Tetrahedron. Vol. 23, pp. 4481 to 4486 Pergamon Press Ltd. 1967. Printed in Great Britain

THE ORD OF GLYCINE PEPTIDES WITH A C-TERMINAL OR N-TERMINAL L-LEUCINE RESIDUE

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(*Received for Tetrahedron Letters* 10 *August* 1966; *accepted for publication* 28 *February* 1967)

Abstract–In neutral aqueous solution the ORD curves from gly-L-leu and gly_2-L-leu are featureless and negative in sign, whereas those from gly_3-L-leu and gly_4-L-leu turn sharply positive below 250 mμ. In both acid and alkaline solution the distinction between the curves from the two pairs of peptides is no longer evident. The assumption that the ORD is dominated by the carboxyl and peptide group chromophores flanking the leucyl α-carbon atom is discussed in light of these facts. L-leugly, L-$leugly_2$, L-$leugly_3$ and L-$leugly_4$ in neutral solution give similar ORD curves which are modified in quantitatively similar fashion by addition of alkali. Rotations are also changed in acid solution, but to an extent which is linear with chain length. It is suggested that, if the principal dichroic chromophore is the peptide bond linking leucine and glycine, the linear relationship implies that the effect of protonation of the carboxylate ion is relayed along the chain.

WHEN L-leucine occupies an internal position in a peptide chain otherwise composed of glycine residues, rotational magnitudes vary both with chain length and with the particular internal site occupied by the leucine, but the ORD curve down to 230 mμ in water is usually featureless and negatively tending.[1] However, those from gly-L-$leugly_2$, gly-L-$leugly_3$ and gly-L-$leugly_4$[2] contain a well-defined minimum at 237 m, which is also present in gly-L-$leugly_2$-L-leugly.[3] A minimum in the same wavelength region is found in the ORD of both gly_3-L-leu and gly_4-L-leu, but no such feature is shown by the di- or tripeptide of this series. The ORD curves, in neutral aqueous solution, are reproduced in Fig. 1. Those of gly-L-leu and gly_2-L-leu are closely

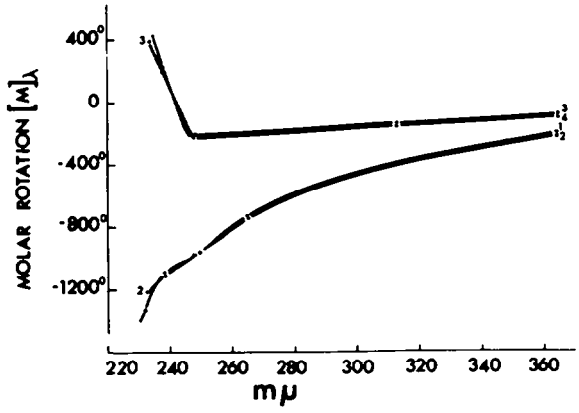

FIG. 1. The ORD in aqueous solution of 1, gly-L-leu; 2, gly_2-L-leu; 3, gly_3-L-leu; 4, gly_4-L-leu. Concentrations are 0·01 M.

[1] A. F. Beecham, *Tetrahedron Letters* 957 (1966).
[2] A. F. Beecham, *Tetrahedron Letters* 211 (1967).
[3] A. F. Beecham, *Tetrahedron Letters* 4757 (1965)

similar, while the marked change produced by incorporation of a third glycine residue in gly_3-L-leu is duplicated in gly_4-L-leu.

In the glycine peptides containing a single internal L-leucine, optical rotatory properties were discussed[1] in terms of the two peptide chromophores flanking the leucine α-carbon atom, since it seemed reasonable to suppose that chromophores more remote from the centre of asymmetry would contribute little directly to the observed rotations. Similarly in these peptides with C-terminal leucine, the carboxylate ion of the asymmetric residue and the peptide group incorporating its nitrogen atom should be the principal dichroic chromophores. There seems to be no reason why the mean disposition of these two planar groups, with respect to each other or to the leucyl side chain, should be affected by chain extension from the amino end of gly-L-leu and, indeed, the similar ORD curves of gly-L-leu and gly_2-L-leu suggest both that the conformational relationships of these two chromophores within the terminal residue are the same in the two peptides and that they do dominate the rotatory characteristics. The additional peptide group in gly_2-L-leu appears to contribute little to the rotation and it seems simplest to assume that the second additional group in gly_3-L-leu will similarly not contribute. Dreiding models indicate no plausible conformation in which its approach to the centre of asymmetry is sufficiently close for it to be affected. The similar ORD curves of gly_3-L-leu and gly_4-L-leu indicate that the sources of optical activity are the same in both. If these are the two chromophoric groups suggested above to be responsible for the dichroic properties of gly-L-leu and gly_2-L-leu, they have been modified in some way.

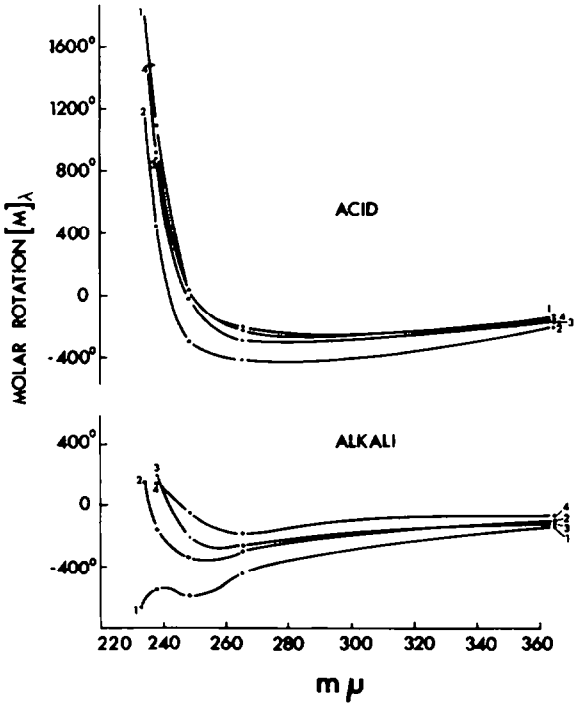

FIG. 2. The ORD in N/10 HCl and in N/10 NaOH of, 1, gly-L-leu; 2, gly_2-L-leu; 3, gly_3-L-leu; 4, gly_4-L-leu. Concentrations are 0·01M.

Fig. 2 shows the ORD curves, in acid and alkali, of the same four peptides. In acid solution, in which the carboxyl group is not ionized, gly-L-leu, gly_3-L-leu and gly_4-L-leu produce very similar curves and, while that of gly_2-L-leu varies quantitatively from the other three, the character of the dispersion is similar. On the long wavelength side of the minima in gly_3-L-leu, negative rotational magnitudes are greater in acid than in neutral solution. It appears, then, that protonation of the carboxylate ion in these two peptides may remove the feature that makes their rotatory characteristics in neutral solution so different from those of gly-L-leu and gly_2-L-leu.

In the absence of more extended data, it is not possible to specify with certainty the source of the positive dichroism affecting the ORD curves in acid solution of these peptides. However, its sign, the wavelength at which it becomes evident and its presence in all four suggest that it is the $n \rightarrow \pi^*$ transition of the uncharged carboxyl group. This transition is probably responsible for the positive extrema near 225 mμ observed in the ORD curves of α-amino and α-hydroxy acids[4,5] of the L-configuration.

It is possible that the difference, in neutral solution, between the rotatory properties of the pairs, gly-L-leu, gly_2-L-leu and gly_3-L-leu, gly_4-L-leu, merely reflects head-to-tail charge interactions affecting conformer populations in the longer peptides, such interactions being removed when the carboxylate ion is protonated. In alkali the possibility of head-to-tail charge interaction is also removed and in this medium, too, the curves from all four peptides show similar characteristics. However, in alkali, the curves of the gly-L-leu and gly_2-L-leu are quite different from those of the same two compounds in water and it is difficult to see, especially in gly_3-L-leu, why removal of charge from the terminal amino group should affect the chromophores of the C-terminal residue.

In the C-terminal leucine peptides, where the observed rotatory dispersion results from circular dichroism within the several transitions of at least two chromophoric groups, the situation is perhaps too complex for useful conclusions to be drawn from the available wavelength-limited data. Where L-leucine occupies the N-terminal position, however, only one obvious dichroic chromophore is present, namely the peptide group linking leucine to glycine, and the close similarity between the ORD curves of L-leugly, L-$leugly_2$, L-$leugly_3$ and L-$leugly_4$, reproduced in Fig. 3 would seem to imply that the rotatory properties of these compounds are, indeed, dominated by dichroic absorption in this group. It would seem to imply, further, that the average disposition of the substituents on the leucine α-carbon atom with respect to the plane of the chromophore varies little as the chain length is increased and, also, that the influence of the carboxylate ion on the rotatory properties of L-leugly is small. This follows from the fact that conversion of the group to a second peptide linkage in L-$leugly_2$ results in only minor changes in rotation, unless it is assumed that the carboxylate ion in L-leugly and the second peptide group of L-$leugly_2$ contribute equally.

However, in acidic aqueous solutions of L-leugly, in which the carboxyl group is uncharged, rotational magnitudes are almost halved. It is difficult to visualize this as a direct chromophoric effect of the carboxyl group, since it is well separated from the centre of asymmetry. A second possibility is that the change in rotation merely

[4] I. P. Dirkx and F. L. Sixma, *Rec. Trav. Chim.* 522 (1964).
[5] D. W. Urry and H. Eyring, *J. Am. Chem. Soc.* **86**, 4574 (1964).

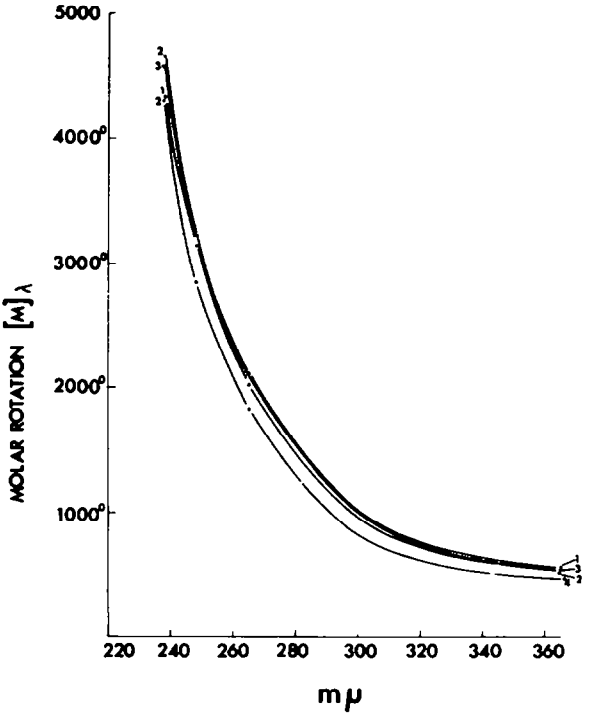

FIG. 3. The ORD in aqueous solution of 1, L-leugly; 2, L-leugly$_2$; 3, L-leugly$_3$; 4, L-leugly$_4$. Concentrations are 0·01M.

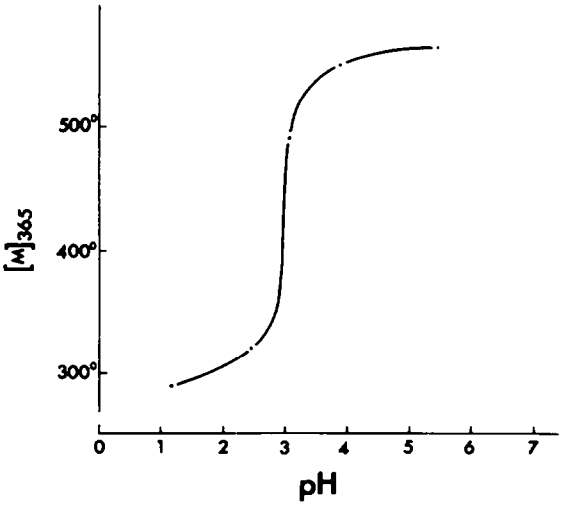

FIG. 4. The change of rotation with pH at 365 mμ in L-leuglv.

reflects a solvation or conformation change in the environment of the peptide chromophore as the surrounding water is replaced by aqueous acid. But, as shown in Fig. 4, the rate of change of rotation with pH is greatest about the pK value of the carboxyl group, suggesting that its protonation and the rotation change are directly linked.

In Table 1 are listed molecular rotation values at four wavelengths for the four N-terminal leucine peptides at neutral, acidic and alkaline pH, together with the water–acid and water–alkali differences expressed as percentages of the water values. The percentage change from water to alkali is constant throughout the series, consistent with the view that, upon removal of charge from the amino nitrogen, the asymmetric environment of the first peptide group changes identically in all four compounds and that this group is the sole asymmetric chromophore. The water to acid percentage change decreases smoothly as chain length increases, again consistent with the view that the first peptide group is the source of the observed optical activity, but with the surprising corollary that dichroic absorption in this group is modified by protonation of the C-terminal glycine even at a separation of four residues.

TABLE 1. MOLAR ROTATIONS OF 0·01 M SOLUTIONS OF N-TERMINAL L-LEUCINE PEPTIDES IN WATER, DECINORMAL AQUEOUS ACID AND DECINORMAL AQUEOUS ALKALI

Compound	Wavelength mμ	$[M]_{H_2O}$	$[M]_{HCl}$	$[M]_{NaOH}$	$\frac{[M]_{H_2O} - [M]_{HCl}}{[M]_{H_2O}}$	$\frac{[M]_{H_2O} - [M]_{NaOH}}{[M]_{H_2O}}$
L-leugly	365	570°	290°	180°	49%	68%
	265	2110°	1090°	560°	48%	73%
	248	3180°	1670°	670°	47%	79%
	238	4330°	2410°	580°	44%	87%
L-leugly$_2$	365	540°	420°	190°	22%	65%
	265	2100°	1680°	560°	20%	73%
	248	3230°	2610°	680°	19%	79%
	238	4650°	3770°	750°	19%	84%
L-leugly$_3$	365	550°	490°	180°	11%	67%
	265	2020°	1790°	580°	11%	71%
	248	3160°	2840°	650°	10%	79%
	238	5470°	4020°	660°	12%	86%
L-leugly$_4$	365	480°	470°	190°	3%	61%
	265	1830°	1760°	520°	4%	72%
	248	2850°	2720°	600°	5%	79%
	238	4300°	3880°	570°	10%	87%

The fall-off in the difference between water and acid values is nearly linear with the number of glycine residues in the chain, which is probably equivalent,[6] to a first approximation, to an inverse square law dependence on the average separation of the

[6] This follows from the interpretation in terms of mean charge separation[7] of the linear dependence of dielectric increment on the number of residues in glycine peptides, if it may be assumed that the dielectric increment of similar peptides with N-terminal leucine is also linear with the number of residues. Such results as are recorded[7-9] are compatible with this assumption.

[7] J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids* Vol. 1; p. 456. Wiley, New York (1961).

[8] W. P. Conner, R. P. Clarke and C. P. Smyth, *J. Am. Chem. Soc.* **64**, 1379 (1942).

[9] P. M. Hardy, G. W. Kenner and R. C. Sheppard, *Tetrahedron* **19**, 95 (1963).

asymmetric residue from the carboxyl group. If the carboxyl group itself were contributing to the optical activity as a chromophore, a much steeper dependence on distance would be expected.[10] This would also be the case if the carboxyl group were influencing directly the asymmetric perturbation of the peptide group linking leucine to glycine. More probably, the effect of protonation is relayed to the dichroic chromophore through the intervening peptide bonds.

Saidel[11] has observed that in acylated amino acids and in dipetides evidence of interaction between the peptide bond and either the uncharged carboxyl group or the carboxylate ion is a general feature of the UV absorption spectra and that in higher peptides[12,13] both carboxyl–peptide and peptide–peptide interactions are suggested by the data. It is well established,[14] both for isotropic and dichroic absorption, that a neighbouring group, absorbing at similar wavelengths but separated by two single bonds, may have an appreciable influence on the electronic transitions of a chromophore. In a peptide chain the possibility exists for such "homoconjugation" to extend over the whole array of chromophores, including the C-terminal carboxyl group, even without enhancement provided by fixed conformational relationships. It seems likely that such an effect is operating here. It must relate to the average mutual disposition of the chromophores and the orbital mixing associated with this, but in a way inaccessible through the available data.

If the ORD of the N-terminal leucine peptides demonstrates chromophoric interaction as suggested and if this is potentially present in all peptide chains, it should, evidently, be taken into account when considering the origin of rotatory characteristics in any such system.

EXPERIMENTAL

Syntheses of the peptides which are new are described elsewhere.[15] They were recrystallized to constant rotation.

Rotations were measured using a Stanley Photoelectric Polarimeter, Serial No. 10,[16] in which the rocking half angle was set at 2·8°. The compensator detector supplied was replaced with a 1P28 photomultiplier. The specified range of the instrument is 300–600 mμ, but rotations accurate to 0·002° were obtained down to 230 mμ. This accuracy required that the voltage on the main detector be 1200 V or less. The short wavelength limit of the instrument appears to be fixed largely by the transparency of the calcite Glan prisms supplied.

Stray light at short wavelengths was estimated by placing a glass cover slip in the light path and noting the residual signal. It was, typically, zero at 240 mμ, $< 1\%$ at 232 mμ and 2–4 % of total signal at 230 mμ.

The monochromator employed was a Zeiss M4QIII and the light sources, Na, Xe (Osram XBO 150 W) and Hg (Philips HB 80 W) arcs.

Cylindrical cells of 1, 2, 5 and 10 cm path length were used. These were selected for absence of birefringence in the silica endplates and were either sufficiently large in cross section for no reflection from the walls to occur during use or had sand-blasted inner wall surfaces. Where a difference in reading was observed between air and an empty cell, the cell was rejected.

Acknowledgement—The author is indebted to Mr. J. J. McNeill of this Division for expert guidance and collaboration in optical matters.

[10] A. Moscowitz, *Advances in Chemical Physics* Vol. IV; p. 67 (1962).
[11] L. J. Saidel, *Arch. Biochem. Biophys.* **54**, 184 (1955).
[12] L. J. Saidel, *Arch. Biochem. Biophys.* **56**, 45 (1955).
[13] L. J. Saidel and H. Lieberman, *Arch. Biochem. Biophys.* **76**, 401 (1958).
[14] K. Mislow, *Ann. N.Y. Acad. Sci.* **93**, 473 (1962).
[15] A. F. Beecham, *Austral. J. Chem.* submitted.
[16] W. F. Stanley and Co. Ltd., New Eltham, London, S.E.9.