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CONFORMATIONAL STUDIES ON ACTINOMYCIN
THE CONFORMATION OF THE ISOLATED PENTAPEPTIDE-LACTONE

P. De Santis, R. Rizzo, G. Ughetto
Laboratorio di Chimica-Fisica - Istituto Chimico - Università di Roma
00185 Rome, Italy

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NMR experiments on some peptide-lactone derivatives of Actinomycin have been recently reported by H. Lackner.[1] On the basis of the interpretation of NMR and of stereochemical models, the author proposed a structure of the peptide-lactone(+) in which all of the peptide groups were in the trans conformation and which contained an intracyclic bond connecting the - NH group of the D-Valine with the C=O group of the Sarcosine residue.

We have been interested in the possible conformations of this peptide--lactone as a step in the conformational analysis of Actinomycin, on which we have carried out theoretical calculations based on NMR, IR, and X-Ray data.[2]

This approach successfully allowed the prediction of the molecular structure of Actinomycin in complete agreement with that determined independently by X-Ray crystal analysis.[3] This structure is shown in Fig. 1 as a projection in a plane normal to the quasi-dyad axis.

We started from the consideration that the most stable conformations which the peptide-lactone may assume are not very different from those we may select on the basis of suitable assemblage of the sterically allowed local conformations of the dipeptide fragments of the cyclopeptide. These were selected by evaluating the van der Waals and torsional conformational energy diagrams corresponding to the dipeptide and tripeptide fragments of the peptide-lactone of Actinomycin D.

Both the cis and trans conformations of the N-substituted peptide groups were examined. In this calculation we used the set of potential functions adopted in the derivation of molecular conformation of Gramicidin S, a natural decapeptide antibiotic.[4]

(+) N-[2-Nitro-3-methoxy-4-methyl-benzoyl]-[cyclo-(L-threonyl-D-valyl-L-prolyl--sarkosyl-N-methyl-L-valyl-O$_{Thr}$)].

Furthermore the rotation angle, χ, about the C_α-C_β bonds of the D-Valine and N-methyl-Valine residue were fixed with the hydrogen atoms in the trans conformation according to the $^3J_{C_\alpha H-C_\beta H}$ coupling constant. However, the low value of $^3J_{C_\alpha H-C_\beta H}$ assigned to the threonine residue in both the Actinomycin as well as in isolated peptide-lactone allows only the gauche conformations with respect to the hydrogen atoms. Finally the rotation angle about the C_α-C chemical bond of the Proline aminoacid residue was fixed at $\Psi = 330^\circ$, corresponding to the only allowed conformation as shown from the van der Waals energy profile in Fig. 2. The result indicates that the conformation proposed by Lackner is not sterically favorable because it involves a considerable steric hindrance between the Sarcosine N-methyl group and the pyrrolidinic ring. However, the Lackner assignement of the hydrogen bond between the D-Valine NH and the Sarcosine C=O groups agrees with our results. The possible assemblage of the sterically allowed local conformers as derived on the basis of the conformational analysis leads to one type structure which is favorable to the ring closure, once the NMR constraints on the angle about the C -N bonds of the D-Valine and the Threonine residues and the formation of an intracyclic hydrogen bond involving the NH of D-Valine were taken into account.

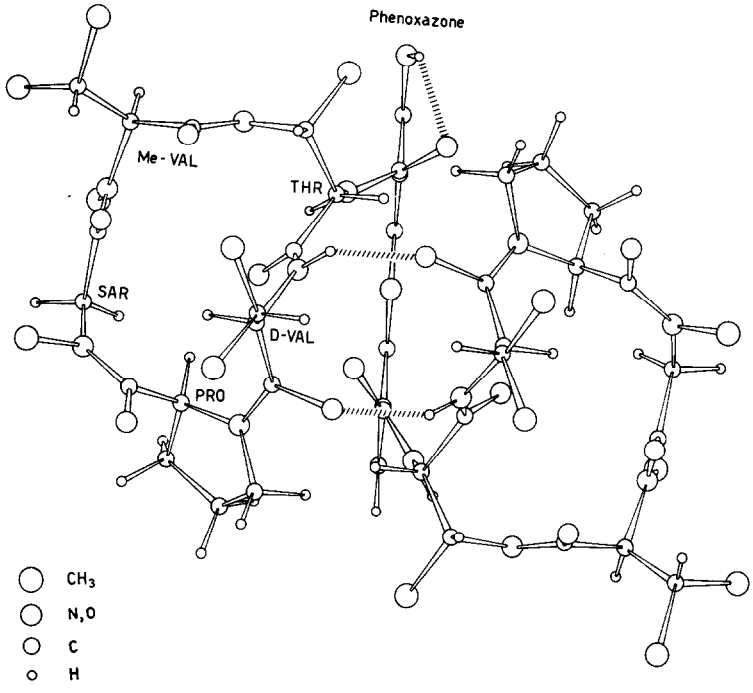

Fig. 1 - Three-dimensional projection of Actinomycin D.

The refinement of the ring closure together with the van der Waals, torsional and hydrogen bond conformational energies was tried using a simultaneous steepest descent mathematical technique satisfactorily adopted in a previous work.[4] As may be seen in Fig. 3, the arrangement about the Proline and Sarcosine

peptide is cis and the C_α-CO-Pro-Sar-N(CH_3)-C_α tripeptide sequence resembles a tripeptide fragment of the Poly-Proline I helical structure.

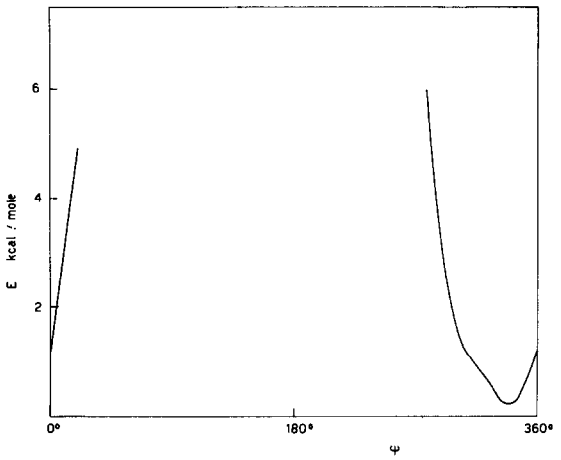

Fig. 2 - Conformational energy profile corresponding to Proline dipeptide unit in terms of rotation angle about C -C bond.

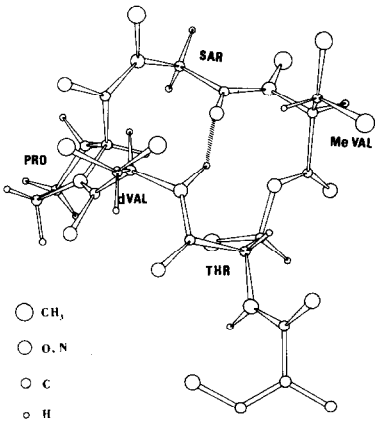

Fig. 3 - Three-dimensional projection of the isolated pentapeptide-lactone of Actinomycin.

In Fig. 4 the representative points of the local conformation of the different fragments of pentapeptide-lactone are reported as "stars" on the pertinent local conformational energy diagrams. As may be noted, they are located in the maps near to the deepest minima and in the allowed ranges of the NMR coupling constant indicated as graphs on the φ axes where the Karplus dependence was assumed.[5] This conformation seems to fit consistently the Lackner experimental data, particularly the NMR coupling constants, the slow proton exchange rate of the D-Valine amide proton, the difference in the chemical shift of the CH_2 Sarcosine proton (one of which lies in the plane of a cis peptide group) and the splitting of N-CH_2 signals.[1]

In conclusion, the model of the isolated pentapeptide-lactone presented in this paper may explain on the one hand the similarity of some NMR signals with those corresponding to Actinomycin and on the other hand the large difference of the chemical shifts (1.5 p.p.m.) assigned to the amide proton of the D-Valine residues. In fact, as may be seen by comparison of Fig. 1 and Fig. 3, the pentapeptide-lactones are very similar in the fragment of the structures containing the Proline and Sarcosine residues whereas are very different in the other zone, especially where the hydrogen bon occurs.[2]

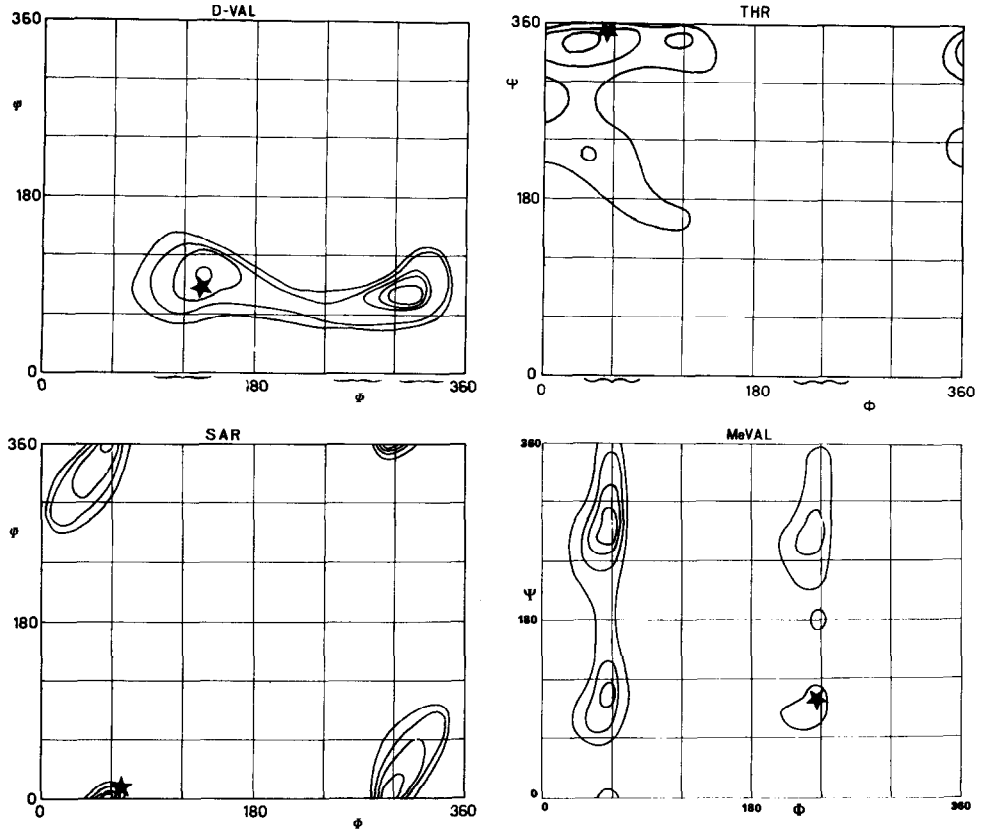

Fig. 4 - Conformational energy diagrams corresponding to: Threonine dipeptide unit (C-CO-Thr-NH-C), D-Valine-Proline tripeptide unit (C-CO-DVal-Pro-N(CH_3)--C), Proline-Sarcosine tripeptide unit (C-CO-Pro-Sar-N(CH_3)-C), N-methyl--Valine-peptide-lactone unit (C-CO-MeVal-O-C). The rotation angles φ and ψ refer to the aminoacid residues indicated at the top of the diagrams. The rotation angle of the Proline residue is fixed at 330° corresponding to the local energy minimum. The rotation angles ablut C_α-C_β bonds are fixed as χ (Thr) = 180°, χ (DVal) = 60°, and χ (MeVal) = 300°. The graphs on the abscissa indicate the allowed ranges from NMR coupling constant.

REFERENCES

1. H. Lackner, Tetrahedron Letters (London), 36, 3189 (1970).
2. P. De Santis, R. Rizzo, and G. Ughetto, Biopolymers, in press.
3. H.M. Sobell, S.C. Jain, T.D. Sakore, and C.E. Nordmann, Nature, 231, 200 (1971).
4. P. De Santis, Biopolymers, 10, 699 (1971).
5. V.T. Bystrov, S.L. Portnova, T.A. Balashova, V.I. Tsetlin, V.T. Ivanov, P.V. Kostetzky, and Yu.A. Ovchinnikov, Tetrahedron Letters (London), 59, 5225 (1969).