A STUDY OF THE CONFORMATIONAL STATES OF CYCLOPEPTIDE SYSTEMS

VI. INFRARED SPECTRA AND DIPOLE MOMENTS OF THE DIASTEREOMERIC CYCLOHEXAALANYLS

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In preceding papers [1-5] we have described the results of the synthesis and physicochemical investigations by the methods of circular dichroism, optical rotatory dispersion, and UV and NMR spectroscopy of a large series of cyclic hexapeptides constructed from L(D)-alanine and glycine residues. The majority of the measurements were performed in polar solvents (water, dimethyl sulfoxide, ethanol), since the compounds studied were sparingly soluble in nonpolar solvents. However, the solubilities of some of them, namely the diastereomeric cyclohexaalanyls (1)-(4) (Fig. 1), proved to be sufficient to obtain their IR spectra, which are being subjected to quantitative interpretation, and for measurements of dipole moments. The results obtained, which permit a number of conclusions concerning the conformational states of the cyclic hexapeptides in nonpolar media, are expounded in the present paper.

There is a series of communications in which it has been shown that the study of the IR spectra of peptides enables fundamental information to be obtained concerning their conformational states [8-11]. Particularly valuable information is given by investigations in dilute solutions of neutral organic solvents which are not proton donors or acceptors in the formation of hydrogen bonds with the dissolved peptides. Since under these conditions, as a rule, no intermolecular association takes place, it is possible to detect the existence of intramolecular hydrogen bonds (IMHBs) and, in a number of cases, to evaluate the ratio of bound and free NH groups [11-20].

The IR spectra of the cyclopeptides (1)-(4) were taken at 25°C in chloroform solutions at concentrations of $\sim 5 \cdot 10^{-4}$ M, i.e., under conditions in which it is possible to neglect the formation of intermolecular H bonds (see [12, 13, 15-19]). As can be seen from Fig. 2, the cyclopeptides (1)-(4) are characterized by extremely similar IR spectra. In the 3300-3470 cm^{-1} region, which corresponds to the stretching vibrations of the NH bond (amide A [9, 10]), there is a strong band with a maximum at ~3340 cm^{-1} and a shoulder in the high-frequency region, and also several bands at 3410-3460 cm^{-1}; the separation of the bands showed that the shoulder mentioned corresponds to a band with a maximum at ~3385 cm^{-1}. In the region of the stretching vibrations of the amide CO groups (amide 1) there is an asymmetric band with a maximum at 1675-1676 cm^{-1}, the separation of which into its individual components does not appear to be possible (Fig. 2).

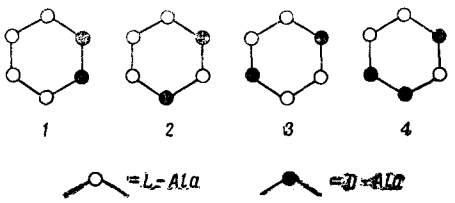

Fig. 1. Structures of the cyclohexapeptides (1)-(4).

The results of a detailed study of the IR spectra of model peptide systems [13, 16, 19] show that the bands at ~3340 and ~3385 cm^{-1} can be ascribed to NH groups participating in IMHBs of the NH· · ·OC type, and the bands with frequencies of 3410-3460 cm^{-1} to various types of free NH groups. The assignment

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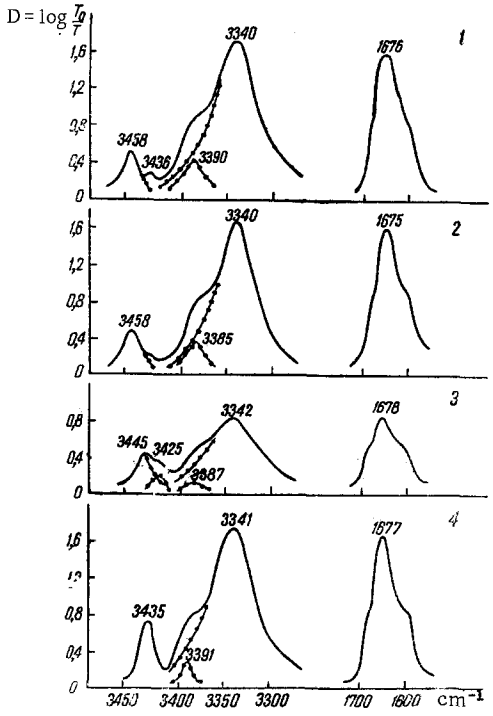
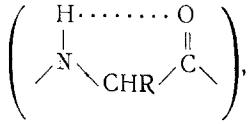


Fig. 2. IR spectra of the cyclopeptides (1)-(4).

of the band at ~3385 cm^{-1} to a frequency appearing as a consequence of the Fermi resonance of an overtone of the amide 1 band and the band at 3340 cm^{-1} is unlikely since in this case it is difficult to explain the considerable fall in its intensity in compound (3) (see Fig. 2 and Table 1) and its complete absence in the case of linear amides and peptides [13, 16, 19]. The assignment of the bands at 3420-3440 cm^{-1} to NH groups participating in H bonds of the 1→1 type  , which was first suggested by Mizushima et al. [13] and has been used by French authors [19], is inadequately substantiated and contradicts calculated figures [21].

For a further analysis of the results obtained it was necessary to evaluate the number of NH groups corresponding to each of the bands found in the 3300-3480 cm^{-1} region. However, the solution of this problem encountered considerable difficulties connected with the absence of definite information concerning the intensities of the bands of the stretching vibrations of various NH groups differing by participation in a hydrogen bond, by the nature of the neighboring groups, by their position, and so on. Thus, it was not possible to make use of the relationships given in the literature between the intensity and half-width of the band of the stretching vibrations of H-bonded XH groups and the energy of the H bond and its length [22-26], since the characteristics of the IMHBs in the cyclopeptides studied were not available. The correlations described previously connecting the shift of the ν_{N-H} frequency in the formation of an H bond with the integral intensity of the band and its half-width [22, 23, 27, 28] could not be used for the present work, either, since the quantitative relationships between the corresponding parameters remained unknown.

The correlations mentioned, and also isolated pieces of literature information on the IR spectra of amides in solutions (see, for example [13, 16, 22, 23, 27-30]), permit the assumption that the integral intensity of the amide A bands, both of free and of H-bonded NH groups, exhibits a tendency to rise with a decrease in the frequency ν_{NH}. The measurements that we have performed under standard conditions (dilute chloroform solutions, 25°C) of the IR spectra of a number of amides and peptides with only one band at 3500-3300 cm^{-1} have shown that, in fact, a definite correlation exists between the frequency of the amide A band and its integral intensity (Table 2 and Fig. 3); in parallel with a rise in intensity there is also a broadening of the absorption band.

We used the empirical relationship found in this way for a quantitative evaluation of the number of NH groups of different types in the cyclopeptides (1)-(4) (see Table 1). Here and below, for convenience of exposition, we have adopted the classification of H bonds of the NH· · ·OC type in peptide solutions according to which frequencies below 3330 cm^{-1} correspond to strong H bonds (energies greater than 3 kcal/mole), ~3350 cm^{-1} to H bonds of medium strength (energies ~3 kcal/mole [16]), and above 3370 cm^{-1} to weak H bonds (energies less than 3 kcal/mole). The calculation of the correlation equation and the number of NH groups corresponding to the individual spectral bands was carried out by the methods of Ramsay [35] and of Wilson and Wells [36]. In the latter case, to decrease the error due to the low optical density in the wings of the absorption band, the calculation was performed by the method of cutting off the contour with respect to the base line [37]. As can be seen from Table 1, the two methods led to results differing insignificantly from one another.

In an analysis of IR spectra, it must be expected that the quantitative evaluation of the free NH groups should give more reliable results than in the case of the H-bonded groups, since in the 3400-3480 cm^{-1} region, unlike the 3300-3400 cm^{-1} region, the contour of the amide A band generally approximates well to a Lorentz function; furthermore, the intensities of the amide A bands of free NH groups vary less on passing from one compound to another than that of groups participating in H bonds (see Table 2). Consequently,

TABLE 1. Characteristics of the IR Spectra of Compounds (1)-(4)*

Compound	Conc. of saturated solutions, $c \cdot 10^4$, M	Free NH groups				$6-n_1^P$	Weakly bound NH groups				H-bonded NH groups					$\frac{R}{n_2}+\frac{R}{n_3}$; $\frac{W}{n_2}+\frac{W}{n_3}$
		ν_{N-H}, cm^{-1}	$\Delta\nu_{1/2}$, cm^{-1}	Ramsay's method A^R n_1^R	Wilson & Wells' method A^W n_1^W		ν_{N-H}, cm^{-1}	$\Delta\nu_{1/2}$, cm^{-1}	Ramsay's method A^R n_2^R	Wilson & Wells' method A^W n_2^W	ν_{N-H}, cm^{-1}	$\Delta\nu_{1/2}$, cm^{-1}	Ramsay's method A^R n_3^R	Wilson & Wells' method A^W n_3^W		
(1)	5,69	3548	20	1,27 1,35	0,63 0,97	4,65 5,03	3390	26	1,30 0,60	0,38 0,28	3340	62,0	14,57	3,62 9,31 4,08		4,22 4,36
(2)	5,34	3458	22	1,82 1,94	0,74 1,16	4,06 4,84	3385	22	1,06 0,46	0,75 0,52	3340	65,0	16,00	3,98 9,90 4,34		4,44 4,86
(3)	3,48	3445	22	2,15 1,95	0,84 1,10	3,27 4,27	3381	—	0,7 0,3	0,3 0,2	3342	74,0	14,02	3,55 7,88 3,52		3,85 3,72
		3425	19	1,07 0,78	0,59 0,63											
(4)	6,45	3435	17	1,42 1,16	0,95 1,13	4,84 4,87	3391	18	0,60 0,28	0,40 0,30	3341	64,0	13,74	3,44 8,91 3,94		3,72 4,24

* A^R and A^W are the integral intensities of the bands calculated by Ramsay's method and by Wilson and Wells' method, respectively ($A \cdot 10^{-4}$ mole · liter · cm^{-2}), and n_1, n_2, and n_3 are the numbers of free, weakly bound, and H-bonded NH groups obtained by dividing A^R and A^W by the integral intensity of the band from one NH group at the given value of ν_{NH} (found from the graphs of Fig. 3).

it is frequently desirable to determine the number of bound NH groups not from the integral intensity of the corresponding bands [n_2+n_3 for the cyclopeptides (1)-(4), see Table 1], but from the difference between the total number of NH groups and the number of free NH groups ($6-n_1$, see Table 1). However, in the cyclopeptides (1)-(4) in the 3410-3470 cm^{-1} region there are several overlapping bands of low intensity (see Fig. 2), which reduces the accuracy of the determination of the number of free NH groups (n_1). In view of this, to determine the bound NH groups in cyclopeptides we used both the methods mentioned (see Table 1).

It follows from Table 1 that in $ChCl_3$ solution in the preferred conformations of the cyclopeptides (1)-(4) there are not less than 3-4 IMHBs of medium strength and, in addition, forms with one or two weak IMHBs also take part in the conformational equilibrium.

In preceding papers [3-5] we have shown that in polar solvents (dimethyl sulfoxide, water, ethanol) cyclic hexapeptides constructed of alanine and glycine residues generally assume the "pleated-sheet" conformation stabilized by two transannular (4→1) H bonds (Fig. 4) and differing from other types of packing of the peptide chain by characteristic CD and ORD curves. On passing to less polar solvents (ethanol–heptane, 1:2), the number, signs, and positions of the Cotton effects scarcely change, but there is some redistribution of their intensities. These results, in combination with the IR spectra, permit the assumption that in nonpolar solvents the cyclic hexapeptides retain the usual type of conformation and H bonds of the 4→1 type. Nevertheless, in the conformational equilibrium, forms differing somewhat from the "polar" forms by the coordinates Φ and Ψ* and having additional H bonds† begin to predominate.

With the condition of the planar trans conjugation of the amide bonds (see [4]), the following possibilities exist for the formation of additional (apart from 4→1 bonds) pairs of IMHBs in the cyclopeptides (1)-(4):‡

(A) H bonds are formed between the NH groups of the $Ala_{(2 \text{ and } 5)}$ residues and the CO groups of the $Ala_{(6 \text{ and } 3)}$ residues (Fig. 5);

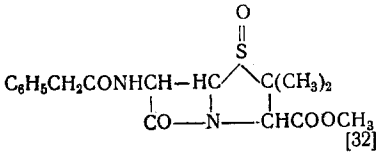
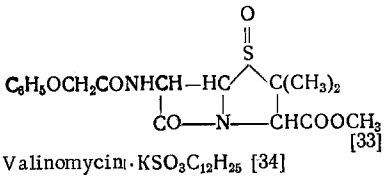
(B) H bonds arise between the NH groups of the $Ala_{(3 \text{ and } 6)}$ residues and the CO groups of the $Ala_{(1 \text{ and } 4)}$ residues (Fig. 6);

*According to the generally accepted nomenclature here and below, in order to describe the conformations of the peptides, the coordinates Φ and Ψ characterizing the rotational states of the NH–C^{α}H and C^{α}H–CO fragments will be used [38].

†At the same time, we cannot exclude the possibility of the appearance of forms of cyclopeptides containing no transannular H bonds. However, this possibility appears unlikely, if one considers the stability of the latter even in such polar solvents as water and dimethyl sulfoxide.

‡For simplicity, only conformations with a second-order axis of symmetry, which are the most probable for compound (3), are considered. In the conformational equilibrium of compounds (1), (2), and (4) one must expect the realization of unsymmetrical forms with different structures of the $Ala_1-Ala_2-Ala_3$ and $Ala_4-Ala_5-Ala_6$ fragments.

TABLE 2. Characteristics of the IR spectra of Compounds Having One Amide A Band in the 3500-3300 cm^{-1} Region

Compound	c, M	ν_{NH}	$\Delta\nu_{1/2}$	$A^R \cdot 10^{-4}$	$A^W \cdot 10^{-4}$
		cm^{-1}		$m^{-1} \cdot liter \cdot cm^{-2}$	
$CH_3CONHCH_3$	$2{,}50 \cdot 10^{-3}$	3472	15,0	0,79	0,58
$C_6H_5CH_2CONHCH$ [31]	$2{,}50 \cdot 10^{-3}$	3467	17,6	0,81	0,62
$CH_3CONHCH(CH_3)COOCH_3$ (*L*) [16]	$2{,}45 \cdot 10^{-3}$	3438	33,0	1,24	0,82
$CH_3CONHCH(CH_3)CON(CH_3)$ (*L*)†	$2{,}92 \cdot 10^{-3}$	3418	37,5	1,42	0,99
$CH_3CONHCH(CH_3)CON(CH_3)CH(CH_3)$-$COOCH_3$ (*LD*) [16]	$2{,}30 \cdot 10^{-3}$	3422	35,2	1,46	0,99
 [32]	$1{,}35 \cdot 10^{-3}$	3399	59,0	1,96	1,29
 [33]	$1{,}26 \cdot 10^{-3}$	3384	62,4	2,32	1,40
Valinomycin·$KSO_3C_{12}H_{25}$ [34]	$8{,}56 \cdot 10^{-5}$	3309	66,0	5,22‡	2,98

*A^R and A^W are the integral intensities of the bands calculated by the methods of Ramsay and of Wilson and Wells, respectively.
†The dimethylamide of acetyl-L-alanine (mp 86-88°C, $[\alpha]_D + 25°$, c 1, $CHCl_3$) was obtained in our laboratory by P. V. Kostetskii by means of a three-stage synthesis starting from benzoxycarbonyl-L-alanine.
‡Calculated to one amide group.

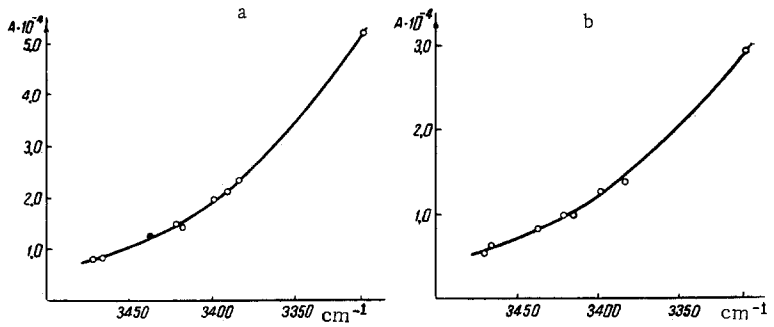


Fig. 3. Dependence of the integral intensity of the amide A band on the frequency ν_{N-H}. a) Calculated by Ramsey's method; b) calculated by Wilson and Wells' method..

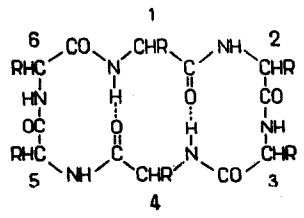


Fig. 4. Position of the intramolecular hydrogen bonds in cyclohexapeptides in polar solvents.

(C) both the possibilities considered above are realized simultaneously (Fig. 7).

In each of the conformations (A), (B), and (C) there are H bonds of the 3→1 type stabilizing seven-membered rings. As the investigation of model amides [12, 13, 19, 20] and of simple linear peptides [16] has shown, NH groups participating in the formation of such H bonds are represented by bands at 3340-3390 cm^{-1}, which is in good agreement with the features of the IR spectra of the cyclopeptides (1)-(4).

In form (A) of the cyclopeptides, the formation of 7-membered rings fixes the conformation of the $Ala_{(1 \text{ and } 4)}$ residues at $\Phi \sim 120°$, $\Psi \sim 240°$ [or $\Phi \sim 240°$, $\Psi \sim 120°$, which corresponds to the mirror form of the peptide chain with the coordinates Φ and Ψ of opposite sign]. In the subsequent discussion, it

TABLE 3. Calculated Values of the Dipole Moments for Various Conformations of the Cyclohexapeptides

Type of conformation	Dipole moment, D
A	1.0–8.0
Conformation A of cyclo-(L-Ala)$_6$, corresponding to a potential energy minimum [41]	3.2
B	5.7–8.0
C	8.0
"Pleated-sheet" conformation of cyclo-(L-Ala)$_6$, corresponding to a potential energy minimum [41]	5.2

TABLE 4. Results of Measurements of the Dipole Moments of the Cyclopeptides (1)-(4)

Compound	α	R	μ(D)
1	12,00	111,1	4,4±0,8
2	16,16	111,1	5,0±0,3
3	24,09	111,1	5,9±0,3
4	12,83	111,1	4,5±1,5

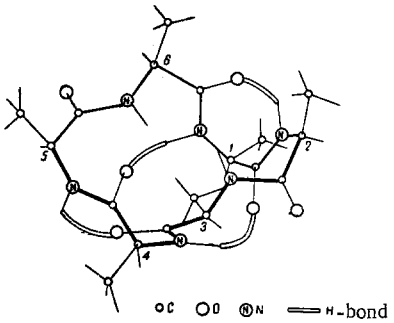


Fig. 5. Form A of cyclohexa-L-alanyl.

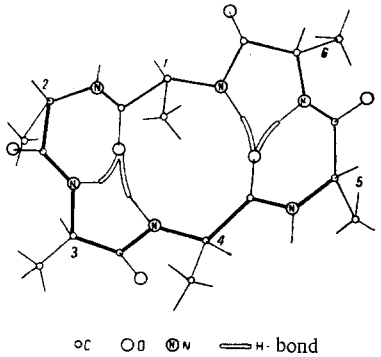


Fig. 6. Form B of cyclohexa-L-alanyl.

must be borne in mind that in addition to the conformations considered below the existence of the mirror forms is possible, particularly in view of the presence of D-alanine residues in the molecules of (1)-(4). So far as concerns the Ala$_{(2\text{ and }5)}$ and Ala$_{(3\text{ and }6)}$ residues, it follows from a theoretical analysis of the conformations of a tripeptide fragment with a hydrogen bond of the 4→1 type [39] that the coordinates Φ and Ψ corresponding to them may vary within extremely wide limits according to the orientation of the amide groups between the Ala$_{(2)}$ and Ala$_{(3)}$ [Ala$_{(5)}$ and Ala$_{(6)}$] residues.

$\Phi_{(2\text{and}5)}$ 110–130; $\Phi_{(3\text{and}6)}$ 45–135 or 225–345;
$\Psi_{(2\text{and}5)}$ 135–180 or 255–315; $\Psi_{(3\text{and}6)}$ 135–325.

However, in an analysis of molecular models of the cyclohexapeptides it can be seen that the formation of a H bond between Ala$_{(2)}$ and Ala$_{(6)}$ [Ala$_{(5)}$ and Ala$_{(3)}$] appreciably narrows the field of possible orientations for the Ala$_{(2\text{ and }5)}$ and Ala$_{(3\text{ and }6)}$ residues. This permits the range of Φ and Ψ for form A of the cyclohexapeptides to be determined in the following way:

$\Phi_{(1\text{ and }4)} \sim 240$; $\Phi_{(2\text{ and }5)} \sim 120$; $\Phi_{(3\text{ and }6)}$ 45–80 or 255–290;
$\Psi_{(1\text{ and }4)} \sim 120$; $\Psi_{(2\text{ and }5)}$ 135–180 or 255–315; $\Psi_{(3\text{ and }6)} \sim 215$.

A theoretical conformational analysis of cyclohexa-L-alanyl has shown that in fact form A corresponds to a local minimum of the potential energy with the coordinates [40]

$\Phi_{(1\text{and}4)}$ 257; $\Phi_{(2\text{and}5)}$ 120; $\Phi_{(3\text{and}6)}$ 70;
$\Psi_{(1\text{and}4)}$ 111; $\Psi_{(2\text{and}5)}$ 140; $\Psi_{(3\text{and}6)}$ 210.

Apparently, a conformation of this type can be realized in cyclopeptides (1)-(4), also. The transannular bonds in this conformation are somewhat weakened (the N_4-O_1 and N_1-O_4 distances are 3.1 Å, while the normal distance is 2.7-2.0 Å), which must lead to a reduction in its energy and to a displacement of ν_{N-H} in the high-frequency direction as compared with the normal frequencies of NH groups participating in strong 4→1 hydrogen bonds (for example, in gramicidin S, ν_{NH} 3314 cm^{-1} [18], valinomycin ν_{NH} 3313 cm^{-1}, and the complex of valinomycin with potassium cations ν_{NH} 3309 cm^{-1} [17, 34]; all figures for solutions in $CHCl_3$). Consequently, in the IR spectra of form A one must expect similar values of ν_{NH} from NH groups participating in H bonds of different types (3→1 and 4→1); this conclusion agrees well with the experimental results (see Fig. 2).

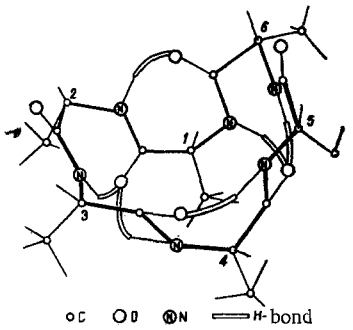


Fig. 7. Form C of cyclohexa-L-alanyl.

The simultaneous formation of H bonds of the 4 → 1 and 3 → 1 types in each of the two tripeptide fragments which is characteristic for conformation B of the cyclohexapeptides has not been detected in peptides hitherto. In an analysis of tripeptide fragments by the method of rigid spheres, S. M. Venkatachalam [39] came to the conclusion that such a type of structure comes into energetically forbidden regions. However, subsequent investigations including the minimization of the potential energy with respect to the valence angles and the angles Φ and Ψ have shown that such conformations correspond to extremely deep energy minima, and their realization in peptides in nonpolar media is completely probable [41]. On this basis, it may be assumed that the band at ~3385 cm^{-1} observed in the IR spectra of the cyclopeptides (1)-(4) corresponds to a 3 → 1 hydrogen bond in conformation B (as weaker than a 4 → 1 bond in the same conformation). In form B, the conformation of $Ala_{(2\text{ and }5)}$ and $Ala_{(3\text{ and }6)}$ residues is fixed to a considerable extent and some free internal rotation is preserved only at the $C_{(1)}$ and $C_{(4)}$ atoms; the following coordinates may correspond to this form:

$$\Phi_{(1\text{and}4)}\,0\text{—}110; \quad \Phi_{(1\text{and}5)} \sim 230^*; \quad \Phi_{(2\text{and}5)} \sim 82^*;$$
$$\Psi_{(1\text{and}4)}\,360\text{—}250; \quad \Psi_{(2\text{and}5)} \sim 110^*; \quad \Psi_{(3\text{and}6)} \sim 152^*$$

It is easy to see that the extreme case of conformation B [$\Phi_{(1\text{ and }4)}$ ~110°, $\Psi_{(1\text{ and }4)}$ ~250°] with six IMHBs rigidly fixed by the form of the peptide chain corresponds to conformation (C); its presence may be assumed in the cyclopeptides (1) and (2) which, judging from the IR spectra (Table 1), have the largest number of IMHBs.

Further information on the structure of the cyclopeptides (1)-(4) was obtained by a comparison of their dipole moments measured in $CHCl_3$ solution with the values of the dipole moments calculated for various conformational forms of the cyclohexapeptides. The calculation was performed by a method developed previously comprising the successive summation of the vectors of the dipole moments of the amide groups along a peptide chain of given conformation [42]. In order to calculate the dipole moments of forms (A), (B), and (C) we used the coordinates Φ and Ψ given above; for comparison we also calculated the dipole moment of the "pleated-sheet" conformation of cyclohexa-L-alanyl with two H bonds of the 4 → 1 type corresponding to a local potential energy minimum [40]. The results of the calculation and the dipole moments determined experimentally are given in Tables 3 and 4. As can be seen from a comparison of them, form C cannot be dominating in solutions of the cyclopeptides (1)-(4) in $CHCl_3$, since its dipole moment (8.0 D) considerably exceeds the observed values (4.4-5.9 D). So far as concerns forms A and B, in spite of the extremely wide and strongly overlapping regions possible for their dipole moments (1.0-8.0 D and 5.7-8.0 D), the experimental results allow form A to be considered as the more likely, although they permit the possibility of the existence of a certain amount of form B, particularly in the case of compound (3).

The results of the present work, in combination with the results of preceding investigations and calculated figures available in the literature, permit us to consider that in the cyclic hexapeptides constructed of L- and D-alanine residues conformations of types A and B with four intramolecular hydrogen bonds are preferred in nonpolar solvents. Both conformations correspond to calculated potential energy minima, and their realization is in agreement with the IR spectra and dipole moments. Furthermore, small amounts of forms with two (4 → 1) hydrogen bonds and also rigid conformations of type C, stabilized by six intramolecular hydrogen bonds, may also take part in the conformational equilibrium.

EXPERIMENTAL

Before the preparation of solutions, the cyclopeptides (1)-(4) were dried over P_2O_5 at 50°C/0.5 mm for 8 h. In order to obtain a saturated solution, a suspension of the cyclopeptide in absolute chloroform was shaken at room temperature for 40 h. The excess of cyclopeptide was filtered off. The concentration of the of the solution was determined by evaporating 10 ml to dryness and weighing the residue after drying over P_2O_5 to constant weight.

* The calculated values of Φ and Ψ corresponding to the most favorable conformation of Ac-L-Ala – L-Ala-NHMe with 4 → 1 and 3 → 1 H bonds [41].

The IR spectra were recorded on a UR-10 instrument with LiF and NaCl prisms. Recording conditions: slit program 4, recording speed 32 cm^{-1}/min, spectral slit width at 3500-3200 cm^{-1} 4 cm^{-1}, at 1750-1610 cm^{-1} ~8.5 cm^{-1}; the thickness of the cell for the measurements in the 3500-3200 cm^{-1} region was 20 mm, and for 1750-1610 cm^{-1} it was 5 mm, the absorption of the chloroform under these conditions not exceeding 60%.

The dipole moments were measured in a Dipol instrument working on the beat principle at a frequency of 1 MHz. The comparatively large error of the measurements is due to the poor solubility of the cyclopeptides (1)-(4) in $CHCl_3$.

The dipole moments were calculated by Hedestrand's method [6]

$$\mu = 0{,}2\sqrt{A(M_2 - M_1\beta) + B\alpha - R},$$

where A and B are empirical constants depending on the solvent and equal to 0.37 and 26.89, respectively, in the case of chloroform;

M_1 and M_2 are the molecular weights of the substance under investigation and of the solvent (426 and 120.5);

R is the molecular refraction calculated from the atomic and group refractions [7] (the correction for atomic polarization was not made);

$\alpha = \frac{\varepsilon_x - \varepsilon_0}{\varepsilon_0 f}$ is the coefficient of the dependence of the dielectric constant of the solution (ε_X) on the concentration of substance (f) expressed in molar fractions; for $CHCl_3$ ε_0^{25} is 4.65; and

$\beta = \frac{d_x - d_0}{d_0 f}$; is the coefficient of the dependence of the density of the solution (d_X) on the concentration of the substance (f); for $CHCl_3$, d_0^{25} is 1.48.

The coefficient β, depending insignificantly on the dipole moment (μ) and taken as the same for all the cyclopeptides (1)-(4), was found as the mean value of two measurements [for compounds (2) and (3)].

SUMMARY

1. An empirical method for the quantitative determination of the number of NH groups of different types in peptides based on measurements of the integral intensity of the amide A bands in the IR spectra has been developed.

2. The IR spectra of the diastereomeric cyclohexaalanyls in $CHCl_3$ solutions have been studied; they indicate the participation of an average of four NH groups in IMBHs.

3. On the basis of a theoretical conformational analysis and of dipole-moment measurements, the system of IMHBs and the type of dominant conformation of the cyclohexapeptides in nonpolar solvents have been established.

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, V. V. Shilin, Ya. Bernat, and Yu. A. Ovchinnikov, Zh. Obshch. Khim., 41, 2318 (1971).
3. V. T. Ivanov, G. A. Kogan, E. N. Meshcheryakova, V. V. Shilin, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 7, 309 (1971).
4. S. L. Portnova, T. A. Balashova, V. F. Bystrov, V. V. Shilin, Ya. Bernat, V. T. Ivanov, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 7, 323 (1971).
5. V. T. Ivanov, S. L. Portnova, T. A. Balashova, V. F. Bystrov, V. V. Shilin, Ya. Bernat, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 7, 339 (1971).
6. G. Hedestrand, Z. Phys. Chem., B2, 428 (1929).
7. B. V. Ioffe, Refractometric Methods in Chemistry [in Russian] GNTI (1960).
8. Yu. N. Chirgadze, Infrared Spectra and the Structure of Polypeptides [in Russian], Moscow (1965).
9. T. Miyazawa, in: Polyamino Acids, Polypeptides, and Proteins (editor M. A. Stahnman), University of Wisconsin Press, Madison (1962), p. 201.
10. T. Miyazawa, in: Aspects of Protein Structure (editor G. N. Ramachandran), Academic Press, New York (1963), p. 257.

11. S. Mizushima, The Structure of Molecules and Internal Rotation, Academic Press, New York (1954).
12. S. Mizushima, T. Shimanouchi, M. Tsuboi, and T. Azakawa, J. Amer. Chem. Soc., 79, 5357 (1957).
13. M. Tsuboi, T. Shimanouchi, and S. Mizushima, J. Amer. Chem. Soc., 81, 1406 (1959).
14. J. E. Shields and S. T. McDowell, J. Amer. Chem. Soc., 89, 2499 (1967).
15. J. E. Shields, S. T. McDowell, J. Pavlos, and G. R. Gray, J. Amer. Chem. Soc., 90, 3549 (1968).
16. S. L. Portnova, V. F. Bystrov, V. I. Tsetlin, V. T. Ivanov, and Yu. A. Ovchinnikov, Zh. Obshch. Khim., 38, 428 (1968).
17. V. T. Ivanov, I. A. Laine, N. D. Abdullaev, L. B. Senyavina, E. M. Popov, Yu. A. Ovchinnikov, and M. M. Shemyakin, Biochem. Biophys. Res. Commun., 34, 803 (1960).
18. Yu. A. Ovchinnikov, V. T. Ivanov, V. F. Bystrov, A. I. Miroshnikov, E. N. Shepel, N. D. Abdullaev, E. S. Efremov, and L. B. Senyavina, Biochem. Biophys. Res. Commun., 39, 217 (1970).
19. M. Avignon and P. V. Huong, Biopolymers, 9, 427 (1970).
20. M. Avignon, P. V. Huong, L. Lascombe, M. Marrand, and J. Heel, Biopolymers, 8, 69 (1969).
21. E. M. Popov, G. M. Lipkind, S. F. Arkhipova, and V. G. Dashevskii, Molekulyarnaya Biologiya, 2, 622 (1968).
22. G. Pimentel and O. MacClennan, The Hydrogen Bond, W. H. Freeman, San Francisco (1960).
23. N. D. Sokolov, The Hydrogen Bond [in Russian], Moscow (1964).
24. L. A. Dement'eva, A. V. Iogansen, and G. A. Kurkchi, Zh. Prikl. Spektrosk., 10, 625 (1969).
25. A. V. Iogansen, Dokl. Akad. Nauk SSSR, 164, 610 (1965).
26. V. Iogansen, G. A. Kurkchi, and B. V. Rassadin, Zh. Prikl. Spektrosk., 11, 1054 (1969).
27. N. N. Khovratovich and N. A. Borisevich, in: Opt. i Spektrosk., Collection 3 on Molecular Spectroscopy [in Russian], Leningrad (1967), p. 123.
28. O. M. Boyarchuk and V. N. Nikitin, Dokl. Akad. Nauk SSSR, 159, 397 (1964).
29. D. N. Shigorin, N.V. Mikhailova, and O. A. Klyueva, Zh. Fiz. Khim., 30, 1591 (1965).
30. I. V. Shablygin, D. N. Shigorin, and N. V. Mikhailova, Zh. Prikl. Spektrosk., 3, 56 (1965).
31. M. H. J. Taverne, Rec. Trav. Chim., 16, 31 (1897).
32. T. Hans et al., The Chemistry of Penicillin, Princeton University Press, Princeton, (1949), pp. 144, 177, 187.
33. R. B. Morin, B. J. G. Jackson, R. A. Mueller, E. R. Lavagnino, B. Scanlon, and S. L. Andrews, J. Amer. Chem. Soc., 85, 1896 (1963).
34. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, E. I. Vinogradova, A. M. Shkrob, G. G. Melenkov, A. V. Evstratov, I. A. Laine, E. I. Melnik, and I. D. Ryabova, J. Membrane Biol., 1, 402 (1969).
35. D. A. Ramsay, J. Amer. Chem. Soc., 74, 72 (1952).
36. B. Wilson and A. J. Wells, J. Chem. Phys., 14, 578 (1946).
37. V. N. Smirnov, Vestn. Mosk. Univ., 1969, No. 1, 61.
38. J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, Biopolymers, 4, 121 (1956).
39. C. M. Venkatochalam, Biopolymers, 6, 1425 (1968).
40. C. Ramachandran and K. P. Sarathy, Int. J. Protein Res., 1, 103 (1969).
41. G. M. Lipkind, S. F. Arkhipova, and E. M. Popov, Molekul. Biol., 4, 509 (1970).
42. G. M. Lipkind, Author's abstract of dissertation [in Russian], Moscow (1970).