A STUDY OF THE CONFORMATIONAL STATES OF CYCLOPEPTIDE SYSTEMS

III. CYCLOHEXAPEPTIDES AS SYSTEMS OF INTERACTING AMIDE CHROMOPHORES

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In the course of our study of the conformational states of peptide systems, we have synthesized a large group of cyclic hexapeptides constructed of L(D)-alanine and glycine residues and have made a detailed physicochemical investigation of them [1-5]. In the present communication we describe the results of an investigation of the ultraviolet (UV) spectra, the circular dichroism (CD) curves, and the optical rotatory dispersion (ORD) curves of the cyclopeptides (2)-(21)† (Fig. 1), and also the UV spectra of cyclohexaglycyl (1) and of two model diamides – the N-methylamide of acetylglycine (20) and the N-methylamide of acetyl-L-alanine (21). Some of the CD and ORD curves discussed (those for compounds 2-4, 6, and 9) were obtained by us previously [1].

The UV spectra of the cyclopeptides (1)-(19), measured in aqueous solutions, are given in Table 1. As can be seen from the table, λ_{max} for the compounds considered is located in the 184-190 nm region, beyond which no maxima or inflections whatever are observed except for the case of compound (14); ε_{max} varies over an extremely wide range (from 3900 to 7100, calculated to one amide bond). Since in the cyclopeptides constructed from alanine and glycine residues the only chromophores absorbing in this region are the amide groups, the assignment of the absorption maximum found to the $\pi \rightarrow \pi^*$ transitions is not a matter of doubt. The maxima corresponding to the $n \rightarrow \pi^*$ transitions and, judging from the ORD and CD results (see below), located at ~215 nm, are extremely weak and are masked by the stronger maxima of the $\pi \rightarrow \pi^*$ transitions. Recently, the question of the possibility of the appearance in the UV spectra and CD and ORD curves of peptides of the $n \rightarrow \sigma^*$ transition of the unpaired electron of the carbonyl atom of oxygen into an excited σ^* orbital (see, for example, [7]), which is detected in the electronic spectra of the amides in the gas phase and is located in the region between the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, has been discussed repeatedly [8, 9]. However, a detailed comparison of the spectra of the amides in the gaseous state and in solutions has shown that this band is connected with a Rydberg transition and does not appear in the spectra of a condensed phase [9]. Consequently, in the present work only the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the amide groups have been taken into account.

On comparing the results obtained with features of the UV absorption of model amides, oligopeptides, and "unordered" polypeptides (Table 2), attention is attracted by some hypochromism, on the average rising with an increase in the number of alanine residues in the ring (for example, Fig. 2).‡ A fall in the intensity

† The enantiomers of compounds (2)-(4), (6), (9), (10), (13), and (18) have actually been obtained and studied. In the present work, for convenience of comparison, the isomers containing the maximum number of L-alanine residues are discussed.

‡ The conclusions that we drew previously in a study of the UV absorption curves of five compounds down to 195 nm [1] proved to be uncharacteristic for the UV spectra taken in the region of the absorption maxima.

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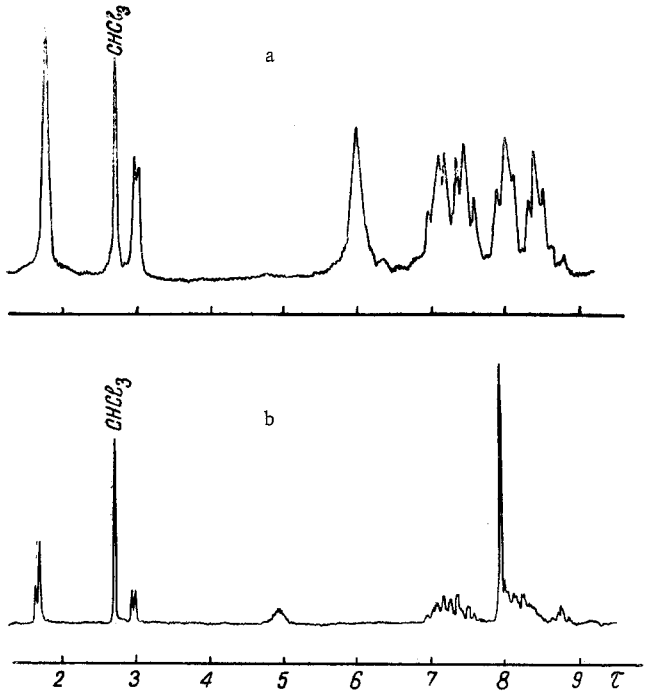

Fig. 1. Structure of the cyclopeptides (1)-(19).

TABLE 1. Features of the IR Spectra of Compounds (1)-(21)

Compound	λ_{max}*	ε_{max}*	D†	f	Δ
(1)	187	6800	8,9	0,22	15,0
(2)	187	7400	9,3	0,23	14,5
(3)	187,5	6700	8,4	0,21	14,5
(4)	187,5	7000	8,8	0,22	14,5
(5)	188	7200	8,7	0,22	14,0
(6)	188	6500	8,4	0,21	15,0
(7)	188	6600	8,5	0,21	15,0
(8)	188	6900	8,4	0,21	14,0
(9)	189	7100	8,9	0,22	14,5
(10)	188	6300	7,9	0,20	14,5
(11)	188,5	7100	8,9	0,22	14,5
(12)	188,5	6400	8,2	0,20	14,0
(13)	189	6300	7,9	0,20	14,5
(14)	184	3900	6,4	0,16	18,5
(15)	189,5	5500	6,8	0,17	14,5
(16)	189	6300	7,9	0,20	14,5
(17)	189	5900	7,1	0,18	14,0
(18)	189	6100	7,6	0,19	14,5
(19)	189	6200	7,5	0,18	14,0
(20)	187	7500	9,1	0,23	14,0
(21)	187	7700	9,1	0,23	13,5

* Calculated to one amide group.
† The dipole strength (D) was calculated on the assumption of a Gaussian form of the absorption curve [7], and the oscillator strength was obtained from an equation borrowed from the literature [13].

of the $\pi \rightarrow \pi^*$ transition in the cyclohexapeptides is also observed in comparison with the UV spectra of the extended forms of Ac-Gly-NHMe (20) and Ac-L-Ala-NHMe (21) in aqueous solutions (see Fig. 1 and Table 1). The effect of hypochromism is particularly pronounced in cyclo-$(L\text{-Ala})_6$, where the fall in ε_{max} in comparison with compound (2) amounts to ~45%, while the shape of the UV absorption curve permits the assumption of the existence of a shoulder at 190-195 nm; a considerable broadening of the absorption band in compound (14) ($\Delta = 18.5$ nm, see Table 1) also shows its nonuniformity.

The results obtained show that in compound (14) and, apparently, in the other cyclopeptides studied, there is an exciton interaction of the electric dipole moments of the transition leading to the splitting of the absorption band and to a reduction in its intensity. Theoretical calculations showed that interaction of the chromophoric groups must be expected where they differ insignificantly in the position of the absorption maxima and are adjacent in space, the nature of the interaction depending to a considerable extent on the mutual orientation of the chromophoric groups [16, 17]. For example, a parallel arrangement of the amide groups must lead to a fall in the intensity of the long-wave absorption band of the $\pi \rightarrow \pi^*$ transition, and a collinear arrangement to its increase [16-19].

Up to the present time, it has been possible to detect the appearance of an interaction of the chromophoric groups in the UV spectra of peptides only in poly(amino acid)s or proteins having the α-helical or β configuration. At the same time, the UV absorption curves of the α-helical polypeptides are extremely similar to the curve of compound (14) (ε_{max}, respectively, 4200 and 3900, shoulder in the long-wave region), although the positions of λ_{max} and the shoulder differ substantially: 191 and 202 nm in the α-helix [19] (see Table 2) and 184 and ~195 nm in the ring (14). So far as concerns polypeptides in the β conformation, for them, conversely, hyperchromism is characteristic [12, 20] (see Table 2).

TABLE 2. Features of the UV Absorption of Amide Groups in Aqueous Solutions of Peptides

Compound	λ_{max}	ε_{max}	Literature data
$CH_3CONHCH_3$	186–187	8800	10,11,13
$HGly_nOH$	187	7800	11
Poly-L-lysine, coil	192	7100	12
α-Helix	192	4400	12
	203 (sh)	2600	
β-Conformation	196	7600	12
Cyclo-$(Gly)_2$	189	6750	13
Cyclo-$(L\text{-}Ala)_2$	188	8250	13
		8150	14

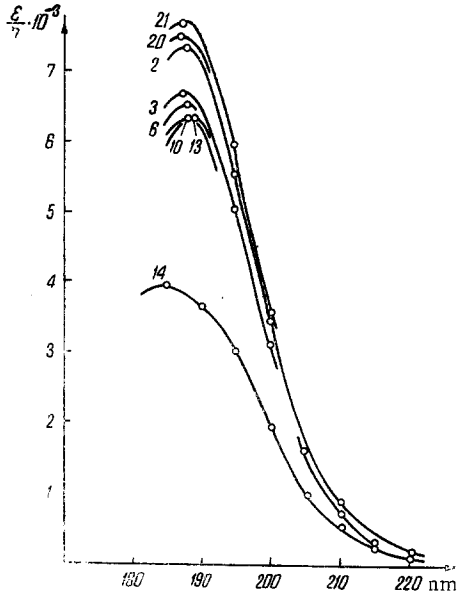

Fig. 2. UV spectra of the cyclopeptides (2), (3), (6), and (14) and also of the diamides (20) and (21) [n is the number of amide links in the molecule; n = 6 for (1)-(19) and n = 2 for (20) and (21)].

What has been said above permits the conclusion that cyclic hexapeptides form a new type of system of interacting amide chromophores having an ordered conformation. In this connection it is interesting to observe that in cyclo-$(Gly)_6$, in which (judging from the results of x-ray structural analysis) irregular conformations of the cyclopeptide skeleton predominate [21], the effect of hypochromism is expressed considerably more feebly than in (14) (see Table 1).

Further information on the structure of the cyclopeptides (2)-(9) was obtained from the ORD and CD curves. In view of the poor solubility of the cyclopeptides in organic solvents, the majority of the measurements was performed in aqueous solutions. In an analysis of the curves obtained (Figs. 2-6), attention is attracted in the first place to their monotypicity [with the exception of the curves for compounds (5) and (19)], showing the similarity of the conformational characteristics of the compounds studied. As can be seen from Figs. 3-6, the curves observed are the result of the superposition of at least three Cotton effects, the absorptions, signs, and intensities of which permit the conclusion that the Cotton effects located in the long-wave region (210-215 nm) relate to the $n \rightarrow \pi^*$ transitions of the amide groups, and the strong effects of opposite sign at 198-200 nm and ~185 nm relate to split components of the $\pi \rightarrow \pi^*$ transitions. This assignment is confirmed by the red shift of the long-wave Cotton effects characteristic for $n \rightarrow \pi^*$ transitions on passing to less polar solvents (see below, Figs. 8 and 9) and by the intermediate position of the UV absorption maximum of the $\pi \rightarrow \pi^*$ transitions (184-189 nm) between the centers of the short-wave Cotton effects. It must be mentioned that the CD curves, as a rule, are considerably less easy to interpret than the corresponding ORD curves, although in individual cases the existence of Cotton effects due to $n \rightarrow \pi^*$ transitions can be seen better on the ORD curves [for example, compounds (6), (9), (13), and (18)].

While in the UV spectra the splitting of the $\pi \rightarrow \pi^*$ absorption bands of the amide chromophores appears clearly only in compound (14), in the ORD and CD curves the splitting of this transition can be seen easily in practically all the cyclopeptides studied. It is interesting to compare the optical properties of compounds (2)-(19) with the corresponding properties for other related amides and peptides containing only amide chromophores and showing splitting of the band of the $\pi \rightarrow \pi^*$ transition: a) the dimer of L-5-methylpyrrolidin-2-one [22], (b) the methylamide of N-acetyl-L-alanine in the convoluted form stabilized by an intramolecular hydrogen bond [1], (c) cyclo-$(L\text{-}Ala)_2$ [14], (d) the hydrogenated analog of gramicidin S [23, 24], (e) poly-L-alanine in the α-helical conformation [25], and f) poly-L-serine in the β conformation [26]. As can be seen from Fig. 7, the nature of the CD curves of the majority of the compounds mentioned differs substantially from the curve of a typical representative of the alanine-glycine cyclohexapeptides – compound (9); cyclo-$(L\text{-}Ala)_2$ has a closer CD curve but does not show the effect of hypochromism (see Table 2). Thus, an analysis of the ORD and CD curves confirms the conclusion drawn in a study of the UV spectra of the realization in cyclohexapeptides in aqueous solutions of a specific conformation in which the

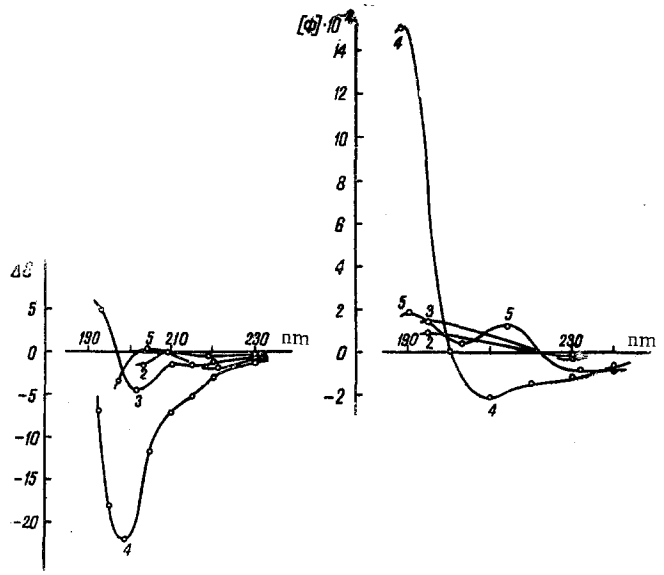

Fig. 3. CD and ORD curves of the cyclopeptides (2)-(5) in H_2O.

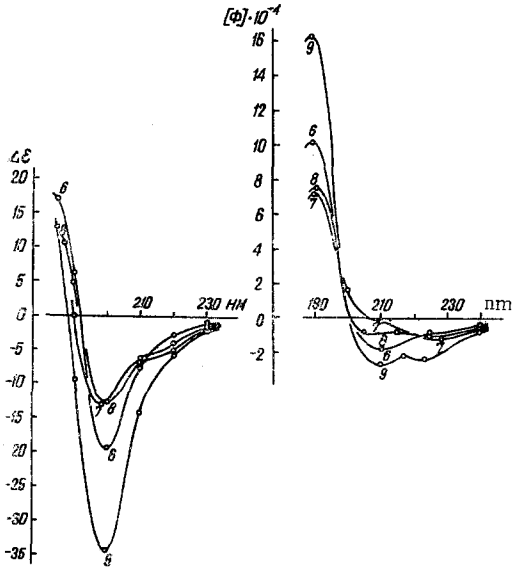

Fig. 4. CD and ORD curves of the cyclopeptides (6)-(9) in H_2O.

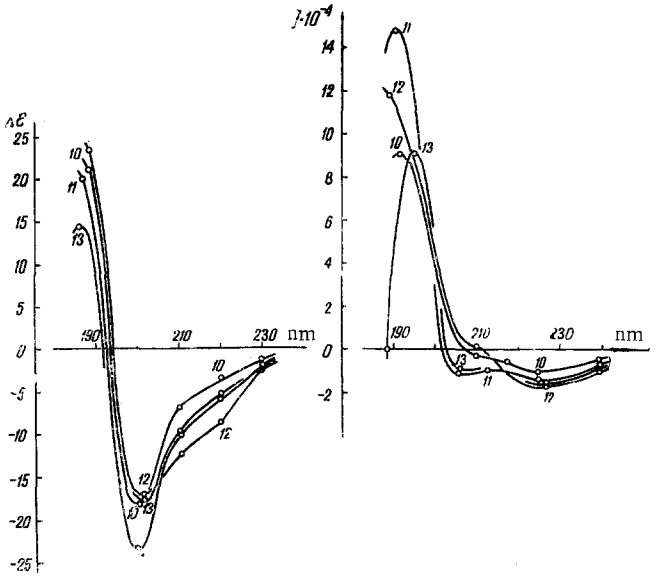

Fig. 5. CD and ORD curves of the cyclopeptides (10)-(13) in H_2O.

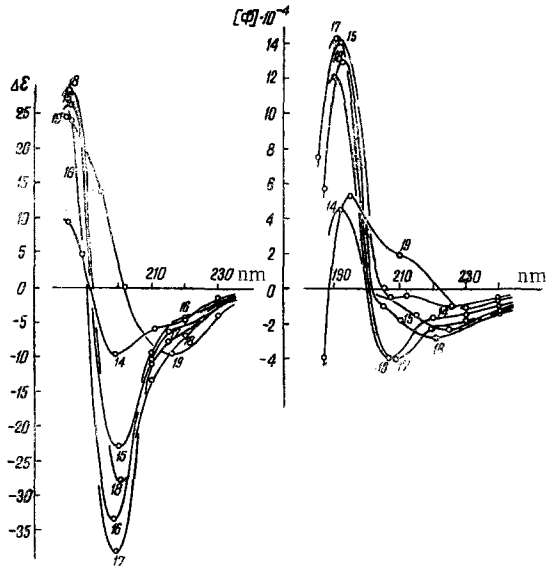

Fig. 6. CD and ORD curves of the cyclopeptides (14)-(19) in H_2O.

amino acid residues possess coordinates Φ and Ψ differing from the corresponding coordinates in other peptide-protein systems with known conformations.

In a preceding paper [1] it was observed that the introduction into a cyclohexaglycyl ring of one or two adjacent residues of L(D)-alanine was still insufficient to stabilize the chiral conformation, as is shown by the low intensity of the Cotton effects in the curves of compounds (2) and (3) [1]. However, the presence in the ring of two L-alanine residues separated by one glycine residue (4) or of three (6-9), four (10-12), etc. alanine residues leads to compounds with intense Cotton effects the absolute values of which are determined mainly not by the number or the configuration of the alanine residues but by their mutual position. On comparing with one another the CD curves of compounds (3)-(14), it may be assumed that the L-Ala–L-Ala fragments are less suitable for the realization of the type of conformation that is common for the cyclohexapeptides than the L-Ala–Gly–L-Ala fragments, as is shown, in particular, by the monotonic decrease in the intensity of the long-wave Cotton effect in the sequence of compounds (9)–(11)–(13)–(14): while (9) contains only L-Ala–Gly–L-Ala fragments, in (11) and (13) they are replaced successively by L-Ala–L-Ala, and (14) has the monotonic sequence $(L\text{-Ala–L-Ala})_3$.

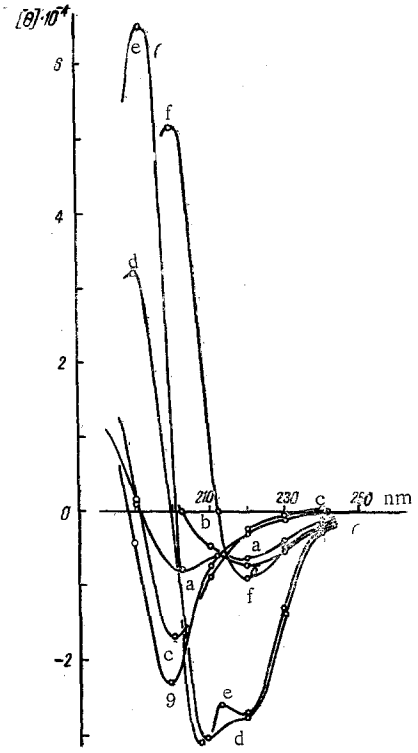

Fig. 7

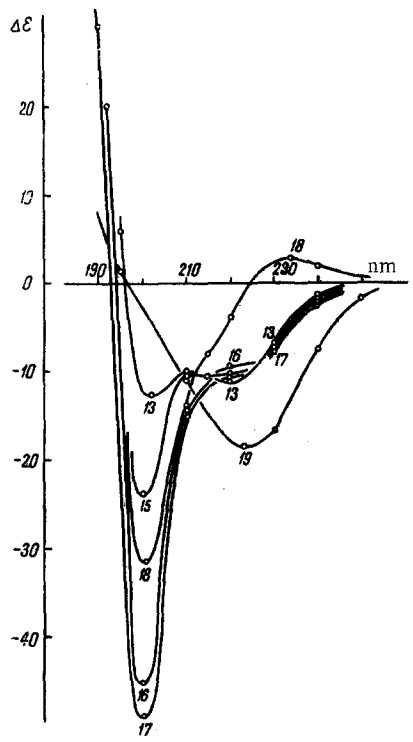

Fig. 8

Fig. 7. CD curves of amides and peptides containing only amide chromophores and showing a splitting of the band of the $\pi \rightarrow \pi^*$ transition.

Fig. 8. CD curves of the cyclopeptides (13) and (15)-(19) in absolute ethanol.

It has been shown previously that the constant b_0 in the Moffitt equation of some cyclopeptides has an extremely high value [for example, +200° for the ring (9)]. This is due to the splitting of the electronic transitions of the amide bond in the dissymmetrical cyclic structure [1]. In order to evaluate the stability of this structure, we measured the ORD curve of compound (9) in trifluoroacetic acid solution. As a rule, in this solvent the secondary structure of linear peptides is completely destroyed ($b_0 = 0$), but in the case of the ring (9) the shape of the ORD curve (measured down to 225 nm) differed only slightly from the curve in water, and the constant b_0 still retained a substantial value (+90°C), which shows the stability of the dominating conformation of the cyclohexapeptides.

The ORD curves of compound (9) in solution in 0.1 N HCl and 0.1 N NaOH do not differ in any appreciable manner from the curve given in Fig. 3. The addition of KCl to a concentration of 0.5 N also causes no change in the ORD curves, which shows the incapacity of the cyclopeptide (9) for forming ion–dipole complexes with the K^+ ion in an aqueous medium. For compound (18), the analogous measurements performed in ethanol also showed that compounds of this type do not form complexes with K^+ ions.

From the following communication [4] on the study of the cyclopeptides (1)-(19) by the NMR method, it can be seen that the presence of trans-annular intramolecular bonds stabilizing the "pleated sheet" structure is characteristic for them. Apparently, the formation of H bonds favors the approach and the fixation of the mutual orientation of the peptide chromophoric groups which, in its turn, leads to the interaction of the monotypical electronic transitions and the appearance of the splitting of the band of the $\pi \rightarrow \pi^*$ transition.

In this connection it is interesting to observe that in all cases where stable H bonds are present in peptides (models a and b in Fig. 7, the linear tetrapeptide BOC–L-Val–L-Val–L-Ala–GlyOEt in methanolic solution [27], gramicidin S [24, 28-32], and its analogs [23, 24, 33], and poly(amino acid)s in the α-helical and β conformations [25, 26]), the CD and ORD curves have a complex nature in the 185-240 nm region. In addition to this, the destruction of the H bonds in these compounds (the monomer of L-5-methyl-

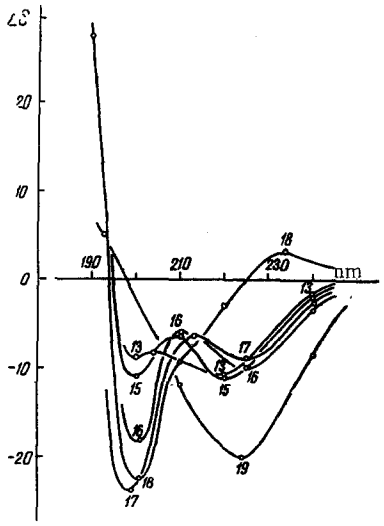

Fig. 9. CD curves of the cyclopeptides (13) and (15)-(19) in a mixture of ethanol and heptane (1:2).

pyrrolidin-2-one [22], the diamide (20), and linear peptides in the convoluted form [1, 27, 34], and the unordered conformation of poly(amino acid)s [19]) leads to the appearance of CD and ORD curves with a single Cotton effect in the region of the $\pi \rightarrow \pi^*$ transitions.

The complexity of the CD and ORD spectra for conformations with H bonds permits an approach to an explanation of the fundamental differences between the results of the ORD and CD methods, on the one hand, and of x-ray structural analysis, on the other hand, on the apparent content of α-helical sections in proteins. (Here we have in view the fact that the lack of correspondence mentioned is not a consequence of differences in the conditions of investigation, since the conformations of proteins in aqueous solutions and moist crystals apparently do not differ in any significant manner whatever [35].) For example, for α-chymotrypsin the amount of α-helices according to x-ray data is only 3% [36], while calculation on the basis of the CD and ORD characteristics has led to a value of ~15% [37]. A substantial increase in the content of helices can be explained by failure to take into account structures with H bonds the conformations of which differ from the α-helix (see, for example, [38]). Cytochrome c [39, 40] and ferricytochrome c [39] may be regarded as still more striking examples; their ORD curves in the UV region are typical for the α-helix. At the same time, x-ray structural analysis has not revealed a significant amount of α-helical sections in these proteins [41]. It appears probable that in these and similar cases the complication of the CD and ORD spectra is connected with the presence of different nonhelical conformations stabilized by H bonds between peptide groups. The features of the ORD curves that have been mentioned are also of value for measurements in the visible region and for the evaluation of the helical content in proteins from the constant b_0.

As already mentioned, cyclohexapeptides of the series studied are soluble in neutral organic solvents to an extremely limited extent, and therefore almost all the results discussed above have related to aqueous solutions. However, it is known that the conformation of oligopeptides can undergo considerable changes on passing from one solvent to another (see, for example, [1, 27, 45]). Consequently, in order to obtain more complete information on the conformational possibilities of the cyclohexapeptides it is desirable to study them over as wide a range of conditions as possible and, in the first place, in solvents of different polarities. With this aim, we have checked the solubilities of compounds (1)-(19) in ethanol and in a mixture of ethanol and heptane (1:2). It was found that for compounds (13) and (15)-(19) it is possible to reach concentrations of 0.1-2 mg/ml in these solvents which are sufficient for measurements of the CD spectra; the curves obtained are given in Figs. 8 and 9. In all cases, a red shift and an increase in the intensity of the Cotton effects of the $n \rightarrow \pi^*$ transitions as compared with aqueous solutions are observed. In the curves of compound (18) it can clearly be seen that in the 210-240 nm region there are at least two Cotton effects, which is apparently connected with the local nature of the $n \rightarrow \pi^*$ transitions [42] and with the independent contribution of the chromophoric groups of the L- and D-amino acid residues to the total CD curve. Nevertheless, it is not excluded that the complex nature of the curves in this region is also connected with the presence of several conformers having Cotton effects of opposite signs.

In the case of compounds (16)-(18), on passing from water to ethanol the intensity of the $\pi \rightarrow \pi^*$ Cotton effect rises, which shows the stabilization of the dominant conformation. However, a further decrease in polarity is accompanied by a fall in the intensity of the $\pi \rightarrow \pi^*$ Cotton effects, possibly because of the appearance in the conformational equilibrium of new conformations possessing a higher symmetry.

Thus, we have shown that the majority of cyclopeptides studied forms a monotypical chiral system in aqueous solutions which differs in its conformation from the other model peptide systems described previously; on passing to nonpolar solvents some conformational rearrangement takes place. Since the CD and ORD methods do not at the present time permit more concrete conclusions to be drawn concerning the spatial structure of the cyclopeptides, we have studied them by the method of NMR (in polar solvents) and IR spectroscopy, and also by means of dipole moments (in nonpolar solvents). The results obtained are given in subsequent communications of the present series [3-5].

In conclusion, it must be observed that until the present work only a small number of CD and ORD curves of cyclic hexapeptides taken in the region of the amide absorption bands had been reported in the literature [42-44]. At the same time, the interpretation of the available results was considerably complicated by the presence of aromatic chromophoric groups of phenylalanine, tyrosine and histidine residues, which have strong absorption bands in the 185-210 nm region. Since the compounds that we have studied are the simplest representatives of the class of cyclic hexapeptides, the facts given above may serve as a basis for spectral assignments in a series of more complex cyclohexapeptide systems, and also for the search for qualitative relationships between the conformation of peptide–protein systems and their optical properties.

EXPERIMENTAL

The synthesis of compounds (1)-(19) and (21) has been described previously [1, 2]; the methylamide of acetylglycine (20) was synthesized in our laboratory by P. V. Kostetskii by the action of methylamine on the methyl ester of acetylglycine, mp 156-157°C (see [6]).

Before the physicochemical measurements, the compounds (1)-(21) obtained in the individual state were dried over P_2O_5 at 50°C/0.5 mm for 16 h. The ultraviolet spectra were taken on Cary-15 and Cary-16 instruments in aqueous solutions with layer thicknesses of 0.01-0.1 cm and concentrations of $(0.1\text{-}3) \cdot 10^{-3}$ M. According to the results of measurements of ε_{max} at different concentrations, the level of scattered light amounted to ~0.001%. The values of λ_{max} and ε_{max} are the means of 3-6 measurements, and the dipole strengths (D), the oscillator strengths (f), and the half-width of the bands (Δ) given in Table 1 were also calculated from the absorption curves. The CD curves were measured on a JOUAN-11 dichrograph and a Cary-60 spectropolarimeter with a Cary-6001 attachment for obtaining CD spectra at concentrations of the solutions of $(0.2\text{-}5) \cdot 10^{-3}$ M and at a temperature of 23-26°C, the thickness of the cell being 0.01-2 cm. The ORD curves were taken on a Cary-60 spectropolarimeter under conditions analogous to those for the measurements of the CD curves. The values of $\Delta\varepsilon$ and $[\Phi]$ given are the means of 2-4 measurements and were calculated without correction for the refractive index of the solvent. The solutions of compounds (13)-(19) in absolute ethanol were obtained by boiling them with stirring for 6 h and filtering off the undissolved excess of cyclopeptide. The concentrations of the solutions were determined by two methods: a) by weighing the residue after the evaporation of the ethanol and drying over P_2O_5 at 50°C/0.5 mm to constant weight, and b) by diluting the ethanolic solution twofold with water, measuring the ORD curve, and comparing it with the ORD curve in a mixture of ethanol and water (1:1) obtained in the usual way. The errors of the measurements of the concentrations did not exceed 10-15%. The solutions in ethanol–heptane (1:2) were obtained by diluting saturated ethanolic solutions with two volumes of heptane. When large amounts of heptane were added, in some cases the cyclopeptides precipitated.

Compound 2, CD, H_2O: $\Delta\varepsilon_{240}$ 0, $\Delta\varepsilon_{230}$ – 0.25; $\Delta\varepsilon_{220}$ – 0.49 (trough); $\Delta\varepsilon_{209}$ 0 (peak); $\Delta\varepsilon_{203}$ – 1.56 (!) ORD, H_2O: $[\Phi]_{235}$ – 1000; $[\Phi]_{230}$ – 2000 (trough); $[\Phi]_{221}$ 0; $[\Phi]_{210}$ + 4500; $[\Phi]_{200}$ + 8000; $[\Phi]_{195}$ + 9000 (peak).

Compound 3, CD, H_2O: $\Delta\varepsilon_{240}$ – 0.08; $\Delta\varepsilon_{230}$ – 0.47; $\Delta\varepsilon_{220}$ – 1.25; $\Delta\varepsilon_{215}$ – 1.41 (shoulder); $\Delta\varepsilon_{210}$ – 1.5; $\Delta\varepsilon_{201,5}$ – 4.5 (trough); $\Delta\varepsilon_{197}$ 0; $\Delta\varepsilon_{193}$ +5 (!) ORD, H_2O: $[\Phi]_{235}$ – 1500; $[\Phi]_{230}$ – 3000 (trough); $[\Phi]_{222}$ 0; $[\Phi]_{220}$ +2000; $[\Phi]_{210}$ + 8000; $[\Phi]_{200}$ + 13 000 $[\Phi]_{195}$ + 14 000 (peak).

Compound 4, CD, H_2O: $\Delta\varepsilon_{240}$ – 0,5; $\Delta\varepsilon_{230}$ – 1.1; $\Delta\varepsilon_{220}$ – 3.0; $\Delta\varepsilon_{215}$ – 5.25 (shoulder); $\Delta\varepsilon_{210}$ – 7.0; $\Delta\varepsilon_{199}$ – 22 (trough); $\Delta\varepsilon_{192}$ – 7.0 (!) ORD, H_2O: $[\Phi]_{240}$ – 7000; $[\Phi]_{230}$ – 13 000 (shoulder); $[\Phi]_{220}$ – 15 000; $[\Phi]_{210}$ – 21 500 (trough); $[\Phi]_{200}$ 0; $[\Phi]_{188,5}$ + 150 000 (peak).

Compound 5, CD, H_2O: $\Delta\varepsilon_{240}$ – 0.23; $\Delta\varepsilon_{230}$ – 1.08; $\Delta\varepsilon_{221}$ – 1.84 (trough); $\Delta\varepsilon_{210}$ 0; $\Delta\varepsilon_{204}$ + 0.25 (peak); $\Delta\varepsilon_{202}$ 0; $\Delta\varepsilon_{197}$ – 3,5 (!) ORD, H_2O: $[\Phi]_{234}$ – 9500 (trough); $[\Phi]_{222}$ 0; $[\Phi]_{214}$ + 12 000 (peak); $[\Phi]_{203}$ + 4000 (trough); $[\Phi]_{190}$ + 18 000 (peak).

Compound 6, CD, H_2O: $\Delta\varepsilon_{240}$ – 0,25; $\Delta\varepsilon_{230}$ – 1,0; $\Delta\varepsilon_{220}$ – 2,75; $\Delta\varepsilon_{210}$ – 14; $\Delta\varepsilon_{200}$ – 19.5 (trough); $\Delta\varepsilon_{192}$ 0; $\Delta\varepsilon_{185}$ + 16.75 (!). ORD, H_2O: $[\Phi]_{240}$ – 4500; $[\Phi]_{227}$ – 9000 (shoulder); $[\Phi]_{220}$ – 11 000; $[\Phi]_{210}$ – 18 000 (trough); $[\Phi]_{200}$ 0; $[\Phi]_{189}$ + 101 000 (peak).

Compound 7, CD, H_2O: $\Delta\varepsilon_{240}$ – 0.20; $\Delta\varepsilon_{230}$ – 1,8; $\Delta\varepsilon_{220}$ – 5,0; $\Delta\varepsilon_{215}$ – 6,0 (shoulder); $\Delta\varepsilon_{210}$ – 6.25; $\Delta\varepsilon_{198}$ – 12.75 (trough); $\Delta\varepsilon_{190}$ 0.

ORD, H_2O: $[\Phi]_{240} - 7000$; $[\Phi]_{228} - 11\,000$ (trough); $[\Phi]_{220} - 9500$; $[\Phi]_{210} - 2000$ (shoulder); $[\Phi]_{205}$ 0; $[\Phi]_{200} + 16\,000$; $[\Phi]_{190} + 72000$ (peak).

Compound **8**, CD, H_2O: $\Delta\varepsilon_{240} - 0.12$; $\Delta\varepsilon_{230} - 1{,}37$; $\Delta\varepsilon_{220} - 3{,}75$; $\Delta\varepsilon_{215} - 5.25$ (shoulder); $\Delta\varepsilon_{210} - 6{,}25$; $\Delta\varepsilon_{200} - 12.5$ (trough); $\Delta\varepsilon_{192}$ 0; $\Delta\varepsilon_{187} + 10{,}75$ (!) ORD, H_2O: $[\Phi]_{240} - 6000$; $[\Phi]_{225} - 10\,500$ (trough); $[\Phi]_{214} - 8000$ (peak); $[\Phi]_{205} - 9000$ (trough); $[\Phi]_{200}$ 0; $[\Phi]_{190,5} + 75\,000$ (peak).

Compound **9**, CD, H_2O: $\Delta\varepsilon_{240} - 0.5$; $\Delta\varepsilon_{230} - 2.0$; $\Delta\varepsilon_{220} - 5{,}75$; $\Delta\varepsilon_{210} - 14.3$; $\Delta\varepsilon_{199,5} - 34{,}25$ (trough); $\Delta\varepsilon_{190} - 8.0$; $\Delta\varepsilon_{187,5}$ 0; $\Delta\varepsilon_{185} + 12{,}75$ (!). ORD, H_2O: $[\Phi]_{240} - 8000$; $[\Phi]_{230} - 18\,000$; $[\Phi]_{223} - 24\,000$ (trough); $[\Phi]_{217} - 22\,000$ (peak); $[\Phi]_{210} - 27\,000$ (trough); $[\Phi]_{200}$ 0; $[\Phi]_{189} + 162\,000$ (peak).

Compound **10**, CD, H_2O: $\Delta\varepsilon_{240} - 0{,}25$; $\Delta\varepsilon_{230} - 1{,}43$; $\Delta\varepsilon_{220} - 3{,}58$ $\Delta\varepsilon_{215} - 5.19$ (shoulder); $\Delta\varepsilon_{210} - 6.98$; $\Delta\varepsilon_{200} - 18{,}5$ (trough); $\Delta\varepsilon_{193}$ 0; $\Delta\varepsilon_{189} + 23$ (!). ORD, H_2O: $[\Phi]_{240} - 4000$; $[\Phi]_{225} - 10\,500$ (trough); $[\Phi]_{220} - 8500$; $[\Phi]_{210} - 2500$ (shoulder); $[\Phi]_{207}$ 0; $[\Phi]_{191} + 91\,000$ (peak);

Compound **11**, CD, H_2O: $\Delta\varepsilon_{240} - 0{,}4$; $\Delta\varepsilon_{230} - 2.17$; $\Delta\varepsilon_{220} - 5.91$; $\Delta\varepsilon_{215} - 7.5$ (shoulder); $\Delta\varepsilon_{210} - 10{,}25$; $\Delta\varepsilon_{199} - 23{,}5$ (trough); $\Delta\varepsilon_{192}$ 0; $\Delta\varepsilon_{187} + 20.7$ (!). ORD, H_2O: $[\Phi]_{240} - 7000$; $[\Phi]_{225} - 15\,000$ (trough); $[\Phi]_{213} - 9500$ (peak); $[\Phi]_{206} - 11\,500$ (trough); $[\Phi]_{201,5}$ 0; $[\Phi]_{190} + 148\,000$ (peak).

Compound **12**, CD, H_2O: $\Delta\varepsilon_{240} - 0.5$; $\Delta\varepsilon_{230} - 2.6$; $\Delta\varepsilon_{220} - 8.75$ (shoulder); $\Delta\varepsilon_{210} - 12.5$; $\Delta\varepsilon_{201} - 17{,}25$ (trough); $\Delta\varepsilon_{194}$ 0; $\Delta\varepsilon_{189} + 23.5$ (!). ORD, H_2O: $[\Phi]_{240} - 10\,000$; $[\Phi]_{227} - 18\,000$; $[\Phi]_{220} - 15\,000$; $[\Phi]_{211,5}$ 0; $[\Phi]_{210} + 1500$ (shoulder); $[\Phi]_{189} + 118\,000$ (!).

Compound **13**. CD, H_2O: $\Delta\varepsilon_{240} - 0.25$; $\Delta\varepsilon_{230} - 2.25$; $\Delta\varepsilon_{220} - 5.5$ (shoulder); $\Delta\varepsilon_{210} - 10$; $\Delta\varepsilon_{200} - 18.5$ (trough); $\Delta\varepsilon_{192}$ 0; $\Delta\varepsilon_{185} + 14{,}5$ (peak). CD, C_2H_5OH: $\Delta\varepsilon_{240} - 1.75$; $\Delta\varepsilon_{230} - 7.0$; $\Delta\varepsilon_{220} - 11$ (trough); $\Delta\varepsilon_{210} - 10$ (peak); $\Delta\varepsilon_{202} - 12.5$ (trough); $\Delta\varepsilon_{196}$ 0; $\Delta\varepsilon_{195} + 6{,}0$ (!). CD, $C_2H_5OH - C_7H_{16}$ (1:2): $\Delta\varepsilon_{240} - 1.75$; $\Delta\varepsilon_{230} - 7.0$; $\Delta\varepsilon_{220} - 10.75$ (trough); $\Delta\varepsilon_{210} - 9{,}25$; $\Delta\varepsilon_{204} - 8{,}25$ (peak); $\Delta\varepsilon_{200} - 8{,}75$ (trough); ORD, H_2O: $[\Phi]_{240} - 8500$; $[\Phi]_{227} - 11\,600$ (trough); $[\Phi]_{213} - 9000$ (peak); $[\Phi]_{205} - 9500$ (trough); $[\Phi]_{203}$ 0; $[\Phi]_{195} + 92\,000$ (peak); $[\Phi]_{188}$ 0 (!).

Compound **14**, CD, H_2O: $\Delta\varepsilon_{240} - 0{,}25$; $\Delta\varepsilon_{230} - 1.75$; $\Delta\varepsilon_{220} - 4.5$ (shoulder); $\Delta\varepsilon_{210} - 6.0$; $\Delta\varepsilon_{199} - 9.5$ (trough); $\Delta\varepsilon_{191,5}$ 0; $\Delta\varepsilon_{185} + 9.25$ (!). ORD, H_2O: $[\Phi]_{240} - 5000$; $[\Phi]_{226} - 10\,000$ (trough); $[\Phi]_{220} - 7500$; $[\Phi]_{212} - 3500$ (peak); $[\Phi]_{207} - 5000$ (trough); $[\Phi]_{201,5}$ 0; $[\Phi]_{192} + 45\,000$ (peak).

Compound **15**, CD, H_2O: $\Delta\varepsilon_{240} - 0{,}3$; $\Delta\varepsilon_{230} - 2{,}53$; $\Delta\varepsilon_{220} - 6.32$; $\Delta\varepsilon_{215} - 8.21$ (shoulder); $\Delta\varepsilon_{210} - 10{,}53$; $\Delta\varepsilon_{200} - 22{,}5$ (trough); $\Delta\varepsilon_{191,5}$ 0; $\Delta\varepsilon_{186} + 26{,}25$ (peak). CD, C_2H_5OH: $\Delta\varepsilon_{240} - 2.0$; $\Delta\varepsilon_{230} - 7.25$; $\Delta\varepsilon_{220} - 10.25$ (trough); $\Delta\varepsilon_{210} - 10{,}2$ (peak); $\Delta\varepsilon_{200} - 23{,}75$ (trough); CD, $C_2H_5OH - C_7H_{16}$; (1:2): $\Delta\varepsilon_{240} - 2.0$; $\Delta\varepsilon_{230} - 7.5$; $\Delta\varepsilon_{220} - 11.0$ (trough); $\Delta\varepsilon_{210} - 6{,}5$ (peak); $\Delta\varepsilon_{200} - 10.75$ (trough); $\Delta\varepsilon_{195}$ 0 (!). ORD, H_2O: $[\Phi]_{240} - 12\,000$; $[\Phi]_{225} - 23\,000$ (trough); $[\Phi]_{220} - 21\,000$; $[\Phi]_{210} - 9000$; $[\Phi]_{207} - 5000$ (shoulder); $[\Phi]_{205}$ 0; $[\Phi]_{192} + 140\,000$ (peak).

Compound **16**, CD, H_2O: $\Delta\varepsilon_{240} - 0.3$; $\Delta\varepsilon_{230} - 1{,}96$; $\Delta\varepsilon_{220} - 4{,}12$ (shoulder); $\Delta\varepsilon_{210} - 9.31$; $\Delta\varepsilon_{198} - 33.3$ (trough); $\Delta\varepsilon_{191}$ 0; $\Delta\varepsilon_{185} + 22{,}5$ (!). CD, C_2H_5OH: $\Delta\varepsilon_{240} - 2.0$; $\Delta\varepsilon_{230} - 7.5$; $\Delta\varepsilon_{220} - 9{,}5$ (shoulder); $\Delta\varepsilon_{210} - 14{,}5$; $\Delta\varepsilon_{200} - 45$ (trough); $\Delta\varepsilon_{194}$ 0; $\Delta\varepsilon_{192} + 20$ (!). CD, $C_2H_5OH - C_7H_{16}$ (1:2): $\Delta\varepsilon_{240} - 3.5$; $\Delta\varepsilon_{230} - 9.0$; $\Delta\varepsilon_{225} - 10$ (trough); $\Delta\varepsilon_{220} - 9.0$; $\Delta\varepsilon_{210} - 6.0$ (peak); $\Delta\varepsilon_{201} - 18.25$ (trough). ORD, H_2O: $[\Phi]_{240} - 10\,000$; $[\Phi]_{230} - 14\,000$; $[\Phi]_{224} - 16\,500$ (shoulder); $[\Phi]_{220} - 17\,500$; $[\Phi]_{207} - 39\,000$ (trough); $[\Phi]_{200}$ 0; $[\Phi]_{190} + 120\,000$ (peak).

Compound **17**, CD, H_2O: $\Delta\varepsilon_{230} - 2.75$; $\Delta\varepsilon_{220} - 5{,}5$ (shoulder); $\Delta\varepsilon_{210} - 11.0$; $\Delta\varepsilon_{199} - 38.5$ (trough); $\Delta\varepsilon_{191}$ 0 (!). CD, C_2H_5OH: $\Delta\varepsilon_{240} - 2.5$; $\Delta\varepsilon_{230} - 7{,}5$; $\Delta\varepsilon_{220} - 10.5$ (shoulder); $\Delta\varepsilon_{210} - 15.0$; $\Delta\varepsilon_{200} - 48{,}75$ (trough); $\Delta\varepsilon_{192}$ 0; $\Delta\varepsilon_{190} + 27.5$ (!). CD, $C_2H_5OH - C_7H_{16}$: (1:2): $\Delta\varepsilon_{240} - 2{,}75$; $\Delta\varepsilon_{230} - 8.0$; $\Delta\varepsilon_{225} - 8.75$ (trough); $\Delta\varepsilon_{213} - 6{,}25$ (peak); $\Delta\varepsilon_{199} - 23{,}75$ (trough); ORD, H_2O: $[\Phi]_{240} - 13\,000$; $[\Phi]_{230} - 19\,000$; $[\Phi]_{225} - 21\,000$ (shoulder);

$[\Phi]_{220} - 23\,000$; $[\Phi]_{208,5} - 40\,000$ (trough); $[\Phi]_{201}$ 0; $[\Phi]_{191} + 142\,000$ (peak).

Compound **18**, **CD, H_2O:** $\Delta\varepsilon_{240} - 0{,}1$; $\Delta\varepsilon_{230} - 2.0$; $\Delta\varepsilon_{220} - 7.1$ (shoulder); $\Delta\varepsilon_{210} - 13.5$; $\Delta\varepsilon_{201} - 28.0$ (trough); $\Delta\varepsilon_{192}$ 0; $\Delta\varepsilon_{186} + 28.25$ (peak). **CD, C_2H_5OH:** $\Delta\varepsilon_{250} + 0.75$; $\Delta\varepsilon_{240} + 2.0$; $\Delta\varepsilon_{234} + 2.75$ (peak); $\Delta\varepsilon_{225}$ 0; $\Delta\varepsilon_{220} - 4{,}0$; $\Delta\varepsilon_{205} - 8{,}0$ (shoulder); $\Delta\varepsilon_{210} - 13.75$; $\Delta\varepsilon_{201} - 31.25$ (trough); **CD, $C_2H_5OH - C_7H_{16}$** (1:2): $\Delta\varepsilon_{240} + 2.0$; $\Delta\varepsilon_{233,5} + 3.0$; $\Delta\varepsilon_{225}$ 0; $\Delta\varepsilon_{215} - 6.25$ (shoulder); $\Delta\varepsilon_{210} - 9.25$; $\Delta\varepsilon_{200,5} - 22.5$ (trough); $\Delta\varepsilon_{194}$ 0; $\Delta\varepsilon_{190} + 27.5$ (!) **ORD, H_2O:** $[\Phi]_{240} - 14\,000$; $[\Phi]_{230} - 22\,000$; $[\Phi]_{221} - 27\,500$ (trough); $[\Phi]_{210} - 18\,000$; $[\Phi]_{205} - 10\,000$ (shoulder); $[\Phi]_{201,5}$ 0; $[\Phi]_{193} + 129\,000$ (peak).

Compound **19**, **CD, H_2O:** $\Delta\varepsilon_{230}$ 4.0; $\Delta\varepsilon_{216} - 9.5$ (trough); $\Delta\varepsilon_{202}$ 0; $\Delta\varepsilon_{187} + 25$ (!). **CD, C_2H_5OH:** $\Delta\varepsilon_{250} - 1.75$; $\Delta\varepsilon_{240} - 7.5$; $\Delta\varepsilon_{230} - 16.5$; $\Delta\varepsilon_{223} - 18{,}25$ (trough); $\Delta\varepsilon_{210} - 11.0$; $\Delta\varepsilon_{200} - 2.5$; $\Delta\varepsilon_{196}$ 0; $\Delta\varepsilon_{195} + 1.25$ (!). **CD, $C_2H_5OH - C_7H_{16}$** (1:2): $\Delta\varepsilon_{240} - 8{,}75$; $\Delta\varepsilon_{230} - 17.5$; $\Delta\varepsilon_{224} - 20$ (trough); $\Delta\varepsilon_{210} - 12.0$; $\Delta\varepsilon_{198}$ 0; $\Delta\varepsilon_{192} + 5$ (!). **ORD, H_2O:** $[\Phi]_{240} - 7000$; $[\Phi]_{230} - 11\,000$ (trough); $[\Phi]_{222}$ 0; $[\Phi]_{210} + 20\,000$ (shoulder); $[\Phi]_{200} + 39\,000$: $[\Phi]_{195} + 53\,000$ (peak); $[\Phi]_{188}$ 0; $[\Phi]_{187} - 40\,000$ (!).

SUMMARY

1. The UV spectra of aqueous solutions of cyclohexapeptides constructed of L(D)-alanine and glycine residues exhibit a considerable (about 45%) hypochromism of the band of the $\pi \rightarrow \pi^*$ transition which has hitherto been observed among peptide-protein systems only in α-helical polypeptides.

2. In water, almost all the polypeptides studied have monotypical CD and ORD curves characterized by two Cotton effects of opposite sign in the region of the $\pi \rightarrow \pi^*$ transition.

3. The features of the optical properties of the cyclohexapeptides are connected with the exciton interaction of the amide chromophores in the "pleated sheet" conformation. The neglect of such interactions may lead to high results in the determination of the degree of helicity in proteins from CD and ORD observations.

4. On passing to less polar solvents, there is a redistribution of the intensities of the Cotton effects connected with a conformational rearrangement of the cyclohexapeptides.

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