CONFORMATIONAL STATES OF METHYLAMIDES OF N-ACETYL α-AMINO ACIDS AND THEIR N-METHYL DERIVATIVES

I. INFRARED SPECTRA

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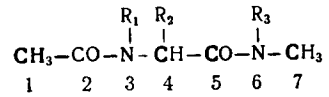
A study of the spatial structure of peptide systems is a necessary stage in the elucidation of the nature and mechanism of their biological action at the molecular level.

The simplest compounds including the main structural elements of a peptide chain are the methylamides of N-acetyl α-amino acids ($CH_3NHCO-CHR-NHCOCH_3$). They are extremely convenient subjects for the study of the nonvalent interactions of the atoms of neighboring peptide groupings and a side chain (R) located between them. The determination of the relationship between the chemical structure of compounds of this type and their conformational states enables the role of this type of interactions in the formation of the structure of more complex peptide–protein systems to be evaluated. The results of a recent theoretical conformational analysis [1, 2] have shown that even in proteins in all local sections of their peptide chain the conformational states of the amino acid residues both in the main chain and in the side chains do not differ significantly from the optimum spatial forms in di- and tripeptide fragments.

In recent years, the methylamides of N-acetyl α-amino acids have become the subjects of careful theoretical analysis (see, for example [3-10]), which has shown that the preferred conformational states of these compounds can be described with the aid of a limited number of canonical forms. Since the conformational states of the amino acid residues in complex peptides also correspond to these forms, the determination of a direct connection between the optimum conformations of model compounds and the experimental results obtained by various physical methods is of fundamental interest. Furthermore, the systematic experimental investigation of the conformations of model compounds will permit a comparison of the experimental results with those of calculations already performed, which is extremely important for the further development of the conformational analysis of peptides and for understanding the nature of the nonvalent interactions and evaluating them quantitatively. And, finally, in view of the fact that the methylamides of N-acetyl α-amino acids and simple peptides related to them [11, 12] are substrates or inhibitors for a series of proteolytic enzymes, the study of the conformational possibilities of such systems is of independent value for enzymology.

The present series of papers [13-16] gives the results of a comprehensive experimental investigation of the conformational states of the following series of methylamides of N-acetyl α-amino acids and their N-methyl derivatives.



I. Ac-L-Ala-NHMe; $R_1=R_3=H$, $R_2=CH_3$
II. Ac-L-Ala-NMe_2; $R_1=H$, $R_2=R_3=CH_3$

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III. Ac-L-MeAla-NHMe; $R_1=R_2=CH_3$, $R_3=H$

IV. Ac-L-MeAla-NMe_2; $R_1=R_2=R_3=CH_3$

V. Ac-L-Val-NHMe; $R_1=R_3=H$, $R_2=CH(CH_3)_2$

VI. Ac-L-Val-NMe_2 $R_1=H$, $R_2=CH(CH_3)_2$, $R_3=CH_3$

VII. Ac-L-MeVal-NHMe; $R_1=CH_3$, $R_2=CH(CH_3)_2$, $R_3=H$

VIII. Ac-L-MeVal-NMe_2; $R_1=R_3=CH_3$, $R_2=CH(CH_3)_2$

IX. Ac-L-Pro-NHMe; R_1, $R_2=(CH_2)_3$, $R_3=H$

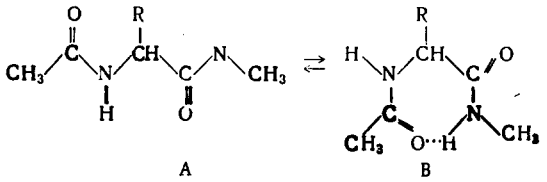
X. Ac-L-Pro-NMe_2; R_1, $R_2=(CH_2)_3$, $R_3=CH_3$

XI. Ac-Gly-NHMe; $R_1=R_2=R_3=H$

XII. Ac-L-Phe-NHMe. $R_1=R_3=H$, $R_2=CH_2C_6H_5$.

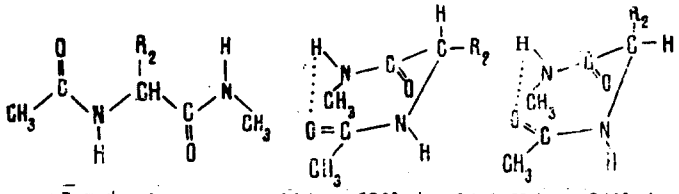
In the investigation of the diamides given in the list, it was necessary to determine their spatial structure and to examine the influence on the position of the conformational equilibrium and on the configuration of the amide groups of the nature of the substituents R_1, R_2, and R_3, the nature of the solvents, and the temperature; to find the relationship between the spatial form of the diamide and the experimental parameters of various methods; and to compare the experimental results with those of a theoretical conformational analysis of analogous compounds.

The IR spectra of a series of related diamides were first described in the fifties by Mizushima et al. [17-26]. In the spectra of dilute solutions of the diamides in CCl_4, these authors found several absorption bands in the 3300-3500-cm^{-1} region corresponding to the stretching vibrations of a NH group. A diffuse band at 3340-3390 cm^{-1} was assigned to the vibrations of a NH group forming an intramolecular H bond of the 3→1 type (third residue connected with the first), which stabilizes a seven-membered ring; bands in the higher-frequency region were assigned to the vibrations of free NH groups. It followed from this that in the diamides in a nonpolar medium there is a conformational equilibrium of extended (A) and folded (B) forms:



A B

Starting from the values of the intensities of the absorption bands at the maxima, Japanese authors, using the methylamide of N-acetyl-DL-N-methylnorleucine as an example, evaluated the amounts of folded and extended forms at various temperatures and determined their entropies and free energies [25]. On the basis of general considerations, for the B form a conformation was proposed [26] with the pseudoequatorial position of the R_2 side chain ($\Phi \sim 120°$ ($C^{\alpha}-N$), $\Psi \sim 240°$ ($C^{\alpha}-C'$), [27]), and for the A form a plane extended structure ($\Phi = \Psi = 0°$) with an intramolecular hydrogen bond of the 1→1 type stabilizing a five-membered ring.



$\Phi = \Psi = 0$ M ($\Phi = 120°$, $\Psi = 240°$) H ($\Phi = 240°$, $\Psi = 120°$)

A B

The extended form of the methylamide of N-acetyl-L-proline, because of the fixation of the dihedral angle Φ at $\approx 120°$, was assigned a structure intermediate between the conformations mentioned above.

Portnova et al. [28] showed that an equilibrium of folded and extended forms is also found in various derivatives of alanine dipeptides. Measurements of the IR spectra at various temperatures and the calculation of the integral intensities of the bands corresponding to the free NH groups permitted a quantitative evaluation of the proportion of the various forms and a determination of the thermodynamic parameters of the transition A⇌B; on the basis of a subsequent analysis of NMR spectra, it was concluded [29] that the pseudoaxial orientation of R_2 in a seven-membered ring ($\Phi \sim 240°$, $\Psi \sim 120°$) was preferred.

The IR spectra of a large number of alkylamides of N-acetyl α-amino acids were investigated by Avignon et al. [30-33]. As in the investigations mentioned above, these authors came to the conclusion of

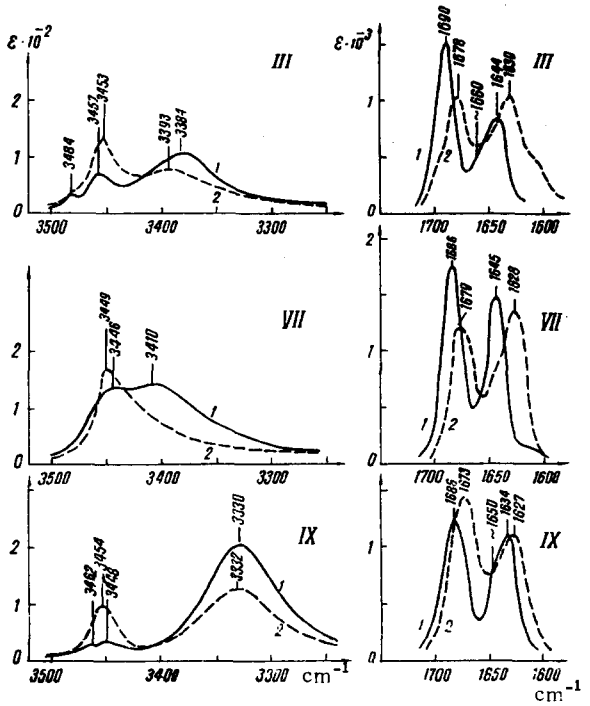


Fig. 1. IR spectra of the methylamides of N-acetyl-N-methyl-L-alanine (III), N-acetyl-N-methyl-L-valine (VII), and N-acetyl-N-methyl-L-proline (IX): 1) in CCl_4; 2) in $CHCl_3$.

the existence of the equilibrium A ⇌ B in CCl_4 solutions. Then extended forms are mainly assigned the conformation with the parameters $\Phi = \Psi = 0°$, and the folded forms those with the parameters $\Phi \sim 120°$ and $\Psi \sim 240°$. On the basis of the peak intensities of the bands corresponding to the extended forms, the French workers in some cases evaluated the proportions of A and B in the equilibrium mixture. However, it must be observed that the assignments of the bands that they adopt differed from the assignments proposed by Mizushima et al. [26], and used by Portnova et al. [28].

On the basis of an analysis of the normal vibrations in the IR spectra in the 60-4000-cm^{-1} range of the crystalline methylamide of N-acetylglycine, Koyama and Shimanouchi [34] came to the conclusion that one stable extended conformation ($\Phi \sim 120°$, $\Psi \sim 0°$) and one metastable conformation ($\Phi \sim 120°$, $\Psi \sim 180°$) were realized. A similar structure in the crystalline state has been ascribed to the methylamides of a number of other amino acids [35].

Thus, in all the investigations performed, the existence in solutions of the diamides of two spatial forms, A and B, has been shown. Nevertheless, there is no single opinion on the concrete geometry of these forms. For each compound the presence of not more than one folded and one extended conformation has been proposed, and these, as a rule, are considered to be identical for a whole series of alkylamides of N-acetyl α-amino acids and their N-methyl derivatives. In the light of the results of a theoretical conformational analysis [6-9, 36, 37], these assumptions do not appear to be obvious. Furthermore, there is no sufficiently strict quantitative information on the relationship between the nature of the equilibrium A ⇌ B, the nature of the solvent, and the temperature; the assignments of the absorption bands of the NH group in the extended and folded forms are contradictory.

In the study of IR spectra, we did not attempt to obtain a detailed idea of the conformational equilibrium and of the number and concrete geometries of the preferred conformations of compounds (I-XII) (this will be done later in a consideration of the whole accumulation of experimental results of various physical methods and the results of theoretical analysis). Our immediate aim was a quantitative investigation of the equilibrium of the two types of forms of diamides in nonpolar (CCl_4) and weakly polar ($CHCl_3$) media. In view of this, the interpretation of the absorption bands of the NH and CO groups was reconsidered. The results obtained from the IR spectra on the A ⇌ B equilibrium are necessary for the further study of the rotational isomerism of the diamides; the assignment of the frequencies made may prove useful in spectroscopic investigations of more complex peptide compounds.

Furthermore, the question, which has not yet been discussed in detail in the literature, of the mutual influence of the amide groups separated in the peptide chain by saturated C_α atoms is also of interest. For this purpose, we have studied additional material on the IR spectra of the corresponding simple amides and have compared their spectral characteristics with the characteristics of the amide groups in the diamides.

The IR spectra of compounds (I-XII) in CCl_4 and $CHCl_3$ solutions are given in Figs. 1-5. The spectra obtained relate to the monomeric forms of the molecules; the absence of any appreciable association of the diamides whatever is shown by the results of measurements of the spectra at higher concentrations of the substances, and also by gas-liquid osmometry [13].

Let us first consider the region of the stretching vibrations of NH groups (3250-3500 cm^{-1}). The assignment of the bands in the IR spectra of compounds (III), (VII), and (IX), containing one secondary amide group (see Fig. 1) is not a matter of doubt. Only one type of H bond (3 → 1) is possible for them, and consequently the two absorption bands observed (3330-3410 cm^{-1} and 3450-3460 cm^{-1}) correspond, respectively,

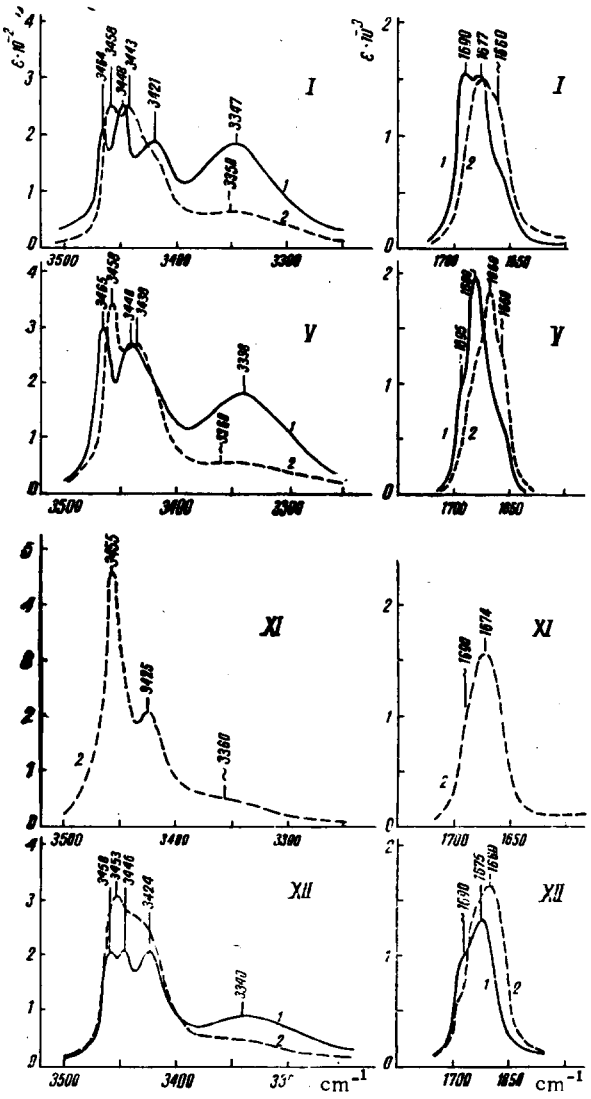


Fig. 2. IR spectra of the methylamides of N-acetyl-L-alanine (I), N-acetyl-L-valine (V), N-acetylglycine (XI), and N-acetyl-L-phenylalanine (XII): 1) in CCl_4; 2) in $CHCl_3$.

to the folded and extended forms. The doublet nature of the low-intensity absorption band of the free NH group in the spectrum of compound (IX) may be connected both with the realization of two extended conformations [38] and with the presence of a form having the cis configuration of the tertiary amide group [13]. A similar explanation can be given for the low-intensity band at 3484 cm^{-1} in the spectrum of compound (III).

In the IR spectra of compounds (I), (V), (XI), and (XII) there is a considerably more complex picture owing to the presence in the molecules of two nonequivalent secondary amide groups (see Fig. 2). In the spectra of compounds (I), (V), and (XII) in CCl_4 in the NH region there are four bands (3338-3360, ~3420, ~3440, and ~3460 cm^{-1}) and in the spectrum of (XI) there are three (~3360, 3425, and 3455 cm^{-1}*). The bands in the 3338-3360-cm^{-1} region are ascribed to the $N^{(6)}H$ bonds in the folded forms of the molecules. In all cases, on passing from solutions in CCl_4 to those in $CHCl_3$, the intensities of these bands decreased, which shows an increase in the amount of the extended forms. In the spectrum of each compound, the intensity of the band at ~3460 cm^{-1} rose, which, on this basis, can be ascribed to the extended form and, more accurately, by analogy with (III), (VII), and (IX) (see Fig. 1), to the vibrations of the $N^{(6)}H$ bond of this form. This assignment agrees completely with the conclusions of the Japanese and French workers. The difference between them relates to the assignment of the bands at ~3440 and ~3420 cm^{-1}, the first of which Mizushima et al. [26] connect with the $N^{(3)}H$ vibrations in the extended forms and the second with the vibration of the free $N^{(3)}H$ bond in the folded forms. The opposite assignment is made in the papers of Avignon et al. [30-33]. Let us consider this question in more detail.

Of the spectra of compounds (I), (V), (XI), and (XII) shown in Fig. 2 (CCl_4 and $CHCl_3$ solutions), the best-resolved bands are found in the spectrum of (I). Passage to $CHCl_3$ is accompanied in the case of (I) by a marked decrease in the intensity of the bands at ~3350 and ~3420 cm^{-1} and by an increase in the intensity of the bands at ~3440 and ~3460 cm^{-1}. Since under these conditions the proportion of folded form decreases, it is natural to assign the band at ~3420 cm^{-1} (in agreement with [26]) to the $N^{(3)}H$ vibration of this form. The correctness of this assignment is confirmed additionally by information on the integral intensities of all the NH bands of compound (I) at various temperatures (see Fig. 3, Table 1) and also by the changes that take place in the spectra of (I) when the concentration is increased (see Fig. 3a). A rise in the temperature of the solutions in CCl_4 and $CHCl_3$ leads to a symbatic increase in the intensity of the bands at ~3440 and ~3460 cm^{-1} and to a decrease in those at ~3350 and ~3420 cm^{-1}. The intensities of the two pairs of bands behave in the opposite way when the concentration of (I) in CCl_4 is increased from $2.5 \cdot 10^{-4}$ (absence of association) to $7.0 \cdot 10^{-4}$ mole/liter (slight intermolecular association [15]); changes of similar nature are observed in the IR spectra of the ethylamide of N-acetylglycine in CCl_4 with variations of the temperature and concentration [30]. The examples given show that the high-frequency NH bands in the spec-

*Results for a solution of (XI) in $CHCl_3$. The low solubility of (XI) did not permit the spectrum of this compound to be obtained in CCl_4.

TABLE 1. Influence of the Temperature on the Frequencies and Integral Intensities of the Absorption Bands of the NH Stretching Vibrations in Ac-L-Ala-$NHCH_3$ in CCl_4*

t°C	ν, cm^{-1}	A·10⁻⁴, $mole^{-1} \cdot liter \cdot cm^{-2}$		ν, cm^{-1}	A·10⁻⁴, $mole^{-1} \cdot liter \cdot cm^{-2}$		ν, cm^{-1}	A·10⁻⁴, $mole^{-1} \cdot liter \cdot cm^{-2}$		ν, cm^{-1}	A·10⁻⁴, $mole^{-1} \cdot liter \cdot cm^{-2}$	
		A_r	A_b		A_r	A_b		A_r	A_b		A_r	A_b
3,5	3447	0,34	0,24	3465	0,19	0,16	3419	0,78	0,60	3338	2,92	1,89
22	3448	0,42	0,34	3467	0,25	0,19	3421	0,60	0,40	3345	2,58	1,75
50	3450	0,39	0,34	3467	0,25	0,25	3422	0,39	0,30	3358	1,35	0,98
70	3449	0,53	0,35	3466	0,36	0,27	3422	0,31	0,26	3366	1,15	0,65

*The values of the frequencies were obtained after the separation of the bands in the calculation of the integral intensities; A_r and A_b are the values of the integral intensities calculated by Ramsay's method and by the base-line method, respectively (see Experimental section).

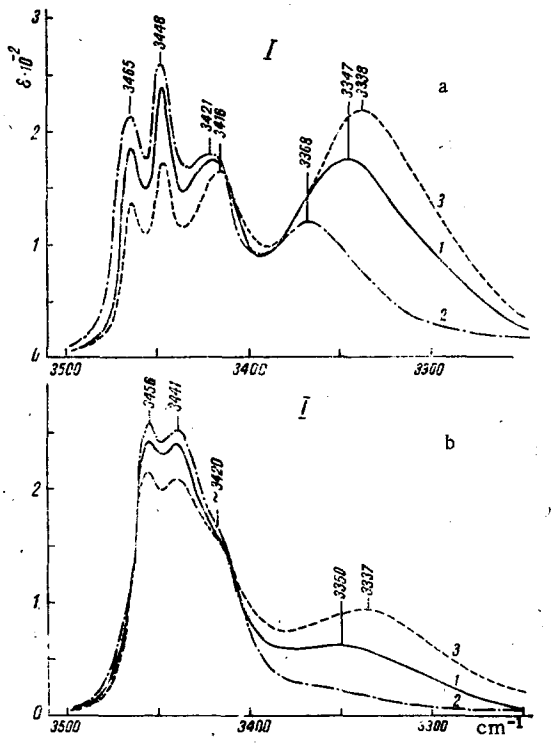


Fig. 3. IR spectra of the methylamide of N-acetyl-L-alanine (I) in CCl_4 (a) and $CHCl_3$ (b) at the following temperatures and concentrations: a) 1, 2, 3) t = 22°, 70, 22°, c = $1.85 \cdot 10^{-4}$, $1.85 \cdot 10^{-4}$, $7.0 \cdot 10^{-4}$ M, respectively; b) 1, 2, 3) t = 22°, 58, 3°, c = $2.3 \cdot 10^{-3}$, $2.3 \cdot 10^{-3}$, $2.3 \cdot 10^{-3}$ M, respectively.

trum of (I) are due to the extended form A and the low-frequency bands to the folded form B. This interpretation does not contradict the spectra of compounds (V), (XI), and (XII), either. In the spectrum of the methylamide of N-acetylglycine (XI) in the extended form, the frequencies of the NH stretching vibrations have extremely close values.

The assignment of the band at ~3420 cm^{-1} to the extended form of the diamides in the literature [30-33] was based (apart from a formal comparison of spectra, see below) on its specific spectral characteristics – great width, high intensity, and comparatively low frequency for a free NH group. All this, in the opinion of the authors concerned [30-33], is due to the formation in the planar structure of a H bond closing a five-membered ring. However, in our opinion, the above-mentioned features of the ~3420-cm^{-1} band are a consequence of the direct interaction of the two amide groups in the folded form of the molecule.

It may be assumed that the formation of the $C^{(2)}=O \cdots H-N^{(6)}$ H bond in the seven-membered ring of form B is accompanied by a shift in the electron density in the direction of the first amide group (the C-terminal group), which affects the properties of both groups and, in particular, of the $N^{(3)}H$ group not directly participating in a H bond. The diffuse nature of the H bond is due to the migration of electron density over the whole system of associated amide groups of form B, which leads to a dependence of the intensity and width of the $N^{(3)}H$ absorption band not only on the vibrational coordinate proper but also on the O ··· H coordinate. If this explanation of the nature of the ~3420-cm^{-1} bond is correct, i.e., if the properties of the NH bond not directly participating in association do actually depend on the formation of a H bond by the carbonyl of the same amide group, then in principle a similar phenomenon must also exist in the association of simple amides and appear, in particular, in their IR spectra with a rise in the concentration. Figure 4 shows the IR spectra of N-methylacetamide obtained at different degrees of dilution; as can be seen, in the spectrum of the most dilute solution (1) there is a single band at 3477 cm^{-1} with a symmetrical contour belonging to the NH vibration of the monomeric molecule. With a rise in the concentration (Fig. 4, curve 2) a diffuse band appears at ~3375 cm^{-1} which shows the formation of associates. The band of free

TABLE 2. Frequencies and Intensities of the Stretching Vibrations of NH and CO in Secondary Amides in CCl_4 Solution

Compound	NH bands		CO bands	
	ν, cm^{-1}	$A \cdot 10^{-4}$, $mole^{-1} \cdot l \cdot cm^{-2}$	ν, cm^{-1}	$A \cdot 10^{-4}$, $mole^{-1} \cdot l \cdot cm^{-2}$
$CH_3—CONH—CH_3$	3477	0,87	1692	3,80
$CH_3CH_2—CONH—CH_3$	3475	0.76	1690	3,33
$(CH_3)_2CHCH_2—CONH—CH_3$	3475	0,62	1690	3,42
$C_6H_5CH_2CH_2—CONH—CH_3$	3474	0,68	1690	4,19
$CH_3—CONH—CH_2CH_3$	3465	0,58	1690	3,80
$CH_3—CONH—CH_2CH(CH_3)_2$	3468	0,45	1691	3,52
$CH_3—CONH—CH_2CH_2C_6H_5$	3462	0,66	1690	3,61
$C_6H_5CH_2CH_2—CONH—CH_2CH_2C_6H_5$	3460	0,79	1687	4,46

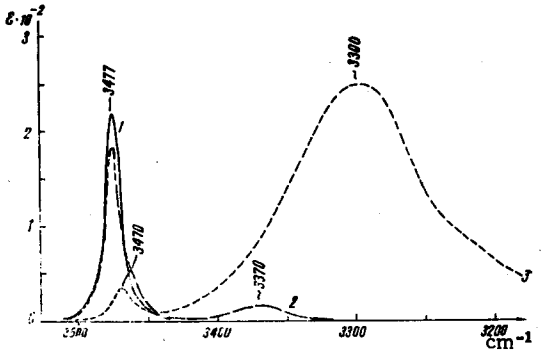


Fig. 4. IR spectra of N-methylacetamide in CCl_4 at concentrations of $1.2 \cdot 10^{-3}$ M (1), $5.9 \cdot 10^{-3}$ M (2), and $5.3 \cdot 10^{-1}$ M (3).

NH bonds at 3477 cm^{-1} decreases in intensity and, which is fundamental, its contour becomes unsymmetrical. This is obviously connected with the fact that at a comparatively low concentration low-molecular-weight complexes (dimers and trimers) in which the frequencies of the vibrations of the free NH bonds have a lower value than the NH frequency of the monomer predominate among the associates. With a further increase in the concentration (Fig. 4, curve 3), the maximum of the band of the associated NH bonds shifts to ~3300 cm^{-1} and its intensity rises. The high-frequency band not only decreases in intensity, but its frequency shifts to 3470 cm^{-1}, and the contour again approximates a symmetrical form. Such changes are perhaps connected with the appearance in this region of the vibrations of free NH bonds due mainly not to isolated but to associated molecules of the N-methylacetamide. Consequently, the intensity of this band, particularly at fairly high concentrations, does not fully reflect the concentration of monomeric molecules in the solution.

Thus, here also, as in the case of diamides in the folded form, the formation of the H bond by one part of the amide groups affects the state of its other part.* In view of this, it is interesting to direct attention to the continuous shift with a rise in the concentration of N-methylacetamide of the maximum of the absorption band of associated NH bonds, which takes place up to the disappearance of the high-frequency band. This also shows the cooperative nature of association (for further details, see [15]). A decrease in the potential energy (calculated to one H bond) in the trimer of N-methylacetamide as compared with the dimer is also shown by the results of a recent quantum-chemical calculation [41]. Just as in the case of N-methylacetamide, we have observed changes in the bands of the stretching vibrations of free and bound NH groups with an increase in the concentration of the solution for the IR spectra of the amides listed in Table 2.

The assignment of the bands at ~3420 cm^{-1} to the $N^{(3)}H$ bonds in the diamides was also based by the French authors [30-33] on the presence of these bands in the IR spectra of a number of dialkylamides of N-acetylglycine and N-acetylalanine in which a H bond of the 1→1 type is possible which stabilizes the planar extended form of the main chain with the parameters $\Phi = \Psi = 0°$. Evidence in favor of the formation of such a H bond, in the opinion of these authors [30-33], is the presence of only one band (3412 cm^{-1}) in the spectrum of $AcGlyNEt_2$ in CCl_4 at 25°C and the appearance of a new band (3450 cm^{-1}) at 70°C†, and also a fall in the intensity of the 3415-cm^{-1} band on passing from AcAlaNMeEt to $AcAlaNEt_2$, which was explained [30] by the destabilization of the H bond in the five-membered ring because of steric hindrance. In actual fact, the IR spectra of compounds (II) and (VI) showed two bands – at about 3420 and 3440 cm^{-1} – which we con-

*On the electronic structure and properties of the amide group, see [39]. A shift of the band of a free NH group in the formation of associates of N-methylacetamide has been reported previously by McLachlan and Nyquist [40].

† The temperature measurements that we performed for compound (II) showed no changes whatever in the spectrum.

nect with the presence of two extended forms (for more details, see [14, 16]). However, as has been shown above, the coincidence of the ~3420 cm^{-1} band in the spectra of (II) and (VI) with one of the bands in the spectra of (I), (V), (XI), and (XII) cannot serve as a basis for its assignment to the $N^{(3)}H$ vibrations in the extended forms. In later papers [16, 14] we shall show that in the conformational equilibrium of compounds (I) and (V) and of (II) and (VI) the dominating extended forms have extremely diverse geometric parameters Φ, Ψ (in all cases, different from $\Phi = \Psi = 0°$). It has recently been shown [42] that in the IR spectra of the cyclic decapeptide N,N'-diacetylgramicidin S in $CHCl_3$ solution there is a band at 3426 cm^{-1} which is assigned to free NH groups of D-phenylalanine residues and to the side chains of acetylornithine residues. The parameters $\Phi = 235°$ and $\Psi = 70°$ correspond to the conformational states of the D-phenylalanine residues; thus, this example too shows that the presence in the spectra of amides and peptides of a band at ~3420 cm^{-1} is not a criterion of a planar structure of the main chain but is connected with the participation of the corresponding carbonyl groups in H bonds.

Hence, the unambiguous assignment of the ~3420-cm^{-1} band to the vibrations of NH bonds participating in H bonds of the 1 → 1 type and the preference that follows from this of the conformation with $\Phi = \Psi = 0°$ in the diamides encounters a number of serious objections. Thus, according to a theoretical analysis [6, 9, 36, 37] the forms of compounds (I), (II), (V), (VI), (XI), and (XII) with $\Phi = \Psi = 0°$ do not correspond to potential-energy minima.

Avignon et al. [30, 31] have suggested that the noncorrespondence of the results of a theoretical analysis of their interpretation of the IR spectra is due to the unsatisfactory nature of the potential of the H bond used in the calculations for a description of the interaction of the atoms in the five-membered ring. It is fairly obvious that at any reasonable potential this weak* H bond cannot change the potential surface of the diamides in such a way that the conformation $\Phi = \Psi = 0°$ becomes predominating. The low probability of this conformation is also shown by the fact that only some of the very many hundreds of amino acid residues in proteins of known structure have angles Φ, Ψ approximating to the values $\Phi = \Psi = 0°$ [44, 45]. Information on dipole moments [14], CD and ORD spectra [16], and the results of calculation [37] show that in nonpolar and weakly polar media compound (II) exists mainly in the γ form ($\Phi \sim 50°$, $\Psi \sim 250°$), and compound (VI) in the γ and δ forms ($\Phi \sim 80°$, $\Psi \sim 320°$). In these conformations, which are favorable from the point of view of all forms of nonvalent interactions, the distance between the $H(N^{(3)})$ and $O(C^{(5)})$ atoms is 2.9-3.1 Å, which corresponds, for example, for the H-bond potential proposed in [45], to a reduction in energy by 0.4-0.2 kcal/mole. This is insufficient to affect the geometry of the molecule appreciably, although in this case small changes in the IR spectra may be expected. Thus, we connect the somewhat lowered values of the frequencies of the stretching vibrations of the $N^{(3)}H$ and $C^{(5)}O$ bonds in the spectra of (II) and (VI) and the increased intensities of the corresponding absorption bands (Fig. 5) not with the realization of a planar structure of the main chains in (II) and (VI) but with the existence of a weak interaction between the H and O atoms in the nonplanar conformations of these molecules.

The analysis performed permits the ranges of frequencies of the NH vibrations of the amide groups in the folded and extended forms of the diamides in solutions in CCl_4 and $CHCl_3$ to be evaluated in the following way (a detailed assignment of the bands is given in Table 3):

$N^{(3)}H$ vibration in extended forms: 3415-3460 cm^{-1};
$N^{(3)}H$ vibration in folded forms: 3415-3430 cm^{-1};
$N^{(6)}H$ vibration in extended forms: 3445-3465 cm^{-1};
$N^{(6)}H$ vibration in folded forms: 3330-3410 cm^{-1}.

The results that we have obtained and those given in the literature permit a number of conclusions concerning the interaction along the chain of two amide groups and the influence of the nature of the side chain and of the spatial form of the molecule.

In the extended forms there is no direct bond between the amide groups. Consequently, the values of the frequencies are determined to a considerable extent by the immediate environment. As can be seen from Tables 2 and 3, the frequencies of the terminal $N^{(6)}H$ groups of the methylamides of N-acetyl α-amino acids in these forms are extremely close to the frequencies of the corresponding secondary amides; in both

*This is shown by the high frequency of the $N^{(3)}H$ vibrations and the large distance (~2.2 Å) between the H and O atoms (the energy minimum of the H bond according to experimental results [43] is at ~1.8 Å). Furthermore, in the five-membered ring of the planar structure the mutual arrangement of the C=O and N−H groups is unfavorable for the formation of a H bond.

TABLE 3. Assignment of the Frequencies of the NH and CO Stretching Vibrations in the IR Spectra of Solutions of Diamides in CCl_4 and $CHCl_3$*

No.	Compound	Form							
		extended				folded			
		$N^{(3)}H$	$N^{(6)}H$	$C^{(2)}O$	$C^{(5)}O$	$N^{(3)}H$	$N^{(6)}H$	$C^{(2)}O$	$C^{(5)}O$
I	Ac-*L*-Ala-NHMe	3448 (3443)*	3464 (3458)	1678 (1660 sh)	1690 (1675)	3421 (3425)	3347 (3350)	1660sh (1660 sh)	1690 (1675)
II	Ac-*L*-Ala-NMe_2	3420 3440 sh (3418)	—	1681 1695 sh (1666, 1680 sh)	1653 1640 sh (1644)				
III	Ac-*L*-MeAla-NHMe	—	3457 3484 (3453)	1660 sh (1640 sh)	1690 (1678)		3384 (3395)	1644 (1630)	1690 (1678)
IV	Ac-*L*-MeAla-NMe_2			1652 1660 sh (1640)					
V	Ac-*L*-Val-NHMe	3440 (3438)	3465 (3458)	1680 (1660 sh)	1695sh (1668)	3425sh (3420sh	3338 (3360)	1660 (1660 sh)	1695sh (1680 sh)
VI	Ac-*L*-Val-NMe_2	3435 3416 sh (3425)		1684 1695 sh (1670, 1680 sh)	1651 (1640)				
VII	Ac-*L*-MeVal-NHMe		3446 (3449)	1649 sh (1643 sh)	1686 (1679)		3410 (3410)	1645 (1628)	1686 (1668 sh)
VIII	Ac-*L*-MeVal-NMe_2			1649 (1633)					
IX	Ac-*L*-Pro-NHMe	3462	3462 3448 (3424)	1650sh (1650 sh)	1686 (1673)		3330 (3332)	1634 (1627)	1686 (1673)
X	Ac-*L*-Pro-NMe_2			1656, 1680 sh (1635, 1660 sh)					
XI	Ac-Gly-NHMe	(3455)		(1674)	(1690 sh)	(3425)	(3360)	(1674)	(1690 sh)
XII	Ac-*L*-Phe-NHMe	3446 (3430sh)	3458 (3453)	1675 (1669)	1690 sh (1683 sh)	3424 (3430sh)	3340 (3345)	1675 (1669)	1690 sh (1683 sh)
†	Ac-*L*-Met-NHMe	3440	3460			3426	3345		
†	Ac-*L*-Met-NHEt	3442				3422	3332		
†	Ac-*L*-Ala-NHEt	3445				3416	3332		
†	Ac-Gly-NHEt	3460	3445			3417	3340		
†	Ac-Gly-NHMe	3444	3462			3424	3348		
†	Ac-*L*-Val-NHEt	3445				3416	3332		

*The frequencies of the bands observed in the spectrum of solutions in $CHCl_3$ are given in brackets.

† The regions of the NH vibrations of the compounds in CCl_4 solution have been described in the literature [30-33].

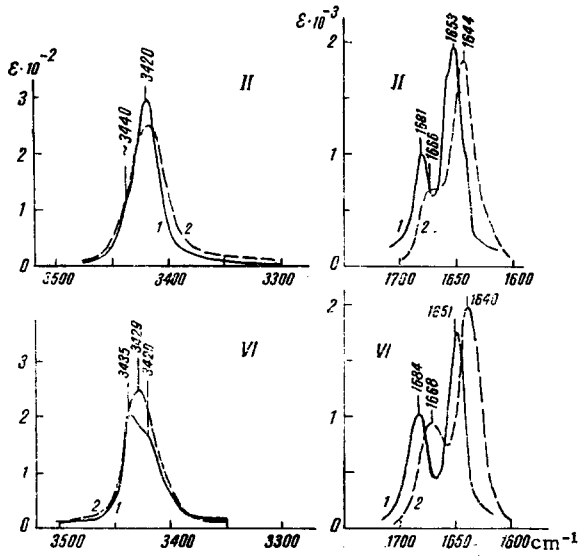


Fig. 5. IR spectra of the dimethylamides of N-acetyl-L-alanine (II) and of N-acetyl-L-valine (VI): 1) in CCl_4; 2) in $CHCl_3$.

cases, the passage from a $-NHMe$ group to a $-NHEt$ group is accompanied by a lowering of the NH frequency by 10-15 cm^{-1}. The mutual influence of the amide groups in the extended forms has a considerable effect on the frequencies of the $N^{(3)}H$ bonds, which have a fairly wide range of possible values (3415-3460 cm^{-1}). The observed values of the $N^{(3)}H$ frequencies depend both on the structure of the adjacent amide group (the passage from secondary amide groupings to tertiary is apparently accompanied by some lowering of the $N^{(3)}H$ frequency) and on the mutual orientation of the two amide groups, i.e., on the parameters Φ and Ψ. As reported above, the mutual influence of the amide groups appears particularly strongly in the extended forms, where they participate in the formation of a H bond. So far as concerns the influence of side chains, judging from the spectra of the Gly, Ala, Val, Phe, and Pro derivatives investigated, their nature has little effect on the electronic structure of the amide groups. In this respect, likewise, analogies have been found with the amides (see Table 2). The passage from solutions in CCl_4 to $CHCl_3$ is accompanied in the IR spectra of the extended forms (as in the case of secondary amides) by a fall in the frequencies of the vibrations of the $N^{(3)}H$ and $N^{(6)}H$ groups by not more than 10 cm^{-1}. The NH frequencies of the folded forms change in a different way; the weakening of the H bond in $CHCl_3$ is accompanied by a rise in the frequencies of both the bound and the free NH groups. The opposite direction of the shifts of the NH vibrational frequencies of the folded and extended forms Of (I), (V), and (XII) leads to a considerable overlapping of the bands in the spectra of solutions in $CHCl_3$, especially at ~3420 and ~3440 cm^{-1}; here a definite role is also played by the reduction in the intensity of the ~3420-cm^{-1} band.

The IR spectra of the methylamides of N-acetyl α-amino acids in the region of C=O stretching vibrations agree with the $A \rightleftharpoons B$ equilibrium. However, because of the closeness of the absorption bands in a narrower spectral interval, the situation here is less clear than in the region of the NH vibrations. The simplest spectra are those of compounds (IV), (VIII), and (X) with only one unsymmetrical band at ~1650 cm^{-1} (Fig. 6), which corresponds to the vibration of two C=O bonds in the extended form. In the spectra of (II) and (VI), in addition to the bands at ~1650 cm^{-1} there are bands at ~1680 cm^{-1}, assigned to the vibrations of the $C^{(2)}O$ bonds in the extended forms. In compound (IX) in CCl_4 solution, the bands at 1634 and 1686 cm^{-1} undoubtedly belong, respectively, to the $C^{(2)}O$ and $C^{(5)}O$ bonds in the folded form. As in the simple amides, N-methylation leads to a lowering of the frequency of the CO stretching vibrations by approximately 30 cm^{-1} and to an increase in the intensity of absorption; the formation of an intramolecular hydrogen bond lowers the CO frequency by 10-15 cm^{-1}. All the CO bands in the spectra of solutions in $CHCl_3$ are shifted by 10-20 cm^{-1} in the long-wave direction as compared with solutions in CCl_4, which is connected with the formation of a H bond of the $C=O \cdots HCCl_3$ type. The most probable assignments of the CO bands are given in Table 3.

For a quantitative evaluation of the amounts of the folded and extended forms of the diamides (I), (III), (V), (VII), (IX), (XI), and (XII) in solutions in CCl_4 and $CHCl_3$, we measured the integral intensities of the bands in the region of the NH stretching vibrations and determined the number of NH groups corresponding to each band. In this process we made use of the correlation relationship found previously [51] between the integral intensities of NH absorption bands and their frequencies. As already mentioned, in published papers the amount of the folded forms of alkylamides of N-acetyl α-amino acids has been determined by measuring the peak intensity and comparing the value obtained with the intensity of the band in the spectrum of some compound or other, taken as standard and containing only the folded or only the extended form. As is clear from the present paper, this approach is unsatisfactory not only because of the low accuracy of the measurement of the peak intensities of band, but also because of the sensitivity of the intensities as also of the NH frequencies to the chemical structure of the diamides and to the mutual orientation of the amide groups (Φ, Ψ).

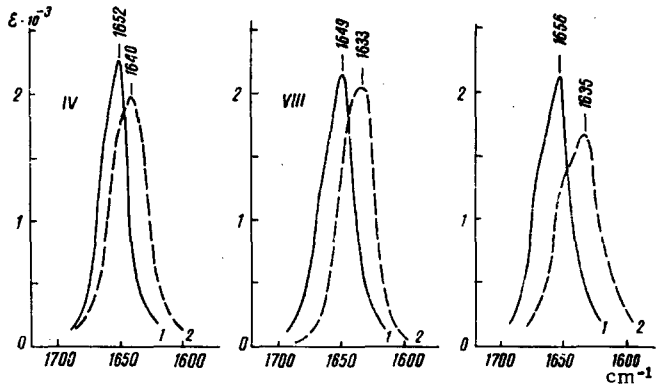


Fig. 6. IR spectra of the dimethylamides of N-acetyl-N-methyl-L-alanine (IV), N-acetyl-N-methyl-L-valine (VIII), and N-acetyl-L-proline (X): 1) in CCl_4; 2) in $CHCl_3$.

TABLE 4. Amounts (%) of the Folded Forms in Solutions of the Diamides in CCl_4 and $CHCl_3$*

No.	Compound	Solvent	
		CCl_4	$CHCl_3$
I	Ac-*L*-Ala-NHMe	65	25
III	Ac-*L*-MeAla-NHMe	75	40
V	Ac-*L*-Val-NHMe	65	20
VII	Ac-*L*-MeVal-NHMe	70	30
IX	Ac-*L*-Pro-NHMe	90	55
XI	Ac-Gly-NHMe	—	10
XII	Ac-*L*-Phe-NHMe	70	30

*Proportion of the folded forms determined with an accuracy of ± 10%.

The approach that we have used appears more reliable, since it is based on a large amount of experimental material on the integral intensities of the bands.

The results of calculations (Table 4) show that the proportion of folded forms varies within extremely wide limits according to the solvent, the N-methylation of the N-terminal amide group, and the type of amino acid. In all cases, on passing from CCl_4 to $CHCl_3$ the proportion of extended forms rises considerably. It is just this direction of the shift in the conformational equilibrium that follows from a theoretical analysis [36]. According to the theoretical estimates of this paper, the proportion of folded forms of the methylamide of N-acetyl-L-alanine (I) in CCl_4 solution should be 70-80%, and in $CHCl_3$ 30-20%. From the point of view of the entropy factor and of the energy of the nonvalent interactions, the folded forms of the methylamides of N-acetyl α-amino acids are less preferred than the extended forms, and their realization in dilute solutions of nonpolar solvents is due to the lowering of the free energy through the formation of a H bond. In polar media, the latter are considerably weakened [46, 47], and the equilibrium shifts in the direction of the sterically more highly preferred extended conformations.

The dependence of the position of the equilibrium on the nature of the substituent on the C^{α} atom is more complex. The results of calculations [6] show that the relative potential energies of the folded forms rise considerably with an increase in the size of the side chain (e.g., on passing from derivatives of Gly to derivatives of Ala, Val, and Pro). However, in this sequence of compounds the entropy of the extended forms decreases simultaneously. The experimental results on the IR spectra of the diamides (I) and (V) show the closeness of the ratios between the values of the free energies of the two forms of these compounds; i.e., the increase in the enthalpy of form B in (V) as compared with (I) is compensated by the decrease in the entropy of form A. A decisive significance of the entropy factor is shown on the position of the equilibrium in the case of compounds (IX) and (XI). Although the relative potential energy of the folded form in the glycine diamide (XI) is smaller than in the other compounds, nevertheless, because of the considerable conformational freedom the amount of the extended forms predominates. In the proline diamide (IX), conversely, the entropies of the folded and extended forms coincide, and the conformational equilibrium in CCl_4 is shifted almost completely in the direction of B. Similar factors explain the high proportion of this form in the case of compounds (III) and (VII).

EXPERIMENTAL

The IR spectra were recorded on a UR-10 instrument with LiF and NaCl prisms. Conditions of recording the spectra: slit program 4, rate of scanning 32 cm^{-1}/min, spectral slit width in the ν_{NH} and the I regions ~ 4 and ~ 8.5 cm^{-1}, respectively. Concentrations of the solutions in CCl_4 ~ $(1.2\text{-}6.1)\cdot10^{-4}$ M and in $CHCl_3$ ~ $(1.1\text{-}4.1)\cdot10^{-3}$ M. Before the calculation of the integral intensity of an overlapped band, the ab-

sorption was divided, for which purpose the curves recorded in the instrument were replotted in the coordinates D versus ν. The integral intensity was calculated by two methods: direct integration according to Ramsay (A_r [48]) and by measuring the effective integral intensity [49] with the averaging of the band with respect to the base line (A_b [50]). The two methods gave similar results. The amounts of free and hydrogen-bound NH groups were determined from the correlation curve $A = f(\nu)$ (intensity as a function of the frequency [51]) starting from the integral intensity of the absorption band of the free NH group of the extended form (~ 3460 cm^{-1}) and of the NH absorption band of the folded form (~ 3350 cm^{-1}). The range of values obtained is shown in Table 4. With pronounced overlapping of the bands, preference was given to the more reliable band.

SUMMARY

1. The IR spectra of methylamides of N-acetyl α-amino acids and their N-methyl derivatives in CCl_4 and $CHCl_3$ solutions have been investigated. An assignment of the bands of the NH and CO stretching vibrations in the folded (with an intramolecular H bond) and the extended conformations has been made.

2. It has been shown that the relative amounts of the folded forms depend on the nature of the amino acid residue and the conditions of the medium. With an increase in the polarity of the solvent, the proportion of folded conformations decreases.

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