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OPTICAL ROTATORY DISPERSION, PART XXXIV

OLIGOPEPTIDES OF ALANINE*

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Optical rotatory dispersion measurements have been used extensively for work on polyaminoacids and proteins (1,2), and in particular to study changes in conformation from random-coil to helical structures (3). Studies on the conformation of small peptides have been made using monochromatic rotations (4) but comparatively few O.R.D. measurements have been made on these smaller molecules (5).

The development of spectropolarimeters capable of penetrating to approximately 210 mμ has now made it possible to study compounds containing the carboxyl and related groups in the region of their low wavelength absorption, and several papers on amino-acids have appeared (6).

The availability of all the possible diastereoisomers of the di-, tri-, and tetra-peptides of alanine and serine (prepared in Liverpool for work on dielectric effects and conformation (7)) has given us the opportunity of studying their O.R.D. curves down to about 225 mμ, and of analysing these experimental

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*Preceding Paper A. LaManna, V. Ghislandi, P.M. Scopes and R.J. Swan, *Il Farmaco*, (*Ed. Sci*.) in press.

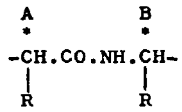
data arithmetically.

Since the amide group (-CONH-) and the carboxylic acid group (-COOH) have their main absorption bands close together in the ultra-violet (8), it is reasonable to assume that the *O.R.D.* curve of a peptide is composed of the Cotton effect curves of the amide chromophores superimposed on that of the carboxyl chromophore. In addition, a contribution to the *O.R.D.* curve will arise from the chirality of the molecule as a whole if it takes up a regular secondary structure cf., recent work on the circular dichroism of peptides at low wavelengths (9). We have now analysed the *O.R.D.* data obtained from the tri- and tetra-peptides of alanine and serine in an attempt to determine whether the contributions of the individual chromophores to the total rotation of the molecule are additive or not.

$$\overset{+}{NH_3}.\underset{\displaystyle R}{\underset{|}{CH}}.CONH.(\underset{\displaystyle R}{\underset{|}{CH}}.CONH)_n.\underset{\displaystyle R}{\underset{|}{CH}}.CO\overset{-}{O}$$

I

For an oligopeptide in water, as Zwitterion, (I) three types of chromophore may be distinguished, the *N*-terminal amide chromophore (in which the residue carries a protonated amino group), a middle chromophore, and the *C*-terminal carboxylate chromophore. There is insufficient evidence available to indicate initially whether the contribution of any particular amide chromophore (II) will be affected only by that asymmetric centre attached to the chromophore at the carbonyl carbon (A), or whether an asymmetric centre attached to the amide nitrogen (B) will also have an appreciable effect.



II

We have assumed that the second centre (B) may be of significance and therefore the '*N*-terminal' and 'middle' types of chromophore may each be further classified as lying between two centres of the *same* or of *different* absolute configuration. This gives five different types of chromophore which may contribute to the total rotation of the molecule. (For symbols see Table 1.)

If the same peptide is dissolved in dilute mineral acid, the asymmetric surroundings of each chromophore remain similar to those in water, except for the *C*-terminal chromophore which is now present as an acid group -COOH and not a carboxylate ion $-COO^-$. By contrast, in alkali, the *N*-terminal chromophore is attached to an asymmetric centre carrying a free NH_2 group and not to the protonated unit; additional terms must therefore be used to describe these chromophores.

TABLE 1

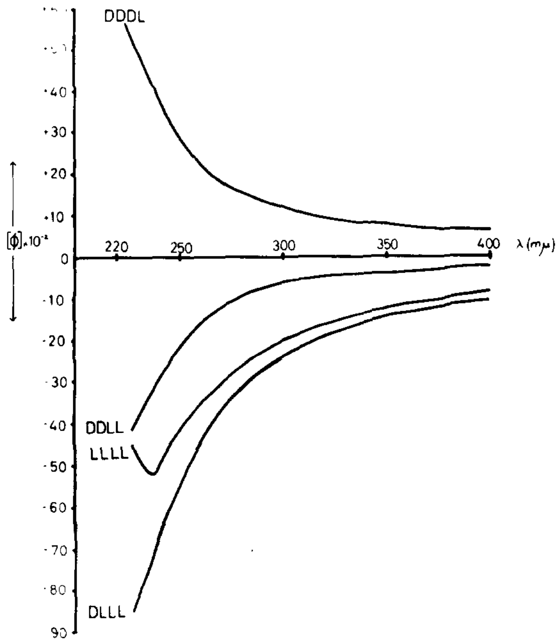
Types of Chromophore

Each type can exist in two enantiomeric forms.

Chromophore	Description of Chromophore	Symbol
$\overset{+}{N}H_3$-CH(R).CONH.CH(R)-	N-terminal amide between two L-centres and with nitrogen protonated	nLL
"	As above between an L-centre (N-terminal) - and a D-centre	nLD
NH_2CH(R).CONH.CH(R)-	N-terminal amide between two L-centres - free amino group	fLL
"	As above between an L-centre (N-terminal) and a D-centre	fLD
-CH(R).CONHCH(R)-	Middle amide unit between two-L-centres	mLL
"	As above between an L-and D-centre	mLD
-CH(R).COO^-	Terminal carboxylate ion chromophore, adjacent to an L-centre	cL
-CH(R).CO_2H	Terminal acid chromophore adjacent to an L-centre	aL

We have examined the four possible tri-peptides and eight possible tetra-peptides of alanine and of serine in water, hydrochloric acid and in alkali, in order to test the hypothesis that the individual chromophore contributions are additive. A typical set of O.R.D. curves (for four of the tetra-alanines in acid) is reproduced in Fig. 1. For each compound in each solvent the values of the molecular rotation have been compared and analysed at eight wavelengths from 400 mμ to 227 mμ. A typical set of experimental results is

FIG. 1.



Optical Rotatory Dispersion
Curves of Tetra-alanine
Peptides in Acid.

shown in Table 2 and expressed in terms of the chromophore contributions. (We have not considered the di-alanines in the following treatment because they contain a unique type of amide chromophore attached through its two asymmetric centres to both NH_2 and CO_2H.)

TABLE 2

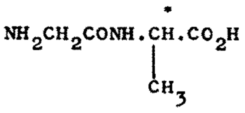
Molecular Rotations of Tri- and Tetra-Alanines in HCl at 227 mμ

Peptide	Molecular Rotation $[\phi] \times 10^{-2}$	Sum of Chromophore Contributions
LLL	-3.6	$n\underline{\underline{LL}} + m\underline{\underline{LL}} + a\underline{\underline{L}}$
LDL	+47.5	$n\underline{\underline{LD}} - m\underline{\underline{LD}} + a\underline{\underline{L}}$
DLL	-42.3	$-n\underline{\underline{LD}} + m\underline{\underline{LL}} + a\underline{\underline{L}}$
DDL	+12.8	$-n\underline{\underline{LL}} - m\underline{\underline{LD}} + a\underline{\underline{L}}$
LLLL	-45.0	$n\underline{\underline{LL}} + 2m\underline{\underline{LL}} + a\underline{\underline{L}}$
DLLL	-84.5	$-n\underline{\underline{LD}} + 2m\underline{\underline{LL}} + a\underline{\underline{L}}$
DDLL	-43.0	$-n\underline{\underline{LL}} - m\underline{\underline{LD}} + m\underline{\underline{LL}} + a\underline{\underline{L}}$
DDDL	+51.6	$-n\underline{\underline{LL}} - m\underline{\underline{LL}} - m\underline{\underline{LD}} + a\underline{\underline{L}}$
LDLL	+ 7.3	$+n\underline{\underline{LD}} - m\underline{\underline{LD}} + m\underline{\underline{LL}} + a\underline{\underline{L}}$
LLDL	+56.8	$+n\underline{\underline{LL}} + a\underline{\underline{L}}$
LDDL	+106.3	$+n\underline{\underline{LD}} - m\underline{\underline{LL}} - m\underline{\underline{LD}} + a\underline{\underline{L}}$
DLDL	+6.1	$-n\underline{\underline{LD}} + a\underline{\underline{L}}$

These equations may be solved to give a value of $m\underline{\underline{LL}}$ (here = -46.2)but beyond this it is necessary <u>either</u> to make an approximation, <u>or</u> to use model compounds in order to determine further chromophore contributions.

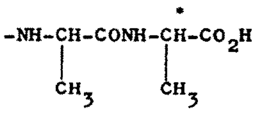
As a first approximation, the most reasonable assumption is that the middle chromophore contribution is independent of the stereochemistry of the residue linked through the nitrogen atom (B* in II) and depends essentially on the configuration of the residue attached to the carbonyl carbon (A* in II), i.e. m*LL* = m*LD*. When this approximation is made, it is possible to solve the equations in Table 2, and to obtain values for the contributions n*LL*, n*LD* and a*L*.

Some support for this approximation may be found if we compare the a*L* values so obtained with the molecular rotation of glycyl-*L* alanine (III). In this compound the acid group is in a situation somewhat similar to that of the *C*-terminal chromophore in the tri- and tetra-alanines (IV), except that there is only one asymmetric centre immediately adjacent to the carbonyl group.



(III)

cf.

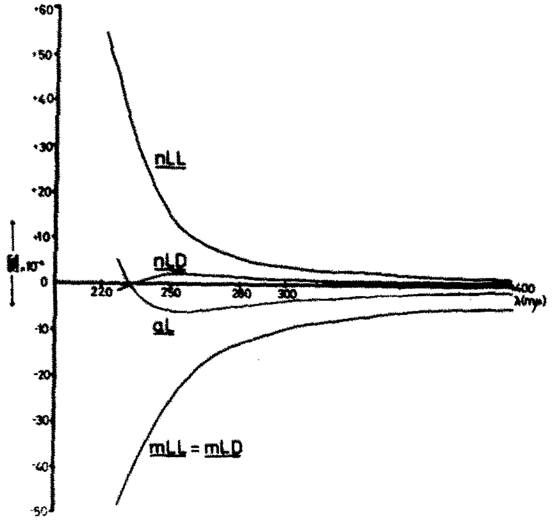


(IV)

Results

With the approximation m*LL* = m*LD* described above, values of all chromophore contributions may be calculated for a given solvent and for a given wavelength. The "calculated" molecular rotation for a given peptide may then be predicted by the addition of the appropriate chromophore contributions and compared with the experimental data.

FIG. 2.



Chromophore Contributions for
Alanine Peptides in Acid.

We have calculated the chromophore contributions for the tri- and tetra-alanines in acid, water and alkali; a comparison of experimental and calculated molecular rotations [Ø] x 10^{-2} for these compounds at 227 mμ, in 1N-HCl is shown in Table 3; graphs of the chromophore contributions in acid are shown in Fig. 2. The observed agreement between experimental and calculated rotations, supports the idea that the contributions of the chromophores are additive in these small peptides.

TABLE 3

	[φ] x 10^{-2}			[φ] x 10^{-2}	
Compound.	Exp.	Calc.	Compound.	Exp.	Calc.
LLL	-3.6	+3.6	DDLL	-43.0	-38.2
LDL	+47.5	+52.8	DDDL	+51.6	+54.2
DLL	-42.3	-41.2	LDLL	+ 7.3	+ 6.6
DDL	+12.8	+ 8.0	LLDL	+56.8	+49.8
LLLL	-45.0	-42.6	LDDL	+106.3	+99.0
DLLL	-84.5	-87.4	DLDL	+ 6.1	+ 5.0

Further confirmation of this idea may be found by comparing residue contributions at different pH values. Middle chromophore contributions (mLL = mLD) would be expected to be the same in water, acid or alkali, N-terminal chromophore contributions (protonated nLL and nLD) should be the same in acid and water, and carboxylate chromophore contributions should be the same in water and alkali.

That this is correct is shown by a comparison of the "middle chromophore" contributions (mLL) derived experimentally and listed below (Table 4).

TABLE 4

Values of mLL at varying pH

in	400	350	300	286	263	250	238	227(mμ)
H_2O	-3.7	-6.4	-10.2	-12.2	-19.5	-27.4	-41.4	-62.8
HCl	-4.6	-6.1	-10.0	-12.1	-18.0	-25.2	-37.2	-46.2
KOH	-4.4	-5.9	-10.8	-13.1	-19.1	-25.7	-36.5	-46.7

Parallel results have been obtained in the serine series, and the results for both alanine and serine peptides will be described in detail elsewhere.

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