A CONFORMATIONAL ANALYSIS OF THE METHYL ESTERS OF α-ACETYLAMINO ACIDS AND METHYLAMIDES OF α-ACETOXY ACIDS

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Many depsipeptides with regularly alternating amino acid and hydroxy acid residues (the antibiotics valinomycin, the enniatins, beauvericin, etc. [1-4]) selectively induce the transport of ions through synthetic and biological membranes. The functional properties of the membrane-active compounds are determined to a considerable extent by their conformational states.

The conformational analysis of various methylamides of N-acetylpeptides carried out previously [5-9] has shown that the spatial structure of compounds with two and three amide groups can be described by means of a limited set of optimum forms, the stability of which is determined mainly by the nature of the α-amino acid and also by the presence of methyl groups on the atoms of the main chain. Furthermore, it has been shown that the canonical forms found for these compounds may act as a conformational code in the analysis of the structure of more complex linear and cyclic molecules of peptide-protein nature [10].

The object of the present work was to determine the stereochemical interactions between neighboring links of a depsipeptide chain. The following methyl esters of α-acetylamino acids (I-III), methylamides of α-acetoxy acids (VI-VIII), and their N-methyl derivatives (IV, V, IX, X) with trans configurations of the amide and ester groups were investigated:

Ac-*Gly*-OMe	(I)	Ac-*Gly*CO-NHMe	(VI)
Ac-*L-Ala*-OMe	(II)	Ac *L-Lac*-NHMe	(VII)
Ac-*L-Val*-OMe	(III)	Ac-*L-Hyiv*-NHMe	(VIII)
Ac-*L*-Me*Ala*-OMe	(IV)	Ac-*L-Lac*-NMe_2	(IX)
Ac-*L*-Me*Val*-OMe	(V)	Ac-*L-Hyiv*-NMe_2	(X)

Compound (V) was also studied with the cis configuration of the amide group and the trans configuration of the ester group. The conformational possibilities of the molecules listed are determined almost completely by the interactions of the atoms of the neighboring amide and ester groups and the intermediate side chain. It may be assumed that the selected series of compounds is adequate for obtaining a set of canonical forms of amino and hydroxy acid residues in a depsipeptide chain suitable for the fragmentary analysis of the spatial structure of various depsipeptides.

The method of calculating the conformations and the functions of the nonvalent interactions of the atoms has been described previously [5]. The idea of a potential surface and of the optimum forms of compounds (I-X) was obtained from Φ-Ψ conformational charts. The charts were constructed with 20° variations of the angles of rotation Φ [C^{α}-N for (I-V) and C^{α}-O' for (VI-X)] and Ψ (C^{α}-C', Fig. 1).

The valence angles of the amide and ester groups were assumed to be constant. The values of the angles were taken from the calculations of N-methylacetamide and methyl acetate [5]. Considering the sensitivity of the –C–C and –O'-C^{α}–C' angles to the volume of the substituent on the C^{α} atom [6], they were taken as 110° in the cases R = H, CH_3, and 107° in the case R = i-C_3H_7. The orientation of the

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$C^{\beta}-H$ bond of the isopropyl side chain is fixed in the trans position with respect to the $C^{\alpha}-H$ bond ($\chi_1 = 180°$). When N-methylamide groups were present, the possibility of rotation round the $N-CH_3$ bond was taken into account. As was shown previously [7], the potential surface and the geometry of the optimum forms of peptides are determined mainly by nonvalence interactions. Consequently, the charts were constructed taking into account only this energy component.

Let us first consider the methyl esters of α-acetylamino acids (I-V). Figure 2 shows a chart of the conformationally most labile methyl ester of N-acetylglycine (I). It is symmetrical with respect to the diagonal of the square and has four broad regions of low energy – R, B, L, and P drawn out along the verticals $\Phi \sim 100°$ and $\sim 260°$. All the minima are approximately equivalent. The barrier separating the R and B (and L and P) regions does not exceed 0.2 kcal/mole, and that separating R and P (and B and L) is ~0.7 kcal/mole. The great similarity of the charts of the methyl ester of N-acetylglycine (I) and the methylamide of N-acetylglycine [6] must be noted. In both cases the area bounded by the 1 kcal/mole countour amounts to ~60%. Thus, the change from an amide group to an ester group does not introduce substantial variations in the interaction of the atoms of the side chain; the geometrical and thermodynamic parameters of the optimum conformations of these compounds are extremely similar.

Characteristic for the potential surface of the methyl ester of N-acetyl-L-alanine (II) is a disturbance of the symmetry and a considerable contraction of the low-energy regions (Fig. 3a). The 1-kcal/mole contour bounds only ~25% of the area of the chart. The potential surface has four nonequivalent minima. The regions cut off in the left-hand part of the chart (R and B) are considerably deeper and wider than on the right-hand side (L and P). Passing to the compound with the isopropyl side chain (III) leads to a further contraction of the low-energy regions; the 1-kcal/mole contour surrounds only ~8% of the area (Fig. 4a). The corresponding peptide analogs of compounds (II) and (III) have extremely similar charts. However, because of the additional contacts with the H atom of the second amide group, there are already low-energy equipotentials.

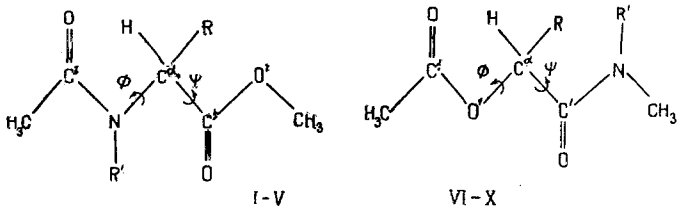

Fig. 1. Models of methyl esters of α-acetylamino acids (I-V) and methylamides of α-acetoxy acids (VI-X) in the $\Phi = \Psi = 0$ conformation; R = H, CH_3, i-C_3H_7, R' = H, CH_3.

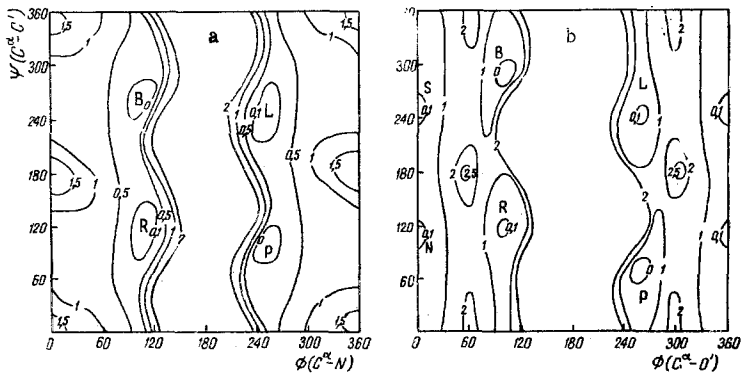

Fig. 2. Conformation charts of the methyl ester of N-acetylglycine (I) (a) and of the methylamide of O-acetylglycolic acid (VI) (b).

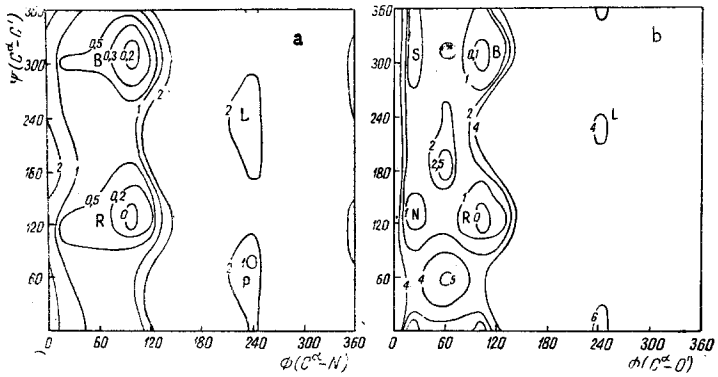

Fig. 3. Conformational charts of the methyl ester of N-acetyl-L-alanine (II) (a) and of the methylamide of O-acetyl-L-lactic acid (VII) (b).

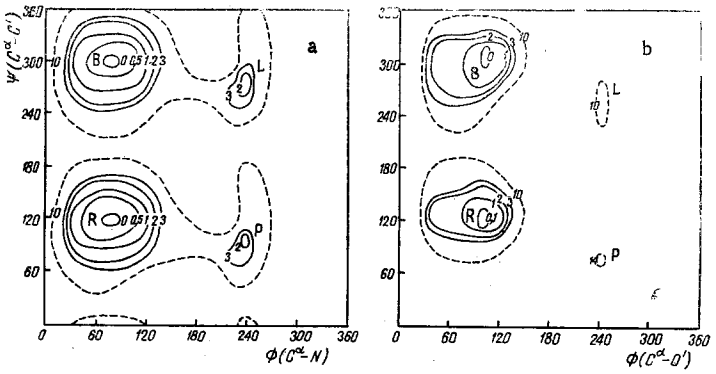

Fig. 4. Conformational charts of the methyl ester of N-acetyl-L-valine (III) (a) and of the methylamide of O-acetyl-L-hydroxyisovaleric acid (VIII) (b).

The conformational charts of the methyl esters of N-acetyl-N-methyl-L-alanine (IV) and N-acetyl-N-methyl-L-valine (V) show the considerable decrease in the accessible regions particularly at $\Phi < 180°$ (Fig. 5a and Fig. 6a). The minima in the left-hand and right-hand parts of the charts become approximately equivalent and the sharp energetic differentiation of the optimum forms characteristic for the N-desmethyl analogs vanishes.

The geometrical parameters and energies of the optimum forms of compounds (I-V) following from the conformational charts are given in Table 1.

It can be seen from Table 1 and Fig. 2a to Fig. 6a that a variation in the side chain and N-methylation affects the conformations and energetic stabilities of the forms. However, it is important to emphasize that at the same time the change in the positions of the local minima in the conformational charts is small. The formation of new regions of low energy does not take place either. Consequently, all the conformational states of the methyl esters of the α-acetylamino acids and their N-methyl derivatives are described by means of the four canonical forms R, B, L, and P. As already mentioned, the charts given in Fig. 2a to Fig. 6a were constructed with only the nonvalent interactions of the atoms taken into account. A consideration of the electrostatic interactions has shown that they stabilize the forms B and P to a greater extent than R and L, the effect increasing with a decrease in the polarity of the medium. So far as concerns the geometrical parameters of the optimum forms, electrostatics makes no appreciable corrections in their values. When the torsional energy is taken into account, the nature of the conformational charts remains unchanged. Compounds (I-V) cannot form intramolecular hydrogen bonds.

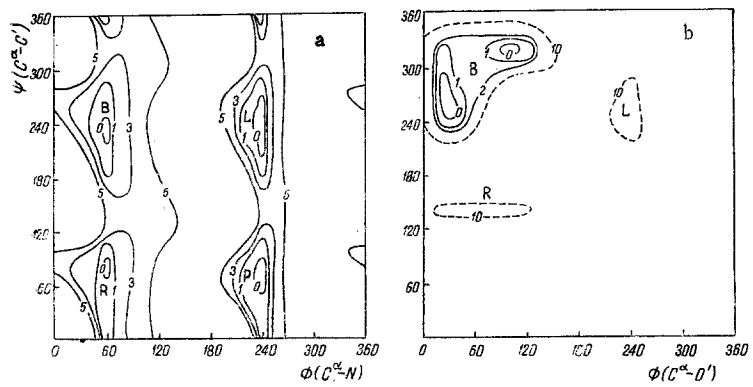

Fig. 5. Conformational charts of the methyl ester of N-acetyl-N-methyl-L-alanine (IV) (a) and of the dimethylamide of O-acetyl-L-lactic acid (IX) (b).

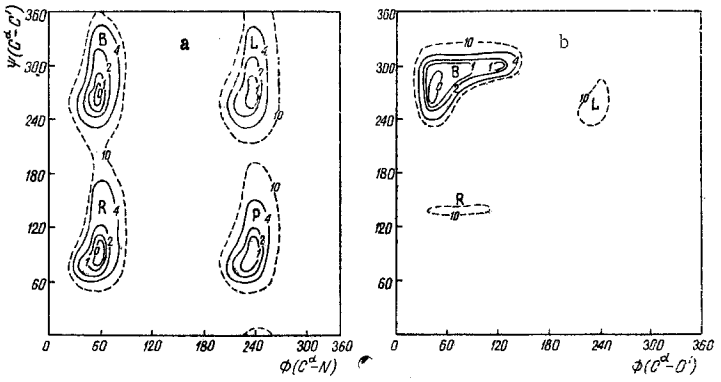

Fig. 6. Conformational charts of the methyl ester of N-acetyl-N-methyl-L-valine (V) (a) and of the dimethylamide of O-acetyl-L-hydroxyisovaleric acid (X) (b).

TABLE 1. Positions (degrees) and Energies (kcal/mole) of the Potential Minima of the Molecules Ac–X–OMe (where X is an amino acid residue)

X	*R*			*L*			*B*			*P*		
	Φ°	Ψ°	*U*	Φ°	Ψ°	*U*	Φ°	Ψ°	*U*	Φ°	Ψ°	*U*
Gly	110	120	0,1	250	240	0,1	100	260	0	260	100	0
L-Ala	100	130	0	240	240	1,2	100	310	0,2	240	80	1,0
L-Val	80	120	0	240	280	1,9	80	300	0,1	240	100	1,9
L-MeAla	60	80	0,1	240	230	0	60	220	0,1	240	70	0
L-MeVal	60	100	0,3	240	280	0,2	60	280	0,4	240	100	0

Let us now consider the features of the nonvalent interactions of atoms in the molecules containing hydroxy acid resideus (VI-X). Figure 2b gives the conformational chart of the methylamide of O-acetylglycolic acid (VI). The chart is symmetrical and resembles those for the methyl ester of N-acetylglycine (I) and the methylamide of N-acetylglycine [6]. A particular difference consists in the existence in (VI) of two additional minima, N and S, at $\Phi \sim 0°$ separated from the other minima by a barrier of ~1.6 kcal/mole. The barriers between N and S amount to ~0.7 kcal/mole and those between R and B (and P and L) to ~1.6 kcal/mole. All six minima are approximately equivalent. Their geometrical parameters and relative energies are given in Table 2.

TABLE 2. Positions (degrees) and Energies of the Potential Minima (kcal/mole) of the Molecules Ac–Y–NR'Me (where Y is a hydroxy acid residue)

R'	Y	R			L			B			P			N			S		
		Φ°	Ψ°	U	Φ°	Ψ°	U	Φ°	Ψ°	U	Φ°	Ψ°	U	Φ°	Ψ°	U	Φ°	Ψ°	U
H	*Glyco*	100	120	0,1	260	240	0,1	100	290	0	260	70	0	0	110	0,1	0	250	0,1
H	*L-Lac*	100	130	0	240	230	4,5	100	310	0,1	240	0	5,9	20	130	0,6	20	320	0,7
H	*L-Hylv*	100	120	0,1	240	260	8	100	310	0	240	80	14	—	—	—	—	—	—
Me	*L-Lac*	100	140	6	240	240	4	100	320	0	—	—	—	—	—	—	—	—	—
								30	260	0									
Me	*L-Hylv*	90	140	9	230	250	7	115	300		—	—	—	—	—	—	—	—	—
								45	280										

The introduction of a methyl side chain (VII, Fig. 3b) substantially changes the nature of the potential barrier. The symmetry disappears, the relief becomes more marked, and the energy difference between the forms rises. Conformations R and B prove to be the most preferred. The region in the right-hand part of the chart (L, P) is characterized by high energy values. The N and S minima noted in (VI) at $\Phi \sim 0°$ are shifted in (VII) to $\Phi \sim 20°$. The energies of these forms rise considerably.

The potential surface for compound (VIII) (see Fig. 4b) presents still higher relief than for (VII); the minima N and S disappear and the energy in the right-hand part of the chart rises sharply. The preferential nature of the two approximately equivalent regions R and B located along the verticals $\Phi \sim 100°$ become dominating. The 1 kcal/mole area is about 16% for (VI) [~6% for (VII) and ~3% for (VIII)].

The conformational freedom of the dimethylamides of O-acetyl-L-lactic acid (IX) and of O-acetyl-L-hydroxyvaleric acid (X) is practically limted to region B in which the two minima are located (Fig. 5b, and Fig. 6b). The R and L regions have a far higher energy; the P region is completely forbidden. The areas within the 1 kcal/mole contour in charts (IX) and (X) are, respectively, ~2.5 and 1%.

Table 2 gives the geometrical parameters and energies of the optimum forms of compounds (VI-X). As can be seen from the table, the regularities observed in the case of the methyl esters of α-acetylamino acids (I-V) are also valid for the methylamides of the α-acetoxy acids (VI-X). There are substantial differences only between the N-methylated derivatives (VI) and (V), and (IX) and (X). These are due mainly to the different positions of the $N-CH_3$ groups in the chain. It is interesting to note the considerable similarity of the conformational charts of the N-desmethyl compounds with isopropyl C^{α} substituents – (III), (VII) – and the methylamide of N-acetyl-L-valine [6]. In this case, the large volume of the side chains dictates the completely determined nature of the potential surfaces in the compounds considered.

In the molecules (VI-X), as in (I-V), electrostatic interactions stabilize form B. In the construction of the charts, the formation of hydrogen bonds was not considered. On taking into account the results of investigations of the methylamides of α-acetylamino acids [6], for compounds (VII) and (VIII) in a nonpolar medium one may expect the realization of a convoluted form of the M type ($\Phi \sim 110°$, $\Psi \sim 250°$) with an intramolecular hydrogen bond in a seven-membered ring.

Let us now consider the influence of the configurational structure of the amide groups on the conformational possibilities of the methyl esters of α-acetylamino acids. This question is particularly important for compounds with N-methylamide groups, which are characterized by electronic equivalence in the trans and cis configurations, and also for cyclic depsipeptides with a small number of links. Thus, an X-ray structural analysis [15] and conformational investigations of the cyclotetradepsipeptides [11, 12] have shown that the most preferred forms of the ring have the amide groups in the cis configuration and the ester groups in the trans configuration.

The conformational chart of the methyl ester of N-acetyl-L-methylvaline with a cis-N-methylamide group is shown in Fig. 7. The change from the trans configuration of the amide group to the cis sharply changes the nature of the potential surface of the molecule (compare Figs. 4a and 7). The number of possible conformations decreases to one half. Only two approximately equivalent regions R and B remain permitted.

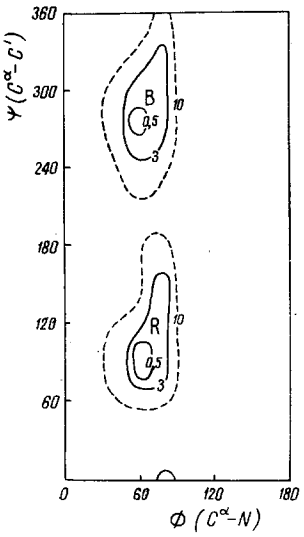

Fig. 7. Conformational chart of the methyl ester of N-acetyl-N-methylvaline with a cis N-methylamide group.

In conclusion, we may note a number of characteristic features following from the conformational analysis of the compounds considered above. It follows from the conformational charts that the substitution of side chains at the C^{α} atoms and the introduction of substituents on the N atoms, while considerably changing the thermodynamic parameters of the molecule, do not lead to the formation of qualitatively new spatial forms. In other words, the geometry of the optimum conformations of (I)-(X) is determined mainly by the interaction of the atoms of the side chain. All the conformational states of the molecules of (I-X) are described by a small number of canonical forms (see Tables 1 and 2). A comparison of charts of (I-V) and (VI-X) shows considerably fewer conformational possibilities of the hydroxy acid residues than of the amino acid residues. This gives grounds for the assumption that the spatial structure of the cyclic depsipeptides that corresponds to the lowest energies of the nonvalent interactions in all local sections is determined primarily by the conformational states of the hydroxy acid residues. The choice of the conformations of the amino acid residues from the number of permitted canonical forms dictates the conditions for ring closure. Of course, the geometrical parameters of the fragments in the ring may undergo deviations (within the limits of the low-energy regions on the conformational charts) from the values optimum for the simplest linear molecules.

SUMMARY

1. The form of the potential surface of the methyl esters of α-acetylamino acids and of the methylamides of α-acetoxy acids is sensitive to the geometry of the side chain and to the presence of N-methyl groups in the main chain.

2. The conformational states of the molecules considered are described by a limitednumber of canonical forms: R, B, L, and P.

3. With respect to the interaction of the atoms of the main chain, the ester group is stereochemically equal to the amide group. The difference between these groups consists in small differences in interactions with the atoms of the side chains.

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