SPECTRA OF DIPEPTIDES WITH AROMATIC AMINO-ACID RESIDUES AND OF OLIGOPEPTIDES WITH ALANINE RESIDUES IN THE FAR INFRARED REGION

I. FAR IR SPECTRA OF THE METHYLAMIDE OF N-ACETYL-L-PHENYLALANINE AND OF DIPEPTIDES WITH PHENYLALANINE RESIDUES

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In previous papers [1, 2] on the investigation of the structure of proteins by IR spectroscopy in the middle and far regions, attempts were made to isolate individual sections of the spectra corresponding to different conformational states (α-helices, β form, and so on). The results obtained show that the spectra in the low-frequency region can be used for conformational analysis of even such a complex system as a protein molecule. At the same time, in the treatment of the spectra considerable difficulties connected with the broadening and diffuseness of the bands due to their overlapping arose. The assignment of the observed bands to different amino-acid residues and protein fragments, and also the establishment of a connection between the measurements obtained in the spectra and the conformational state of the system become extremely complex. Consequently, it is particularly desirable to study simple model compounds – for example, methylamides of N-acetyl-L-amino acids and oligomeric short peptides with different sequences of amino-acid residues in the chain as a preliminary stage in the investigation of more complex molecules.

A detailed assignment of the bands in the IR spectrum of the model compound N-acetylglycine methylamide and information on the two possible conformational states of this compound were first obtained by Koyama and Shimanouchi [3]. In addition, other methylamides of acetylamino acids [4, 5] the IR spectra of which show a connection between the position of the bands in the far IR region and the conformation of the molecule have been studied. Recently, the long-wave spectral characteristics of a conformation of the "pleated sheet" type for model cyclohexapeptides with alanine and glycine residues have been obtained [15]. Thus, the number of model compounds investigated is continually expanding, which will permit us to obtain the characteristics of new conformational states encountered in proteins and not described previously.

The question of the assignment of the bands in the conformationally sensitive region of the spectrum of aromatic amino-acid residues (phenylalanine, tyrosine, tryptophan, and histidine) deserves particular attention, since these compounds exist in many globular proteins and give overlapping absorption bands. The study of the IR spectra of short peptides with a lengthening of the chain also presents interest.

The present paper describes the results of a study of the methyl esters of acetyl derivatives of dipeptides constructed from alanine and phenylalanine residues (LL and LD isomers) with different sequences (1)-(3). For the unambiguous assignment of the bands in the spectra of compounds (1)-(3) we have investigated N-acetyl-L-phenylalanine methylamide, its isotope-substituted derivatives, and compounds of similar structure (4)-(10). The IR spectra of the oligomeric alanine peptides were obtained for compounds (11)-(12) and their N-deuterium-substituted derivatives (13)-(14). The formulas of the compounds mentioned are given below.

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(1) Ac-*L-Ala-L-Phe* OMe, (2) Ac-*L-Ala-D-Phe* OMe,
(3) Ac-*D-Phe-D-Ala* OMe, (4) Ac-*L-Phe*NHMe,
(5) $CH_3CONDCHC_6H_5CONDCH_3$, (6) $CD_3CONHCHC_6H_5CONHCH_3$,
(7) $CD_3CONDCHC_6H_5CONDCH_3$, (8) $CH_3CONHCHC_6D_5CONHCH_3$,
(9) Ac-*L-Phe* OMe, (10) $C_6H_5CH_2CH_2CONHCH_3$,
(11) Ac-*L-Ala-L-Ala* NHMe, (12) Ac-*L-Ala-L-Ala-L-Ala*NHMe,
(13) $CH_3CONDCHCH_3CONDCHCH_3CONDCH_3$,
(14) $CH_3CONDCHCH_3CONDCHOH_3CONDCHCH_3CONDCH_3$.

For a more complete assignment of the bands in the IR spectra of compounds (1)-(3) it is desirable in the first place to consider information on the spectra of N-acetyl-L-phenylalanine methylamide (4) and its deuterium-substituted derivatives (5)-(8). Figure 1 gives the spectra in the 800-840 cm^{-1} region. The assignment of the amide vibrations in compound (4) was performed by a published method [3-5]. In the region of the amide vibrations IV-VI (750-500 cm^{-1}) there should be six bands for compound (4) [3], while ten bands are actually observed in the spectrum. The increase in the number of bands is connected with the vibrations due to the aromatic ring. The bands corresponding to the amide vibrations may be arbitrarily divided into two groups: *a* and b, respectively differing by the position of the CONH grouping relative to the C^{α} atom [3, 4]. The inclusion of the amide bands in groups *a* and b follows from the results of a comparison of the spectra of the isotope-substituted compounds (5)-(7) and of the model compounds (9) and (10) containing *a* and b CONH groupings with the spectra of compound (4). The strong and broad band at 715 cm^{-1} is assigned, by analogy with previous work [3, 4], to the amide vibration V*a*, b. This is confirmed by the results of a comparison of the spectra of compounds (5) and (7) in which the amide band is shifted into the ~510 cm^{-1} region. In compounds (6) and (8)-(10) the amide vibration V is represented by a broad and intense band at ~700 cm^{-1}.

Since on N-deuteration the amide IV band is not significantly displaced and the amide VI band is shifted strongly in the low-frequency direction, it is possible to assign both types of vibration unambiguously. In compounds (5) and (7) the amide IV vibration may be represented by the two bands at 700 and at 620 cm^{-1} to which bands at 702 and 615 cm^{-1} correspond in compound (4). In the 620 cm^{-1} region in the spectrum of compound (4) there are two adjacent bands, at 621 and 615 cm^{-1}. The assignment of the 615 cm^{-1} band to the amide IV vibration is based on the fact that the 621 cm^{-1} band is considerably displaced when the aromatic ring is deuterated (see Fig. 1), and this permits it to be assigned to the vibrations of a monosubstituted aromatic ring (symmetry type B_1 [9]), while the 615 cm^{-1} band in compound (8) is displaced insignificantly (612 cm^{-1}). The separation of the amide IV vibrations into groups follows from an analysis of the IR spectra of compounds (6), (7), (9), and (10). On C-deuteration in group *a* the 615 cm^{-1} band is displaced considerably – to 565 cm^{-1} – for compounds (6) and (7), while the 702 cm^{-1} band scarcely changes its position. Consequently, the band at 702 cm^{-1} may be assigned to the amide IVb vibration, and the band at 615 cm^{-1} to the amide IV*a* vibration. This conclusion is confirmed by the results of a comparison of the spectra of the model compounds (9) and (10) having *a* and b groups, respectively, with the spectrum of compound (4). In compound (9) the amide IV*a* vibration is represented by a band at 633 cm^{-1}, and in compound (10) the amide IVb vibration by a band at 699 cm^{-1}, which probably coincides with the broad amide V band.

Let us pass to the assignment of the amide VI bands. In the region of this vibration in the case of compound (4) there are three bands of medium intensity (600, 567, and 541 cm^{-1}), one of which (567 cm^{-1}) is connected with the vibration of an aromatic ring, since in the spectrum of compound (8) it has undergone displacement. Thus, the other two bands, at 600 and 541 cm^{-1}, may be assigned to the amide VI vibration. The assignment of the amide VI bands to groups also follows from the spectra of the C-deuterated compounds (6) and (7). It can be seen from a comparison of the spectra that the bands at 600 cm^{-1} in compound (4) are shifted to ~540 cm^{-1}, and the bands at 541 cm^{-1} in compounds (6) and (7) to the 525 cm^{-1} region. From the difference in the size of the shifts, the 600 cm^{-1} band must be assigned to the amide VI*a* vibration and the 541 cm^{-1} band to the amide VIb vibration. In the model compound (9), the amide VI*a* band appears clearly at 595 cm^{-1} and, furthermore, this compound has no absorption in the 540 cm^{-1} region, while in compound (10) there is no characteristic strong band at 600 cm^{-1}. The band at 540 cm^{-1} in compound (10) may be overlapped by the broad and strong band at 499 cm^{-1}. The assignment of the bands and the order of their arrangement (IVb, IV*a*, VI*a*, VIb) that we have found agree well with information published recently [5] on a series of acetylamino acid methylamides.

The bands in compound (4) that remain in the amide IV-VI region may be assigned on the basis of an analysis of the spectrum of compound (8) and literature information [9] to the vibrations of an aromatic ring (the type of symmetry is shown in brackets): 755 (B_2), 736 (A_1), 702 (B_2), 621 (B_1), 567, and 526 cm^{-1}.

The region of the skeletal vibrations begins with a strong band at 477 cm^{-1} and bands of medium intensity at 415 and 410 cm^{-1}. The first of them, as follows from a comparison of the spectrum of compound (4) with those of compounds (5) (7), and N-acetylglycine methylamide [3], relates to the CCN vibration. This assignment is confirmed by the results of a comparison of the spectra of the two forms of N-acetyl-DL-phenylalanine methylamide [5] in which changes in structure lead to a change in the frequency of the vibrations of the amide group but not of the aromatic ring. In this case, it is again possible to determine the inclusion of the vibrations of the CCN angle to the individual groups. It follows from the spectra of compounds (6), (7), (9), and (10) that the band at 477 cm^{-1} is connected mainly with the vibrations of the (CCN) *a* angle, to which a band at 495 cm^{-1} corresponds in the spectrum of compound (9). A weaker band at 415 cm^{-1} relates to the (CCN) b vibration. The latter is represented by a band at 426 cm^{-1} in the spectrum of compound (10). The remaining band, at 410 cm^{-1}, undergoes no displacement on N- and C-isotope substitution, and therefore must be assigned to the vibration of the aromatic ring, which is shown by the spectrum of compound (8).

Below the region of vibrations of the CCN angles there are other skeletal vibrations characteristic for the peptide skeleton. To these may be assigned the bands at 371, 334, 314, and 255 cm^{-1}. This assignment is confirmed by the small shifts of these bands in the spectra of the N- and C-isotope-substituted molecules, and also by the results of a comparison with the spectra of acetylamino acid methylamides [3-5].

Two strong and broad bands at 227 and 173 cm^{-1} lying in the region of torsional vibrations probably relate to the amide VII vibration. The stretching vibration of the hydrogen bond is also apparently shown by two bands at 132 and 115 cm^{-1}. The presence of two bands in this region, as of two torsional vibrations, is possibly explained by the different nature of the intermolecular bonds in the associated molecules as a consequence of the large volume of the substituent. The assignment of the 135, 115, and 89 cm^{-1} bands relating to the vibration of the crystal lattice was performed by analogy with the work of Koyama and Shimanouchi [3].

On the basis of what has been said above, we have made an assignment of the bands in the spectra of compound (1)-(3) (see Fig. 2). Below we give the assignments of the frequencies in the IR spectra of the dipeptides (1)-(3)

cm^{-1}	Assignment	cm^{-1}	Assignment	cm^{-1}	Assignment
760	?			745	Aromatic ring
744	Aromatic ring	750	Aromatic ring	724	Amide V
718	Amide V	728	Amide V	701	Aromatic ring or amide IV
700	Aromatic ring or amide IV	702	Aromatic ring or amide IV	620	Aromatic ring
622	Aromatic ring	620	Aromatic ring	615	Amide IV
610	Amide IV	606	Amide IV	597	Amide VI
599	Amide VI	600	Amide VI	568	Aromatic ring
568	Aromatic ring	566	Aromatic ring	559	Amide VI
550	Amide VI	548	Amide VI	505	?
501	CCN	507	CCN	497	CCN
461	Ester group	460	Ester group [7, 8]	459	Ester group [7, 8]
420	CCN	414	CCN	422	CCN
406	Aromatic ring	409	Aromatic ring	406	Aromatic ring
400		369		395	
365		340		373	
338	Deformation vibration of	324	Deformation vibration of	329	
322	the skeleton	307	the skeleton	298	Deformation vibration of
270		250		262	the skeleton
244				241	
224	Amide VII	225	Amide VII	222	
204	Ester group [7, 8]	214	Ester group	202	Ester group
155	Aromatic ring	155	Aromatic ring	191	Amide VII?
136	Hydrogen bond	134	Hydrogen bond	155	Aromatic ring
126	Lattice vibration	127	Lattice vibration	134	Hydrogen bond
114	Hydrogen bond	114	Hydrogen bond	116	Hydrogen bond
102	Lattice vibration	102	Lattice vibration		

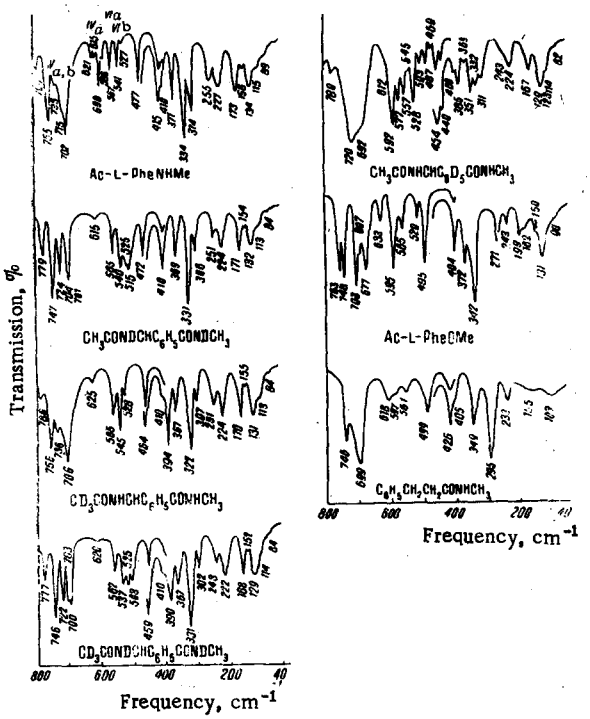

Fig. 1. IR absorption spectra of compounds (4)-(10) (KBr tablets down to 400 cm^{-1} and paraffin oil below cm^{-1}).

To evaluate the changes in the IR spectra with an increase in the peptide chain, compounds (11) and (12) were selected. This question arose in connection with the refinement of the assignment of the amide vibrations in the regular structures of polypeptides. A gradual increase in the length of the peptide chain permits the tendency to a displacement in the spectrum of bands of a structurally repeating element (CONH group) to be followed and the laws obtained to be used for interpreting the IR spectra of more complex molecules. Figure 3 gives the spectra of compounds (11) and (12) and their N-deuterium-substituted analogs (13) and (14) in the 800-840 cm^{-1} region, from which it follows that the amide V vibrations are grouped in the region of a broad and strong band at 710-715 cm^{-1}. On comparing the spectra of compounds (11) and (12) with the spectrum of the first member of the series under investigation – N-acetyl-L-alanine methylamide [4, 5] – and of compounds (13) and (14), it can be seen that the order of arrangement of the amide IV-VI bands remains the same as in the simplest diamide – Ac-L-Ala-NHMe. Consequently, in the spectrum of (12) the band at 638 cm^{-1} must be assigned to the amide IV vibration and several bands in the 615-540 cm^{-1} region to the amide VI vibration. The smaller number of bands in this region for compound (11) is apparently due to the fact that it exists in a somewhat different crystalline modification, and this will be discussed below.

The bands of the vibrations of the CCN angles lie in the lower-frequency region (500-400 cm^{-1}) and so do the vibrations of the peptide skeleton. We may note that the band belonging to an intermolecular hydrogen bond shifts successively from the diamide ($\approx$ 150 cm^{-1}) to the tripeptide ($\approx$ 120 cm^{-1}). The frequencies obtained in the IR spectrum for compound (12) already coincide fairly closely with the frequencies of the β form of poly-L-alanine which has been studied previously [10].

The results of a comparison of the frequencies of the amide vibrations and the vibrations of the CCN angle for Ac-L-Phe-NHMe, Ac-Gly-NHMe, Ac-L-Ala-NHMe, and Ac-L-Va-NHMe in the 700-400 cm^{-1} region permit the conclusion that these diamides have some conformational differences. Particularly pronounced changes in the spectra of Ac-L-Phe-NHMe and Ac-Gly-NHMe, on the one hand, and of Ac-L-Ala-NHMe and Ac-L-Val-NHMe, on the other hand, are observed in the region of the vibrations of the CCN angle (appearance of strong bands at 477 and 481 cm^{-1} in Ac- L-Phe-NHMe and Ac-Gly-NHMe in comparison with the medium-intensity bands at $\approx$ 420 cm^{-1} for Ac-L-Ala-NHMe and Ac-L-Val-NHMe). It is interesting that the appearance of a strong band at 470-490 cm^{-1} is accompanied by some shift in one of the amide VI bands in the high-frequency direction, while the amide V and amide IV bands are displaced to an extremely small extent. This indicates that the conformational differences between the diamides are slight and the values of the angles of internal rotation (Φ and Ψ) are roughly in the same region of the conformational chart [11]. The features that we have noted in the changes of the frequencies of the amide VI bands and of the CCN angle are also seen in the spectra of the methylamides of N-acetyl-DL-leucine, N-acetyl-DL-norleucine, and N-acetyl-L-aspartic acid in comparison with the spectrum of α-acetylamino-n-butyric acid methylamide, which belongs, according to the nature of the spectrum, to diamides of the type of Ac-L-Ala-NHMe and Ac-L-Val-NHMe, which have been studied in detail by Japanese workers [5]. Thus, the series of short peptides may be divided into two classes according to the nature of their IR spectra in the far region:

1) with the amide VI band in the 540-570 cm^{-1} region and intense absorption at 470-490 cm^{-1}; and
2) with the amide VI band at 530-520 cm^{-1} and bands of medium intensity at 410-430 cm^{-1}.

A similar classification using the available x-ray structural data for short peptides enables the angles Φ and Ψ to be determined fairly correctly for both classes, the first of which also includes Ac-L-Phe-NHMe.

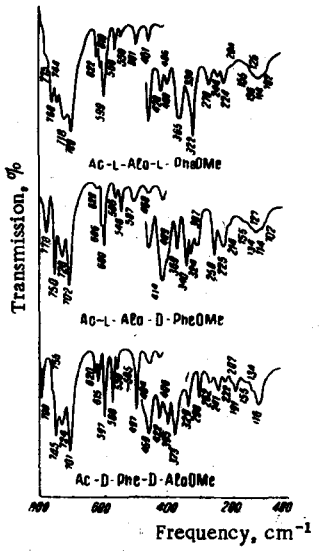

Fig. 2

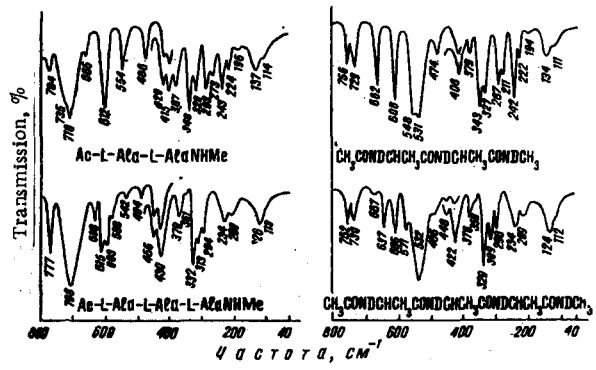

Fig. 3

Fig. 2. IR absorption spectra of the dipeptides (1)-(3) (KBr tablets down to 400 cm^{-1} and paraffin oil below 400 cm^{-1}).

Fig. 3. IR absorption spectra of compounds (11) and (12) and their N-deuterated derivatives (13) and (14) (KBr tablets down to 400 cm^{-1} and paraffin oil below 400 cm^{-1}).

It follows from the x-ray structural results for N-acetyl-DL-leucine methylamide [12] that the values of the angles Φ and Ψ in this compound are 86 and 319°, respectively. Moreover, somewhat different values – 60 and 300° – have been found for α-acetylamino-n-butyric acid methylamide [5]. The first pair of angles is in the range corresponding to the known conformation of the type of polyglycine II, polyproline II, and of a number of amino-acid residues included in peptides and proteins [13], while the second belongs to the conformation of peptides and polypeptides most closely approaching the β form [14]. Consequently, the spectra of the diamides of the first class, which includes Ac-DL-Leu-NHMe and Ac-L-Phe-NHMe correspond to the type of conformation with $\Phi \simeq 100°$ and $\Psi \simeq 330°$ and the spectra of the second class to the conformation with the angles $\Phi \simeq 60°$ and $\Psi \simeq 300°$. The question arises of some lack of correspondence between the results of the conformational analysis of the N-acetylglycine methylamide molecule (in the B form) [3] and the present investigation. The values of the Φ and Ψ for Ac-Gly-NHMe obtained by Koyama and Shimanouchi are 120° and 360°, respectively. This difference from our results is probably due to the inadequate definiteness of the criteria developed by these authors [3], which has already been mentioned in a study of cyclohexapeptides [15]. In addition to this, the results of empirical and quantum-chemical calculations of diamides with phenylalanine residues [16, 17] and also a complete analysis of the conformational chart of the dipeptide Gly-Ala performed by Scheraga's deflation method [18] does not show any region whatever of the existence of a low energy for angles of 120 and 360°.

Since the IR spectra of the dipeptides (1)-(3) are similar to the spectrum of Ac-L-Phe-NHMe in the region of the amide vibrations and of those of the CCN angle (see above), it may be assumed that the conformation of the phenylalanine fragment in these compounds is similar to the conformation of Ac-L-Phe-NHMe, which is confirmed by the PMR spectra for the opened-out form of the dipeptides in solutions which have been studied previously [19]. The vibrations of the aromatic ring in compounds (1)-(4) appear fairly well in the 750-500 cm^{-1} region and are practically absent in the low-frequency region, with the exception of two weak bands at 400 and 155 cm^{-1}. A change in the sequence and configuration of the amino-acid residues in the dipeptides (1)-(3) affects only the frequencies of the skeletal vibrations (below 400 cm^{-1}) which indicates their sensitivity to small geometrical changes in the structure of the molecule not leading to changes in the angles of internal rotation [20].

What has been said above provides the possibility of establishing certain conformational changes with an increase in the length of a peptide chain. The spectrum of compound (11) differs from the spectra of Ac-L-Ala-NHMe [4] and of compound (12), which shows the predominant arrangement of the amino-acid residues in a conformation with the angles $\Phi \simeq 100°$ and $\Psi \simeq 330°$, while the IR spectrum of compound (12)

corresponds to a considerable extent to the spectrum of the β form of poly-L-alanine [10] both in the region of amide and skeletal vibrations and in the region of the stretching vibration of a hydrogen bond. This is probably explained by the fact that in compound (11) the terminal amide groups have a greater possibility of internal rotation than in the diamide, and with an increase in the number of CONH groupings in compound (12) their contribution to the conformational state becomes less considerable because of the formation of sections with a regular structure.

EXPERIMENTAL

The synthesis of the dipeptides (1)-(3) was performed as described by Portnova et al. [6]. The other compounds were synthesized by the usual methods of peptide synthesis. The constants of the compounds obtained are given below.

Compound	mp °C	$[\alpha]_D$, deg	Solvent
(1)	140	+8	2, $CHCl_3$
(2)	138	−70	2, $CHCl_3$
(3)	190	+20	1, C_2H_5OH
(4)	226	−23	1, C_2H_5OH
(9)	88,5	—	—
(10)	45–50	—	—
(11)	310 (subl.)	−105	1, H_2O
(12)	Amorph.	−78	1, CH_3COOH

The IR spectra in the 800-400 cm^{-1} region were measured on Perkin-Elmer 257 and Hilger H-800 instruments (discs with KBr) and in the 500-540 cm^{-1} region on a Hitachi FIS-21 spectrometer with diffraction gratings (paraffin oil) between polyethylene windows. The accuracy of the measurements of all the bands observed was ± 2 cm^{-1} (in the 800-400 cm^{-1} region) and ± 1 cm^{-1} (in the 500-540 cm^{-1} region).

The instruments were calibrated from the absorption bands of polystyrene and of water vapor. The degrees of deuteration of compounds (5), (6), (7), (8), (13), and (14) were evaluated from the disappearance of the bands corresponding to stretching vibrations for the N- and C-deuterated derivatives and of those corresponding to the deformation vibrations in the 750-700 cm^{-1} region where the aromatic ring was deuterated.

Samples (1)-(3) were kindly given to us by P. V. Kostetskii.

SUMMARY

1. Two types of conformational states differing in the nature of their spectra in the far IR region have been found for a number of methylamides of N-acetyl-L-amino acids (in the solid state).

2. The changes in the IR spectra in the far infrared with an increase in the length of the peptide chain and with a change in the sequence and configuration of the amino-acid residues have been discussed.

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