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

V. ULTRAVIOLET SPECTRA AND CIRCULAR DICHROISM AND OPTICAL ROTATORY DISPERSION CURVES

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In preceding papers we have given the results of an investigation of the spatial structure of a series of methylamides of N-acetyl α-amino acids based on IR [1] and NMR [2] spectroscopy, dipole moments [3], and gas-liquid osmometry [4]. It has been found that in all compounds it is mainly the trans configurations of the amide groups that are formed, although in the derivatives of the N-methylated amino acids and proline, particularly in polar media, a considerable proportion (up to 35%) of forms in which the N-terminal tertiary amide groups are present in the cis configuration exists. The compounds studied were

Ac-L-Ala-NHMe (I), Ac-L-Ala-NMe_2 (II), Ac-L-MeAla-NHMe (III), Ac-L-MeAla-NMe_2 (IV), Ac-L-Val-NHMe (V), Ac-L-Val-NMe_2 (VI), Ac-L-MeVal-NHMe (VII), Ac-L-MeVal-NMe_2 (VIII), Ac-L-Pro-NHMe (IX), Ac-L-Pro-NMe_2 (X).

It follows from the IR spectra of these compounds that in the diamides $R^3 = H$ there is an equilibrium of the extended and folded conformations; in the latter there are intramolecular H bonds of the $3 \rightarrow 1$ type closing a seven-membered ring. The amount of the closed conformations depends greatly on the nature of the amino acid residue and on the solvent; in nonpolar media (CCl_4), as a rule, they are dominating, but their amount falls sharply even on passing to weakly polar media ($CHCl_3$).

The results of measurements of dipole moments have permitted a substantial limitation of the range of possible values of the dihedral angles $\Phi(C^{\alpha}-N)$ and $\Psi(C^{\alpha}-C')$ of the preferred conformations of compounds (I-X) in CCl_4 and $CHCl_3$ solutions, and also a determination of the tendency to a shift in the conformational equilibrium with an increase in the polarity of the medium. The concentration dependences of the molecular weights, dipole moments, and specific rotations of compounds (I-X) in $CHCl_3$ solution have been obtained as discussed [4]. The stepwise change in the properties of (I) and (V) with a rise in the concentration can be explained by the successive formation of different types of dimeric associates of the closed forms H and M.

The present work was a continuation of investigations of the conformations of compounds (I-X) by the methods of UV spectroscopy, circular dichroism (CD), and optical rotatory dispersion (ORD). The main aims of this investigation consisted in obtaining a more detailed idea of the spatial structure of compounds (I-X) and in determining the influence of the medium (particularly a polar medium) on the position of the conformational equilibrium. In addition, the results obtained from the UV, CD, and ORD spectra may prove to be useful in comparison with the results of other methods used in conformational analysis and in calculating the optical properties of peptides.

In a consideration of the optical properties of compounds (I-X) it is desirable to divide them into four groups: 1) compounds (I) and (V) with secondary amide groups; 2) compounds (III), (VII), and (IX) with sec-

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TABLE 1. UV Spectra of Compounds (I-X) and of Secondary and Tertiary Amides

No. group	No. compound	Compound	Heptane (cyclohexane) λ_{max}, nm	Heptane (cyclohexane) ε_{max}, liter · mole^{-1} · cm^{-1}	Heptane (cyclohexane) Δ, nm	Water λ_{max}, nm	Water ε_{max}, liter · mole^{-1} · cm^{-1}	Water Δ, nm
		Secondary amides [24]	184–186	—	15,5	186–188	—	15
		Tertiary amides [24]	196–203	—	14,5–18	196–202	—	17
1	I	Ac-L-Ala-NHMe	188	—	16,5	187,5	15 400	13,5
	V	Ac-L-Val-NHMe	—	—	—	185	15 300	17,5
2	III	Ac-L-MeAla-NHMe	189	12 400	21	192	14 900	18,5
	VII	Ac-L-MeVal-NHMe	190	12 300	21	192	13 600	19
	IX	Ac-L-Pro-NHMe	191	12 000	20,5	192,5	15 800	19
3	II	Ac-L-Ala-NMe_2	192	15 800	17,5	192	15 100	20
	IV	Ac-L-Val-NMe_2	193	13 900	19	192,5	15 500	21
4	IV	Ac-L-MeAla-NMe_2	198	12 900	18,5	198	15 300	18,5
	VIII	Ac-L-MeVal-NMe_2	199	14 800	18,5	198	15 400	20,5
	X	Ac-L-Pro-NMe_2*	198	12 700	18,5	199	16 290	17

* Literature data [25]: cyclohexane – λ_{max} = 199 nm, ε_{max} = 13,300, Δ = 17 nm; water – λ_{max} = 198 nm, ε_{max} = 16,000, Δ = 17 nm.

ondary C-terminal and tertiary N-terminal amide groups; 3) compounds (II) and (VI) with secondary N-terminal and tertiary C-terminal amide groups, and, finally, 4) compounds (IV), (VIII), and (X) with two tertiary amide groups. As we shall see later, this division corresponds to the conformational possibilities of these compounds.

The parameters of the UV spectra of compounds (I-X) agree with the results obtained previously for secondary and tertiary amides (Table 1). Thus, the absorption maxima connected with $\pi\pi^*$ transitions in the amide groups in compounds of the first class are located in the 185-188-nm region, i.e., extremely close to λ_{max} for secondary amides (184-186 nm), in compounds of the fourth class they are at 198-199 nm (in tertiary amides 196-203 nm), and in compounds of the second and third classes with secondary and tertiary amide groupings they occupy an intermediate position (189-193 nm). In the majority of spectra taken in heptane solution, there is a shoulder in the 220-230-nm region which corresponds to a combined $n\pi^*$ transition from two amide groups; in aqueous solutions the band of the $n\pi^*$ transition is shifted in the short-wave direction and is masked by the considerably stronger band of the $\pi\pi^*$ transition.

The UV spectra of compounds (I), (III), (VII), and (IX) in heptane solutions permit the assumption that a band is present in the 200-210-nm region. This is indicated by the fairly large half-width of the combined band of the $\pi\pi^*$ transition of these compounds in the spectra of heptane solutions as compared with those of aqueous solutions. In our opinion, the appearance of a shoulder and the change in the half-width are connected with an effective exciton interaction of the spatially adjacent amide chromophoric groups connected by hydrogen bonds in the closed conformations of the molecules. Kaya and Nagakura [7] have observed a similar shift of the band of the $\pi\pi^*$ transition by 10 nm in the long-wave direction in the dimerization of a N-methylamide.

In the present paper we do not discuss the possibility of assigning the bands of the absorption and of the chiral-optical effects of the $n\pi^*$ transition [8, 9]. This Rydberg transition has not been observed for peptides in the region of the spectrum under discussion until recently. It is not excluded that it appears only in the spectra of gaseous amides [10].

According to a calculation by Bayley, Nielsen, and Schellman [12], in the UV spectra of compounds of the first and fourth groups one must expect the splitting of the band of the $\pi\pi^*$ transition and the appearance on the CD curve of two bands of dichroic absorption of opposite sign located on different sides of the initial band. On the CD curves of the compounds of the second and third groups, which have amide groups with different degrees of alkylation, again two bands of dichroic absorption should be present which correspond to the $\pi\pi^*$ transitions of the secondary and the tertiary amide groups. Because of the electronic interaction of the spatially adjacent chromophoric groups, the distance between the CD bands may be somewhat greater than the difference in the frequencies of the $\pi\pi^*$ transitions of secondary and tertiary amides.

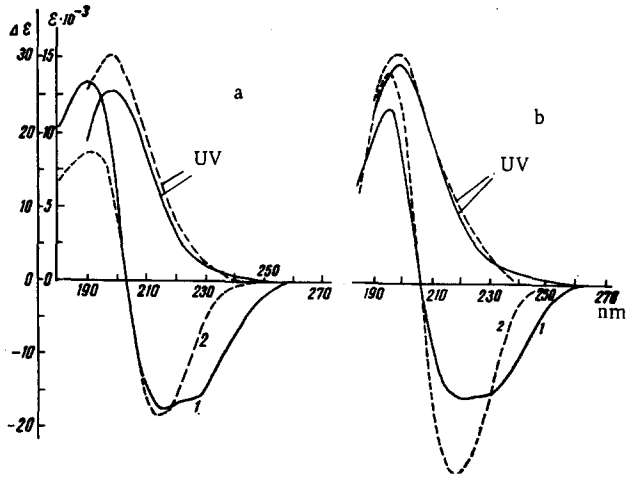

Fig. 1. UV spectra and CD curves of the dimethylamides of N-acetyl-N-methyl-L-alanine (IV) (a) and of N-methyl-L-valine (VIII) (b) in heptane (1) and in water (2).

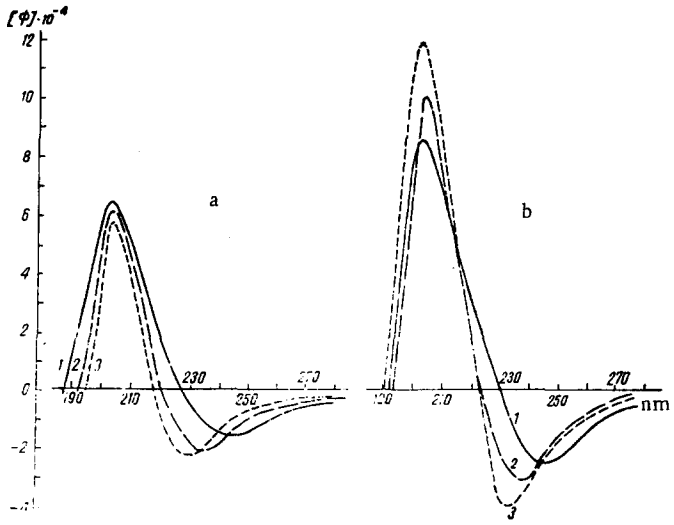

Fig. 2. ORD curves of the dimethylamides of N-acetyl-N-methyl-L-alanine (IV) (a) and of N-methyl-L-valine (VIII) (b) in heptane (1), ethanol (2), and water (3).

So far as concerns the region of $n\pi^*$ transitions, for this it is theoretically possible to predict (in contrast to the $\pi\pi^*$ transition) the appearance of only one combined band from the two chromophoric groups.

Thus, in the simplest case one must expect three bands of dichroic absorption on the CD curves of compounds (I-X): two in the region of the $\pi\pi^*$ transition and one in the region of the $n\pi^*$ transition. The assumptions expounded above are valid for individual conformations of the diamides under investigation. In preceding papers, however, it was shown that these compounds exist in a complex conformational equilibrium and, consequently, the CD curves must be the result of the superposition of the curves of all the conformations participating in the equilibria with the corresponding contributions to the intensities of the bands. It is extremely likely that the CD curves of the individual conformations of compounds (I-X) differ substantially from one another in position, sign, and intensity of the bands of dichroic absorption. Consequently, the experimental curves may have a considerably more complex form than in the simplest case mentioned.

In considering the CD curves with the aim of obtaining information on the spatial structure of the molecules of compounds (I-X), we started from a number of assumptions.

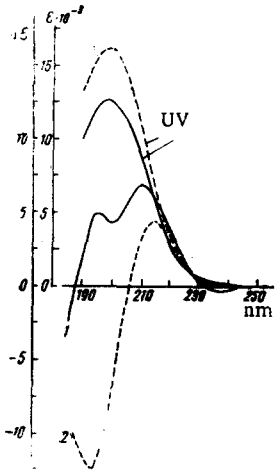

Fig. 3. UV spectra and CD curves of the dimethylamide of N-acetyl-L-proline (X) in heptane (1) and in water (2).

1. The change in the CD curves with a change in the solvent reflects the degree of conformational mobility of the compound. It must be borne in mind that the shape of the CD curve is sensitive to the medium even for diamides with a rigidly fixed conformation [11]. The transition to polar solvents is accompanied by a blue shift of the band of the $n\pi^*$ transition and by an increase in the intensity of the corresponding CD band because of the more effective interaction with the $\pi\pi^*$ transition [12]. However, the general form of the CD spectrum changes insignificantly, since the signs and relative positions of the chiral-optical effects remain unchanged.

2. The results of a comparison of the CD curves of compounds within each of the groups mentioned permits an idea of the similarity or difference of their conformational parameters Φ and Ψ. A comparison of the CD curves of compounds belonging to the various groups for this purpose must be performed with great care because of the somewhat different electronic structures of secondary and tertiary amide groups.

3. In the assignment of the CD curves to a definite conformation or set of conformations of compounds (I-X), the results that we have obtained previously [1-4], the results of a theoretical analysis of diamides, and also the maps of the rotational forces calculated by Bayley, Nielsen, and Schellman [12] for the $n\pi^*$ and near $\pi\pi^*$ bands of the dichroic absorption of compounds of the first and second groups were taken into account. The maps of the rotational forces reproduced in Figs. 8 and 11 were obtained by quantum-chemical calculation based on known experimental results on the statistical distribution of the charges, the energies of the electronic transitions, the magnitudes and directions of the dipole moments of the transition in the amide groups, and so on, taking into account the contribution to the total rotation of all the mechanisms of optical activity considered in the literature [13-16]. It is natural that the maps obtained of the rotational forces have a qualitative nature and cannot be used for an accurate determination of the parameters Φ and Ψ of different conformations of the diamides or of their relative proportions in the equilibrium. Nevertheless, as the present investigation has shown, in many cases the maps agree satisfactorily with the results of other theoretical and experimental methods of conformational analysis and have proved to be useful for determining the type of spatial forms of the diamides, especially those of the first and second groups, that are dominating under the conditions of measurement.†

Let us begin a consideration of the CD and ORD spectra with compounds of the fourth group – (IV), (VIII), and (X) – which possess the least conformational freedom. The CD curves of the dimethylamides (IV) and (VIII) in heptane solution (see Fig. 1) show negative bands of the $n\pi^*$ transitions at 227 nm and, in each case, two bands of opposite sign at ~ 212 and ~ 192 nm, which are characteristic for split $\pi\pi^*$ transitions. In aqueous solutions, the CD curves change their shape only insignificantly, mainly as a result of a blue shift of the band of the $n\pi^*$ transition. The ORD curves of compounds (IV) and (VII) in solutions in heptane, ethanol, and water (see Fig. 2) also differ little from one another. Thus, the CD and ORD spectra show the identity of the spatial structures of (IV) and (VIII) and also the retention of the conformational state of these compounds in different media. The results of a calculation of the rotational forces (Fig. 11) permit the conclusion that, of the four conformations corresponding to local minima of the potential energy on the conformational maps of compounds (IV) and (VIII) [17-19], only one, namely the γ conformation ($\Phi \sim 40°$, $\Psi \sim 250°$), which has the minimum energy, corresponds to the experimental CD spectra, i.e., has negative bands of the $n\pi^*$ and near $\pi\pi^*$ transitions [12]. The dipole moments of (IV) and (VIII) in CCl_4 and $CHCl_3$ solutions that we have found (2.9 and 2.57 D, respectively, in CCl_4) also agreed with the values calculated for this conformation (3.00 and 2.50 D). Consequently, it can be stated that the dimethylamides (VI) and (VIII) possess conformations of the γ type, which are stable both in polar and in nonpolar media. It is interesting to note that the CD curves of poly(N-methyl-L-alanine) in CF_3CH_3OH solution are extremely similar to those of compounds (IV) and (VIII) [20]. This obviously shows a similar mutual orientation of the amide groups in the diamide and in the polymer. In this case, notwithstanding the theory [21], the nature of the CD curve is determined primarily by the parameters Φ and Ψ of the amino acid residue and depends only slightly on the length of the peptide chain.

† The analysis of the ORD curves is based essentially on the same assumption. However, because of the smaller resolving capacity of the ORD-curves method, they do not yet permit basically new information to be obtained concerning the spatial structure of the peptides, as compared with CD.

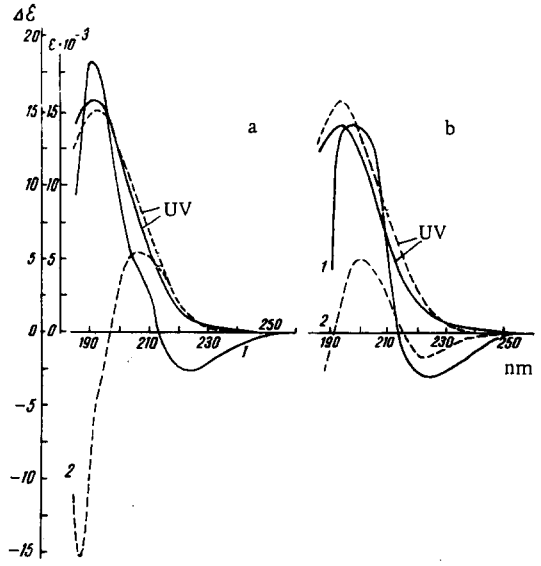

Fig. 4. UV spectra and CD curves of the dimethylamides of N-acetyl-L-alanine (II) (a) and -L-valine (VI) (b) in heptane (1) and water (2).

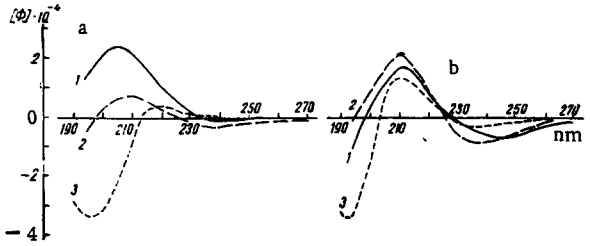

Fig. 5. ORD curves of the dimethylamides of N-acetyl-L-alanine (II) (a) and of -L-valine (VI) (b) in heptane (1), ethanol (2), and water (3).

In contrast to the compounds (IV) and (VIII) considered so far, in the dimethylamide of N-acetyl-L-proline (X), the fixation of the angle Φ at 120°C excludes the existence of the γ form. The results of a calculation of the conformational states of proline diamides [17, 22, 26] and of a proline dipeptide [23] have shown that in the case of the trans configuration of the amide group the most preferred conformation from the point of view both of the potential energy and of the entropy factor is the δ conformation with the parameters $\Phi \sim 120°$ and $\Psi = 280\text{-}360°$. The other optimum form – the R form ($\Phi \sim 120°$, $\Psi = 120\text{-}130°$) – has a sharply delimited potential minimum located 5-10 kcal/mole higher than the potential plateau of the δ conformation. The observed CD spectrum of compound (X) in heptane (Fig. 3) does not contradict the δ form, which has, according to the maps of rotational forces (Figs. 11 and 12), a negative band of the $n\pi^*$ transition and a stronger band of the $\pi\pi^*$ transition. The experimental dipole moment of (X) in CCl_4 solution (4.17 D [3]) also agrees well with the calculated value (4.0 D) for this form with the parameters $\Phi \sim 120°$ and $\Psi \sim 300°$. Compound (X) behaves somewhat unexpectedly on passing from heptane solution to aqueous solution (see Fig. 3). The changes in its CD curves in the 240-210-nm region with a change in the solvent are insignificant and could be due to a blue shift of the negative $n\pi$ band, strongly overlapped by the positive $\pi\pi^*$ band, but in the 195-nm region the transition is accompanied by a sharp change in the CD curve which generally indicates a considerable shift in the conformational equilibrium. However, in the present case this apparently does not take place. In view of the profile of the potential surface of compound (X), we assume that the reversal of the sign of the far band of the dichroic absorption of the $\pi\pi^*$ transition is caused by a change in the parameter Ψ within the limits of the same δ conformation, namely a change in the angle Ψ to 360°, which leads to an increase in the dipole moment of the molecule to 5-6 D [3]. A confirmation of the hypothesis put forward is the extremely close similarity of the dichroic curves of compound (X) and of poly-L-proline (XI) in aqueous solution. While the CD curves of compound (X) and of poly-L-proline (XI) are completely identical in water, only a shift of the dichroic absorption of (X) by 8-10 nm in the short-wave

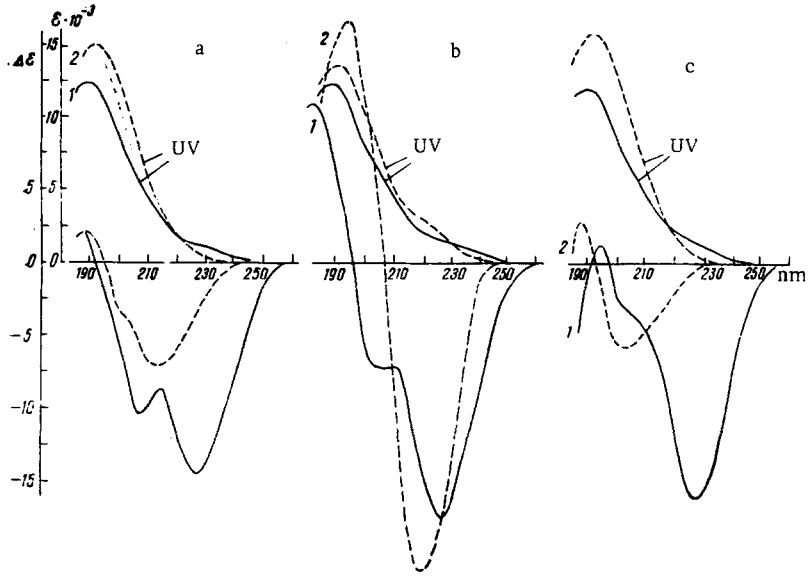

Fig. 6. UV spectra and CD curves of the methylamides of N-acetyl-N-methyl-L-alanine (III) (a), of N-methyl-L-valine (VII) (b), and of-L-proline (IX) (c) in heptane (1) and in water (2).

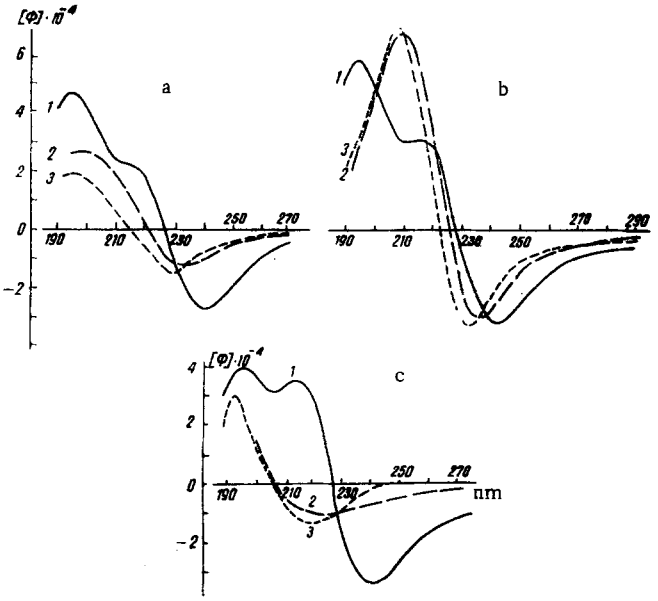

Fig. 7. ORD curves of the methylamides of N-acetyl-N-methyl-L-alanine (III) (a), of N-methyl-L-valine (VII) (b), and of -L-proline (IX) (c) in heptane (1), ethanol (2), and water (3).

direction is observed. As is well known [27], poly-L-proline (XI) exists in the form of a left-handed δ helix ($\Phi \approx 120°$, $\Psi \approx 330°$) and has the trans configuration of the amide groups. In addition, a special investigation of the NMR spectra of compound (X) in solutions containing CD_3OD ($CD_3OD - C_6D_5CD_3$) (1 : 1) and $CD_3OD - CF_2Cl_2$ (1 : 2) at low temperatures (down to −130°C) has not led to the detection of the second possible conformation, R [2].

The compounds of the third group – the dimethylamides of N-acetyl-L-alanine (II) and of -L-valine (VI) – are sterically less hindered than the completely methylated derivatives considered above. On the basis of the results of a theoretical analysis of the molecule of (II) the γ conformation ($\Phi \sim 50°$, $\Psi \sim 250°$) may be predicted to be the most stable conformation in nonpolar media, after which δ and L follow with only

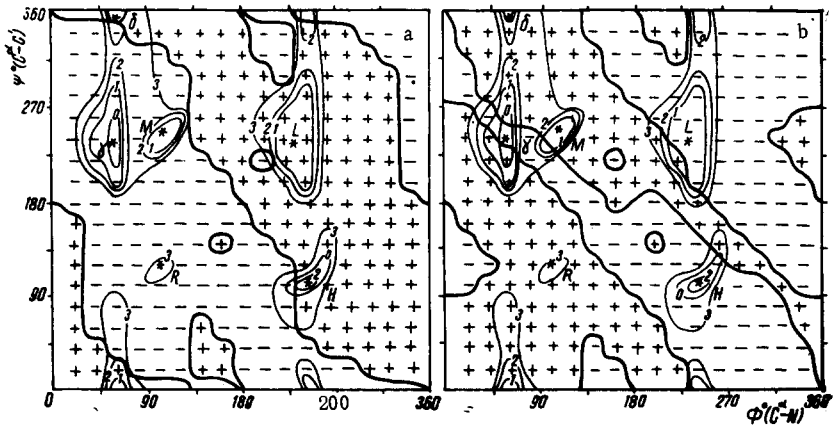

Fig. 8. Maps of the rotational forces of the methylamides of N-acetyl-N-methyl-L-alanine (III), of N-methyl-L-valine (VII), and of -L-proline (IX) [12], combined with the conformational map of (III) [17]: a) $n\pi^*$ transition; b) $\pi\pi^*$ transition.

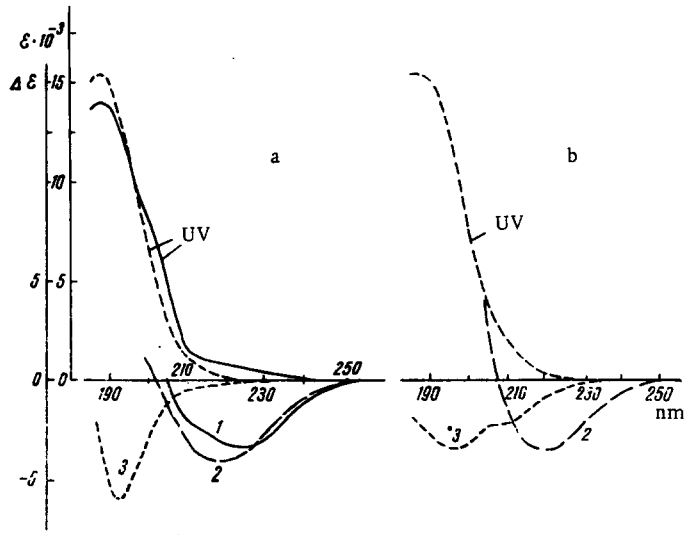

Fig. 9. UV spectra and CD curves of the methylamides of N-acetyl-L-alanine (I) (a) and of-L-valine(V) (b) in heptane (1), dioxane (2), and water (3).

a slight energy difference. In polar media, the existence of the L conformation ($\Phi \approx 230°$, $\Psi \approx 250°$) and of the γ conformation is most probable. Such conformational states are obviously also characteristic for compound (VI). One may expect only a slightly greater energy differentiation of the forms in favor of the γ conformation. The high values of the spin–spin coupling constants of the protons in the NH–CH fragments measured from the NMR spectra [$^3J_{NH-CH} = 9.2$ Hz (in chloroform) and 9.4 Hz (in dimethyl sulfoxide)] show that in the valine derivative (VI) in all media those forms which have the trans orientation of the protons of the NH and CH groups, i.e., $\Phi \sim 60°$, effectively predominate [2]. In dimethyl sulfoxide solution, compound (II) has the lower value of $^3J_{NH-CH} = 8.2$ Hz, which satisfies both the trans and the cis orientations, i.e., $\Phi \sim 60°$ and $\Psi \sim 240°$.

The dipole moments of these compounds, which we have studied previously [3], agree completely with the results of calculation [17]. The experimental values of the moments of (II) and (VI) in CCl_4 solution (2.80 and 1.99 D, respectively) coincide with the values calculated for the γ form (2.7 and 2.0 D). On passing to a weakly polar solvent ($CHCl_3$), a considerable increase in the moment of (II) is observed, which shows a shift in the conformational equilibrium to more polar forms; the dipole moment of (VI) remains practically constant. Thus, the CD and ORD curves of compounds (II) and (VI) in heptane correspond to the γ conformation (Figs. 4 and 5). On passing to aqueous solutions, the CD and ORD curves of compound (II) change

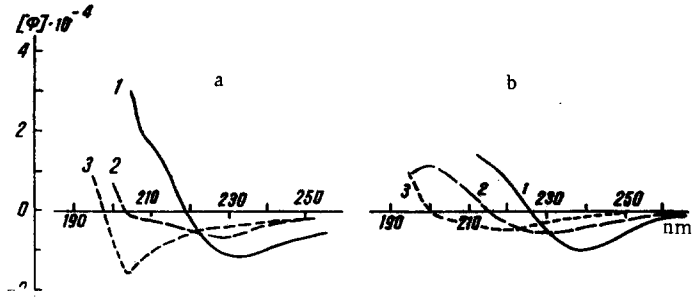

Fig. 10. ORD curves of the methylamides of N-acetyl-L-alanine (I) (a) and of-L-valine (V) (b) in heptane (1), ethanol (2), and water (3).

sharply and now correspond to a different conformational state. In water, apparently, the L form predicted by calculation [17] and satisfying the features of the NMR spectra and the dipole moments is now the dominating form. It is interesting to observe that the CD curve of (II) in water is similar to the curve of poly-β-methyl-L-aspartate, which forms a left-handed α-helix [28, 29], and is also extremely similar to the mirror image of the curve of the right-handed helix of poly-L-alanine [30].

The similarity of the CD curves of compound (II) and of poly-β-methyl-L-aspartate, of compounds (IV) and (VIII) and of poly-N-methyl-L-alanine, and also of compound (X) and poly-L-proline (XI), the amino acid residues of which are present in the equivalent conformational state, is not fortuitous. The similarity that has been observed of the CD curves of diamides that may be considered as isolated unit fragments of a peptide chain and the corresponding polypeptides shows the decisive contribution to the nature of the dichroic absorption of the mutual orientation of the closest amide groups, i.e., the values of Φ and Ψ of one residue. Thus, we conclude that compound (II) exists in the γ conformation in heptane and in the L conformation in water, while in ethanol the γ and L forms are present in approximately equal amounts.

The CD curve of compound (II) in heptane shows signs of the dichroic absorption of the L conformation, and the CD curve of an aqueous solution of (II) shows signs of the γ conformation. It may be concluded from them that the amount of the impurity forms is low. The valine derivative (VI) exists almost completely in the γ conformation in heptane and ethanol, and only in water is the equilibrium shifted appreciably in the direction of the L conformation. If the intensities of the CD bands at 223 and 198 nm are considered, the $\gamma \rightarrow$ L transition for this compound takes place to the extent of ~30%.

Let us consider the CD and ORD curves of diamides of the second group: the methylamides of N-acetyl-N-methyl-L-alanine (III), of N-methyl-L-valine (VII), and of L-proline (IX).

The results of an investigation of the IR spectra [1], in agreement with calculation [17, 31], show the dominating presence for these compounds in an inert medium of the folded forms with an intramolecular H bond of the 3 → 1 type. The proportion of folded forms in solutions of (III) and (VII) in CCl_4 solution is 70%, and for (IX) it is 95% [2]. In actual fact, in heptane the diamides (III), (VII), and (IX) give extemely similar CD and ORD curves (Figs. 6 and 7). Since under these conditions compound (IX) is present almost completely in the folded M conformation (Φ ~ 120°, Ψ ~ 240°), the closeness of the curves of the three compounds shows the existence of folded forms of the same type (i.e., the M type) in (III) and (VII) as well. This conclusion corresponds to the maps of rotational forces (Fig. 8), according to which for the M conformation of the diamides (III), (VII), and (IX) CD curves are predicted with strong negative bands of the $n\pi^*$ transitions and very low-intensity positive bands of the $\pi\pi^*$ transitions of the tertiary amide groups [12, 22]. Thus, in compounds (III) and (VII) of the two folded conformations possible in principle (M and H) the M form is the preferred one. The H forms, being even more favorable in these compounds with respect to nonvalent interactions [17], apparently possess a considerably lower entropy.

In addition to the bands mentioned, the CD spectra of compounds (III) and (VII) in heptane show negative dichroic absorption at ~ 205 nm, which obviously relates to the near $\pi\pi^*$ transition of the open forms. According to the maps of rotational forces (see Fig. 8) conformations with the angles Ψ in a narrow range of values from 230 to 250°, i.e., in the region of the most preferred among the open forms of the γ conformation (Φ = 54°, Ψ = 238° [17]), may have a negative sign of the $\pi\pi^*$ transitions. Therefore, we assume the existence in compounds (III) and (VII) in heptane of the conformational equilibrium $M \rightleftarrows \gamma$, shifted in the direction of the folded conformation. On the CD curve of compound (IX) in heptane there is a shoulder in this

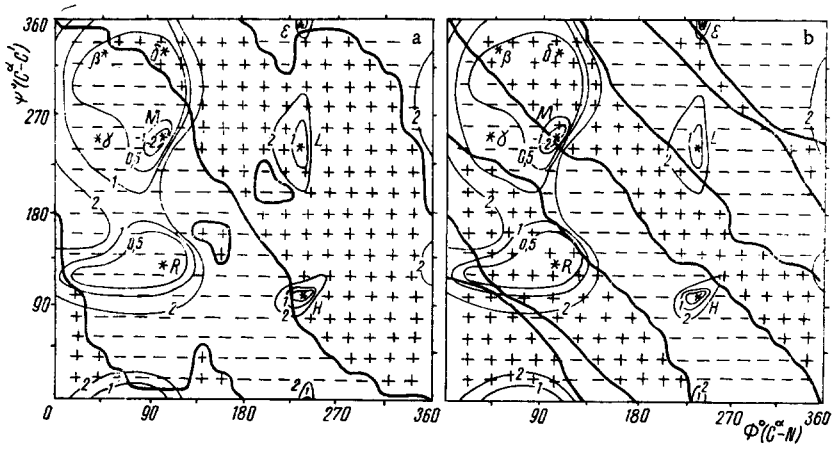

Fig. 11. Maps of the rotational forces of the methylamides of N-acetyl-L-alanine (I) and of-L-valine (V) [12] combined with the conformational map of (I) [40]: a) $n\pi^*$ transition; b) near $\pi\pi^*$ transition.

region. It possibly relates to the extended R form, the amount of which is ~ 10% and which we consider to be the dominating form in aqueous solution.

On passing to aqueous solutions, the CD and ORD spectra of the diamides (III), (VII), and (IX) change sharply (compare Figs. 6 and 7). The exceptionally strong negative band of the $n\pi^*$ transition in the CD spectrum of compound (VII) in water permits preference to be given to the first (see Fig. 8) of the three possible extended conformations, $\gamma(\Phi \approx 50°, \Psi \approx 240°)$, $L(\Phi \approx 230°, \Psi \approx 240°)$ and δ $(\Phi \approx 60°, \Psi \approx 350°)$, this form having the lowest potential energy [17]. Attention is attracted by the similarity of the CD and ORD curves of (VII) in water to the curves of compounds (IV) and (VIII) which exist, as has been shown above, in the γ conformation. In the CD spectrum of the alanine derivative (III), the low intensity of the negative band of the $n\pi^*$ transition shows (in full agreement with calculation [17]) the appearance in aqueous solution of appreciable amounts of the L form, in addition to the γ form. So far as concerns the proline diamide (IX), the negative band at ~ 204 nm (and, apparently, a weak band in the 210-215-nm region) observed on its CD curve under these conditions may correspond to the R conformation ($\Phi \sim 120°$, $\Psi \sim 140°$) or the δ ($\Phi \sim 120°$, $\Psi \sim 350°$) [22, 26, 31-33]. An analysis of the CD curve of this compound is further complicated by the presence in its aqueous solution of 30% of a form with the cis configuration of the tertiary amide groups [2] for which the nature of the dichroic absorption is unknown. In our opinion, the R conformation with a high dipole moment, which exists in the crystalline state [34], is the most probable one for the diamide (IX) in water.

The methylamides of N-acetyl-L-alanine (I) and of-L-valine (V), which belong to the first group, are the most conformationally labile compounds of the series of diamides considered. The results of an analysis of the IR spectra that we performed previously [1] have shown that in (I) and (V) in a nonpolar medium more than 60% of the folded forms exist. The CD curves of these compounds in heptane and dioxane (Fig. 9) have negative bands of dichroic absorption at 220-225 nm. It can be seen from the map of the rotational forces of the $n\pi^*$ transition (Fig. 11) that of the two possible conformations under these conditions, M ($\Phi \sim 120°$, $\Psi \sim 240°$) and H ($\Phi \sim 240°$, $\Psi \sim 120°$), only for the former do the results of the calculation agree with the experimental figures. While, therefore, giving preference to the M conformation, it must nevertheless be borne in mind that these results do not exclude the presence in the equilibrium of appreciable amounts of the H conformation, which is similar to M in enthalpy [31, 36, 37]. In particular, this is indicated by the comparatively high values of the $^3J_{NH-CH}$ constants [2].† The existence in dilute solutions of (I) and (V) of the folded M and H conformations is also shown by the results of an investigation of the associative properties of these compounds in $CHCl_3$ [4]. The low intensity of the band of the dichroic absorption of the $\pi\pi^*$ transition (shoulder at 210 nm) agrees well with the corresponding map of rotational forces (Fig. 11b), in which the low intensity of the near $\pi\pi^*$ dichroic absorption corresponds to both folded forms.

It is not excluded that the presence of a band at 210 nm is connected with the formation of associates in saturated heptane solution (see [11] and [42]). In this case, it is quite impossible to detect chiral-optical effects connected with the $\pi\pi^*$ transition on the CD curve.

† The results obtained permit the assumption that the M form is the more probable also for the alanine dipeptides investigated previously by IR and NMR spectroscopy, the folded forms of which were ascribed the H conformation [35]. As Ramachandran and Chandrasekaran [36] have recently shown, this conclusion does not contradict the $^3J_{NH-CH}$ constants found for dipeptides.

TABLE 2. Conformational States of the Methylamides of N-Acetyl α-Amino Acids and Their N-Methyl Derivatives

No. groups	No. compounds	Compound	Conformational state* heptane, CCl_4	Conformational state* water
1	I	Ac-L-Ala-NHMe	M, H, B, (R)	B, (R, L)
	V	Ac-L-Val-NHMe	M, H, B, (R)	B, (R, L)
2	III	Ac-L-MeAla-NHMe	M, γ	γ, L
	VII	Ac-L-MeVal-NHMe	M, γ	γ
	IX	Ac-L-Pro-NHMe	M, R	R, (δ)
3	II	Ac-L-Ala-NMe_2	γ, L, (δ)	L, γ, (δ)
	VI	Ac-L-Val-NMe_2	γ, (L)	γ, L
4	IV	Ac-L-MeAla-NMe_2	γ	γ
	VIII	Ac-L-MeVal-NMe_2	γ	γ
	X	Ac-L-Pro-NMe_2	δ	δ

* The figures in the table characterize the spatial forms of compounds (I-X) with trans configurations of the amide groups. The sequence of listing the conformations corresponds to their proportions in the equilibrium. Conformations the realization of which does not follow directly from the experimental results are shown in parentheses. However, according to the conclusions of a theoretical conformational analysis, their presence in small amounts is quite likely.

The CD and ORD curves of the diamides (I) and (V) in aqueous solution differ sharply. Thus, in the CD spectra the intensity of the band of the dichroic absorption of the $n\pi^*$ transition decreases and a negative band of the $\pi\pi^*$ transition appears at 192 nm. These changes are connected with the instability of the M and H conformations in a polar medium and with a shift in the equilibrium in the direction of the extended forms. According to the high $^3J_{NHCH}$ constants, the most preferred of them are the configurations with $\Phi = 40\text{-}80°$ [2], among which the negative CD bands of the near $\pi\pi^*$ transition are expected for the conformations with $\Psi = 220\text{-}300°$, i.e., the γ and β conformations. Since a strong negative effect of the $n\pi^*$ transition corresponds to this region (taking into account the low intensity of the dichroic absorption at 210-215 nm), one must assume the presence of other conformations with a positive band of the $n\pi^*$ transition in the equilibrium. This condition is satisfied by the δ and L forms. However the realization of the L form is unlikely, since in this case the presence of a considerable amount of the R form, which is more favorable than the L form from the points of view both of enthalpy and entropy [17], must be assumed. It can be seen from Fig. 11 that the R form has a strong negative band of the $n\pi^*$ transition and a positive $\pi\pi^*$ transition, i.e., the presence of a considerable proportion of it may lead to the compensation of the dichroic absorption in the 200-240-nm region. Thus, we come to the conclusion that in aqueous solutions of the diamides (I) and (V) conformations with the parameters Φ and Ψ corresponding to region B of the conformational map ($\Phi = 0\text{-}120°$, $\Psi = 240\text{-}360°$) are realized. According to the results of the calculation of the methylamide of N-acetyl-L-alanine as applied to a polar medium [39], the energy surface of the molecule in this region consists of a fairly broad level plateau; the B region possesses the highest entropy and a somewhat lower enthalpy than the R region following it. In view of the absence from the B regions of clear potential troughs, the isolation of the individual extended forms (γ, δ, etc.) of compounds (I) and (V) is difficult and hardly desirable.

EXPERIMENTAL

The synthesis of compounds (I-X) has been described elsewhere [5]. The UV spectra of compounds (I-X) in heptane and in water were obtained on a Cary-15 instrument at 23-26°C with layer thicknesses of 0.01-5 cm and concentrations of $(0.01\text{-}5)\cdot 10^{-3}$ M. From the results of measurements of ε_{max} at various temperatures, the level of scattered light amounted to 0.003%. The values of λ_{max} and ε_{max}, and also the half-width of the bands (Δ) calculated from three or four measurements, are given in Table 1. The CD and ORD curves of (I-X) in heptane, ethanol, and water were taken on a Cary-60 spectropolarimeter with an

attachment for obtaining the CD curves under conditions analogous to those for the measurement of the UV spectra. In view of the poor solubility of compounds (I) and (V) in heptane, their CD curves were measured in dioxane solution. In order to obtain the CD curve of (I) in heptane, a solution was prepared by boiling a suspension of (I) for 30 min, cooling the mixture, and filtering. The concentration of the solution ($1.85 \cdot 10^{-5}$ M) was determined from its optical density at λ_{max} (188 nm), ε_{max} being taken as 14,000.

We have studied the spatial structure of a series of methylamides of N-acetyl α-amino acids and their N-methyl derivatives in various media, using a number of physical methods. The selected diamides were derivatives of alanine, valine, and proline. The results of a comparison of the alanine and valine derivatives give an idea of the influence on the conformational state of the main peptide chain of the volume of the substituent on the C^{α} atom. Proline and N-methylated derivatives were used to investigate the spatial structure of the main chain under the conditions of a more limited conformational freedom. The investigation of the latter is also of interest for understanding the laws of the formation of the spatial structures of biologically active compounds containing N-methyl amino acid residues (especially cyclopeptide antibiotics [43]).

The basis of the interpretation of the experimental material was a hypothesis of the possibility of describing the conformational states of the methylamides of N-acetyl α-amino acids and the N-methyl derivatives with the aid of a limited set of canonical forms [17]. The close correlation between the conclusions of theoretical analysis, optical data, and the results of the calculation of maps of dipole moments and rotational strengths of the dichroic absorption has been discussed. A direct connection has been established between the canonical forms of model compounds and the experimental results of various physical methods; a tendency has been detected for a shift in the conformational equilibrium on passing from a nonpolar to a polar solvent.

The final results of the investigation of the conformational states of compounds (I-X) in solutions are given in Table 2. As can be seen from this table, when, in the diamides, an alanine side chain is replaced by a valine side chain [compare (I) with (V), (II) with (VI), (III) with (VII), and (IV) with (VIII)], the formation of no new spatial forms whatever takes place and there is only a displacement of the equilibrium within the limits of the same conformations. Naturally, the alanine derivatives show a greater conformational mobility. Among the open forms of these molecules the most preferred are the conformations with the parameters Φ and Ψ located in region B. The same conformational states are obviously characteristic of leucine and isoleucine diamides. From the point of view of nonvalent interactions the derivatives of the other natural α-amino acids (with the exception of Gly and Pro) belong to the stereochemical type of Ala that has been considered [40, 41]. In view of this, the presence in them of other types of interactions will affect the selection of preferred conformations among those permitted by nonvalent interactions in the methylamide of N-acetyl-L-alanine (I).

A change in the spatial structure of the methylamide and dimethylamide of N-acetyl-L-proline is possible only through one parameter $-\Psi$. However, in this case, also, a definite generality of the conformational states of (IX) and (X) and the other diamides is observed. The conformations realized for compounds (IX) and (X) correspond to minima on the potential curves which are extremely similar in their profile to the sections of the conformational maps of the diamides (III) and (IV) at $\Phi = 120°$. Among the extended forms of (IX) and (X), the most favorable again prove to be those with conformations having the parameters Φ and Ψ in the B region (δ).

The replacement in the diamides of NH groups by NCH_3 groups, particularly in the C-terminal position, considerably decreases the conformational mobility of the molecules. Nevertheless, the conformations realized in the N-methylated derivatives are present on the conformational maps of the corresponding methylamides of N-acetyl α-amino acids in low-energy regions.

Passage from nonpolar to polar solvents leads to a cleavage of intramolecular H bonds and to a displacement of the conformational equilibrium towards the extended forms. In those cases where there are several extended forms with similar energies, a tendency is found to an increase in the proportion of the conformations with higher dipole moments.

SUMMARY

1. The UV spectra and CD and ORD curves of methylamides and dimethylamides of acetylamino acids have been studied in polar and nonpolar media.

2. On the basis of an analysis of the spectropolarimetric results and those of other methods, the predominating types of spatial forms in media of different polarities have been determined.

3. It has been shown that the conformational state of the main peptide chain is affected both by the volume of the substituent on the C^{α} atom and by the presence of tertiary amide groups.

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