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A MINIMUM AT 237 mμ IN THE O.R.D. OF A HEXAPEPTIDE

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The residue rotation of an amino acid in a peptide chain is known to vary widely according to whether the residue concerned is N-terminal, internal, or C-terminal (1). The rotational contribution of a residue in any of these categories sometimes seems roughly constant (2), sometimes variable (3), as the remainder of the molecule is altered. One possible cause of variation is interaction between optically active neighbours. It, therefore, seemed worth studying the rotational properties of peptides in which optically active residues were separated by glycines (4). The molar rotation of any such peptide should be the sum of the residue rotations of the asymmetric units it contains. These would be expected to vary only slightly from peptide to peptide, in the absence of associative or conformational factors. Any large departure from additivity might then imply the intervention of such effects. Some results are listed below.

		$[M]_D$ obs.	$[M]_D$ calc.
I	Z-L-leugly$_3$ (4a)	-76°	
II	Z-gly$_3$-L-leu (4c)	-39°	
III	Z-L-leugly$_2$-L-leu (4c)	-114°	-115° (I+II)
IV	Z-gly$_2$-L-leugly$_3$ (4a)	-80°	
V	Z-L-leugly$_3$-L-leugly$_3$ (4a)	-144°	-156° (I+IV)
VI	Z-L-leugly$_3$-L-leugly$_3$-leugly$_3$ (4a)	-213°	-224° (IV+V)
VII	L-leugly$_3$ (4a)	+145°	
VIII	gly$_2$-L-leugly$_3$ (4c)	-120°	
IX	L-leugly$_3$-L-leugly$_3$ (4a)	+23°	+25° (VII+VIII)

Rotations of the benzyloxycarbonyl (Z-) compounds were determined in aqueous $KHCO_3$, those of the free peptides in water, all at comparable concentrations and at ambient temperatures. As may be seen the rotational contributions of the leucyl residues appear to be additive.

However, the hexapeptide gly-L-leugly$_2$-L-leugly (4b) was found to have $[M]_D^{22} = -164°$. This is considerably less than twice that of either of the model compounds which were available for assessing the residue rotation of internal L-leucyls, namely gly-L-leugly (4b) with $[M]_D^{23} = -108°$ and VIII above with $[M]_D^{25} = -120°$.* Moreover, while, as expected, the rotation of gly-L-leugly was identical in water and in aqueous HCl, that of the hexapeptide increased in the latter solvent.

*Literature values for two other possible model compounds are gly$_2$-L-leugly (5), $[M]_D^{24} = -130°$ (2½% H_2O) and gly$_3$-L-leugly (5), $[M]_D^{24} = -120°$ (2½% H_2O). The difference between these figures is interesting in itself.

The dispersion of optical rotation in aqueous solution of these two compounds was then examined over the range 589–230 mμ. It was found that, while that of gly-L-leugly was featureless, that of the hexapeptide included a well defined minimum at 237 mμ.

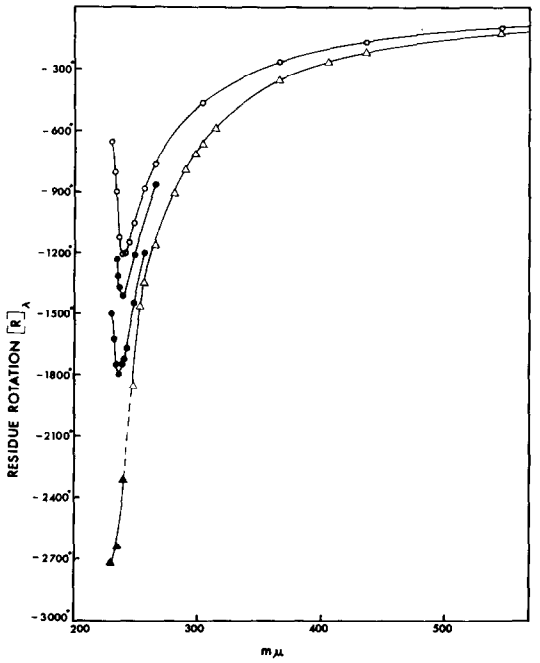

FIG.

The residue optical rotatory dispersion of gly-L-leugly$_2$-L-leugly: ○ , 0.002M in water; ⊙ , 0.01M in 0.8M aqueous urea; ● , 0.02M in N HCl; and of gly-L-leugly: △ , 0.04M in water and (589–265 mμ) 0.1M in N HCl; ▲ , 0.005M in water.

These measurements were made on a Stanley Photoelectric Polarimeter, the wavelength limit of which appears to be fixed largely by the transparency of the calcite Glan prisms supplied. Since, in the region of interest, the instrument is operating near the limit of its range and absorption due to the peptide chromophore is appreciable, the possibility of the minimum being an artefact had to be examined. The curve from an 0.004M solution of the hexapeptide showed the minimum, while that from an 0.01M solution of gly-L-leugly (similarly absorbing) was smooth and negatively tending through the same region. Changes in molar rotations at various wavelengths as the concentrations were varied were similar in the two compounds. The light beam at 237 mμ contained no detectable stray light and less than 1% at 232 mμ. Finally, curves for the tri- and hexapeptides closely similar to thse in the figure were obtained using a JASCO Automatic Spectropolarimeter equipped with quartz optics.

The coincidence in location of this minimum and that associated with the α-helix (6) might appear significant, despite the tenfold difference in rotational magnitudes. However, one cell dimension of the hexapeptide, 4.9Å, as determined by X-rays on a single crystal was too small to accommodate a folded structure and suggested an extended form. Tritium exchange measurements revealed no slowly exchangeable hydrogen. The dielectric increment in aqueous solution, 220±25 at 21°C was comparable with that of hexaglycine; 240±25 at 25°C (7). The optical rotatory dispersion was simple over most of the range covered, fitting a single term Drude equation with λ_c = 198 mμ from 546-246 mμ (8). For these reasons it has been concluded that the minimum in the curve does not reflect the α-helical conformation. Further, in polypeptides and proteins the O.R.D. curves of the α-helical and randomized forms

invariably cross as the short wavelength region is approached (8) and will do so no matter how much the helical data is diluted with that from the random coil. The curve of the hexapeptide lies above that of gly-L-leugly at all accessible wavelengths. This appears to eliminate the possibility that the observed dispersion of the hexapeptide in aqueous solution results from a mixture of conformational states among which the α-helix is energetically favoured. This conclusion is reinforced by the persistence of the minimum in aqueous urea and HCl.

In a recent paper (9) Holzwarth and Doty have provided convincing evidence that the observable rotatory properties of both helical and random polypeptides are dominated by dichroic transitions of the peptide chromophore lying between 180 and 245 mμ. The minimum at 233 mμ in the O.R.D. curve of the helical form results from two negative bands at 222 and 206 mμ. These are followed by a positive one of much greater rotational strength at 190 mμ which has a large effect on visible rotations. The random coil, on the other hand, shows weak positive circular dichroism from 235-210 mμ followed by strong negative dichroism at 200 mμ, accounting for the flattened region between 240 and 230 mμ in the negatively tending dispersion curve. The observed dichroism may be the result of two strong overlapping bands of opposite sign near 200 mμ, or of a single strong negative band at 200 mμ and a weak positive one at 220 mμ. If the latter is correct, the 220 mμ band is almost certainly assignable to the $n \rightarrow \pi^*$ transition of the peptide oxygen. By making use of the opposite shifts in wavelength of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions with changing solvent polarity, Litman and Schellman (10) have recently isolated a positive $n \rightarrow \pi^*$ Cotton effect in L-3-aminopyrrolid-2-one.

With these facts in mind, despite the limited nature of the available data, perhaps it is permissible to speculate on the origin of the minimum in the hexapeptide curve. It is highly unlikely that the α-helical conformation is involved. It is at least possible, however, that the minimum results from the superposition of a positive Cotton effect near 220 mμ on a negatively tending curve, as may be the case in the random polymers. This would suggest that the $n \rightarrow \pi^*$ transition is implicated, although why this should have a large effect on the dispersion of the hexapeptide and no obvious one on that of gly-L-leugly is not clear. There is no evidence to suggest conformationally induced interaction between the asymmetric chromophores of the hexapeptide. If these are acting in isolation, both the average local conformations associated with them and their locations relative to the ends of the chain may be important.

However that may be, the results reported here suggest that a study of simple peptides may well help to clarify the rotatory properties of the random polypeptide chain. To this end we propose to collect rotational and other data from a series of such compounds.

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