination, in equal weights, of the alanine and valine spectra. The composite spectrum is clearly very similar to the CD of the norvaline heptamer (Figure 3). In the composite spectrum, the first crossover is at 212 nm, the positive CD band is at 202 nm, there is a slight shoulder near 190 nm, the second crossover is at 185 nm, the second negative band is near 176 nm, the third crossover is at 162 nm, and there is weak dichroism near 150 nm. The intensities of some of the features of the calculated composite spectrum differ from the intensities in the observed norvaline spectrum, but the intensities are largely determined by the polarizabilities of the side chains, which differ in the three heptamers. In terms of the positions of the extrema and crossovers, which are most reflective of the conformation, the composite spectrum is virtually identical with the CD of the norvaline heptamer.

A β conformation in which about 50% of the strands have antiparallel neighbors might well be expected to show infrared absorption at 1692 cm^{-1}, as Palumbo et al. have observed.[2] The deblocked leucine heptamer shows a weak infrared shoulder near 1690 cm^{-1} rather than a well-resolved band, which may indicate that there is a mixture of the two sheets in that case as well. The deblocked isoleucine heptamer shows neither a band nor shoulder near 1690 cm^{-1}. What the minimum amount of antiparallel sheet is that can be detected by infrared absorption is not known. It may turn out that by the CD criteria the leucine heptamer will be shown to have a small portion of antiparallel strands. The suggestion made here that mixtures of parallel and antiparallel sheets have characteristic CD can, therefore, be further tested especially by studying the leucine and isoleucine series, work which is now underway.

It is clear that the differences we find here with alkyl oligopeptides may not be identical with variations found with chromophoric side chains. CD measurements on the methionine, phenylalanine, and S-substituted cysteine series, for example, would be needed for such an extension.

Within the alkyl oligopeptides, it is interesting to consider what factors determine the type of β sheet which is favored. The explanation might lie in two factors: the bulkiness of the side chain, and the presence of β branching. If it is supposed that both factors contribute toward favoring the parallel sheet in approximately additive fashion, then in valine the parallel sheet would be favored on both grounds, but in norvaline only on one. Extension of that notion to the case of leucine would lead to the expectation that the parallel sheet would be favored more than in the case of norvaline, which is, perhaps surprisingly, consistent with the appearance in the infrared spectrum of the deblocked leucine heptamer of a shoulder at 1690 cm^{-1} rather than a resolved band. In isoleucine the parallel sheet would be especially favored, also as indicated by the infrared data.

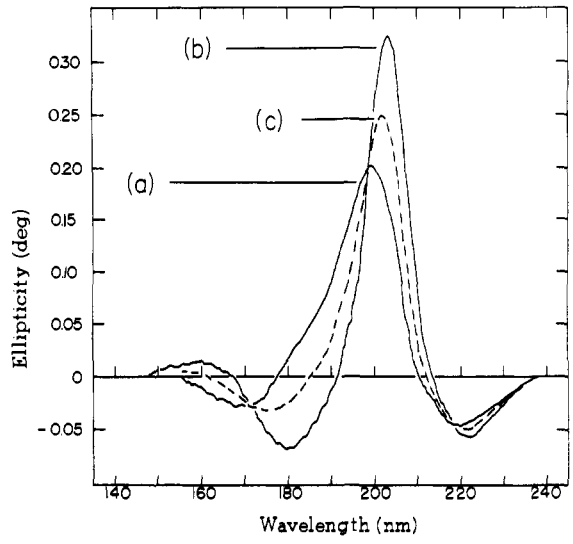

Figure 4. (a) CD of BOC(L-Ala)$_7$OMe; (b) CD of BOC(L-Val)$_7$OMe; (c) composite CD calculated by combining curves (a) and (b) in equal weights.

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(1) (a) Brown University; (b) University of Padova.
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