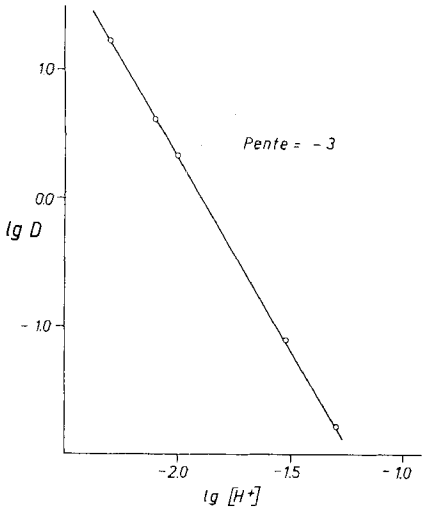

Fig. 2. *Variation du coefficient d'extraction D en fonction du pH de la phase aqueuse*

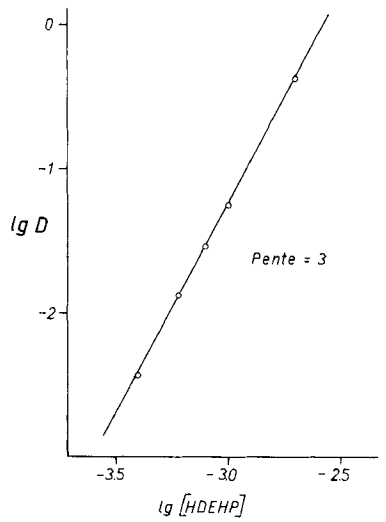

Fig. 3. *Variation de D en fonction de la concentration en échangeur*

Nous remercions l'Institut de Radiophysique Appliquée (Dir: Prof. *P. Lerch*), ainsi que le Centre de Calcul électronique de l'*EPFL*. – Ce travail fait partie d'un projet subventionné par le *Fonds National Suisse de la Recherche Scientifique*, que nous remercions de son aide précieuse.

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187. Conformational Studies of Cyclic Pentapeptides by Proton Magnetic Resonance Spectroscopy

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Summary. The proton magnetic resonance spectra of *c*-(-Gly-L-Ala-Gly-Gly-L-Pro-) (I) and four analogous cyclopentapeptides are presented. At ambient temperature the spectra contain two sets of resonances which correspond to two different molecular conformations of the peptides. The relative concentrations of the two forms depend on the peptide, the solvent, and the temperature. For the two molecular species of peptide I in DMSO solution, the NMR. data imply that the peptide linkage involving the nitrogen of proline is respectively in the *cis*- and the *trans*-form, and both

conformations contain intramolecular hydrogen bridges. Replacement of L-alanine in I by L-cysteine leaves the molecular conformations essentially unaltered. On the other hand substitution of L-proline by D-proline, or replacement of the two glycines in positions 3 and 4 by two sarcosyl residues gives rise to markedly different types of peptide backbone conformation.

Introduction. – Over the last two decades much knowledge on organisation and readout of biological information concerning peptides has been accumulated [1] [4], and for several peptides quite detailed information concerning structure-function relations has been obtained from comparative studies of analogous compounds [1]–[3]. Quite often, however, these studies have also led to the conclusion that a detailed knowledge of the molecular conformations would probably be an important prerequisite for a better understanding of the modes of action on the molecular level. On the other hand, mainly due to experimental difficulties, molecular conformations in oligopeptides have so far been rather elusive. This lead to an increased interest in model compounds which might mimic some of the functional properties of physiological peptides, and yet be more amenable to conformational studies. It was in connection with a model study on group Ia and IIa metal ion binding to peptides [2] [4] that we became interested in the cyclic pentapeptides discussed in this paper.

Among the various techniques applied to the investigation of molecular conformations[1]) in peptides, high resolution nuclear magnetic resonance (NMR.) spectroscopy has evolved as a very promising approach, in particular for studies of cyclic peptides [5]–[8]. Two NMR. spectral features are of particular interest. First, the positions and the temperature dependences of the amide proton resonances are markedly different depending on whether these groups are freely accessible to the solvent, or, on the contrary, shielded from interactions with the solvent by their environment in the peptide molecule [5] [9]. Second, it appears that the dependence on the dihedral angle of the vicinal spin-spin coupling constant between the amide and C_α-protons ($J_{H_nH_a}$) obeys a *Karplus*-type relation [10]–[12]. For all the common amino acid residues except prolyl, one out of every pair of torsion angles ϕ and ψ[2]), which define the peptide backbone conformation, can thus, in principle, be determined. Combined with the observations on the solvent accessibility of the different amide protons, this suffices in many cases to single out a small number of 'allowed conformations' [14] which would be compatible with the NMR. data. The present paper describes NMR. studies of a series of five analogous cyclic pentapeptides.

Experimental. – *Peptide Syntheses.* – The synthesis of *cyclo*-Glycyl-(S-diphenylmethyl)[3])-L-cysteyl-glycyl-glycyl-L-prolyl ('Peptide II') has been described previously (4). *cyclo*-Glycyl-L-alanyl-glycyl-glycyl-L-prolyl ('Peptide I') was obtained from Peptide II by treatment with *Raney* nickel: To 100 mg of the peptide in 20 ml of dry dimethylformamide a suspension of 1 g of freshly prepared *Raney* nickel in absolute ethanol was added and the mixture was hydrogenated for 24 h at room temperature. The suspended nickel was then filtered off, the solvent evaporated in high vacuum at 35° and the product was subsequently crystallized from chloroform/ethanol: 45 mg (75%). The elemental analysis gave C 49.51% (calc. 49.5%); H 6.26% (6.20%); N 21.05% (20.64%). A thin layer chromatogram of the product on *Merck* 'Silicagel' in butanol/acetic acid/water

[1]) In the following the term 'Conformation' will stand for 'Ensemble average seen by the NMR. technique'.

[2]) Definitions according to the IUPAC recommendations (13) are used for the description of the peptide molecules.

[3]) Abbreviated S-DPM.

10:2:3 (v) gave a single *Reindel-Hoppe*-positive spot with Rf 0.22. As will be seen in the following section, the NMR. data imply that no racemization occurred at the C_α of alanine during hydrogenation. *cyclo*-Glycyl-L-alanyl-glycyl-glycyl-D-prolyl ('Peptide III') was obtained by the same procedure from *cyclo*-Glycyl-(S-DPM)-L-cysteyl-glycyl-glycyl-D-prolyl.

The procedure for the synthesis of *cyclo*-Glycyl-(S-DPM)-L-cysteyl-sarcosyl-sarcosyl-L-prolyl ('Peptide IV') and *cyclo*-Glycyl-(S-DPM)-L-cysteyl-sarcosyl-sarcosyl-D-prolyl ('Peptide V') was quite similar to that for peptide II (4). Experimental details will be described in a forthcoming paper.

NMR. measurements. – For the proton NMR. studies the peptides were dissolved in dimethylsulfoxide-d_6 (DMSO-d_6), to which a trace of TFA (trifluoracetic acid) was added for some of the measurements. The peptide concentration was ca. 0.1 to 0.3 M.

The proton NMR. spectra were recorded on the *Varian* spectrometers HR-220 and XL-100, equipped with the standard *Varian* variable temperature control unit. In some spectra the signal-to-noise ratio was improved by spectrum accumulation either in a *Varian* C-1024 computer of average transients, or in a *Varian* spectrosystem 119 using the *Fourier* transform method. The resonance assignments are based mostly on observations in homonuclear double irradiation experiments performed on the XL-100 spectrometer.

Results. – The proton NMR. spectra at 220 MHz of five cyclic pentapeptides are shown in Fig. 1. In *c*-(-Gly-L-Ala-Gly-Gly-L-Pro-) (I) the alanyl methyl protons are at 1.2 ppm, the β- und γ-methylene protons of proline at 1.9 to 2.1, all the C_α-protons and the C_δ-methylene protons of proline between 3.4 and 4.6 ppm, and the amide protons between 6.7 and 8.8 ppm (see Table 1). Overall the resonances of the amino acid residues in peptide II, which differs from I in that L-alanyl is replaced by (S-DPM)-L-cysteyl, are quite similar to those of I. In addition there are the resonances of the aromatic protons of the DPM group at around 7.3 ppm, and the methine proton resonance of DPM at 5.3 ppm.

In spectrum III, *c*-(-Gly-L-Ala-Gly-Gly-D-Pro-), four strong and three less intense amide proton resonances are readily recognized in the spectral region between 7.3 and 9.2 ppm. A fourth weak amide resonance is at 7.75 ppm at 22°, and therefore buried under one of the stronger lines [15]. The pattern of the amide proton resonances is largely different from that for peptide I. The two spectra are also quite different in the region from 1.8 to 4.6 ppm. In particular the resonances at approximately 2.0 and 4.5 ppm, which in peptide I were assigned to protons of L-proline (Table 1), are markedly different in the two spectra.

In *c*-(-Gly-L-Cys(DPM)-Sar-Sar-L-Pro-) (IV) the N-methyl resonances of the two sarcosyl residues are at 2.78 and 2.93 ppm, the DPM resonances at 5.3 and 7.3 ppm, and the amide proton lines between 7.7 and 9.3 ppm. The proline resonances at ca. 2 ppm and the C_α-proton resonances between 3.5 and 4.5 ppm are quite markedly different from the corresponding spectral regions in peptide II. All the spectral differences between compounds II and IV cannot be explained by the differences between the resonances of sarcosyl [7] and glycyl residues, which indicates that conformational differences between the two peptide molecules are also manifested in the NMR. spectra. Finally, the spectrum of *c*-(-Gly-L-Cys(DPM)-Sar-Sar-D-Pro-) (V) is essentially identical with spectrum IV.

In each of the five spectra of Fig. 1 two forms of the cyclic pentapeptide are observed. In peptide I eight resonances correspond to the four amide protons. There are four resonances with relative intensities 0.65, and four weaker lines with intensities 0.35. The amide proton resonances in peptides II and III present a very similar

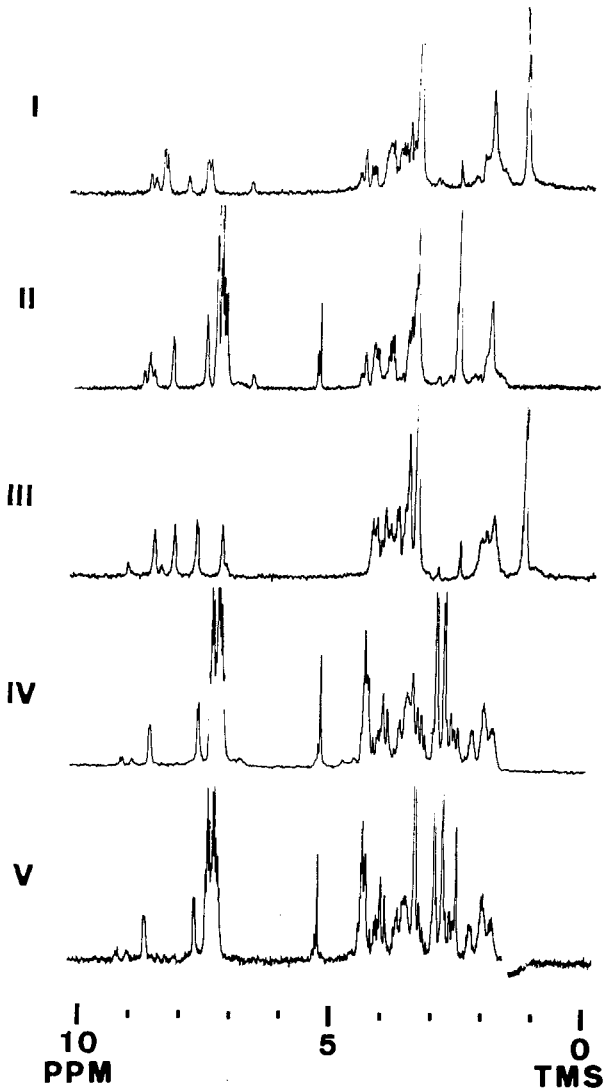

Fig. 1. *Proton NMR. spectra at 220 MHz of five cyclic pentapeptides in DMSO-d_6. T = 22°C*

I *c*-(-Gly-L-Ala-Gly-Gly-L-Pro-); II *c*-(-Gly-L-Cys(DPM)-Gly-Gly-L-Pro-); III *c*-(-Gly-L-Ala-Gly-Gly-D-Pro-); IV *c*-(-Gly-L-Cys(DPM)-Sar-Sar-L-Pro-); V *c*-(-Gly-L-Cys(DPM)-Sar-Sar-D-Pro-). Two resonances at 2.51 and 3.31 ppm correspond to the residual protons in DMSO-d_6 and to H_2O. The different intensities of these lines in the different spectra are due to the use of different solvent qualities

situation, the relative intensities for the two species being again 0.65 and 0.35, and 0.80 and 0.20, respectively. In peptide II the resonance of the methine proton of the DPM group consists also of two lines with relative intensities 0.65 and 0.35. In peptides IV and V the two amide protons give rise to two groups of two lines with relative intensities 0.85 and 0.15, and the intensities of the two lines at 5.3 ppm, which

Table 1. *NMR. Parameters for the two molecular species M (65%) and m (35%) observed in a solution of* c-(*-Gly-*L*-Ala-Gly-Gly-*L*-Pro-*) *in DMSO at 22°*

The coupling constants J are to within ±1 Hz

Residue	Type of NMR. Spectrum	Conformation M		Conformation m	
Gly (1)	*ABX*	$\delta_A^M = 3.64$	$J_{AB}^M = -16.0$	$\delta_A^m = 3.55$	$J_{AB}^m = -16.0$
		$\delta_B^M = 3.75$	$J_{AX}^M = 5.5$	$\delta_B^m = 3.81$	$J_{AX}^m = 4.0$
		$\delta_X^M = 8.51$	$J_{BX}^M = 5.5$	$\delta_X^m = 8.07$	$J_{BX}^m = 5.5$
Ala (2)	A_3PX	$\delta_A^M = 1.22$	$J_{AP}^M = 7.0$	$\delta_A^m = 1.25$	$J_{AP}^m = 7.0$
		$\delta_P^M = 4.05$	$J_{AX}^M = 0.0$	$\delta_P^m = 3.95$	$J_{AX}^m = 0.0$
		$\delta_X^M = 8.57$	$J_{PX}^M = 5.5$	$\delta_X^m = 8.85$	$J_{PX}^m = 4.5$
Gly (3)	*ABX*	$\delta_A^M = 3.39$	$J_{AB}^M = -14.0$	$\delta_A^m = 3.74$	$J_{AB}^m = -14.0$
		$\delta_B^M = 4.32$	$J_{AX}^M = 3.0$	$\delta_B^m = 3.88$	$J_{AX}^m = 3.5$
		$\delta_X^M = 7.70$	$J_{BX}^M = 7.5$	$\delta_X^m = 6.79$	$J_{BXm} = 6.5$
Gly (4)	*ABX*	$\delta_A^M = 3.37$	$J_{AB}^M = -15.0$	$\delta_{Am} = 3.58$	$J_{AB}^m = -16.0$
		$\delta_B^M = 3.98$	$J_{AX}^M = 4.0$	$\delta_B^m = 3.81$	$J_{AX}^m = 4.5$
		$\delta_X^M = 7.63$	$J_{BX}^M = 6.5$	$\delta_X^m = 8.75$	$J_{BX}^m = 6.5$
PRO (5)	*ABCDPQX*	$\delta_A^M = 1.90$		$\delta_A^m = 1.79$	$J_{DX}^m = 7.0$
		$\delta_B^M = 1.90$		$\delta_B^m = 1.91$	
		$\delta_C^M = 1.87$		$\delta_C^m = 1.68$	
		$\delta_D^M = 2.05$		$\delta_D^m = 2.27$	
		$\delta_P^M = 3.41$		$\delta_P^m = 3.35$	
		$\delta_Q^M = 3.44$		$\delta_Q^m = 3.40$	
		$\delta_X^M = 4.49$		$\delta_X^m = 4.59$	

correspond to the methine proton of DPM, are also approximately 0.85 and 0.15. The relative intensities of the resonances corresponding to the different species are quite sensitive to changes of solvent and temperature [15]. On the other hand the amide proton resonances were found to be insensitive to variation of the peptide concentration in the range 0.003 to 0.3 M.

The dependence on temperature of the amide proton resonance positions in peptide I is shown in Fig. 2. One resonance of the less abundant species (m) at 6.7 ppm is essentially independent of temperature, and two resonances of the more abundant species (M) at 7.6 and 7.7 ppm show only little variation with temperature. The temperature behaviour of the remaining five resonances is similar to what is generally observed for amide protons which are freely accessible to the solvent [9].

The rate of exchange of the amide protons in a mixed solvent of DMSO-d_6 and D_2O has been studied at 28°. On addition of 20 μl of neutral D_2O to a solution of 16 mg of peptide I in 0.3 ml of DMSO-d_6, which corresponds to a ratio of deuterium in D_2O to amide protons in peptide of approximately 10:1, the exchange of all the amide protons was completed in less than five minutes.

Corresponding amide proton resonances in the species M and m of peptide I (Fig. 3A) have been identified from observations in INDOR experiments. The C_α-proton resonances corresponding to the different amide proton lines were then identified by

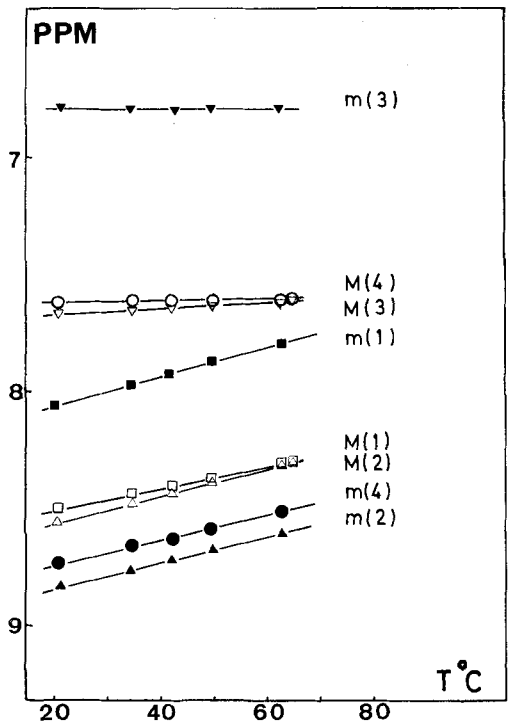

Fig. 2. *Dependence on temperature of the amide proton resonances in Fig. 1, I*
The resonance assignments correspond to the numbering of the amino acid residues in Fig. 6

various double resonance experiments. The spectrum obtained after deuteration of the amide groups was also studied in detail. In this way the experimental spectrum I (Fig. 1) could be decomposed into the two spectra corresponding to the species M and m. The NMR. parameters for these two species are given in Table 1, and in Fig. 4 the spectra computed with these parameters are compared with the experimental spectrum.

The following comments may be useful for a further qualification of the data in Table 1 and Fig. 4. First, only one out of a total of at least 26 significant spin-spin coupling constants between the protons of proline in the two species M and m could be determined with reasonable certainty from our spectra. In the computation of the prolyl resonances we therefore started with the coupling constants given by *Deber et al.* [16], which had to be modified only slightly in order to obtain the spectra in Fig. 4. Second, peptide I has been synthesized so far three times in our laboratory. The different preparations gave rise to two slightly different NMR. spectra (Fig. 3, A and B). Analysis of these spectral differences showed that there were small chemical shifts between corresponding resonances in the two spectra, but that the spin-spin coupling constants in Table 1 are valid for both spectra. Thus the interpretation of the data in the following section applies to all the preparations of peptide I which we have studied.

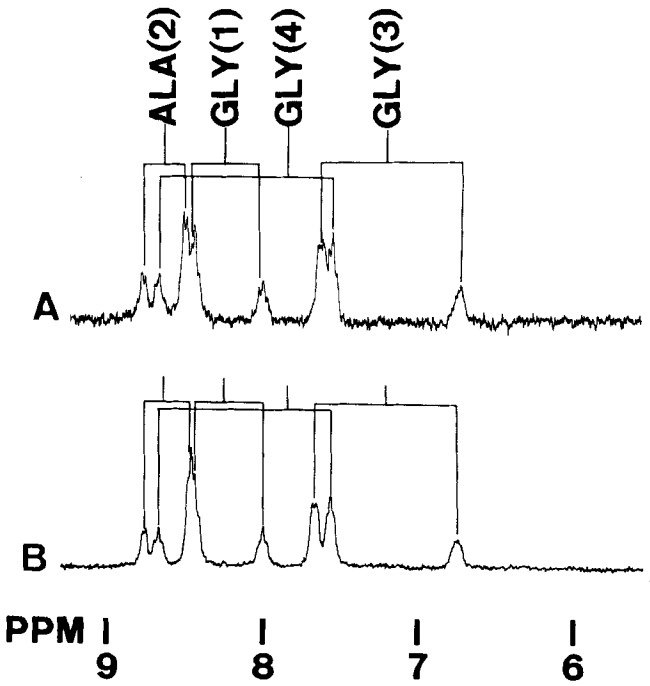

Fig. 3. *Spectral region from 6 to 9 ppm in the proton NMR. spectrum of* c-(-*Gly*-L-*Ala*-*Gly*-*Gly*-L-*Pro*-) *in DMSO-d_6. T = 22°*

The two spectra A and B were obtained from two different preparations. The resonance assignments correspond to the numbering of the amino acid residues in Fig. 6

Discussion. – The two sets of resonances in the NMR. spectra of the cyclic pentapeptides (Fig. 1) correspond to two different molecular conformations which are simultaneously present in the solutions in DMSO. This is borne out by the observation that the relative intensities of the two sets of resonances depend on solvent and temperature. For example, in a solution of peptide V in chloroform, only one set of proton resonances is observed [15]. Limiting values for the life times τ with respect to interchange between the two molecular forms were obtained on the one hand from the occurrence of separate NMR. lines for the two species, on the other hand from the observation in INDOR experiments of double resonance effects mediated between corresponding resonances of the two species by the interchange process [17]. For peptide I it was found that τ_M, the life-time in the major conformation, has to be longer than ca. 2×10^{-2} sec, but shorter than ca. 3×10^{-1} sec. These values, which are probably representative for all the five peptides in Fig. 1, make it understandable that even the amide groups, which, from their resonance positions and temperature dependences, appear to be not freely accessible to the solvent in either of the two species M and m, are rapidly deuterated in a mixed solvent of DMSO and D_2O.

The observation that the NMR. spectra in Fig. 1 are essentially not influenced by variation of the peptide concentration indicates that the two spectra M and m (Fig. 4).

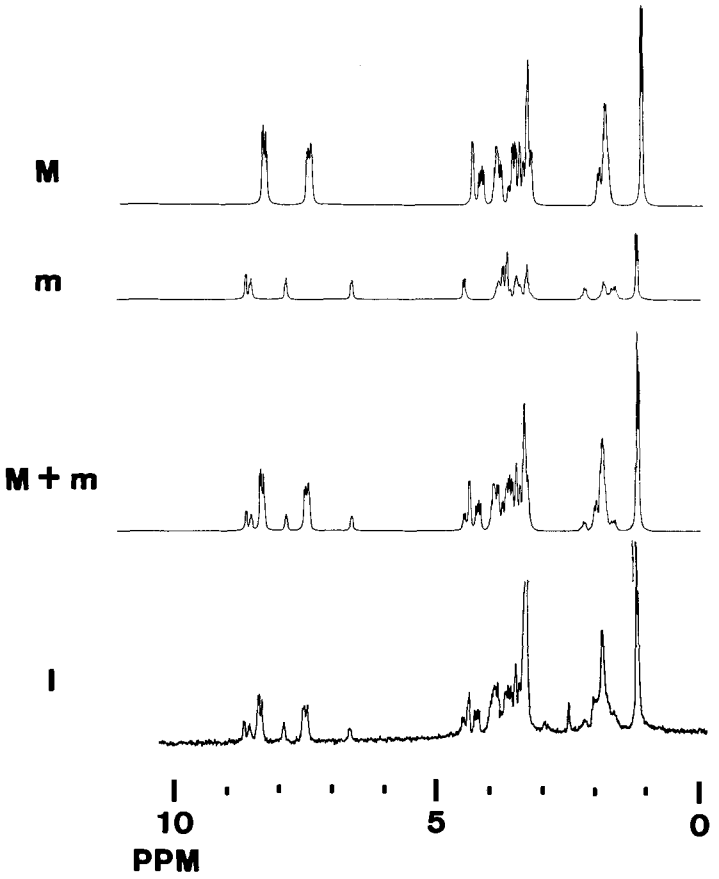

Fig. 4. *Comparison of the spectra calculated using the parameters of Table 1 with the observed NMR. spectrum at 220 MHz of* c-(-*Gly*-L-*Ala*-*Gly*-*Gly*-L-*Pro*-)

A half-width of the lines at half-height of 2.3 Hz was used in the computations. M: major conformation; m: minor conformation; M + m: computed spectrum which is to be compared with the experimental spectrum I

correspond to two different monomeric species. In the following we shall first discuss the conformations of the two monomeric forms M and m in solutions of *c*-(Gly-L-Ala-Gly-Gly-L-Pro-) (peptide I), and then investigate how these molecular conformations are affected by the amino acid substitutions in peptides II to V (Fig. 1).

trans *cis*

Fig. 5. cis- *and* trans-*Proline. Conformations involving the proline nitrogen peptide bond*

In the NMR. spectrum of peptide I (Fig. 1, Table 1) the C_α-H resonance of proline in the less abundant conformation m is located at lower field than the corresponding resonance in M. On the basis of earlier observations in the model compound *t*-Boc-Gly-L-Pro-OH [18] we conclude that the peptide bond involving the nitrogen of proline is in the *cis* conformation in m, and in the *trans* conformation in the species M (Fig. 5). Since the amide proton resonance at 6.7 ppm, which from its fine structure could be assigned to one of the glycyl residues, is essentially independent of temperature (Fig. 2), it appears that in the conformation m one glycyl amide proton is thoroughly shielded from interactions with the solvent. Examination of the molecular models shows that such a shielding effect in a monomeric species of peptide I is only possible if the amide proton is involved in transannular hydrogen bonding. If proline is considered to be in the *cis*-conformation (Fig. 5), only the hydrogen bridge between the amide proton of the glycyl residue in position 3 and the carbonyl oxygen of proline (Fig. 6 m) can readily be formed in the molecular models. We therefore assign the resonance at 6.7 ppm to the amide proton of glycine (3).

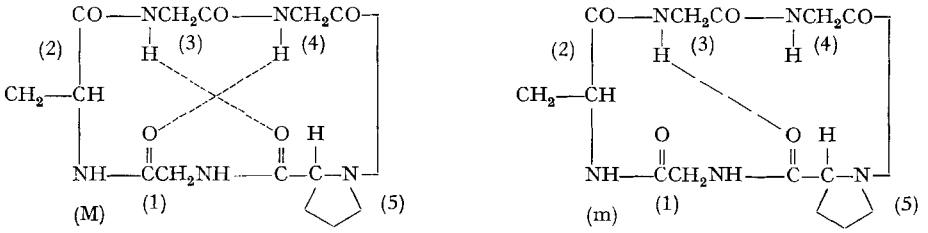

Fig. 6. c-(*-Gly-L-Ala-Gly-Gly-L-Pro-*) *with the intramolecular hydrogen bridges proposed on grounds of the NMR. data in Figs. 1, 2 and 3*

M: Major conformation with two favourable hydrogen bonds in a dynamic equilibrium. m: Minor conformation with one preferred hydrogen bridge

In the more abundant conformation M the NMR. data (Fig. 1 and 2) show that there are two partially shielded glycyl amide protons. As in the species m this shielding from the solvent might come about through intramolecular hydrogen bonding. Fig. 3 further shows that the amide proton of glycine (3) is involved in the hydrogen bonds in M. With proline in the *trans*-conformation (Fig. 5) and one hydrogen bridge corresponding to that in Fig. 6m, we find that only a conformation of the type shown in Fig. 6 M could conceivably be compatible both with the NMR. data and the molecular models. This accommodation of the proton NMR. data to conformations which are 'allowed' in the space-filling CPK. molecular models thus leads to the resonance assignments in Fig. 3 and Table 1.

From the molecular models it appears that the most favorable spacial arrangements of the peptide backbone atoms in the two types of conformation shown in Fig. 6 are probably very similar to those described by the dihedral angles[2]) in Table 2. The prolyl residues in the two molecular species would then be respectively in the *cis-trans'* (m) and *trans-trans'* (M) conformation [19].

The two types of conformation in Fig. 6 contain two particularly satisfactory features. First, since they lead exclusively to the formation of 10- and 11-membered rings, all the transannular hydrogen bonds can be formed without imposing undue

strains on the molecular skeleton. Second, since an activation energy of approximately 20 Kcal/Mol appears to be characteristic of the *cis-trans* isomerism in peptide bonds [20] the occurrence of *cis*- and *trans*-proline in the species m and M, respectively, provides also an explanation for the occurrence of two separate NMR. spectra at ambient temperature. To ascertain the resonance assignments in Fig. 3 and Table 1, and hence the validity of the proposed conformations (Fig. 6 and Table 2), we are

Table 2. *Dihedral angles ϕ and ψ defined according to the IUPAC recommendations (13) in the two conformations proposed for the Species M and m in* c-(*-Gly-*L*-Ala-Gly-Gly-*L*-Pro-*)

Conformation M		Conformation m	
$\phi_1^M = 0$	$\psi_1^M = -90$	$\phi_1^m = 40$	$\psi_1^m = -90$
$\phi_2^M = -80$	$\psi_2^M = 0$	$\phi_2^m = -50$	$\psi_2^m = -30$
$\phi_3^M = -160$	$\psi_3^M = 40$	$\phi_3^m = -160$	$\psi_3^m = 30$
$\phi_4^M = 150$	$\psi_4^M = -130$	$\phi_4^m = 60$	$\psi_4^m = 100$
$\phi_5^M = -60$	$\psi_5^M = 150$	$\phi_5^m = -60$	$\psi_5^m = 160$

in the course of synthesizing derivatives of peptide I in which one or both of the glycyl residues-(1) and -(4) will be deuterated. Work is also in progress to refine the conformations M and m (Table 2) using conformational energy calculations [11] [14].

In principle the structure of peptide I could accommodate four different, potentially equally favorable, hydrogen bonds. These are the two bonds shown in Fig. 6, and those between the amide proton of glycine (1) and the carbonyl oxygen of glycine (3) and between the amide proton of alanine and the carbonyl oxygen of glycine (4). It appears, at this stage of the investigation, that at least three of these potentially favorable hydrogen bridges are present in at least one of analogous pentapeptides I to V (Fig. 1).

In peptide II, *c*-(-Gly-L-Cys(DPM)-Gly-Gly-L-Pro-), the C_α-H-resonance of proline is again at lower field in the less abundant species, which contains also a glycyl amide proton line at 6.7 ppm. Overall, with the exception of some chemical shift differences which appear to arise entirely from the introduction of the S-DPM group into the side chain at position 2, and not from changes of the peptide backbone arrangements, the spectra I and II (Fig. 1) are very similar. The relative concentrations of the two molecular forms M and m are also the same in the two compounds. The NMR. data thus show that the two types of conformation in Fig. 6 apply also to peptide II. This is of particular relevance for the investigation of bicyclic peptides of the type S,S'-Bis-*c*-(-Gly-L-Cys-Gly-Gly-L-Pro-) [2] [4], which will be further discussed below.

In the spectrum of peptide III, *c*-(-Gly-L-Ala-Gly-Gly-D-Pro-), the amide proton resonances of alanine and of one of the glycyl residues are in positions at rather high fields and change only slightly with temperature [15]. Molecular models indicate that this might be most readily compatible with a conformation in which the transannular hydrogen bridges of the amide protons of glycine (3) and alanine with the carbonyl oxygens of glycines-(1) and -(4) are more favoured than the bonds shown in Fig. 6.

In the peptides IV, *c*-(-Gly-L-Cys(DPM)-Sar-Sar-L-Pro-), and V, *c*-(-Gly-L-Cys-(DPM)-Sar-Sar-D-Pro-), the formation of the two hydrogen bonds as in Fig. 6 is not possible. Hence a new type of conformation is to be expected. The NMR. data (Fig. 1) indicate that in the more abundant species there is one transannular hydrogen

bond involving the amide proton of cysteine, whereas both amide protons appear to be freely accessible to the solvent in the less abundant conformation. The substitution of L-proline by D-proline[4]) appears not to affect the molecular conformations as far as they are manifested in the proton NMR. spectra. A possible explanation for this was found in the molecular models which indicate that in both structures IV and V only one of the two possible transannular hydrogen bridges can readily be formed. A more detailed discussion of the molecular conformations encountered in the peptides III. IV, and V will be given in a forthcoming paper.

As mentioned in the introduction, this investigation was initiated in connection with studies on the complex formation, with group Ia and IIa metal ions, of bicyclic peptides of the type S,S'-Bis-*c*-(-Gly-L-Cys-Gly-Gly-L-Pro-) [2] [4]. Comparison of the peptides I and II (Fig. 1) with the corresponding bicyclic compound has shown that the conformational properties of the latter, in as far as they are manifested in the proton NMR. spectra, are essentially entirely determined by the conformation of the individual pentapeptide rings [21]. It will now be of interest to investigate how the conformational differences between the cyclopentapeptides I–V (Fig. 1) affect the metal binding properties of the corresponding bicyclic peptides. In the long run one may hope to be able to devise particularly potent metal chelating compounds on the basis of such studies, and thus perhaps acquire a more profound knowledge of biologically active group Ia and IIa cation-specific polypeptide compounds.

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188. Synthèses dans la série des bis-indéno-fluorènes, VII[1])
Le trioxo-5,12,13-dihydro-12,13-5*H*-bis-indéno[2.1-*a*; 2′.1′-*g*] fluorène

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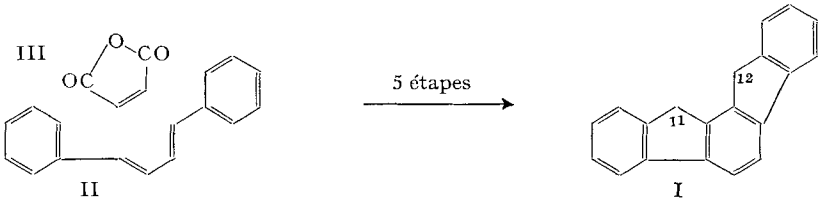
(15 VII 72)

Summary. Starting from 9-oxo-fluorene-1-carbaldehyde, the title compound, XII, a trioxo-derivative of a new biangular diindenofluorene system, has been synthesized in 5 steps (overall yield 37,5%). The corresponding hydrocarbon could not be obtained by reduction of XII. Attempts to synthesize the isomer XX of the trioxoderivative XII are described.

Le dihydro-11,12-indéno[2.1-*a*]fluorène (I) (*endo-cis*-fluorénaphène en nomenclature abrégée [*2*]) a été synthétisé en cinq étapes à partir du diphényl-1,4-butadiène-1,3 (II) [3]: l'addition d'anhydride maléique (III) au diène II donne l'anhydride diphényl-3,6-tétrahydro-1,2,3,6-phtalique, celui-ci par aromatisation au soufre l'anhydride diphényl-3,6-phtalique, qui, par une double cyclisation en deux étapes, fournit le dérivé dioxo-11,12 de I; une réduction finale donne I.



L'emploi, au départ, d'un phényl-1-[fluorénonyl-(x)]-4-butadiène-1,3 devait permettre, par un chemin analogue, la construction d'un système bis-indéno-fluorénique nouveau.

Nous sommes partis d'abord du fluorénone-carbaldéhyde-1 (IV), commodément accessible par ozonation du fluoranthène [4]. La condensation de IV avec le chlorure de cinnamyl-triphénylphosphonium [5] selon *Wittig* donne le phényl-1-[fluorénonyl)-(1)]-4-butadiène-1,3 (V). Par addition d'anhydride maléique à V on obtient l'anhydride phényl-3-[fluorénonyl-(1)]-6-tétrahydro-1,2,3,6-phtalique (VI), dont l'aromatisation fournit l'anhydride phényl-3-[fluorénonyl-(1)]-6-phtalique (VII). Cet anhydride, chauffé dans la quinoléine avec du chromite de cuivre donne la (biphénylyl-4)-1-fluorénone (VIII), qui, réduite selon *Wolff-Kishner*, fournit le (biphénylyl-4)-1-fluo-

[1]) VI[e] Communication: voir [1].