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CONFORMATIONAL STUDIES OF CYCLIC PEPTIDES IN SOLUTION. NMR SPECTRA OF CYCLOHEXAPEPTIDES CONSISTING OF L(D)-ALANINE AND GLYCINE RESIDUES *.

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In the course of systematic conformational studies of cyclic peptides and depsipeptides we have carried out an NMR investigation of two series of cyclohexapeptides synthesized in our laboratory (2); namely,1) those with all possible combinations of L-alanine and glycine residues and 2) those with all possible diastereomeric cyclohexaalanyls (exepting the antipodes) (Fig.1)**

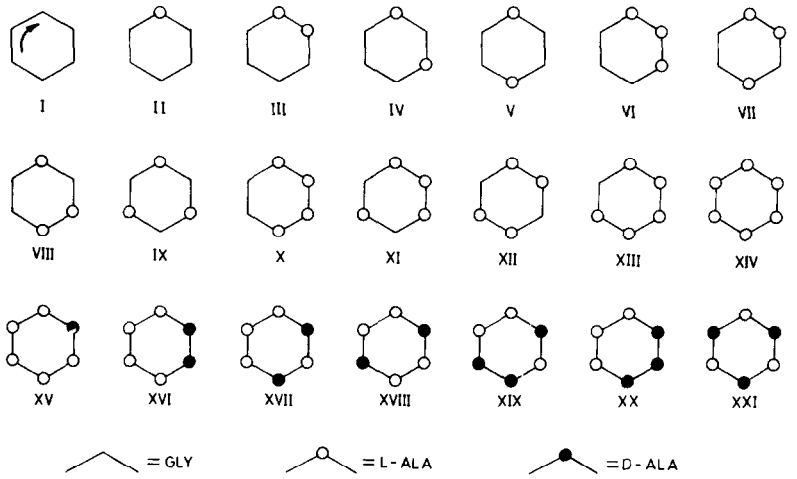


Fig.1

In $(CD_3)_2SO$ at 25° the spectra of all the cyclopeptides revealed two groups of NH signals: at 7.3-8.0 ppm given by two protons, and the other, due to the remaining four protons, being at lower field (7.9-8.6 ppm).Temperature studies showed the $\Delta\delta/\Delta t$ values to be lower for the protons resonating at higher field (Table 1). The temperature dependence indicated that the two higher

* For details see (1)

** An NMR investigation of cyclohexapeptides containing Leu,Tyr, His and Pro residues has been carried out previously (3).

Table 1

Compound	Chemical shifts (ppm) and in parenteses temperature gradients ($\Delta\delta/\Delta t$ 10^{-3} ppm/°C) of the NH protons					
III	8.05(3.9)	8.14(5.4)	7.88(1.4)*	7.92(3.1)*	8.23(4.0)*	8.41(5.7)*
IV	8.19(5.0)	8.21(5.0)	7.80(1.7)*	7.84(2.1)*	8.34(4.4)*	8.38(5.7)*
V	8.46(5.9)	8.46(5.9)	7.55(0.0)*	7.55(0.0)*	8.39(4.2)*	8.39(4.2)*
VI	7.87(2.9)	8.08(3.3)	8.24(5.1)	8.01(3.3)*	8.15 (a)*	8.48(5.8)*
VII	8.02(4.2)	8.35(6.5)	8.35(6.5)	7.59(0.0)*	7.72(1.5)*	8.46(6.3)*
VIII	7.97(3.0)	8.10(5.6)	8.25(6.4)	8.12(3.4)*	8.21(4.8)*	8.28(4.8)*
IX	8.00(3.9)	8.00(3.9)	8.00(3.9)	8.27(4.7)*	8.27(4.7)*	8.27(4.7)*
XI	7.85(2.1)	7.95(4.2)	8.10(4.0)	8.12(4.4)	8.30(6.6)*	8.39(5.6)*
XII	7.80(2.4)	7.80(2.4)	8.32(4.8)	8.32(4.8)	8.12(5.0)*	8.12(5.0)*
XIII	7.78(1.4)	7.81(2.8)	7.90(3.6)	7.97(3.4)	8.29(5.1)	8.49(6.4)*
XIV	8.19(3.0)	8.19(3.0)	8.19(3.0)	8.19(3.0)	8.19(3.0)	8.19(3.0)
XVI	7.56(5.4)	7.87(1.7)	7.87(4.3)	7.96(3.2)	8.06(3.4)	8.36(6.1)
XVIII	7.38(0.0)	7.38(0.0)	8.20(7.0)	8.20(7.0)	8.56(6.4)	8.56(6.4)
XIX	7.38(2.0)	7.47(1.7)	7.96(5.3)	8.03(6.0)	8.34(6.3)	8.44(6.3)

* NH Protons of Gly residues; (a) When raising the temperature the signal hidden by other NH signals.

field resonating protons form intramolecular hydrogen bonds (IHB), whereas the four remaining NH protons interact effectively with the solvent (4). Accordingly the molecules are in a "pleated sheet" form, proposed by Schwyzer (5) in which the two NH protons are engaged in transannular IHB of the type 4→1, each proton closing a 10-membered ring (Fig. 2). The lower $\Delta\delta/\Delta t$ value of the NH protons of XII at 7.80 ppm are accompanied by lower deuterio exchange rates in $(CD_3)_2SO$ - H_2O (9:1). However, other cyclopeptides show no differentiation in the deuterio exchange rates, which is explained by fast conformational equilibrium of the type A⇌B⇌C as shown on Fig.3. Indeed, cooling the solution of IX causes the broadening of the CH_2 signals (Fig.4), an indication of mixture of rapidly interconverting conformers.

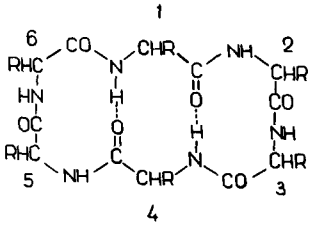
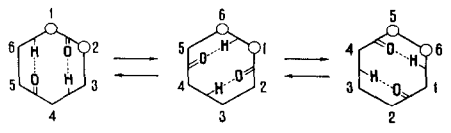


Fig. 2

On the basis of the NH chemical shifts and temperature gradients the predominant structural types of the cyclic peptides of series 1) should be such as shown on Fig.5. It follows from the figure that the cyclopeptides investigated do not display any regularity in the formation of IHB. For instance,



A B C

Fig.3

in compounds IV and VII the IHB are formed by Gly residues whereas in XII they are formed by Ala residues. This nonspecificity is apparently due to the small energy differences between the A, B and C forms. For the symmetric compounds IX and XIV the alternate A, B and C forms are equally possible. Hence they show intermediate $\Delta\delta/\Delta t$ values (Table 1). In the "pleated sheet" model two groups of amino acid residues can be distinguished. To the first group belong the two residues (1 and 4) forming IHB and situated at the "fold" of the "sheet". The second group comprises four residues (2, 3 and 6) not participating in IHB and situated at the "corners". The alanine methyls at positions 1 and 4 can be pseudo-axial or pseudo-equatorial with respect to the C_2 axis, the angles Φ being 0-60° and 300-360° respectively, which corresponds to dihedral angles H-N-C^{α} and N-C^{α}-H of 120-180° and 60-120°.

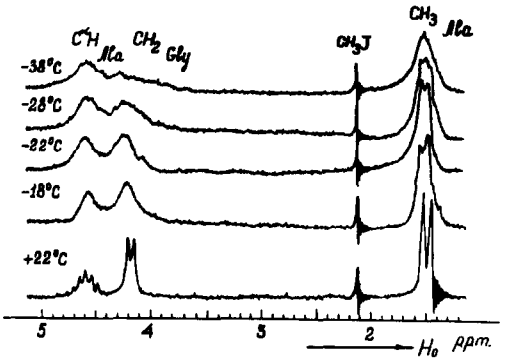


Fig. 4
Temperature dependence of the NMR spectrum of IX in CF_3COOH: H_2O (7:1). Recorded with JEOL CO JNM 4H-100 instrument, 100 MHz, TMS as internal standard.

From the stereochemical dependence of the $^3J_{NHCH}$ constant (6) it follows that for the pseudo-axial position one would expect higher $^3J_{NHCH}$ (3.0-10.7 Hz)

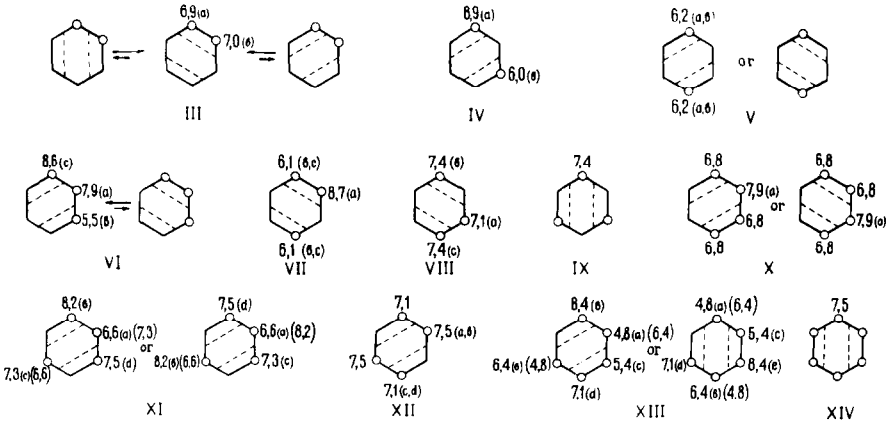


Fig. 5

than for the pseudo-equatorial ones (0-3.0 Hz). The values actually found (4.8 - 8.2 Hz, Fig.5) refer to the first possibility. As for the "corner" Ala residues they seam to prefer the conformations with $\Phi_{(2,5)}$ 110-130° (θ 130-110°) and $\Phi_{(3,6)}$ 50-90° (θ 150-180°) or 260° ($\theta \sim 20°$) as follows from energy computations (7) and available experimental data (8) on peptides with 4 1 IHB. Therefore the Ala residues in positions 2 and 5 should have rather small $^3J_{NHCH}$ values (< 5 Hz) whereas for those in positions 1 and 3 more probable are the higher $^3J_{NHCH}$ values (7-10 Hz). This is consistent with the experimental $^3J_{NHCH}$ values and allows assignment of the most of alanine NH signals as shown on Fig. 5. A typical cyclopeptide conformation with pseudo-equatorial methyl groups in positions 2 and 5 and pseudo-axial methyls in positions 1, 3, 4 and 6 is presented on Fig. 6 for compound XIII.

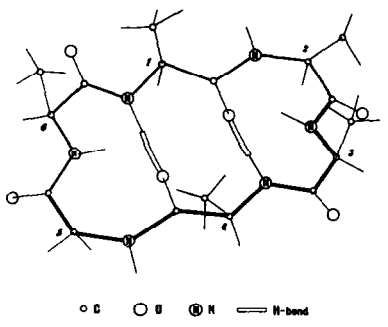


Fig. 6

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