A MOLECULAR ORBITAL STUDY ON THE CONFORMATION OF ENNIATIN B.

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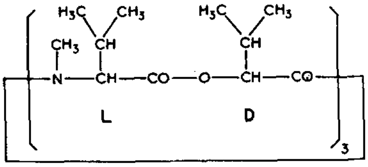
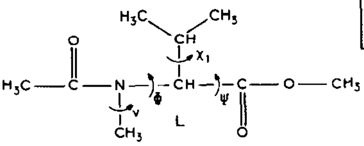
Summary. Molecular orbital calculations using the PCILO method are performed on the conformation of the symmetrical form of enniatin B. The values of the Φ and ψ angles found for the preferred conformation agree closely with the results of X-ray study of the K^+ complex of enniatin B.

The structure, conformation and metal ion binding properties of cyclic peptide and depsipeptide antibiotics are one of the most actively investigated subject of research (For general references see (1)-(7)). The present note inaugurates the application of quantum-mechanical methods to this problem.

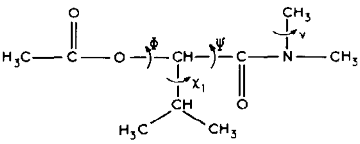
Enniatin B is a cyclohexadepsipeptide formed, as indicated in I, from alternating N-methyl-L-valine and D-α-hydroxyisovaleric acid residues. RMN(8) and ORD(9) studies in solution point to the existence of this molecule in two forms : 1) a symmetrical one with a C_3 symmetry axis, predominant in polar solvents, said to be identical to the K^+ complexing form observed by X-ray crystallography(10), having a pseudo-equatorial orientation of the isopropyl groups and a central cavity and 2) an asymmetrical one, predominating in non-polar solvants, devoid of the central cavity and whose isopropyl groups have a pseudo-axial orientation.



I. Enniatin B



II



III

We have limited our attention to the symmetrical, "complexing" form. Our theoretical investigation has been carried out by the PCILO (Perturbative Configuration Interaction using Localized Orbitals) molecular orbital method(11) which has been extensively used beforehand for similar studies on the conformation of proteins and nucleic acids(12). We have benefited, however from a recent important technical development of the method, into the so-called Differential-PCILO(13), which greatly increases its computational rapidity. As input data we have used the bond lengths and angles found recently in a related cyclotetradepsipeptide(14). ν = 60°, χ_1 = 300° for the L-valyl residue and χ_1 = 180° for the D-α-hydroxyisovaleryl residue (H^{α} and H^{β} *trans* in both cases) have been adopted. The peptide and ester bonds have been taken as planar-*trans*. The results of our computations may be compared with those of empirical calculations carried out by Popov *et al.* (8)(15).

Starting from the usual and rather well substantiated hypothesis that short-range interactions are the essential ones in determining the conformation of oligo- and polypeptides we have investigated in the first place the conformational possibilities of the two residues which enter into the structure of enniatin B. For this sake the (Φ, Ψ) conformational energy maps have been constructed for the two model compounds :N-acetyl-N-methyl-L-valinate II and O-acetyl-D-α-hydroxyisovaleryl-N-dimethylamide III. The results are presented in figures 1 and 2.

Figure 1 indicates the existence of four nearly equivalent low energy regions, labelled K, L, M, N to indicate the overall analogy to the results found by empirical computations(8), although there are differences in details : in particular the N energy minimum of the empirical computations is split in the quantum-mechanical ones into N_1 and N_2. Fig. 2 presents three energy minima, labelled P, R, S with one of them significantly more stable than the remaining

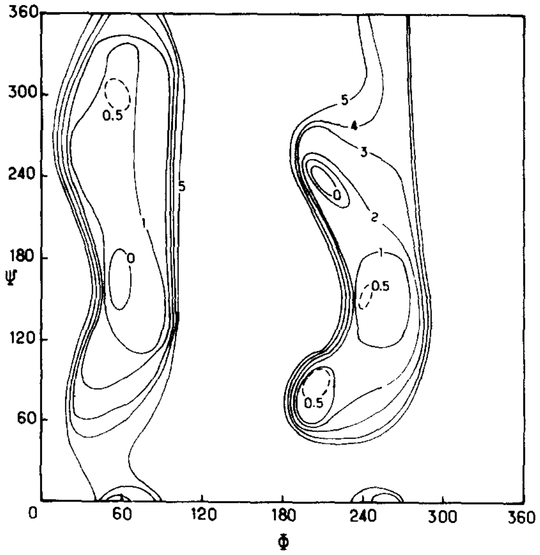


Figure 1. – Conformational energy map of I.Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero.

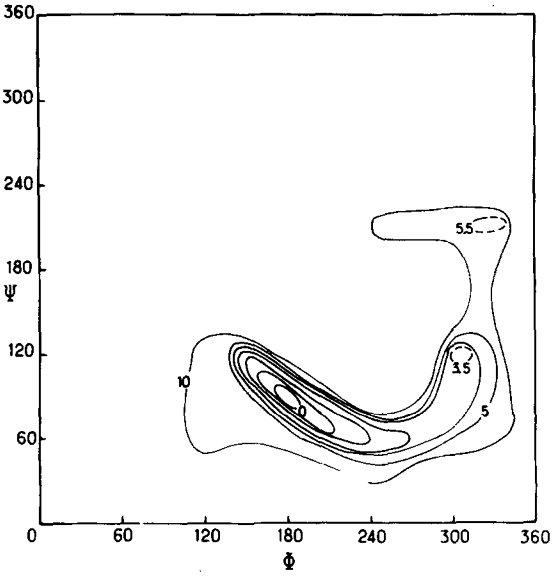


Figure 2. - Conformational energy map of II. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero.

two. In this case the quantum-mechanical results are significantly different from the empirical ones. In particular, the global minimum which in the empirical computations corresponds to P is shifted in the PCILO ones to R. A more detailed comparison of the quantum-mechanical and empirical results is given in Table I.

In the zero order approximation the hypothesis of the predominance of short range interactions implies that the starting Φ, Ψ values for the study of the conformation of enniatin should be chosen in the low energy regions of the individual amino acid and ester residues.

Now, one of the main results of the computations for these residues is to indicate that the amino acid unit has much more conformational freedom than the ester one. We may therefore reasonably suppose that the conformational possibilities of the ester residues play a decisive role in the overall conformation of the cyclic compound. Otherwise speaking, the selection of the (Φ, Ψ) combination for the ester residues imposes to a large extent the possible choice of the (Φ, Ψ) combinations for the amino acid residues. In fact, the examination of models shows that, for the values $\Phi_1 \Psi_1$, $\Phi_3 \Psi_3$, $\Phi_5 \Psi_5$ given to the ester units, the values of $\Phi_2 \Psi_2$, $\Phi_4 \Psi_4$, $\Phi_6 \Psi_6$ of the amino acid units must correspond, grosso modo, to the combination $\Psi_1 \Phi_3$, $\Psi_3 \Phi_5$, $\Psi_5 \Phi_1$ (15). At this point, it may be useful to adopt Popov's notation in which the values of Φ and Ψ in the region 0° - 180° are depicted by the symbol (↑) and their values in the region 180° - 360° by the symbol (↓). Following this convention the K region is represented

TABLE I. - Comparison of the PCILO and empirical results for I and II

Compound		Quantum-mechanical computations					Empirical computations				
	Minima	Φ	Ψ	χ_1	ν	$E_{kcal/mole}$	Φ	Ψ	χ_1	ν	$E_{kcal/mole}$
	K	60	300	300	60	0.7	61	270	304	67	0
	L	60	150	300	60	0.1	56	104	301	37	0.1
	M	210	240	300	60	0	241	266	301	60	0.2
	N_1	210	90	300	60	0.5	238	89	304	60	0.1
	N_2	240	150	300	60	0.5					
	P	300	120	180	60	4.1	311	90	303	55	0
	Q	-	-	-	-	-	260	50	303	64	0.3
	R	180	90	180	60	0	128	120	313	74	3.5
	S	330	210	180	60	6.0	262	220	315	37	5.3

TABLE II. - PCILO computations on the conformation of enniatin B

Presumed form	Residue	Before minimization			After minimization		
		Φ	Ψ	$E_{kcal/mole}$	Φ	Ψ	$E_{kcal/mole}$
$(RK)_3$	Ester	180	90	14.3	226	72	0.
	Amino acid	60	300		92	302	
$(PK)_3$	Ester	300	120	8.1	295	98	4.3
	Amino acid	60	300		75	315	
$(SM)_3$	Ester	330	210	38.4	308	224	26.1
	Amino acid	210	240		218	261	
$(RL)_3$	Ester	180	90	0.	206	63	14.7
	Amino acid	60	150		83	251	

by (↑↓), the L by (↑↑), the M by (↓↓), the N by (↓↑), the P by (↓↑), the R by (↓↑) and the S by (↓↓). Then using the above stated rule the possible "starting" conformations for the symmetrical form of enniatin B are $(R\ K)_3$, $(PK)_3$, $(SM)_3$

TABLE III. - Preferred theoretical and experimental conformation of enniatin B.

Angle	PCILO computations		Empirical computations		Values in crystallographic complex with K^+
	Before minimization	After minimization	Before minimization	After minimization	
Φ_{ester}	180	226	320	254	240
Ψ_{ester}	90	72	60	44	60
$\Phi_{\text{amino acid}}$	60	92	60	77	120
$\Psi_{\text{amino acid}}$	300	302	300	351	300

and $(RL)_3$. The calculation of the total energy of each of these four possible forms (Table II) indicates that at this stage the $(RL)_3$ form apears as the most stable one.

The next step in the calculation consists of carrying out starting from these combinations an energy minimization process. This is done by the Simplex method (16). The results are indicated in the last columns of Table II. The refinement reverses the relative stabilities of the combinations indicating that now the most stable conformation is of the $(RK)_3$ type.

It may be useful to compare the results of the quantum-mechanical calculations and of the empirical ones with the experimental values of the Φ and Ψ angles in the crystallographic form of enniatin B complexed with K^+ which is said to be identical with the symmetrical form in solution. This comparison is carried out in Table III. It may be seen that before minimization the two theoretical results are nearly equidistant, although differently, from the experimental data but that after minimization the PCILO results are significantly closer to the experimental values than the empirical results, in particular as concerns the angles of the amino acid residues.

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