STUDY OF THE CONFORMATIONAL STATES OF CYCLOPEPTIDE SYSTEMS

X. SPATIAL STRUCTURE OF CYCLOHEXAPEPTIDES CONSTRUCTED OF L-VALINE, L-LEUCINE, L-NORVALINE, AND GLYCINE RESIDUES

V. T. Ivanov, I. A. Lavrinovich,
G. A. Kogan, E. A. Meshcheryakova,
S. L. Portnova, M. D. Isabaev,
P. V. Kostetskii, and Yu. A. Ovchinnikov

UDC 547.96

In communications [1-6] of the present series we have given the results of an investigation of the conformational states of cyclic hexapeptides containing L(D)-alanine and glycine residues in polar media (water, dimethyl sulfoxide) [1-4], in nonpolar media [chloroform, heptane–ethanol (2:1)] [2, 5], and in the crystalline state [6]. For all the compounds of this series several main conformers existing in rapid equilibrium have been found.

It has been established that in polar media and in the crystalline state "pleated sheet" structures with two intramolecular hydrogen bonds (intraHBs) of the 4 → 1 type closing 10-membered rings ("β inflections") and formed by the CO and NH groups of oppositely located amino-acid residues (Fig. 1) predominate. It has also been shown that all three possible "pleated sheet" structures differing by the position of the intraHBs (i.e., formed by the pairs 1-4, 2-5, and 3-6) participate in the conformational equilibrium. At the same time, it follows from the NMR spectra (in association with a theoretical conformational analysis) that each of the three structures can exist in the following two basic forms: I) with the orientation of the carbonyl groups located between the "angular" C_α atoms (C_2 and C_3, C_5 and C_6) downwards with respect to the plane of Fig. 1;

	Ala (1,4)	*Ala* (2,5)	*Ala* (3,6)
Φ	−150±30	−60±20	−90±30
Ψ	150±30	−30±30	0±40

II) with the orientation of the same carbonyl groups above the mean plane of the ring*

	Ala (1,4)	*Ala* (2,5)	*Ala* (3,6)
Φ	−150±30	− 60±20	80±30
Ψ	150±30	100±30	0±40

In the crystalline state form (I) is preferred (results of x-ray structural analysis [7, 8] and IR spectroscopy [6]), and in nonpolar media two additional intraHBs of the 3 → 1 type are formed [5].

In the present paper we describe the conformational states of the cyclopeptides (1)-(7), differing from those considered above by the presence of more voluminous side chains. In this connection it was borne in mind that an increase in the size of an aliphatic radical can, on the one hand, increase the solubility of the cyclopeptide in nonpolar solvents and can, on the other hand, decrease the number of conformers participating in the equilibrium and thereby greatly facilitate the interpretation of spectral characteristics. As will be shown below, this was confirmed for compound (4) (increased solubility in $CHCl_3$) and for the valine-containing cyclopeptides (2) and (7) (conformational "uniformity").

* For the conformational nomenclature of the peptide, see [22].

M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 202-211, March-April, 1975. Original article submitted August 27, 1973.

Cyclo-(-L-Nva-Gly-Gly-L-Nva-Gly-Gly-) (1)
Cyclo-(-L-Val-Gly-Gly-L-Val--Gly-Gly-) (2)
Cyclo-(-L-Nva-Gly-L-Nva-Gly-L-Nva-Gly-) (3)
Cyclo-(-L-Leu-Gly-L-Leu-Gly-L-Leu-Gly-) (4)
Cyclo-(-L-Val-Gly-L-Val-Gly-L-Val-Gly-) (5)
Cyclo-(-L-Nva-L-Nva-Gly-L-Nva-L-Nva-Dly-) (6)
Cyclo-(-L-Val-L-Val-Gly-L-Val-L-Val-Gly-) (7)

Let us first consider information on compounds (3)-(7) (Figs. 2 and 3). By comparing the corresponding CD curves it is easy to see that the replacement of methyl side chains (Ala) for propyl (Nva) of isobutyl (Leu) side chains containing no branchings at the C^{β} atoms scarcely affects the conformational states of cyclopeptides (8) and (9) in aqueous solutions. The replacement of the methyl groups of compound (9) by sterically hindered isopropyl groups likewise does not change the overall form of the CD curve, but it leads to a sharp increase in its intensity (curve 3 in Fig. 3). This effect is probably due to a decrease in the number of conformers in the equilibrium mixture and to the displacement of the equilibrium in the direction of some particular "pleated sheet" structure;* the tendency to an increase in the energy differentiation of the forms in the presence of valine residues has been observed previously in linear peptides [9, 10].

The behavior of cyclopeptide (5) is extremely peculiar. In aqueous trifluoroethanol (~70 mole % of water), its CD curve (curve 3 in Fig. 2) is of the same type as the curves of the alanylglycine cyclopeptides, but transition to pure trifluoroethanol is accompanied by a marked change in the nature of the curve. The curve formed practically coincides in the position and sign of the dichroic absorption bands, and also the ratio of their intensities, with the CD curves of polypeptides in the antiparallel β conformation [11]. Such a close similarity of the CD curves is a strong piece of evidence in favor of the association of the molecules of the cyclopeptide (5) with the formation of polymeric chains constructed in the manner of the antiparallel β structure (Fig. 4). The presence of associates was shown by measuring the CD curves of compound (5) in trifluoroethanol at various concentrations: Dilution of the solution led to an appreciable change in the curve and to an approach of its shape to that of the CD curve in aqueous solutions (Fig. 5). The tendency to association explains the lower solubility of this compound than of the other cyclopeptides. The considerably higher tendency of the valine peptides to intermolecular interaction than of the alanine peptides has been demonstrated by using as examples the diamides AcValNHMe and AcAlaNHMe [12], and also the oligopeptides $BOCIle_nOMe$ and $BOCAla_nOR$ [13, 14]. It is difficult to state to what extent the spatial structure of the cyclopeptide (5) changes when it is included in a polymeric formation. In any case, the change in the shape of the curve still does not show great conformational rearrangement, since the increase in the number of interacting chromophoric groups itself leads to a sharp change in optical activity [15].

By considering the facts presented it is possible to explain a number of features of the CD curves which have hitherto not been capable of interpretation. The nature of the change in the CD curves of compounds (3), (4), (6), and (7) on passing from aqueous solutions to trifluoroethanol or [with the exception of compound (4)] ethanol also shows association, although not so effectively as in the case of [(L-Val-Gly)$_3$] [5]. Intermolecular interactions are observed only in an extremely narrow range of polarities of the solvent, since the addition of dioxane or heptane to the alcohols leads to a destruction of the antiparallel β-structure (compare curves 1, 2, and 7 in Fig. 2a; 1, 8, 9 in Fig. 2b; 1 and 6 in Fig. 2c; and 1 and 5 in Fig. 3b). In these circumstances, the intermolecular H bonds are apparently replaced by intramolecular H bonds, as is indicated by the similarity of the CD curves arising to the curves of the previously studied diastereomeric cyclohexaalanyls in heptane–ethanol (2 : 1) [2], in which connection, likewise, the presence of additional intraHBs is assumed [5].

The IR spectrum of compound (4), the only one of the whole series (1)-(7) giving sufficient solubility in $CHCl_3$, is also similar to the spectra of the cyclohexaalanyls mentioned [for example, the cyclopeptide (11), Fig. 6] and shows the presence of 3-4 intraHBs (from a calculation of integral intensities, as described previously [5]).

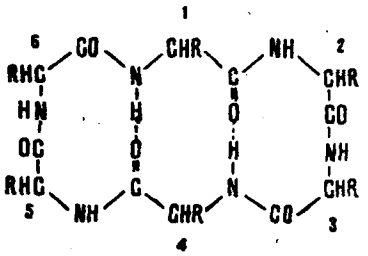

Fig. 1. Positions of the intramolecular hydrogen bonds in the "pleated sheet" structure.

*It is not excluded that the increase in the intensity of the CD curve in this case is partially connected with the presence of appreciable amounts (~30 mole %) of trifluoroethanol. In the case of compounds (3) and (6) (~15 mole % of trifluoroethanol), and also of (4) (~30 mole % of ethanol) no such effect is observed.

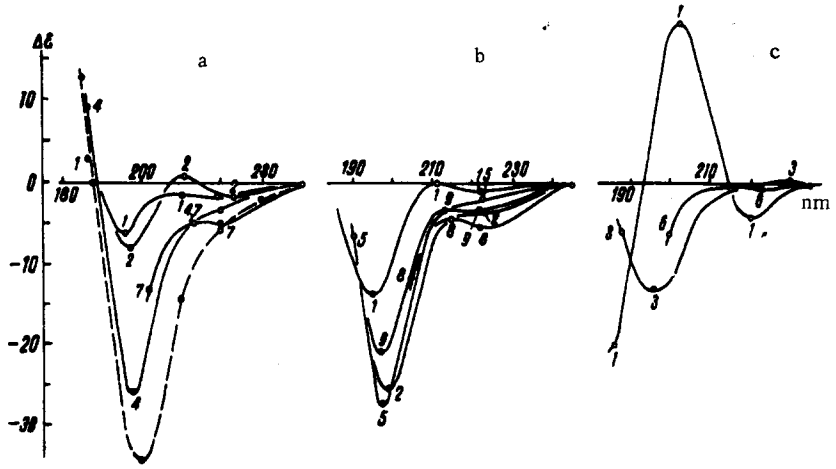

Fig. 2. CD curves of the cyclopeptides: a) compound (3); b) compound (4); c) compound (5): 1) CF_3CH_2OH, 2) C_2H_5OH, 3) $CF_3CH_2OH-H_2O$ (3 : 1), 4) $CF_3CH_2OH_2-H_2O$ (4 : 3), 5) $C_2H_5OH-H_2O$ (4 : 3), 6) $CF_3CH_2OH-C_4H_8O_2$ (3 : 1), 7) $CF_3CH_2OH-C_4H_8O_2$ (4 : 3), 8) $C_2H_5OH-C_4H_3C_2$ (1 : 3), 9) $C_2H_5OH-C_7H_{16}$ (4 : 3). The dashed lines show the CD curve of the cyclopeptide [(L-Ala-Gly)$_3$] (8) in water [2].

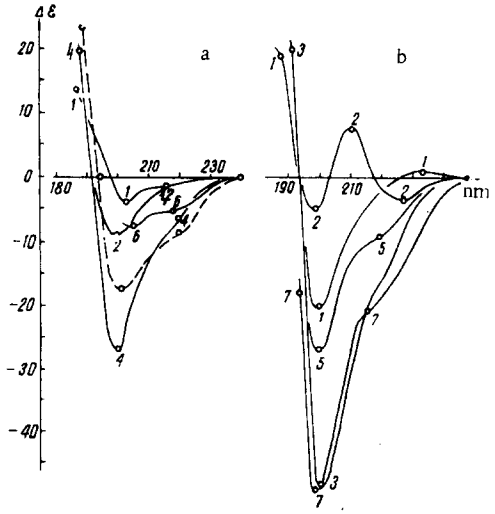

Fig. 3. CD curves of the cyclopeptides: a) compound (6); b) compound (7): 1) CF_3CH_2OH, 2) C_2H_5OH, 3) $CF_3CH_5OH-H_2O$ (3 : 1), 4) $CF_2CH_2OH-H_2O$ (4 : 3), 5) $CF_3CH_2OH-C_4H_8O_2$ (3 : 1), 6) $CF_3CH_2OH-C_4H_8O_2$ (4 : 3), 7) $CF_3CH_2OH-CF_2COOH$ (6 : 1). The dashed line shows the CD curve of the cyclopeptide [(L-Ala-L-Ala-Gly)$_3$] (9) in water [2].

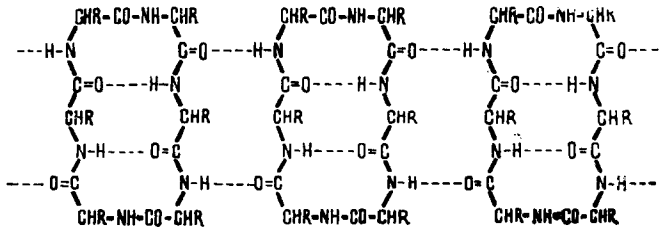

Fig. 4. Association of cyclohexapeptides in trifluoroethanol.

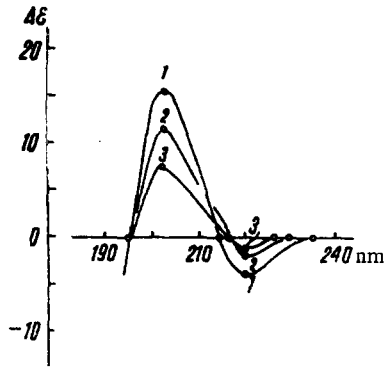

Fig. 5. CD curves of compound (5) in trifluoroethanol: 1) $c = 3.11 \cdot 10^{-4}$ M; 2) $c = 7.78 \cdot 10^{-5}$ M; 3) $c = 1.95 \cdot 10^{-5}$ M.

The CD curves of cyclo[−(L−Nva−Gly_2)$_2$−] (1) differ only insignificantly from the curve of the corresponding alanine cyclopeptide cyclo[−(L−Ala−Gly_2)$_2$−] (10); in cyclo[−(L−Val−Gly_2)$_2$−] (2) the general form of the CD curve is preserved but the intensity of the dichroic absorption bands has increased (Fig. 7). The results obtained show the retention of a common conformational type in the series of compounds (10) and (1)-(2); this structure, substantially differing from the structure of the other cyclopeptides, is most clearly expressed in the last-mentioned compound. Some similarity of the CD curves of the associated form of the cyclopeptide (5) (curve 1 in Fig. 2c) with the curves of compound (2) (Fig. 7b) is connected with the association of the latter, as is shown by the weak dependence of its CD curves on the solvent. The NMR spectra of compounds (1) and (2), which are considered below, likewise do not show the presence of strong intermolecular H bonds in them.

NMR SPECTRA

Because of the poor solubility of compounds (1)-(7), the majority of the NMR spectra were taken in trifluoroacetic acid. Compounds (1), (2), and (4) were also studied in dimethyl sulfoxide (Table 1). The general pattern of the spectra of compounds (3)-(7) and the $^3J_{NH-CH}$ constants found are extremely similar to the parameters

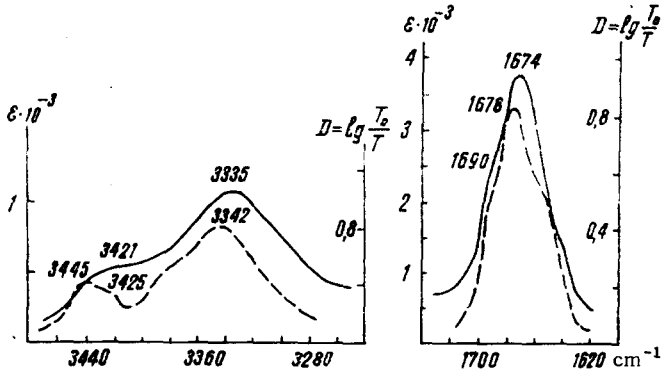

Fig. 6. IR spectrum of the cyclopeptide (4) in $CHCl_3$ (on the scale of molecular extinction, ε). The dashed line shows the IR spectrum of the cyclopeptide [(L−Ala−L−Ala−D−Ala)$_2$] (11) (in the scale of optical densities, D) [5].

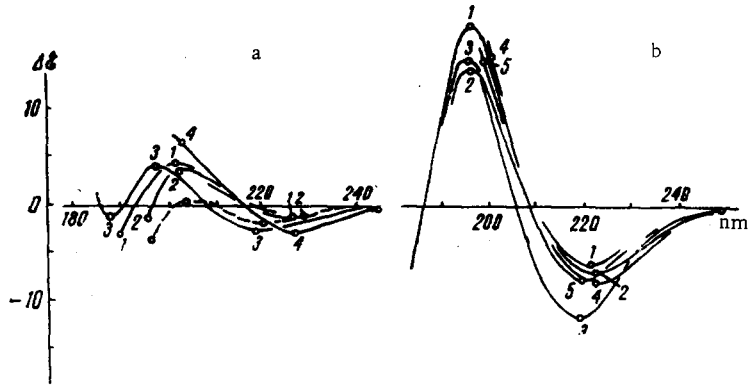

Fig. 7. CD curves of the cyclopeptides: a) compound (1); b) compound (2): 1) CF_3CH_2OH, 2) C_2H_5OH, 3) H_2O, 4) $CF_3CH_2OH-C_4H_8O_2$ (4 : 3), 5) $CF_3CH_2OH-CF_3COOH$ (6 : 1). The dashed line shows the CD curve of the cyclopeptide [(L−Ala−Gly−Gly)$_2$] (10) in water [2].

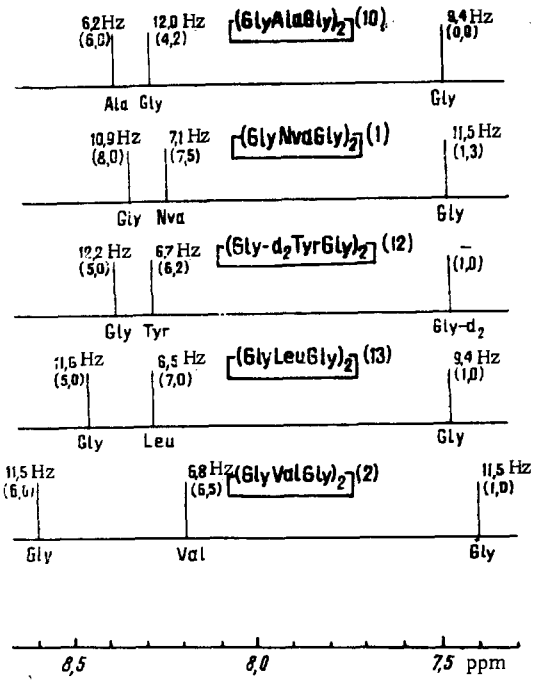

Fig. 8. Chemical shifts (δ, ppm) and their temperature dependence ($\Delta\delta/\Delta T \cdot 10^3$, ppm/deg, given in parentheses) and the $^3J_{NH-CH}$ constants of the proteins of the NH compounds (1), (2), and (10) [3], and also of [(L−Tyr−Gly−Gly)$_2$] (12) and [(L−Leu−Gly−Gly)$_2$] (13) [16].

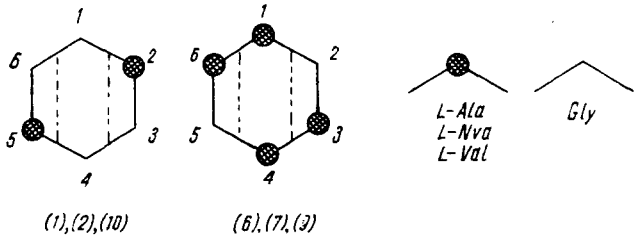

Fig. 9. Positions of the intraHBs in cyclic hexapeptides of types [(X−Gly−Gly)$_2$] and [(X−X−Gly)$_2$].

of the NMR spectra of the alanine cyclopeptides (8) and (9) [3, 4]. In both solvents, compounds (1) and (2) also have similar spectra. It may be assumed that trifluoroacetic acid, in spite of its capacity for protonating amide groups, does not destroy the "pleated sheet" structure, which is also shown by the slight change in the CD curve on the addition of trifluoroacetic acid to trifluoroethanol (curves 7 in Fig. 2 and 5 in Fig. 7).

As in the alanine derivative (10), one pair of glycine NH groups in each of compounds (1) and (2) forms an intraHB ($\Delta\delta/\Delta T \cdot 10^3 = 1.0$-$1.3$ ppm/deg, Table 1). The NMR spectra of two analogous cyclopeptides, cyclo[−(L−Tyr−Gly$_2$)$_2$−] (12) and cyclo[−(L−Leu−Gly$_2$)$_2$−] (13) have been investigaged by Kopple et al. [16]; the CD curve for the second compound has also been described [17]. A comparison of the results obtained by the American authors with those of the present work shows the similar spatial structure of cyclopeptides of the type [(X−Gly−Gly)$_2$]. This is convincingly shown, in particular, by the similar NMR parameters (chemical shifts δ, their dependence on the temperature, $\Delta\delta/\Delta T$, and the $^3J_{NH-CH}$ constants) in the NH signals, which are the most sensitive to conformational changes, in all five cyclopeptides: (1), (2), (10), (12), and (13) (Fig. 8). The NMR spectra of the deuterated derivative of a tyrosine cyclopeptide showed that in these compounds the intraHBs are formed by the glycine residues (positions 1 and 4 in Fig. 9) preceding the residues of optically active amino acids (positions 2 and 5) [16]. The closeness of the conformational states of cyclopeptides of the [(X−X−Gly)$_2$] type [(6), (7), and (9)] was mentioned above in a discussion of the CD curves. The positions of the intraHBs in these compounds that are shown in Fig. 9 follow from the NMR spectra of the deuterium-labelled alanine cyclopeptide (9) [3, 4].

TABLE 1. Parameters of the NMR Spectra of Compounds (1)-(7)

Compound	Solvent	Chemical shifts, ppm								$^3J_{NH-CH}$ with correction for the electronegativity of the substituent	
		CH_3	$C^\alpha H$	$C^\alpha H_2$	$C^\beta H$	$C^\beta H_2$	$C^\gamma H_2$	NH(Gly)*	NH (Nva, Val, Leu)*	Gly(Σ)	Nva, Val, or Leu
1	CF_3COOH	0,98	4,57	4,30	—	1,48	1,92	8,00 8,95	7,72	11,5 11,5	7,0
1	$(CD_3)_2SO$	0,87	3,80	~3,50	—	1,54	1,70	7,50 (1,3) 8,36 (8,0)	8,26 (7,5)	11,5 10,9	7,1
2	CF_3COOH	1,11	4,60	4,38	2,23	—	—	~8,02	7,77	12,5	7,3
2	$(CD_3)_2SO$	0,92	3,80	~3,50	1,88	—	—	7,41 (1,0) 8,64 (6,0)	8,21 (6,5)	11,5 11,5	6,8
3	CF_3COOH	0,98	4,58	4,26	—	1,45	1,85	8,04	7,79	13,0	7,3
4	CF_3COOH	1,03	4,63	4,28	—	1,78	1,55‡	8,13	7,80	11,4	7,4
4	$(CD_3)_2SO$	0,87	4,21	3,73	—	1,57	1,54‡	8,26 (4,4)	8,00 (4,2)	10,2	7,6
5	CF_3COOH	1,09	~4,50	~4·30	2,25	—	—	8,11	7,85	13,1	7,6
6	CF_3COOH	1,02	4,69	4,25	—	1,45	1,91	8,08	7,83 7,86	11,3	6,8 7,4
7	CF_3COOH	1,11	4,52	~4,25	2,17	—	—	~7,98	7,77 ~8,05	†	7,1 †

*The values of $\Delta\delta/\Delta T \cdot 10^3$ (ppm/deg) are given in parentheses.
†The $^3J_{NH-CH}$ constants could not be determined because of the overlapping of the signals.
‡ The signals of C^γ-H of Leu are given.

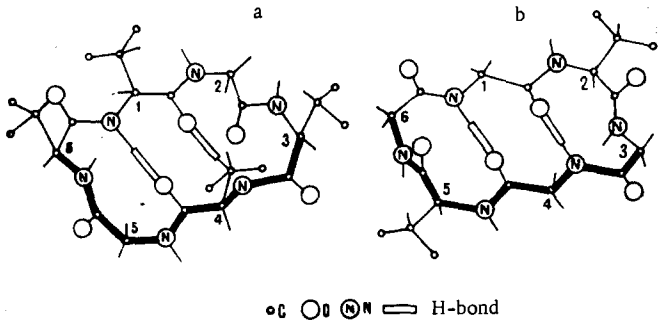

Fig. 10. Conformations of the cyclopeptides [(L-Val-L-Val-Gly)$_2$] (a) and [(L-Val-Gly-Gly)$_2$] (b) in aqueous solutions.

CONFORMATIONAL ANALYSIS

Thus, the material accumulated on cyclopeptides shows the existence of two types of "pleated sheet" structures in aqueous solutions. Both types have characteristic CD curves. One of them is distributed extremely widely, while the second is predominant only in compounds with the general formula [(X-Gly-Gly)$_2$]. These structures are realized in the purest form in the valine-containing cyclopeptides (2) and (7).

It is not a matter of doubt that the conformations found correspond to the forms (I) and (II) of the "pleated sheet" structure given above, which differ by the angles Φ and Ψ of the amino-acid residues in positions 2, 3, 5, and 6.* It remains to be established which of the two conformations has the parameters Φ and Ψ of form I and which has those of form II. For this purpose, let us consider two groups of cyclopeptides belonging to different conformational types (Fig. 9). The values of the $^3J_{NH-CH}$ constants of compounds (1), (2), (6), (7), (9), and (10) (Table 1, Fig. 8, and [3, 4]) agree with the parameters $\Phi_{(2,5)}$ and $\Phi_{(3,6)}$ of both forms (I) and (II) and do not permit preference to be given to either of them. A choice could be made by comparing the structure of the "β-inflections" of the compounds shown in Fig. 9 with the known conformational properties of the individual amino-acid residues. It is natural that in both groups of compounds the L-amino-acid residues will tend to adopt the conformations most favorable for them. Numerous ex-

*The participation in the equilibrium of centrosymmetrical structures with the opposite orientations of the carbonyl groups in the two "β inflections" is not excluded. However, for compounds (1), (2), (6), and (7) with C_2-symmetrical formulas they are unlikely.

periments and calculations (see, for example, [10]) show that such conformations are represented by the coordinates of region B of the conformational maps, i.e., the region of the energy minimum located in their top left-hand quadrants and corresponding to the β form of polypeptides. Since the angles of rotation of form I correspond to the $\underline{R}$† $_{(2)}$ and $\underline{B}_{(3)}$ regions, and forms II to the $\underline{B}_{(2)}$ and $\underline{L}_{(3)}$† regions, it is easy to see that compounds (10), (1), and (2) which have L-amino-acid residues in position 2, must adopt the "pleated sheet" conformation II, and compounds (9), (6), and (7) with a L-amino-acid residue in position 3 must adopt the "pleated sheet" conformation I. The increased stability of the forms found in the case of the valine derivatives (2) and (7) is explained by the better-defined tendency of the valine residue to adopt a conformation of type B [9, 10, 18, 19]. The conformations of compounds (2) and (7) corresponding to the two main types of "pleated sheet" structure are shown in Fig. 10.

Thus, the conformation of the "β inflection" (type I) found in the crystalline cyclopeptide [D-Ala-D-Ala-Gly$_4$] [8] is present in the overwhelming majority of cyclic hexapeptides in solution. A conformation of type (II) is encountered relatively more rarely and is predominating only for one combination of amino-acid residues. The same structure has been detected in the L-Ser-L-Ser section in crystalline ferrichrome A [20], where it is apparently due to the presence of hydroxymethyl side chains or to the steric limitations imposed by the complex part of the molecule.

EXPERIMENTAL METHOD

The synthesis of compound (1)-(7) has been described previously [21]. Before the physicochemical measurements, the compounds (1)-(7) obtained in the individual state were dried over P_2O_5 at 50°C/0.5 mm for 16 h. The CD curves were measured on a Cary-60 spectropolarimeter with a Cary-6001 attachment for obtaining CD curves at concentrations of the solutions of $(0.1\text{-}3)\cdot 10^{-3}$ M and a temperature of the solutions of 23-26°C; the cell thickness was 0.01-1 cm. In view of the limited solubility of compounds (1)-(7), especially the valine derivatives, both in water and in nonpolar solvents, to measure the CD curves the cyclopeptide sample was usually dissolved in trifluoroethanol or ethanol, and the resulting solution was then diluted with water, trifluoroacetic acid, dioxane, or heptane. The IR spectra were recorded on a UR-10 instrument with LiF and NaCl prisms. The thickness of the cell in measurements in the 3500-3200 cm^{-1} regions was 20 mm and in the 1750-1610 cm^{-1} region 5 mm; the concentration of the solutions was $1.2\cdot 10^{-4}$ M. The NMR spectra were taken on a JNM-4H-100 instrument with a working frequency of 100 MHz and with stabilization of the resonance conditions on one sample. Tetramethylsilane was used as internal standard ($\delta = 0.00$ ppm). The chemical shifts were determined with an accuracy of ±0.005 ppm, the spin-spin coupling constants with an accuracy of about ±0.1 Hz, and the temperature with an accuracy of ±2°C. The concentrations of the solutions were 0.08-0.12 M.

The authors thank L. B. Senyavina for measuring the IR spectra and for calculating the integral intensities of the bands.

SUMMARY

1. The CD, IR, and NMR spectra of the cyclohexapeptides (1)-(7) have been studied.

2. It has been shown that in trifluoroethanol and ethanol the compounds investigated show a tendency to association which is particularly strong in the case of [(L-Val-Gly)$_3$] (5).

3. In aqueous media, cyclohexapeptides of the type of [(X-Gly-Gly)$_2$] adopt the "pleated sheet" II conformation, and the other cyclopeptides the "pleated sheet" I conformation.

4. In nonpolar media, conformations with three or four intramolecular hydrogen bonds predominate.

LITERATURE CITED

1. Yu. A. Ovchinnikov, V. T. Ivanov, V. V. Shilin, and G. A. Kogan, Molekul. Biol., 3, 600 (1969).
2. V. T. Ivanov, G. A. Kogan, E. A. Meshcheryakova, V. V. Shilin, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 309 (1971).
3. S. L. Portnova, T. A. Balashova, V. F. Bystrov, V. V. Shilin, Ya. Bernat, V. T. Ivanov, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 323 (1971).

†The R region is located in the bottom left-hand quadrant of the conformational map and corresponds to a right-handed α helix, and region L is located in the top right-hand quadrant and corresponds to a left-hand α helix.

4. V. T. Ivanov, S. L. Portnova, T. A. Balashova, V. F. Bystrov, V. V. Shilin, Ya. Bernat, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 339 (1971).
5. V. T. Ivanov, L. B. Senyavina, E. S. Efremov, V. V. Shilin, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 347 (1971).
6. G. A. Kogan, V. M. Tul'chinskii, V. V. Shilin, and V. T. Ivanov, Khim. Prirodn. Soedin., 361 (1972).
7. I. L. Karle and J. Karle, Acta Cryst., 16, 969 (1963).
8. I. L. Karle, J. W. Gibson, and J. Karle, J. Amer. Chem. Soc., 92, 3755 (1970).
9. E. M. Popov, G. M. Lipkind, S. F. Arkhipova, and V. G. Dashevskii, Molekul. Biol., 2, 622 (1968).
10. V. T. Ivanov, P. V. Kostetskii, E. A. Meshcheryakova, E. S. Efremov, E. M. Popov, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 363 (1973).
11. F. Quadrifoglio and D. W. Urry, J. Amer. Chem. Soc., 90, 2760 (1968).
12. E. S. Efremov, P. V. Kostetskii, V. T. Ivanov, E. M. Popov, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 354 (1973).
13. U. Widmer and G. P. Lorenzi, Chimia, 25, 236 (1971).
14. M. Goodman, F. Naider, and C. Toniolo, Biopolymers, 10, 1719 (1971).
15. R. W. Woody and I. Tinaco, J. Chem. Phys., 46, 4927 (1967).
16. K. D. Kopple, A. Go, R. H. Logan, Jr., and J. Savrda, J. Amer. Chem. Soc., 95, 973 (1972).
17. S. M. Zigdler and C. A. Bush, Biochem., 10, 1330 (1971).
18. P. K. Ponnuswamy and V. Sasisekharan, Biopolymers, 10, 565 (1971).
19. B. Maigret, D. Perahia, and B. Pullman, Biopolymers, 10, 491 (1971).
20. A. Zalkin, J. D. Forrester, and D. H. Templeton, J. Amer. Chem. Soc., 88, 1810 (1966).
21. V. T. Ivanov, I. A. Lavrinovich, V. V. Lapshin, P. V. Kostetskii, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 63 (1975).
22. J. C. Kendrew, W. Klyne, S. Lifson, T. Miyazawa, G. Nemethy, D. C. Philips, G. N. Ramachandran, and H. A. Scheraga, J. Mol. Biol., 52, 1 (1970); Biochem., 9, 3471 (1970); Arch Biochem. Biophys., 145, 405 (1971).