CONFORMATION OF ENNIATINE B IN NONPOLAR SOLVENTS

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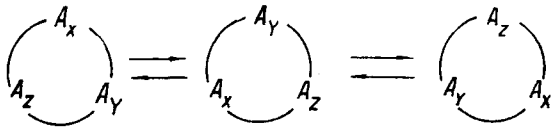
In preceding papers giving information on the spatial structure of the membrane-active antibiotic enniatine B (Fig. 1) it was shown that this cyclodepsipeptide can assume conformations of two types – "polar" (P) and "nonpolar" (N) [1, 2]. The results of a comparison of the corresponding optical rotatory dispersion curves [1] and circular dichroism curves [3] show that the complexes of enniatine B with alkali-metal ions have the same conformation as the antibiotic itself in polar media. The parameters of this conformation (P) – Φ and Ψ – have been determined previously in the crystalline state and in solution [1, 4] and have also been calculated theoretically [2] as a conformational analysis of a molecule related to enniatine B – the cyclohexadepsipeptide $\overline{\text{(L-MeAla-D-Lac)}_3}$.* So far as concerns the N form, on the basis of NMR spectra taken at low temperatures [1] it is possible to suggest for it a spatial structure in which the three chemically equivalent fragments –L-MeVal-D-Hylv– will have different conformations (A_X, A_Y, and A_Z):



A rapid establishment of equilibrium between these conformers at room temperature will lead to an averaging of the chemical shifts (CSs) and to the formation of simple spectra.

Using the absorption of ultrasound, Grell et al. [7] have found a conformational transition of enniatine B in hexane. Although the German authors do not give an accurate value of the barrier to this transition, which is observed at a frequency of 100 MHz, the free energy of activation ($\Delta G^{\neq}$) can be evaluated by means of the following approximate formula:

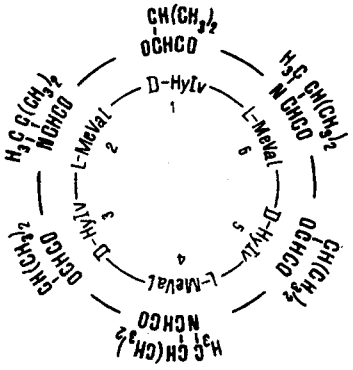


Fig. 1. Structure of enniatine B (the residues are numbered in the inner circle).

$$\tau_p = \tau_0 \cdot e^{-\frac{\Delta G^{\neq}}{RT}} \text{ [8]},$$

where $\tau_p = 1/2\pi\nu$; $\tau_0 \approx 10^{-12}$ sec; and ν is the frequency of the ultrasound at which the conformational transition is observed.

Then, for ν = 100 MHz, $\Delta G^{\neq}$ = 4.6 kcal · mole^{-1}. In view of the low value of the barrier, we consider it most probable that an equilibrium between rotamers with respect to the $C^{\alpha}-C^{\beta}$ bands (i.e., rotamers

*In this paper the abbreviated designations of amino-acid residues are used in agreement with the recommendations of the IUPAC nomenclature commission [5]: Lac and Hylv are, respectively, the residues of lactic and α-hydroxyisovaleric acids. For the conformational nomenclature of peptides, see [6].

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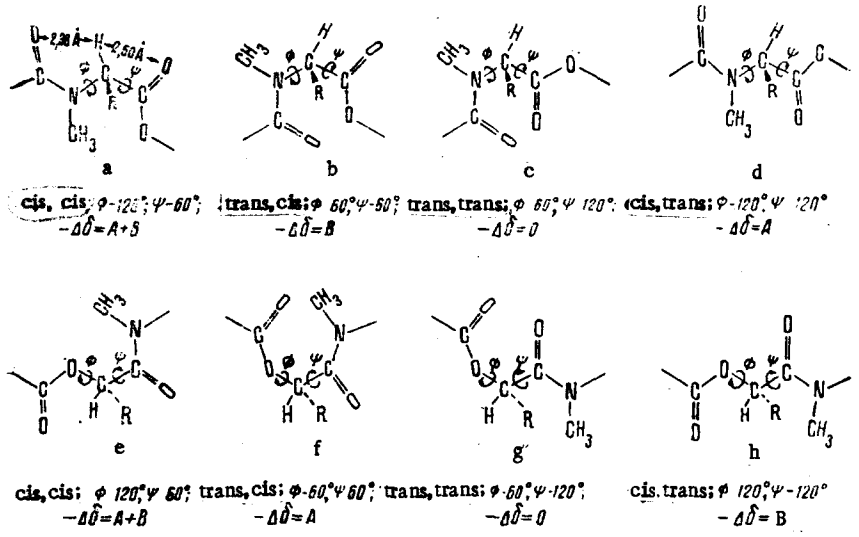


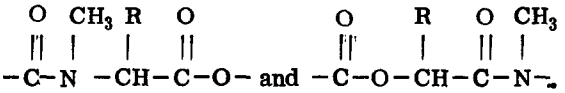
Fig. 2. Main types of orientation of the amide (A) and ester (B) carbonyl groups relative to the amino-acid (a-d) and the hydroxy-acid (e-h) α-protons.

differing in the orientation of the isopropyl groups of the side chains) is responsible for the observed conformational transition.

As a result of the theoretical analysis mentioned above, a number of optimum conformations of enniatine B (N_1-N_5, see the table in [2]) have been found which, in pyridine, could correspond to the N form actually existing; at the same time, the conformation originally put forward on the basis of the results of a consideration of conformational maps of Ac-L-MeVal-OMe and Ac-D-Hylv-NMe_2 and molecular models [1] was not confirmed. However, in view of the approximate nature of the calculation (fixed bond lengths and valence angles at C^α, simplified side chains, etc.) its possible error exceeded the energy differences between the individual conformations. Consequently, it is impossible solely on the basis of calculated figures to come to a definite conclusion on the parameters of the N conformation, although some preference was given to the N_1 conformation, which has the lowest energy in media with a low dielectric constant.

The present paper gives the results of a determination of the preferred conformation of enniatine B in nonpolar media, for which is was necessary to make a choice between structures N_1-N_5. In order to solve this problem, we made use of a new approach to the determination of the spatial structure of the peptides based on a theoretical evaluation of the Δδ values of the CSs of the signals of the $C^\alpha H$ and $N-CH_3$ protons in the ^1H NMR spectra of different conformers and a comparison of the results obtained with the experimental results. We started from the fact that, in view of the well-defined magnetic anisotropy of N-methylamide and ester groups, the CSs of the signals from the $C^\alpha H$ and the $N-CH_3$ protons are determined mainly by their orientation with respect to the groups mentioned, i.e., by the conformational parameters and Φ and Ψ. Paulsen and Todt [9, 10], and also Franclin et al. [11, 12], have shown that the amide group, as a whole, with the cis orientation of the carbonyl with respect to the α-methine proton, shows an anisotropic descreening influence, and its signal appears in a weaker field than with the trans orientation of the carbonyl and the proton. In these circumstances, the maximum differences in the CSs of the protons in the cis and trans orientation with respect to the amide carbonyl amounted to 1.0 ppm. It was also established [9] that in the case of two neighboring amide groupings the anisotropic effect has an additive nature.

Taking as a basis the experimental results on the anisotropic screening of the amide group, we have considered (Fig. 2a-h) four limiting types of mutual orientation of the α-proton and the two carbonyl groups



in the depsipeptide fragments $-C(=O)-N(CH_3)-CH(R)-C(=O)-O-$ and $-C(=O)-O-CH(R)-C(=O)-N(CH_3)-$. In the case of the cis,cis orientation (Fig. 2a, e), the descreening influence of the amide (A) and the ester (B) groups is summed, causing the maximum downfield shift of the signals of the α-protons, i.e., $-\Delta\delta = A + B$. With an arrangement of the trans,trans type (Fig. 2c, g) the anisotropic effect is zero ($-\Delta\delta = 0$). In the trans,cis (Fig. 2b, f)

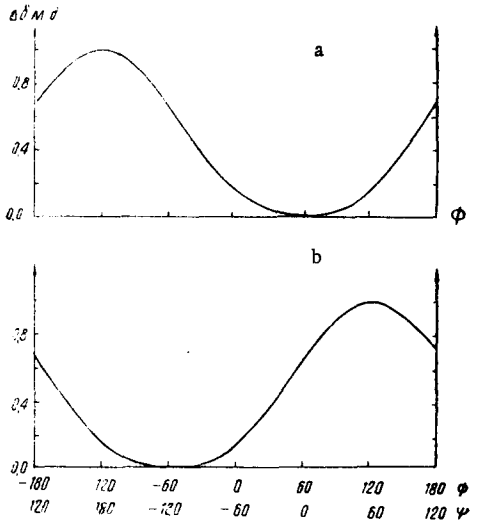


Fig. 3. Influence of the angles Φ and Ψ on the CSs of the α-protons in the residues of N-methyl-L-valine (a) and in the residues of D-α-hydroxy-isovaleric acid (b).

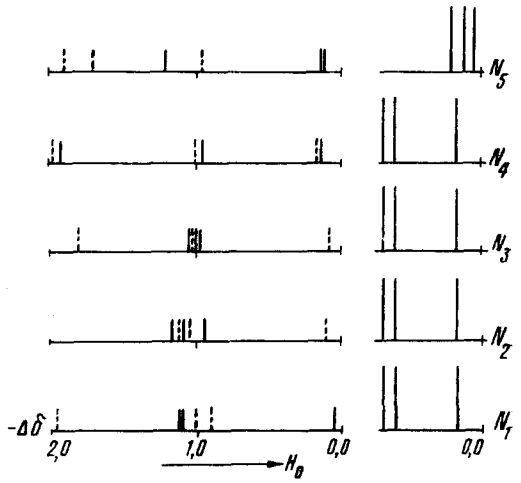


Fig. 4. Calculated form of the ^{1}H NMR spectra for the conformations N_1-N_5 of enniatine B (region of the C^{α} and the N-methyl protons). The signals from the α-protons of the hydroxy-acid residues are shown dashed.

and cis,trans (Fig. 2d, h) orientations the values of Δδ are determined by the descreening influence of only one of the groups (ester or amide), i.e., $-\Delta\delta$ = B or $-\Delta\delta$ = A. As shown in Fig. 2a, for the cis orientation of the α-proton with respect to the neighboring carbonyl groups, the H···O distances between the proton and the oxygens of these groups are extremely close – 2.50 and 2.38 Å, respectively, i.e., just as in the compounds described by Paulson and Todt [9, 10]. Hence, taking into account also the similarity of the electronic structures of the amide and ester groups [13] we can take A ≈ B ≈ 1 ppm.

To evaluate the change in the CSs in intermediate orientations of the α-protons and the CO groups we adopted a sinusoidal form of the dependence of the magnitude $-\Delta\delta$ on the angles Φ and Ψ (Fig. 3a, b), see [9, 14], and the initial values of the CSs (at $-\Delta\delta$ = 0) for the C^{α} protons of the amino-acid and hydroxy-acid residues were taken to be the same. The descreening of the N-methyl protons by the amide carbonyl group is constant (all the amide groups in the molecule of enniatine B have the trans orientation), and therefore the CS of these protons is determined solely by the orientation of the ester carbonyl.

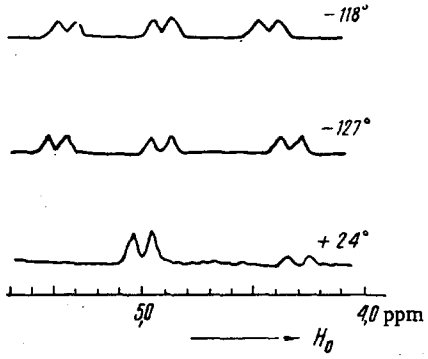


Fig. 5

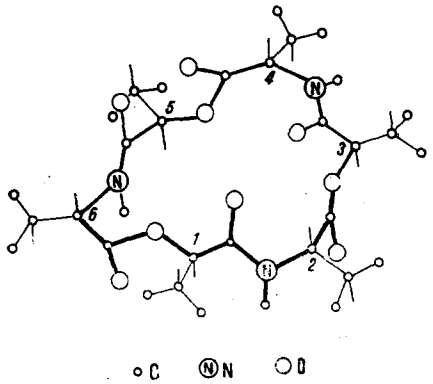


Fig. 6

Fig. 5. ^{1}H NMR spectra (region of the α-protons) of a labelled sample of enantio-enniatine B in CS_2 (top spectrum) and in a mixture of CS_2 and $CD_3C_6D_5$ (2 : 1) at various temperatures.

Fig. 6. Conformation of enniatine B in nonpolar solvents ($\Phi_1 = 89$, $\Psi_1 = -109$; $\Phi_2 = -118$, $\Psi_2 = 92$; $\Phi_3 = 154$, $\Psi_3 = 32$; $\Phi_4 = 73$, $\Psi_4 = -55$; $\Phi_5 = -58$, $\Psi_5 = -92$; $\Phi_6 = -133$, $\Psi_6 = 140$).

The results of an estimate of the CSs of the $C^{\alpha}H$ and $N-CH_3$ signals in the ^{1}H NMR spectra of the conformations N_1-N_5 of enniatine B obtained in this way are given in Fig. 4. In a comparison of the theoretical and experimental spectra it can be seen that the spectrum observed in CS_2, in which there are three groups of signals in the region of $C^{\alpha}H$ protons with an intensity ratio of 1 : 4 : 1 and two groups in the $N-CH_3$ region with a ratio of 6 : 3, corresponds to the calculated spectra of two conformations – N_1 and N_3. The equidistant (0.31 ppm) position of the three $N-CH_3$ signals for the spectrum in a mixture of CS_2 and $CD_3C_6D_5$ (2 : 1) does not agree with any of the calculated spectra which is apparently due to the specific interaction of one of these groups with the aromatic solvent. In view of the significantly different distributions of the signals from the $C^{\alpha}H$ amino-acid and hydroxy-acid residues with respect to the three groups mentioned in the spectra of N_1 and N_3 (see Fig. 4) it is possible to make a choice between these two conformations if the appropriate spectral assignments are obtained. For this purpose we performed the synthesis of a labelled enantiomer of enniatine B in which in the N-methyl-D-valine residues were replaced by N-methyl-D-valine -C^{α}-d residues [15] (the conclusions based upon its investigation are, of course, fully transferable to enniatine B). As can be seen from Fig. 5, the NMR spectrum of the deuterated analog in the 2.4-5.6 ppm region corresponds to the calculated figures for the N_3 conformation, which gives grounds for considering it to be demonstrated for enniatine B in nonpolar media. The calculated dipole moment of this conformation (3.80 D, see the table in [2]) also agrees well with the experimental value of 3.35 ± 0.1 D in CCl_4) if the approximate nature of the method of calculation is taken into account.

For the conformation of enniatine B in a nonpolar medium that has been found, which is shown in Fig. 6, as for the P form [1], the pseudoequatorial orientation of all the isopropyl groups is characteristic. Two of its amide and one of its ester carbonyl groups are oriented above the mean plane of the ring, and the other three carbonyl groups below it. Formally, the $N_3 \rightarrow P$ transition can take place by the rotation of the methylamide bond located between C_4 and C_5 through 180°. The energy barriers of these transitions also, possibly, determine the kinetics of the conformational transformations of the antibiotic: a rough estimate of the barrier to the $N \rightarrow P$ transition gives a value of 6.9 kcal · mole^{-1} (from the results on the absorption of ultrasound). A theoretical conformational analysis shows that in relation to nonvalent interactions the N form is less suitable than the P form. However, the latter is destabilized because of the electrostatic repulsion of the similarly charged and spatially close carbonyl groups. The interaction of these groups with a polar solvent or with a cation reduces this repulsion and leads to the realization of the P form.

Thus, the results of the present work show that the enniatine cyclodepsipeptides belong to the substances the spatial structure of which in solutions cannot be determined by any single physicochemical or theoretical method. Apparently, only by the combined use of a combination of experimental and mathematical methods is it possible to count upon success in the study of the conformational states of such labile peptide systems in solutions.

CONCLUSIONS

1. The relative positions of the $C^{\alpha}H$ and $N-CH_3$ signals in the ^1H NMR spectra of different conformers of enniatine B have been evaluated.

2. The ^1H NMR spectra [tri-(N-methyl-D-valyl-C^{α}-d)] enniatine B have been investigated.

3. The spatial structure of enniatine B in nonpolar media has been determined.

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