THEORETICAL CONFORMATIONAL ANALYSIS OF CYCLIC HEXADEPSIPEPTIDES. ENNIATINS

E. M. Popov, V. Z. Pletnev,
A. V. Evstratov, V. T. Ivanov,
and Yu. A. Ovchinnikov

UDC 615.779.90

The interest in the study of the spatial structure of antibiotics of the cyclodepsipeptide group (enniatins [1], beauvericin [2], valinomycin [3], etc.) is due to their specific influence on the transport of ions of the alkali metals through artificial and biological membranes [4, 5].

In a preceding paper [1] in which the results of a study of the conformations of the membrane-active antibiotic enniatin B (Fig. 1) were given, we showed that for this compound there is a conformational equilibrium of two forms which shifts with a change in the polarity of the medium [4]. One of the forms (P), which is dominating in polar solvents and is also found in complexes of the enniatin antibiotics with alkali-metal ions [6-8], has a third-order axis of symmetry and is characterized by a pseudoequatorial orientation of the lateral isopropyl groups; the ester and N-methylamide carbonyl groups in it are oriented on different sides of the mean plane of the ring with the formation of an opening with a diameter of 3.4 Å in the center of the molecule. The second form (N) is dominating in nonpolar solvents. The results of measurements of the NMR spectra at low temperatures show the nonequivalence of all the amino acid and hydroxy acid residues. Having analyzed molecular models in the light of the results of a calculation of the conformational patterns of the amino acid and hydroxy acid fragments of the antibiotic (Fig. 2), for the "nonpolar" form we proposed a compact conformation with no elements of symmetry in which the values of the angles Φ and Ψ were similar for all the hydroxy acid fragments and the corresponding parameters for the amino acid residues differed substantially from one another. The dipole moment calculated for this conformation proved to be close to the experimental value. However, the available data were insufficient to consider the conformation as definitively proved.

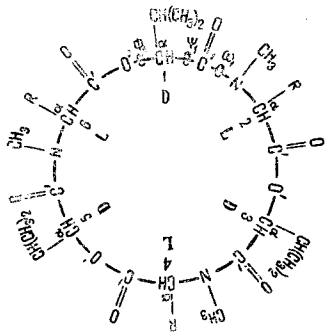

Fig. 1. Structural formulas of the cyclodepsipeptides: R is the side chain. Enniatin A, $CH(CH_3)C_2H_5$. Enniatin B, $CH(CH_3)_2$. Enniatin C, $CH_2CH(CH_3)_2$. Beauvericin, $CH_2C_6H_5$. Φ_i and Ψ_i are the angles of rotation around the $C^\alpha-N(C^\alpha-O')$ and $C^\alpha-C'$ bonds; ω_i is the angle of rotation around the $C'\cdots N$ and $C'\cdots O'$ partial multiple bonds.

The aim of the present investigation, which is a logical development of our work on the theoretical conformational analysis of the cyclodepsipeptides, is the development of a general approach to the analysis of the spatial structure of the cyclic hexadepsipeptides and a further study based upon it of the conformational states of enniatin antibiotics.

Method of Calculation. The formulas of enniatins A, B, and C and of beauvericin, and also the symbols used, are shown in Fig. 1. The calculation of the conformations was performed by searching for the minimum potential energy in the light of the nonvalence interactions of the atoms, the elasticity of the valence angles, the electrostatic interactions, and the torsional energy. As the potential describing the nonvalence interactions we selected Kitaigorodskii's function [9, 10]

Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 616-622, September-October, 1970. Original article submitted July 10, 1970.

© 1973 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for $15.00.

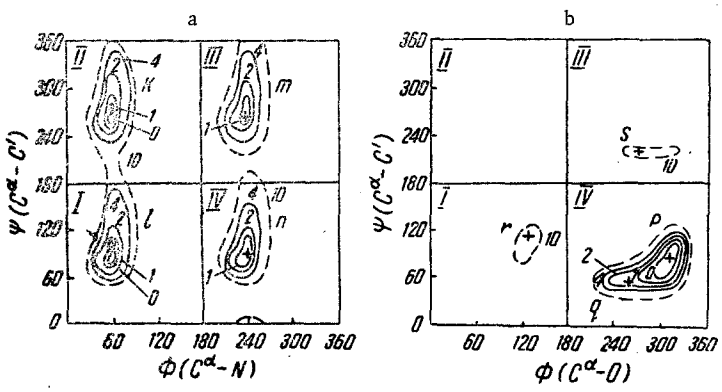

Fig. 2. Conformational patterns of the methyl ester of N-acetyl-N-methyl-L-valine (a) and of the dimethylamide of O-acetyl-D-hydroxyisovaleric acid (b).

with a published set of van der Waals radii (K_1) [11]. The potential functions of the other types of interactions were taken from the literature [12, 13]. The charges on the atoms were selected in such a way that the dipole moments of the trans and cis amide and ester groups calculated from them agreed with the experimental values of μ of the corresponding lactams and lactones [14, 15]. The following charges were obtained for an amino acid residue (in electron units): −0.280 (O), +0.040 (C'), +0.380 ($HC^{\alpha}R$), −0.230 (N), +0.150 (Me); and for a hydroxy acid residue: −0.415 (O), +0.275 (C'), +0.270 ($HC^{\alpha}R$), −0.190 (O'). The charges on H (C^{α}) and R (C^{α}) were taken as +0.05. With these charges, the dipole moment of the trans amide group is 3.7 D and forms an angle of 38° with the C'−N bond, while for the cis configuration of this group the moment is 3.8 D and is directed at an angle of 41°; in the trans and cis ester groups, the dipole moments are, respectively, 1.8 and 4.2 D and have directions (with respect to the C'−O' bond) of 54 and 78°. The values of the dipole moments of the optimal forms of enniatin were determined by the vectorial combination of the moments of the amide and ester groups.

One of the bonds of the ring ($C_6^{\alpha} - C_6'$) can be represented in the form of a resilient spring drawing together the ends of the molecule. At the function ensuring the closure of the ring we used the Scott potential as modified by ourselves [12].

$$U_{\text{ring}} = a(r - r_0)^2 + b(2 - \cos\alpha_1 - \cos\alpha_2) + C(1 - \cos\beta),$$

where r_0 is the equilibrium length of the $C_6^{\alpha}-C_6'$ bond; r is the distance between the C_6^{α} and C_6' atoms; and α_1, α_2, and β are the deviations of the $N_6-C_6^{\alpha}-C_6'$ and the $C_1-C_6'-O_1'$ angles and of the angle of rotation ω_6 from their equilibrium values.

The barriers to rotation around the C'⋯N and C'⋯O' bonds were chosen as 14 and 9 kcal/mole, respectively [16, 17]. The parameters a, b, and c are given small values at the beginning of the iteration process ($< 10^2$) and at the end were increased to values of the order of 10^6, which ensured a more effective search for a local minimum.

As the variables we took the five pairs of dihedral angles Φ and Ψ (the sixth pair is dependent), the six angles ω, and the $N-C^{\alpha}-C'$ valence angle, which is equal to the $O'-C^{\alpha}-C'$ angle. In the first stage of minimization, only the angles Φ and Ψ were varied. Close to the potential energy minimum, all 17 variables were changed. The values of the fixed valence angles for the amide and ester groups were obtained from the calculation of N-methylacetamide [18] and methyl acetate: $C^{\alpha}-C'-N = 118°$, $C'-N-C^{\alpha} = 123°$, $C^{\alpha}-C'-O' = 117°$, $C'-O'-C^{\alpha} = 114°$, $C^{\alpha}-C'-O = 119°$, and $C'-N-Me = 121°$. The angle $H-C^{\alpha}-R = 107°$. The lengths of the bonds were taken from the literature [19]. The trans configuration of the methylamide and ester groups, which is characteristic of enniatin B and its analogs and its complexes with alkali-metal cations [1, 8], was assumed for calculation. For the ester bonds the trans form is preferable even for the highly strained cyclotetradepsipeptides [19, 20].

Choice of Zero Approximations. The results of conformational analysis of peptide [21] and depsipeptide [20] compounds show that variations in the substituents on the C^{α} atoms changing the thermodynamic parameters of the optimum conformations do not lead to qualitatively new spatial forms of the molecules.

The greatest conformational freedom is possessed by compounds consisting of Gly and Glyco residues. Their replacement by other residues causes a shift in the conformational equilibrium within the limits of the forms permitted for the corresponding Gly and Glyco derivatives. This rule, which has been checked for a large number of peptide and depsipeptide compounds, enables the solution of the conformational problem for the enniatins to be simplified and to be reduced to the calculation of the model of the molecule $(L-MeAla-D-Lac)_3$.

This molecule is highly labile, and therefore its investigation gives an idea of the maximum conformational possibilities of the enniatins. The choice of the model $(L-MeAla-D-Lac)_3$ for calculation does not contradict known experimental facts. Thus, enniatins A, B, and C, with differing side chains, have similar optical rotatory dispersion and circular dichroism curves in solution in heptane or trifluoroethanol, which shows that the rings have similar conformational structures [4-7].

In the investigation of derivatives of the cyclic tetradepsipeptides [20] we established that the most suitable conformations of the ring have low energies of the nonvalence interactions between neighboring peptide and ester groups and the intermediate side chain. Because of the local nature of these interactions, the selection of preferred cyclic forms out of all those possible for $(Gly-Glyco)_2$ was made on the basis of the steric patterns of the corresponding linear depsipeptide fragments. It is obvious that this approach is completely justified also for rings containing a larger number of links. Figure 2 gives the conformational patterns of the linear molecules modelling the amino acid and hydroxy acid fragments of enniatin B with the trans configurations of the Ac–L–MeVal–OMe (A) and Ac–D–Hyiv–NMe_2 (B) amide and ester bonds [1].

The potential surface of A has energetically similar minima in each of the four quadrants (k, *l*, m, n). In the case of compound B, the region of lowest energy (p, q) is found in quadrant IV. The minima r and s in quadrants I and III are several kcal/mole higher; they are also less preferable from the point of view of entropy; the region II is forbidden. Thus, the amino acid residues possess considerably greater conformational possibilities than the hydroxy acid residues. This gives grounds for assuming that the conformations of the hexadepsipeptide rings with low energies of the nonvalence interactions in all the local sections of the ring are determined primarily by the conformational states of the hydroxy acid residues. In the enniatins, the most energetically favorable forms are probably those in which the maximum number of hydroxy acid residues are located on the conformational chart of B in quadrant IV.

The choice of the angles Φ and Ψ for the three hydroxy acid residues predetermines the geometry of the amino acid residues to a considerable extent. An analysis of molecular models shows that with not very large deviations of the C^{α} atoms from the plane of fixation of the angles Φ and Ψ, the three hydroxy acid residues determine the range of forbidden values for all the pairs of angles Φ and Ψ of the amino acid residues, i.e., their quadrants on the conformational chart (I). Thus, with given values of Φ_1, Ψ_1; Φ_3, Ψ_3; and Φ_5, Ψ_5 for the hydroxy acid residues, the angles of the amino acid residues Φ_2, Ψ_2; Φ_4, Ψ_4; and Φ_6, Ψ_6 will be found, respectively, in the quadrants corresponding to the coordinates $\Psi_1\Phi_3$, $\Psi_3\Phi_5$, and $\Psi_5\Phi_1$.

Thus, in the selection for the minimization of the zero approximations we assume that, in the first place, the cyclic hexadepsipeptides have low energies of the nonvalence interactions in all local sections of the ring, in the second place that the structure of the ring is determined primarily by the conformational states of the hydroxy acid residues, and, in the third place, that the positions of the hydroxy acid and amino acid residues in this cyclic system are interdependent.

For a clear idea of the forms selected it is desirable to introduce the following symbols. Let us express the angles Φ and Ψ in the range from 0 to 180° by the symbol (↑), and in the range from 180 to 360° by (↓). Then quadrant I is determined by two arrows directed upwards (↑ ↑), II by (↑↓), III by (↓ ↓), and IV by (↓ ↑). The fixation of the hydroxy acid residues in definite quadrants means the selection of three pairs of arrows. When arranged according to the numbering adopted (see Fig. 1), they simultaneously determine Φ and Ψ quadrants of the amino acid residues. For example, the arrangement of all the hydroxy acid residues in quadrant IV corresponds to the set (↓ ↑ ↓ ↑ ↓ ↑) and in quadrants I, III, and IV to the set (↑ ↑ ↓ ↓ ↓ ↑). In each set, the pairs of arrows 2 and 3, 4 and 5, and 6 and 1 reflect the state of three amino acid residues. In addition, the arrows show the predominant direction of the C=O bonds relative to the mean plane of the ring.

The initial types of conformations for minimization in the symbols adopted have the following form: P (↓ ↑ ↓ ↑ ↓ ↑), N_1 (↓ ↓ ↓ ↑ ↓ ↑), N_2 (↑ ↑ ↓ ↑ ↓ ↑), N_3 (↓ ↑ ↓ ↓ ↑ ↑), N_4 (↓ ↑ ↑ ↑ ↓ ↓), N_5 (↓ ↓ ↓ ↓ ↓ ↑). The

TABLE 1. Optimum Conformations of L−MeAla−D−Hylv)$_3$

Φ, ψ, deg	Conformations					
	P	N_1	N_2	N_3	N_4	N_5
Φ_1	254	298	125	269	282	268
ψ_1	44	235	108	71	89	310
Φ_2	77	224	84	52	31	239
ψ_2	351	277	306	272	120	259
Φ_3	254	332	306	334	107	308
ψ_3	44	62	38	212	48	202
Φ_4	77	60	67	253	85	243
ψ_4	351	257	264	125	293	266
Φ_5	254	318	304	122	301	319
ψ_5	44	79	111	88	222	61
Φ_6	77	49	113	47	246	120
ψ_6	351	291	14	320	332	204
U_{tot} (ε=1)	3,9	0	7,2	4,4	10,9	34,6
(kcal/mole) (ε=4)	0	2,8	5,5	3,0	12,0	31,0
(ε=10)	0	4,8	5,9	9,5	13,0	32,5
μ (D)	7,15	2,55	8,85	3,80	4,20	7,25

Note. The labelling of the angles Φ, Ψ corresponds to the nomenclature in the literature [23].

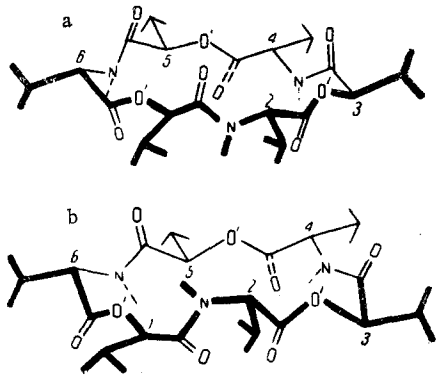

Fig. 3. Conformation (P) of enniatin B in a polar medium (a) and (N_1) of enniatin B in a nonpolar medium (b).

zero approximations selected thus correspond to forms of the cyclohexadepsipeptides in which three (P), two (N_1, N_2), or one (N_3-N_5) hydroxy acid residues are present in the most favorable conformation (p, q). The form N_6 (↑ ↑ ↑ ↑ ↓ ↓), in which there are two hydroxy acid residues with a conformation of type r (quadrant I), was not studied in detail since even with one hydroxy acid residue in the conformation the corresponding form of the cyclohexadepsipeptide (N_2) possesses an extremely high energy (Table 1). The form P corresponds to the experimentally found conformation of enniatin B that is dominant in polar solvents and in complexes [1, 8], while the forms N_1-N_5 have no elements of symmetry and, in all probability, relate to the conformations of enniatin B characteristic for nonpolar solvents. Besides those listed, several zero approximations were also taken in which the mutual dependence of the conformational states of the hydroxy acid and amino acid residues in the ring was not taken into account. In particular, the structure with the three hydroxy acid residues in quadrant IV and the amino acid residues in quadrants I and III that has been proposed for enniatin B in a nonpolar medium [1] was considered.

Results and Discussion. The calculation confirmed the correctness of the approach described above. The optimum conformations of the molecule (L−MeAla−D−Lac)$_3$ do actually correspond to the P and N_1-N_5 approximations selected for minimization. At the same time, the calculation of other approximations did not lead to satisfactory results; in the minimization process the values of their geometrical parameters were transformed into structures of the N_1-N_6 types.

Table 1 gives the values of the potential energy, the dipole moments, and the values of the angles Φ and Ψ of the optimum conformations P−N_5. In the conformations found the trans-N-methylamide and ester groups have almost a planar structure, the maximum deviation ω not exceeding 2°; the values of the N−C^{α}−C′ and O′−C^{α}−C′ angles in all the conformations are about 100°.

Of the optimum conformations considered, the most satisfactory are P and N_1, their relative energies being extremely sensitive to the electrostatic component (see Table 1): the energy of the P form falls with an increase in the dielectric constant of the medium, i.e., on passing to a more polar solvent, while in the N_1 form the opposite tendency is observed – its tendency is a minimum in a neutral medium (see [23]).

The conformation P (Fig. 3a) completely corresponds in its parameters to the "polar" form of enniatin B found experimentally: it belongs to the C_3 symmetry group, and all the hydroxy acid residues possess conformations of the p, q type and the amino acid residues those of type k.

In the N_1 form (Fig. 3b) two hydroxy acid residues assume the p, q conformation and one the s conformation; the conformations of the amino acid residues correspond to the minima k (two) and m (one). The N_1 conformation lacks elements of symmetry. The carbonyl groups are directed away from the center of the molecule. The side chains of the hydroxy acid and amino acid residues approximately retain the pseudo-equatorial orientation. The dipole moment of the form (2.55 D) agrees satisfactorily with the moment found for enniatin B in CCl_4 solution (3.35 D) [1]. To a first approximation the transition of the P form of the enniatin cyclodepsipeptide into the N_1 form can be represented as the result of the rotation of the N-methyl-amide bond located between the C_1^{α} and C_2^{α} asymmetric atoms; the orientation of the lateral groups changes only very slightly during this process.

Taking into account the results of the present work and those of experimental studies [1, 8], we may regard the conformation of the "polar" form of enniatin B to be definitively established; a conformation of type N_1 is most probable for the "nonpolar" form.

We are using the above-described approach to the conformational analysis of the cyclodepsipeptides to study the spatial structure of diastereomers of enniatin B differing from the natural antibiotic in the configuration of the asymmetric centers.

CONCLUSIONS

The spatial conformation of the "polar" form of enniatin B has been established. A conformation of the N_1 type has been proposed for the "nonpolar" form.

LITERATURE CITED

1. Yu. A. Ovchinnikov, V. T. Ivanov, A. V. Evstratov, N. D. Abdullaey, E. M. Popov, G. M. Lipkind, S. F. Arkhipova, E. S. Efremov, and M. M. Shemyakin, Biochem. Biophys. Res. Commun., 37, 668 (1969).
2. R. L. Hamill, C. E. Higgens, H. E. Boaz, and M. Gorman, Tetrah. Let., 4255, No. 49 (1969).
3. V. T. Ivanov, I. A. Laine, N. D. Abdullaev, L. B. Senyavina, E. M. Popov, Yu. A. Ovchinnikov, and M. M. Shemyakin, Biochem. Biophys. Res. Commun., 34, 803 (1969).
4. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, E. I. Vinogradova, A. M. Shkrob, G. G. Malenkov, A. V. Evstratov, I. D. Ryabova, I. A. Laine, and E. I. Melnik, J. Membr. Biolog., 1, 402 (1969).
5. M. M. Shemyakin, V. K. Antonov, L. D. Bergelson, V. T. Ivanov, G. G. Malenkov, Yu. A. Ovchinnikov, and A. M. Shkrob, The Molecular Basis of Membrane Function, Prentice-Hall, New Jersey (1969).
6. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, A. M. Shkrob, I. I. Mikhaleva, A. V. Evstratov, and G. G. Malenkov, Biochem. Biophys. Res. Commun., 29, 834 (1967).
7. M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, Angew. Chem. Internat. Edit., 8, 492 (1969).
8. M. Dobler, J. D. Dunintz, and J. J. Kraewski, Mol. Biol., 42, 603 (1969).
9. A. I. Kitaigorodsky, Tetrah., 14, 230 (1969).
10. A. I. Kitaigorodsky, Acta Cryst., 18, 585 (1965).
11. M. Venkatachalam, G. N. Ramachandran, in: Conformation of Biopolymers, Vol. 1, Academic Press, London-New York (1967), p. 83.
12. H. A. Scheraga, in: Advances Phys. Org. Chem, Vol. 6, Academic Press, London-New York (1968), p. 103.
13. G. N. Ramachandran and V. Sasisekharan, in: Advances Protein Chem., Vol. 23, Academic Press, New York-London (1968), p. 283.
14. R. Huisgen and H. Walz, Chem. Ber., 89, 2616 (1956).
15. R. Huisgen and H. Ott, Tetrah., 6, 253 (1959).
16. O. Buchardt, P. L. Kumler, and C. Lohse, Acta Chem. Scand., 23, 1155 (1969).
17. J. F. Piercy and S. V. Subrahmanyam, J. Chem. Phys., 42, 1475 (1965); Subrahmanyam and Piercy, J. Acoust. Soc. Am., 37, 340 (1965).
18. E. M. Popov, V. G. Dashevskii, G. M. Lipkind, and S. F. Arkhipova, Molek. Biol., 2, 622 (1968).
19. J. J. Konnert and I. L. Karle, J. Am. Chem. Soc., 91, 4888 (1969).
20. V. Z. Pletnev and E. M. Popov, Izv. AN SSSR, Ser. Khim., 991 (1970).
21. E. M. Popov, G. M. Lipkind, S. F. Arkhipova, and V. G. Dashevskii, Molek. Biol., 2, 622 (1968).
22. J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and M. A. Scherga, J. Mol. Biol., 15, 339 (1966); J. Biol. Chem., 241, 1004 (1966); Biopolymers, 4, 121 (1966).
23. G. M. Lipkind, S. F. Arkhipova, and E. M. Popov, ZhSKh, 11, 121 (1970).