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CONFORMATIONAL STUDIES ON PEPTIDES—I
CIRCULAR DICHROISM OF CYCLO-γ-OLIGOGLUTAMIC ACIDS

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Abstract—CD properties of three cyclo-γ-oligoglutamic acids and their t-butyl esters are reported. The assignments of the different Cotton effects are discussed. The CD of the cyclodipeptide is interpreted on the basis of a conformation with two *trans* amide groups in *anti* positions. A phenomenological correlation between chiroptical properties and conformation is presented for the other cyclooligopeptides.

INTRODUCTION

THE PRIMARY STRUCTURE of bacterial γ-polyglutamic acids[1, 2] is characteristically different from that of α-polyamino acids and proteins, the amide groups in the peptide chain of the former polymer being separated from each other by three instead of only one carbon atom and the α-carboxyl groups functioning as side chains. The question has arisen whether γ-polyglutamic acid can also adopt some sort of ordered secondary structure, similar to the α-helix or the β-conformation of α-polypeptides, or whether the conformation of this polypeptide is entirely random.

Earlier physico-chemical investigations of Edelhoch *et al.*[3, 4] led to the conclusion that γ-polyglutamic acid forms a random coil in aqueous solution, but the molecules are more compact in the undissociated than in the ionised form. From the results of ORD measurements made between 600 and 250 nm, Rydon[5] suggested a helical structure for the undissociated form of the polyacid. Recently Watanabe *et al.*[6] measured a negative ORD extremum at 230 nm in the acidic aqueous solution of γ-poly-D-glutamic acid and proposed a β-conformation for it on the basis of analogy to the nearly identical trough in the ORD spectra of proteins in the β-form. On the other hand, Kajtár and Bruckner[7] have pointed out in an earlier communication that there is no substantial difference in the ORD properties between the γ-polyglutamic acid and the γ-oligoglutamic acids of low molecular weight. According to these experimental results the "complex dispersion" found by Rydon[5] cannot arise from an ordered secondary structure of the polymeric molecule, but it should be an inherent property of the chromophoric system in a γ-glutamyl residue. Rydon's former reasoning was based on a formal analogy between the rotatory data of γ-polyglutamic acid and of α-polypeptides in the helical conformation. The same criticism can be held to the conclusion of the Japanese authors.[6] They mention themselves that the D-enantiomer of γ-polyglutamic acid exhibits an ORD extremum of the same sign (negative) as that of the L-enantiomers of α-polyamino acids and proteins in the β-conformation. The absolute values of the two extrema are rather different, too.

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For the purpose of investigating the relationship between chiroptical properties and conformation of ω-linked peptides of α-amino-dicarboxylic acids we have synthesized oligo- and polypeptide derivatives containing γ-glutamyl[8–10] and β-aspartyl[11] residues and measured their circular dichroism (CD) and optical rotatory dispersion (ORD).[12] From all these data, however, we have not come to a clear-cut conclusion concerning the problem mentioned above, because the preferred conformation of the very flexible oligo- and polypeptide molecules could not be predicted. It became obvious that we need model substances of more rigid conformation. The cyclo-γ-oligopeptides of glutamic acid synthesized by us recently[13] have proven more appropriate models for our conformational study.

In the present communication we report the results of CD measurements made on the cyclo-γ-diglutamic, cyclo-γ-triglutamic and cyclo-γ-tetraglutamic acids (**1a–3a**) and their corresponding t-butyl esters (**1b–3b**).

R	n		
	2	3	4
H	**1a**	**2a**	**3a**
Bu^t	**1b**	**2b**	**3b**

RESULTS

TABLE 1. CD DATA OF CYCLO-γ-OLIGO-L-GLUTAMIC ACIDS AND ESTERS

Comp.	Solvent	λ, nm (Δε)†			
		I	II	III	IV
1a	H_2O			204 (+2·90)	186 (−2·5)
	0·1N-HCl			203 (+3·57)	
	0·1N-KOH			205 (+4·90)	
1b	EtOH	243 (−0·08)		202 (+3·30)	
	TFE	244 (−0·02)		200 (+4·75)	
	Cyclohexane			201 (+1·80)	
2a	H_2O		212 (+4·80)	204 (+5·30)	186! (−18)
	0·1N-HCl		213 (+4·62)	204 (+5·35)	
	0·1N-KOH		215 (+2·75)	positive	
2b	EtOH	251 (−0·02)	218 (+3·30)	208 (+2·80)	190! (−10)
	TFE	249 (−0·01)	214 (+4·83)	205 (+3·80)	187! (−17)
	Cyclohexane		222 (+0·73)	201 (+1·45)	negative
3a	H_2O		211 (+3·60)	202 (+5·50)	186! (−8·4)
	0·1N-HCl		213 (+2·73)	202 (+5·19)	
	0·1N-KOH		216 (+1·81)	positive	
3b	EtOH	245 (−0·08)	212 (+2·47)	203 (+2·58)	186! (−6)
	TFE	245 (−0·03)	210 (+2·70)	204 (+3·00)	187! (−6)

† The Δε values relate to one γ-glutamyl residue

The data presented in Table I summarize the positions, signs and magnitudes of the Cotton effects of compounds **1a–3a** and **1b–3b** in different solvents. The $\Delta\varepsilon$ values are given per γ-glutamyl residue.

In the CD spectra up to four bands of varying rotational strengths can be found. Band I is a weak negative Cotton effect between 240 and 250 nm. (The signs of all bands correspond to L-enantiomers.) Both band II and band III are positive and of medium intensity at 210–220 nm and 200–205 nm, respectively. Band IV lies at short wavelengths below 190 nm. In most of the cases the maximum of this band could not be reached, but its sign was found to be negative (Figs. 1–6).

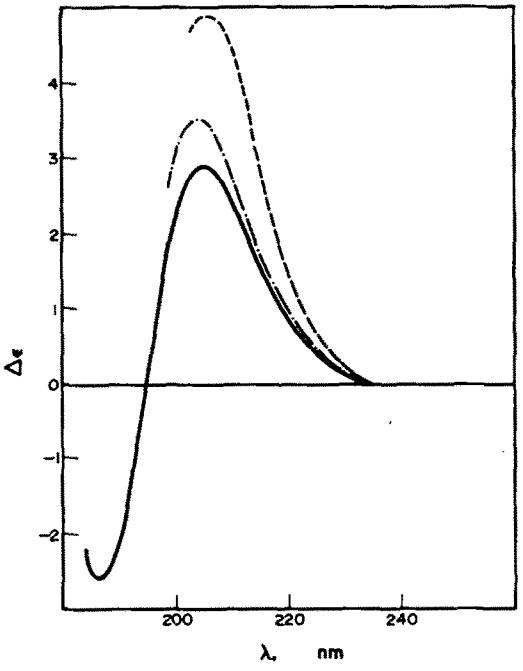


FIG 1. CD of cyclo-γ-di-L-glutamic acid (**1a**) in water (——), 0·1N HCl (–·–) and 0·1N KOH (– – –).

Band I is absent from the spectra of the cyclopeptide acids **1a–3a** in aqueous solutions. Neither was it observed in the spectra of the esters **1b–2b** measured in cyclohexane. As these spectra had to be taken, however, in rather dilute solutions owing to poor solubility, the absence of band I does not strictly exclude the existence of a weak Cotton effect in this wavelength region.

The most striking difference between the CD of the homologous cyclooligopeptides is the fact that band II is absent from the spectra of the cyclodipeptide **1a** and its ester **1b** (Figs. 1 and 2), whereas the cyclotripeptides **2a** and **2b** (Figs. 3 and 4) and the cyclotetrapeptides **3a** and **3b** (Figs. 5 and 6) exhibit both bands II and III, though of different relative intensities. The ratio of the rotational strength of band III to that of band II is always larger in the tetrapeptide than in the tripeptide. In the spectra of the cyclotripeptide acid **2a** in aqueous solutions (Fig. 3) the two bands are of about the

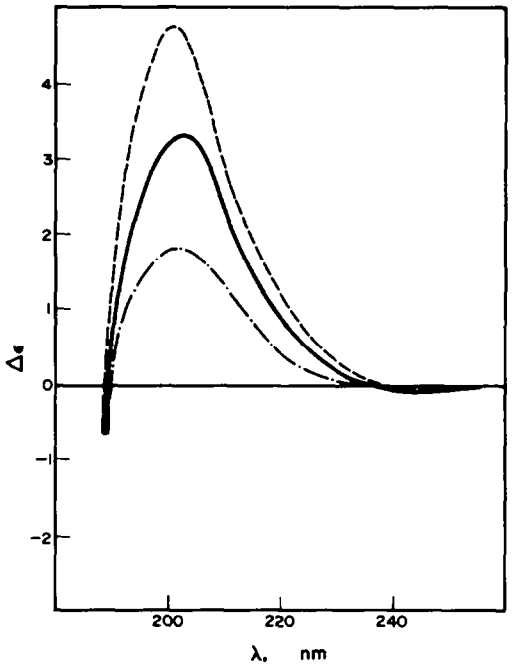


FIG 2. CD of di-t-butyl cyclo-γ-di-L-glutamate (**1b**) in EtOH (——), trifluoroethanol (– – –) and cyclohexane (–·–).

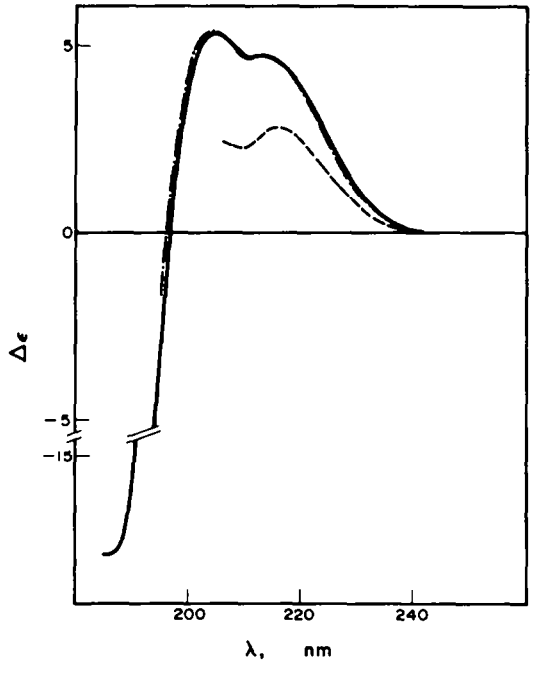


FIG 3. CD of cyclo-γ-tri-L-glutamic acid (**2a**) in water (——), 0·1N HCl (–·–) and 0·1N KOH (– – –).

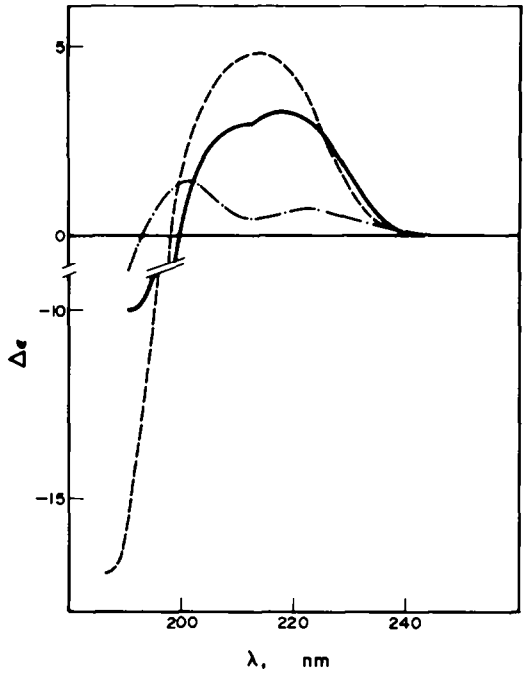


FIG 4. CD of tri-t-butyl cyclo-γ-tri-L-glutamate (**2b**) in EtOH (——), trifluorethanol (– – –) and cyclohexane (–·–).

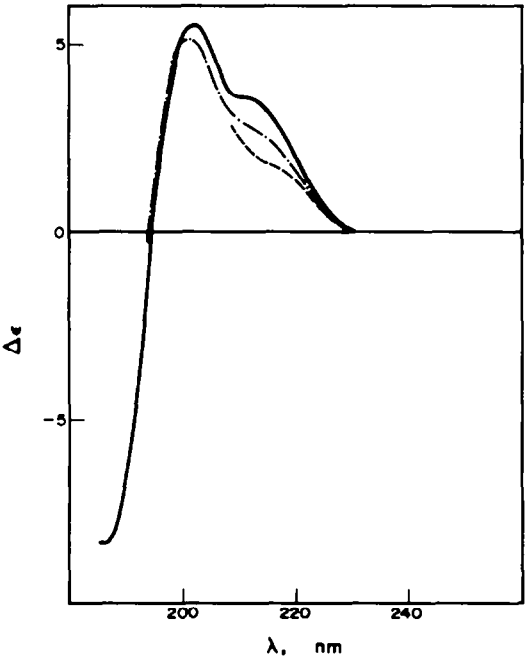


FIG 5. CD of cyclo-γ-tetra-L-glutamic acid (**3a**) in water (——), 0·1N HCl (–·–) and 0·1N KOH (– – –).

same intensity, while in those of the cyclotetrapeptide acid **3a** (Fig. 5) band III is stronger than band II. No pronounced peak was observed in the wavelength area of band III in the spectra of **2b** in alcohols (Fig. 4). From the shape of the CD curves one can assume, however, that this band also gives some contribution to the CD of this substance. In contrast to this, there is a pronounced maximum present in the spectra of the cyclotetrapeptide ester **3b** in the same solvents around 203 nm (Fig. 6). In cyclohexane solution of **2b** band III is more intense than band II (Fig. 4). Unfortunately, the cyclotetrapeptide ester **3b** does not dissolve in cyclohexane, thus its spectrum could not be compared with those of the smaller homologues in this solvent.

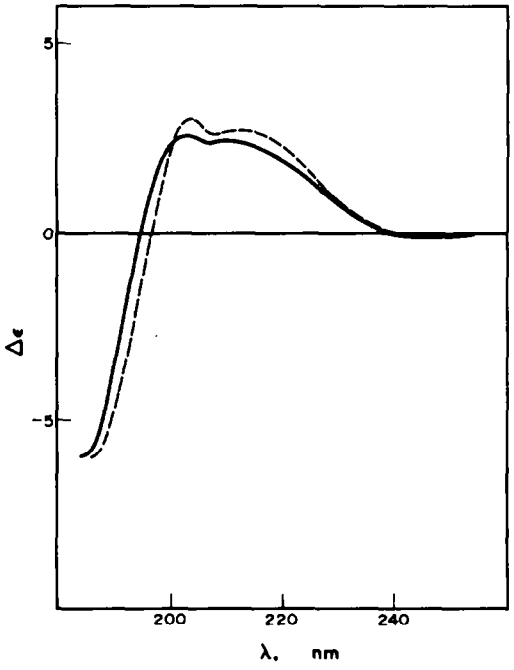


FIG 6. CD of tetra-t-butyl cyclo-γ-tetra-L-glutamate (**3b**) in EtOH (——) and trifluoroethanol (– – –).

Although it was not possible to measure the CD through the maximum of band IV, one nevertheless can see that the CD is of rather high negative values at short wavelengths, especially in the case of the cyclotripeptides **2a** and **2b** (Figs. 3 and 4). The position of the maxima can be estimated to be around 185 nm. High absorption of the solvents prevented measurements below 200 nm in acidic or alkaline aqueous solutions.

No substantial changes in the shapes of the curves or in the magnitudes of the Cotton effects could be observed in the concentration range of about 10^{-2}–10^{-5} mole/l.

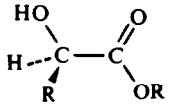
In the UV absorption spectra of the cyclooligopeptide acids **1a**–**3a** in aqueous solution only one maximum is present at 188–189 nm. The molar extinction coefficients calculated per glutamic acid residue are 3960, 6330 and 6930 for **1a**, **2a** and **3a**, respectively; the band widths are about 30 nm.

The IR spectra of the cyclooligopeptide esters **1b–3b** measured in $CHCl_3$ solutions show the N—H stretching vibrations at 3410–3420 cm^{-1} (non associated) and at 3300–3340 cm^{-1} (associated), and the amide-I and amide-II bands at 1650–1670 and 1500–1515 cm^{-1}, respectively, which are characteristic for *trans* amides.[14–16]

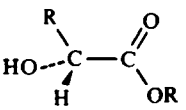
DISCUSSION

In order to interpret the CD spectra and to correlate them with conformation, it is necessary to assign the different Cotton effects to the electronic transitions of the chromophores. The assignments of band IV and band I seem to be almost unequivocal. All our cyclopeptides exhibit UV absorption maxima at 189 nm in water. This band corresponds to the $\pi \rightarrow \pi^*$ transition of the secondary amide group,[17] therefore the Cotton effect below 195 nm, i.e. band IV can be assigned to this transition.[18–21]

Band I is very probably analogous to the weak band around 240–250 nm found in the CD spectra of many α-hydroxy-,[22–30] α-amino-[28–32] and α-halogenocarboxylic[33] acids and esters in addition to a stronger band at about 210 nm. Both bands have been assigned to the $n \rightarrow \pi^*$ transition of the carboxyl or ester group in different conformations of the molecules.[23–27, 29, 30] Listowsky *et al.*[26] suggested rotamer **A** of an α-hydroxycarboxylic acid (in which the carbonyl group eclipses the C^{α}—O bond)



A



B

to be responsible for the shorter wavelength intense band and rotamer **B** (containing the carbonyl group and the C^{α}—C^{β} bond in eclipsed position) for the weak band at 240 nm. Analogous conclusions have been made by Gaffield and Galetto[33] in correlating the two CD bands of α-halogenocarboxylic acids to their conformation. We assign, therefore, band I to the $n \rightarrow \pi^*$ transition of the ester group in a conformation similar to **B** (see later). It is worthwhile mentioning that according to our present results not only amino- and alkylamino-, but also acylamino-carboxylic acids and esters exhibit this long-wavelength weak Cotton effect (*cf.* also [12]).

Band II is one of the two bands which seem to be the most important for the correlation of the CD of our cyclooligopeptides to their conformation. This band can very probably be ascribed to the $n \rightarrow \pi^*$ transition of the amide group. Cotton effects in this wavelength region were found in the ORD and CD spectra of simple cyclic amides containing no other chromophoric groups[34–39] and also of small peptides,[40] cyclopeptides[41] and proteins.[18–21] In all these cases the corresponding band was assigned to the amide $n \rightarrow \pi^*$ transition.[42] The small red shift of band II with decreasing polarity of solvent is in accordance with its $n \rightarrow \pi^*$ character.

The origin of band III remains rather problematic; in analogy to assignments made in the literature for similar CD-bands of acid derivatives, some possibilities are discussed in the following.

(a) Madison and Schellman[43, 44] have calculated theoretical CD spectra for a number of N-acetyl-L-proline derivatives. In the case of the N-acetyl-L-proline methyl ester, i.e. of a compound having a similar chromophoric system to that of our cyclooligopeptides, they took into consideration the following transitions: amide $n \rightarrow \pi^*$

at 212 nm, ester n → π* at 205 nm, tertiary amide π → π* at 198 nm and ester π → π* at 167 nm. The wavelengths corresponding to the first three transitions are about the same as those of bands II, III and IV. (The secondary amide transition should appear at somewhat shorter wavelength than the tertiary).[17] According to this, band III could tentatively be assigned the n → π* transition of the carboxyl or ester group. Such a n → π* transition should be blue-shifted upon ionization,[45] which has not been observed in our cases. This may, however, be due to technical reasons, as the maximum found for the dipeptides at 205 nm appeared just at the cut-off of the measurement, thus it is perhaps no real maximum.

(b) A band of very similar position to that of band III was found in the CD spectra of cyclic amides.[34–36] This band disappeared[35] or at least considerably decreased[34, 36] by diluting the solutions. It was, therefore, concluded[36] that it arises from the association of the lactam molecules. The intensity of band III was, however, not diminished by dilution of the solutions by a factor of about 1000, consequently it is very improbable that band III originates from some sort of intermolecular association. Anyway, a strong association in acidic or basic aqueous solution is very unlikely.[46, 47]

(c) A third possibility would be the consideration of a n → σ* transition, the so-called "mystery band" of the amide group.[48, 49] However, until now no conclusive evidence has been found concerning the role of this band in the CD of amides.[34–36,38,39]

(d) As all our cyclopeptide molecules contain more than one amide group supposedly in a rather rigid conformation, exciton splitting[50–52] of the π → π* bands of these groups may obtain. In this case the two bands of opposite sign, i.e. band III and band IV may be the two wings of an exciton couplet.[51] The UV maxima of the cyclo-oligopeptides lie between these two CD bands and their band widths are rather large, which would be in agreement with this assumption.

Despite the fact that without further experimental and theoretical investigations no unequivocal assignment for band III can be made, we, nevertheless, can use it for phenomenological correlations.

As it has been pointed out, the most striking feature of the CD properties of the cyclodipeptides **1a** and **1b** is the absence of band II, i.e. the Cotton effect of the n → π* transition of the amide chromophores. We believe that the reason for this fact is a special conformation of the molecule. The ring of the cyclo-γ-diglutamic acid can adopt only a few conformations, and as according to the IR spectrum the amide groups have *trans* configuration, we propose the conformation given in Fig. 7 as the most preferred. The two amide groups are assumed to be in "*anti*" position owing to the electrostatic interaction of their dipoles.

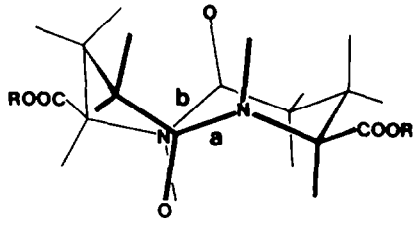


FIG 7. Preferred conformation of **1a** (R = H) and **1b** (R = Bu^t). For better legibility the hydrogen atoms have been omitted.

The conformation of ten-membered cyclic diesters and diamides has been discussed by Dale[53] on the basis of the known preferred conformation of cyclodecane.[54] The *trans* amide groupings can be positioned only in the places of the two pairs of C-atoms of type III of the cyclodecane.[54] In this conformation each C=O and N—H bond is staggered to the adjoining CH_2 group. Assuming such a conformation for the cyclodipeptides **1a** or **1b** will force one of the two COOR groupings into a semi-axial[54] position; this unfavourable arrangement may be relieved by flipping over the neighbouring methylene group of type I into the other possible conformation, which leaves unchanged the conformation of the rest of the molecule. In this way we introduce one N—H/C—H and one C=O/C—H eclipsing. The first is destabilizing, the second, however, stabilizing such a conformation and these two effects may cancel themselves to a large extent. Thus there remains a net energy gain for the conformation given in Fig. 7.

In this conformation there is no possibility for intramolecular hydrogen bonding. In consequence of the rather short distance of the two amide groups, however, a transannular interaction can be effective between them even more stabilizing this conformation. Such electrostatic interactions between a nitrogen[55] or an oxygen[54, 56] atom and a carbonyl group and between two amide groups[57, 58] have been suggested to be present in medium ring compounds. The proposed conformation (Fig. 7) can be characterised by torsional angles ϕ[59] of about 60° (amide group *a*) and 240° (amide group *b*) for the two N—C^{α} bonds.

A special feature of this conformation is that the environments of the two amide carbonyl groups are almost enantiomeric (Fig. 8). This fact can result in a substantial compensation of their contributions to the amide n → π* Cotton effect. We therefore believe that this is the reason for the absence of band II from the spectra of the cyclodipeptides **1a** and **1b**.

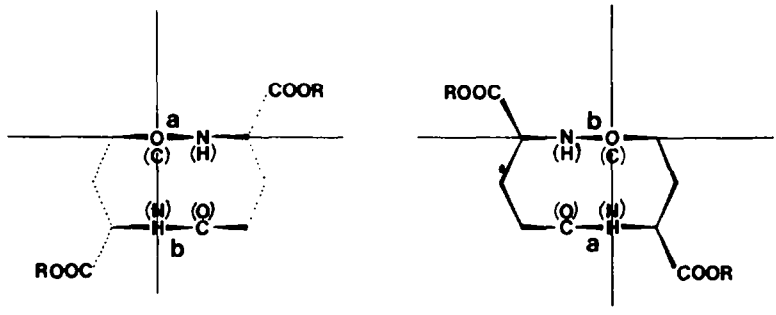


FIG 8. Projections of the preferred conformation of the cyclodipeptides from oxygen to carbon of the amide groups *a* and *b*, respectively. Atoms of the amide groups which are lying directly behind those shown in the formula are written in parentheses.

The rigid position of the two amide groups can in principle give rise to an appreciable splitting of their π → π* transitions. This would be in agreement with one of the possible explanations for the origin of band III considered above. As, however, molecular models (Fig. 7) show, the two electric transition moments are almost parallel and thus no prediction can be made for the sign and magnitude of the resulting couplet without exactly knowing the relative positions of the interacting chromophores.

Exact parallel alignment of the two vectors can, of course, not give rise to an exciton couplet, but just to a blueshift of the absorption band.[60] It should, nevertheless, be kept in mind that small deviations from the parallel orientation are sufficient for band splitting.[52]

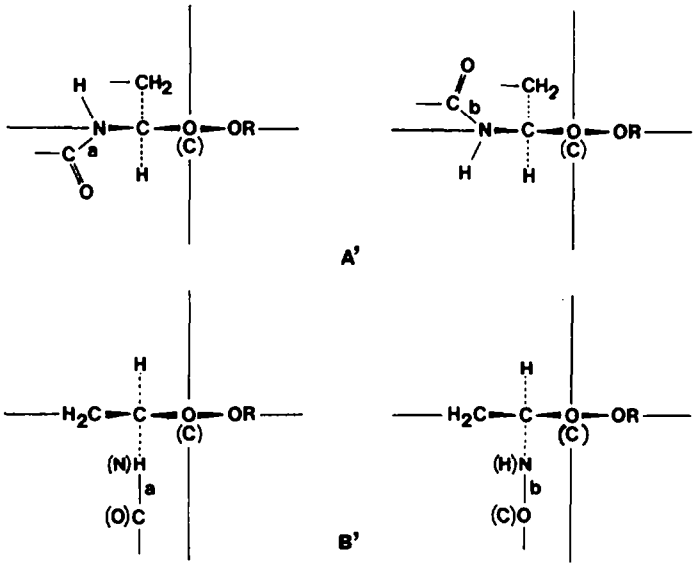


FIG 9. Projections of the environments of the two ester groups in the neighbourhood of amide groups *a* and *b* of **1b** for conformation **A'** (top) and **B'** (bottom). Meaning of letters in parentheses like in Fig. 8.

Another possibility for the appearance of band III would be its assignment to a $n \rightarrow \pi^*$ transition of the carboxyl chromophore. This explanation can also be in accordance with the proposed model. The carboxyl or ester groups can adopt three conformations in which the C=O bond eclipses the C^α—N, the C^α—C^β or the C^α—H bond. Of the three rotamers it is the first (**A'**, Fig. 9) which should be preferred owing to electrostatic compensation of the carboxyl C=O and the amide dipoles. This conformation was found to be the most favoured in the crystals of small peptides by X-ray diffraction.[61] Owing to its diminished polarity, it is expected to be stable especially in nonpolar solvents. The other rotamer to be considered (*cf.*[26, 33, 62]) is supposedly the second one mentioned (**B'**, Fig. 9). As can be seen from Fig. 9, the nearest environments of the two carboxyl (or ester) groups (the one is in the neighbourhood of amide group *a*, the other in that of amide group *b*) are about the same both in conformation **A'** and in conformation **B'**. The rotatory contributions of the carboxyl groups to the $n \rightarrow \pi^*$ transition should, therefore, be of the same sign in either of the two conformations. Applications of our sector rule[63] leads to a positive Cotton effect in conformation **A'** and to a negative one in conformation **B'**. In conformation **A'** the π-orbital of the amide group can overlap with the n-orbital of the carbonyl oxygen of the carboxyl group, resulting probably in the stabilization of the n electronic state of the latter. In conformation **B'**, however, such a stabilization can be

effective between the π-orbital of the amide and the π*-orbital of the carboxyl group. One may expect, as a consequence, that the n → π* transition should be blue-shifted in the first and red-shifted in the second rotamer. We can suppose that these two transitions of opposite sign result in the two bands I and III in the CD-spectra of the cyclodipeptide ester **1b**. The real separation of these bands is, however, very probably much smaller than the difference in the positions of the two "virtual" Cotton effects arising from their overlap (*cf.*[64, 65]). It should, however, be mentioned that even this detailed discussion of the CD-bands does not resolve the problem of unequivocally assigning the parentage of band III.

The molecules of the cyclotripeptides **2a** and **2b** and of the cyclotetrapeptides **3a** and **3b** should be much more flexible. The interpretation of their CD properties can be based on the simplifying assumption that band III is characteristic for a rather rigid conformation similar to that of the cyclodipeptides, whereas band II which can also be found in every open-chain derivative measured by us[12] correlates with the "most stable conformation" of a system built up of an amide and a carboxyl (or ester) group bound to the same carbon atom of *S*-chirality. The molecules of the larger cyclopeptides may adopt local conformations both of the "cyclodipeptide type" and of the "open-chain type". The four γ-glutamyl residues of the cyclotetrapeptides can better accommodate to the "cyclodipeptide type" conformation than the three residues in the cyclotripeptide which, therefore, should contain a higher extent of the "open-chain type" conformation. This explains the higher ratio of the intensities of band III to band II in the CD-spectra of the cyclotetrapeptides (Figs. 5 and 6) compared to that of the cyclotripeptides (Figs. 3 and 4).

For these large-membered ring cyclo-γ-oligopeptides of glutamic acid the essential chiroptical properties can thus be satisfactorily rationalized on the basis of conformational analysis. We believe also that this can give a good starting point for the discussion of similar problems in the case of the open-chain γ-oligo- and polypeptide derivatives of glutamic acid.

EXPERIMENTAL

The synthesis and the physical properties of the cyclo-γ-oligoglutamic acids and esters have been published elsewhere.[13]

The CD measurements were made on a Roussel–Jouan Dichrographe model 185 at 20° in concentrations of about 0·5 to 2 mg/ml in cells of path lengths of 0·01 to 2·0 cm. For the measurements below 210 nm the instrument was purged with dry nitrogen. The CD data are not corrected for the refractive index of the solvent.

The UV spectra were measured on a Cary-15 spectrophotometer purged with dry nitrogen at room temp in a 0·01 cm cell at concentrations of approximately 10^{-3} mole/l.

The IR spectra were obtained in $CHCl_3$ solution (concentration about 10 mg/ml) in a 0·01 cm NaCl cell using a Perkin–Elmer model 221 instrument with prism-grating interchange unit.

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