Tetrahedron Letters No. 38, pp 3385 - 3388, 1977. Pergamon Press. Printed in Great Britain.

THE GLOBAL MINIMUM ENERGY CONFORMATION OF CYCLOTETRAGLYCYL

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(Received in UK 25 July 1977; accepted for publication 4 August 1977)

Cyclic peptides fulfil a diversity of biologically important functions by acting as hormones, toxins, antiobiotics, ion carriers, inhibitors etc. and *in vivo* activity is intimately related to their molecular conformations.[1] We have investigated the possibility of systematically exploring the potential energy hypersurface of such molecules by means of empirical valence force field calculations. Previous approaches to this problem have met with limited success and suffer either from lack of generality[2,3,4] or include several gross approximations.[2]

We have now developed an almost automatic general procedure, based on interactive computer graphics, for the location of all low-energy conformations of cyclic peptides containing up to ten or so amino acid residues. The computer programs have been developed in FORTRAN for use on a DEC GT44 system and the algorithm is illustrated by the following description of calculations on c-Gly_4.

A Ramachandran map of potential energy as a function of the torsion angles ω and ψ was calculated for GlyGly, and six (ω,ψ) pairs evenly distributed in the extensive low-energy conformational space were selected as "generators" for the next step in the proceedings. The tetrapeptide chain was then folded up into a series of low-energy conformations corresponding to all possible combinations of generators for each of the six configurational sequences of *cis*- and *trans*- amide groups; the initial cartesian coordinates were obtained from an X-ray analysis of c- Sar_8[5] by removing an arbitrary tetrapeptide fragment and replacing the N-methyl groups by hydrogen atoms. The fragment contained non-planar amide groups with a variation in geometry from residue to residue.

Ring closure of the folded chains was attempted by means of a simple pattern

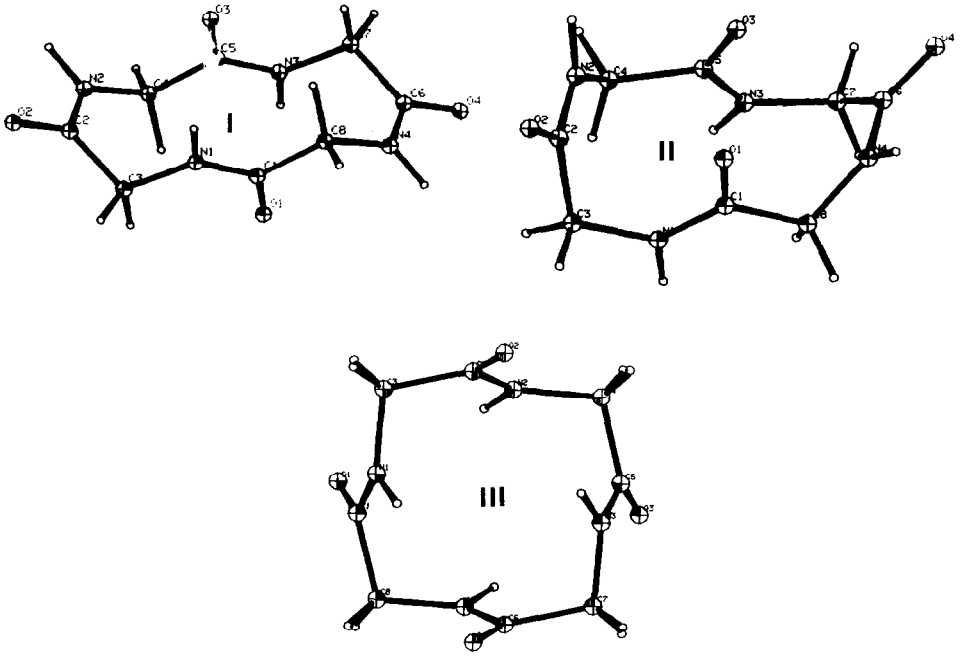
search procedure which depended on geometric rather than energetic criteria to effect closure. Each individual ring torsion angle (φ, ψ and ω) and a α-carbon ring valency angle was allowed to vary by up to $\pm$ 20° from its starting value and conformations which did not then close to within $\pm$ 0.1Å of the optimum bond length were rejected. Variation of bond lengths and angles as well as torsion angles etc. during subsequent energy minimization[6] was sufficient to close the small remaining gap. Because the calculations were performed on a homopolymer it was possible to generate repeat conformations related to the original by advancing the pattern of torsion angles by one or more residues. All such repetitions were deleted at this stage. The connectivities of the remaining conformations were calculated on the basis of a maximum bonded distance of 1.6Å and those with ten or more "bonds" in excess of the proper value, due to very short non-bonded intramolecular distances, were also rejected. Notice that the hydrogen atoms were ignored for the purposes of this test as apparently gross stereochemical irregularities involving these atoms are frequently simple to remedy. Further pruning of the survivors from the above battery of tests was accomplished by examination of the individual conformations on the cathode ray tube of the minicomputer graphics system in order to eliminate sterically unreasonable structures.

The original 7776 conformations had been reduced to around 100 cyclic and stereochemically reasonable structures which were capable of refinement by energy minimization. It would have been feasible at this stage, or earlier, to arrange the conformations in order of decreasing probability so that only the most likely proportion of the full set need be considered further. However, in this instance we preferred to continue with all of the possibilities. We have implemented the first step of our two-stage Newton-Raphson energy minimization procedure[6] on the GT44 as part of the Glasgow University Chemical Graphics System (GUCGS) and all 3N-6 atomic cartesian coordinates of each conformation were adjusted until the root-mean-square values of the first derivatives of potential energy w.r.t. the coordinates were sufficiently small for the second stage of minimization to converge. At this point it became obvious that some groups of superficially different conformations were converging upon identical minima and redundant conformations were again deleted. Stage two minimizations were completed on a

mainframe computer and yielded a final set of conformations which had not been artifically depleted by insisting upon fixed bond lengths or angles, standard Pauling-Corey geometry or exact ring closure of trial conformations.

Differences between the backbone conformations of cyclic peptides composed of glycyl residues and other cyclic peptides can be interpreted in terms of the extra steric requirements of the side-chains of non glycyl residues.[7] We should therefore expect to find that the conformations of all known cyclic tetrapeptides form a subset of the final group of calculated c-Gly_4 conformations. This is indeed the case and I, II and III are ORTEP drawings, produced by GUCGS, of the calculated analogues of c-Sar_4[8], c-(-D-HyIv-L-MeIleu-D-HyIv-L-MeLeu-)[9] and c-(-Iabu-L-Phe-D-Pro-L-X-)[10] -type conformations, respectively. Conformation III is calculated to be the global minimum followed by, I at 1.3 kcal.mole^{-1} above and, II at 4.2 kcal.mole^{-1} above III in potential energy.

Grathwohl et al. have studied NMR spectra of c-Gly_4 in various solvents and interpret their results in terms of an S_4-symmetric conformation identical to III.[11] Dale and Titlestad, on the other hand, appear to have studied a protonated form of c-Gly_4, in TFA as solvent, and find in favour of conformation I.[12] In order to understand these apparently conflicting results consider first the effect of protonating the amide groups of III. The double bond character of the amide bonds will be enhanced, thereby raising the barrier to free rotation from the unprotonated value of $\approx$ 20 kcal.mole^{-1} to somewhere in the region of the 60 kcal.mole^{-1} typical of a C=C bond. Ring closure of III involves significant non-planarity of the trans- amide groups as well as a reduction of the ring valency angles at the α-carbon atoms, resulting in appreciable Pitzer and Baeyer strain, respectively. This situation is exacerbated to the extent of $\approx$ 10 kcal.mole^{-1} on protonation. Conformation I is calculated to be only 1.3 kcal.mole^{-1} less stable than III and is essentially free of Baeyer and Pitzer strain so that protonation of III results in a conformational interconversion to I!



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