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49. ^{1}H- and ^{13}C-NMR. Studies of the Molecular Conformations of Cyclo-Tetraglycyl [1]

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(5. XII. 74)

Summary. The ^{1}H- and ^{13}C-NMR. spectra of cyclo-tetraglycyl show that the four peptide groups are magnetically equivalent, and different from either a standard *trans* or a standard *cis* peptide group. It is suggested that the observed NMR. features correspond to a non-planar form of the peptide groups. On the one hand these data confirm the earlier conclusions from theoretical investigations of the molecular geometry, that cyclic tetrapeptides could not contain four standard *trans* peptide groups. On the other hand they are not consistent with a previously suggested alternative molecular conformation according to which cyclo-tetraglycyl would adopt a conformation similar to cyclo-tetrasarcosyl, with two *cis* and two *trans* peptide bonds. The different

behaviour of glycine and sarcosine under the steric strains of tetrapeptide ring closure would appear to suggest that with the exception of the X-Pro bonds, *transoid* peptide groups in polypeptide chains of the common amino acids should be more likely to occur than the *cis* form, which has as yet apparently not been observed for N-unsubstituted peptide groups in natural peptides or proteins.

Introduction. – The concept of the standard planar *trans* peptide group as a structural entity has been a great asset for the investigations of polypeptide conformations over the past twenty years [2] [3]. Today, in the light of the details of peptide conformation which are accessible to observation by the modern experimental techniques, it appears no longer to be universally adequate. The occurrence of *cis* peptide groups has been demonstrated in numerous polypeptide chains containing proline or other N-substituted amino acid residues [1] [4–11], and experimental and theoretical studies of model compounds indicated that non-planar peptide groups might also be quite common in peptides and proteins [12–14]. In this context the molecule c-tetraglycyl, which had been synthesized by *Schwyzer et al.* in 1956 [15], is of particular interest because it is the only presently known cyclo-tetrapeptide which contains four N-unsubstituted peptide groups, and which can thus be used to investigate the behavior of the peptide groups under the sterical strain imposed on them as a consequence of the ring closure [16] [17]. Because the corresponding N-methylated compound, c-tetrasarcosyl, has previously been studied [18], the present investigation will also enable us to compare the free and the N-methylated peptide groups under essentially identical conditions of sterical strain.

Experimental. – The synthesis of c-tetraglycyl had been described previously [15]. For the present experiments, we used some material from the original synthesis [15], and a new batch which was obtained by a slightly modified procedure: Z-Gly-Gly-Gly-Gly-OH was converted to the *p*-nitrophenol active ester with *p*-nitrophenol and dicyclohexylcarbodiimide in pyridine by stirring for 2 h at 0°, and further reacting overnight at room temperature. The Z protecting group of Z-Gly-Gly-Gly-Gly-ONP was then removed by treatment with HBr in acetic acid. The so obtained product was taken up in a small volume of dimethylformamide and then cyclised in pyridine at 60°. The product c-[-Gly_4-] was recrystallized from ethanol/water. For the NMR. experiments, *ca.* 0.03 M solutions of c-[-Gly_4-] in TFA, d-TFA, and d_6-DMSO were prepared. TMS was added as an internal standard.

For purposes of comparative studies and resonance identifications, the following compounds were also investigated: c-[-Gly-Gly-] obtained as a gift from *Senn Chemicals*, Dielsdorf, Switzerland, c-[Gly-L-Ala-] and c-[L-Ala-L-Ala-] obtained as a gift from Dr. *B. Donzel* [19], H-Gly-Gly-Gly-OH, H-Gly-Gly-L-Ala-OH, H-Gly-L-Ala-L-Ala-OH, TFA-Gly-Gly-Gly-L-Ala-OCH_3, and TFA-Gly-Gly-L-Ala-L-Ala-OCH_3 purchased from *Bachem* AG, Liestal, Switzerland. For the ^{13}C-NMR. studies, 0.1 M solutions of these peptides in d-TFA or d_6-DMSO (see table 1) were prepared, with TMS added as an internal reference.

^{13}C-NMR. spectra at 25.16 MHz were obtained with the *Fourier* transform (FT) technique using a *Varian* XL-100 spectrometer. Sample tubes with 12 mm outer diameter were used. The sample temperature was *ca.* 35°. ^{1}H-NMR. spectra were recorded at 60, 100 and 220 MHz, using *Varian* T-60, XL-100 and HR-220 spectrometers, respectively.

Results. – The ^{1}H-NMR. spectrum of c-tetraglycyl in TFA-solution is shown in Fig. 1. It consists of a resonance with relative intensity corresponding to one proton at 7.98 ppm, and a resonance with intensity two protons at 4.32 ppm. From their chemical shifts, these lines can be assigned to the amide protons of the four glycyl residues, and the glycyl C^α-protons, respectively. The multiplet structures of the

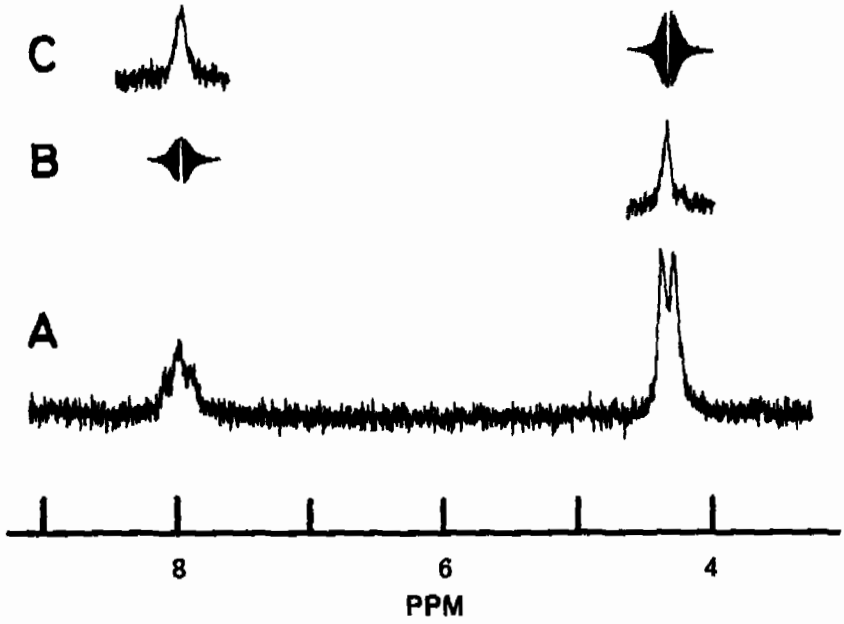

Fig. 1. A. ^1H-*NMR. spectrum* at 60 MHz *of a solution of c-tetraglycyl in trifluoroacetic acid.* B. and C.: *Homonuclear spin decoupling experiments.* $T = 35°$

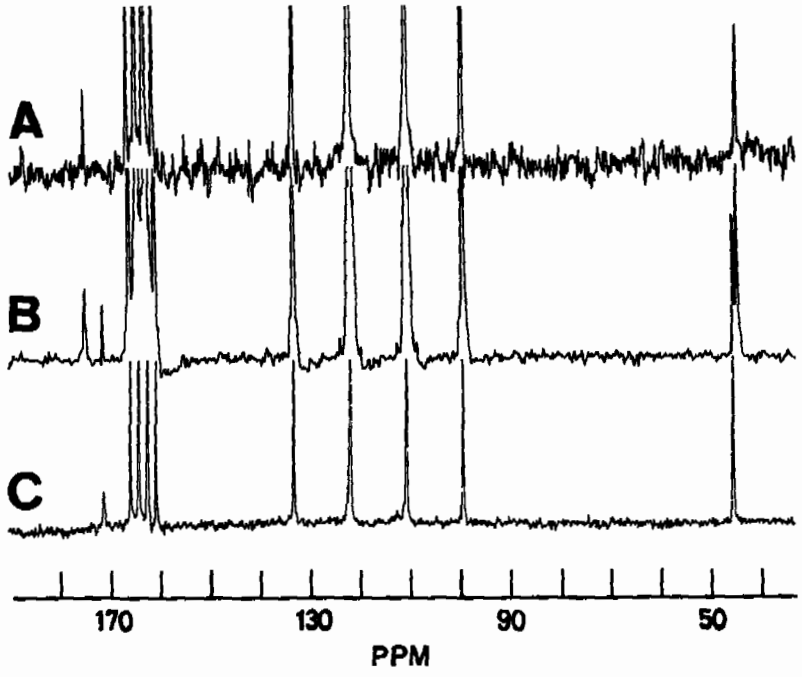

Fig. 2. ^{13}C-*NMR. spectra at 25.16 MHz of TFA solutions of* A. *cyclo-tetraglycyl* B. *a mixture of cyclo-tetraglycyl and cyclo-diglycyl in a molar ratio of ca. 3:2.* C. *cyclo-diglycyl*

resonances provide additional support for these assignments. If the spectrum (Fig. 1) is interpreted in terms of an A_2X spin system, $J_{vic} = 6.0 \pm 1$ Hz is obtained. A similar spectrum was observed in d_6-DMSO solution, where the amide proton chemical shift is 8.07 ppm, and the C^α-protons are at 3.75 ppm. The temperature dependence of the spectrum in DMSO was studied over the range from 20° to 80°. The C^α-proton resonance is independent of temperature, whereas the amide proton line shows a temperature coefficient of *ca.* -0.005 ppm/deg.

In Fig. 2, the ^{13}C-NMR. spectra of c-tetraglycyl and c-diglycyl are compared. Both compounds give rise to a proton-decoupled spectrum consisting of two lines. The chemical shifts of the resonances in Fig. 2, and of the corresponding spectra in d_6-DMSO solution are given in Table 1. The table includes further the ^{13}C chemical shifts for a number of model peptides which were used for the structural interpretation of the spectral parameters of c-[-Gly_4-]. For the cyclic peptides, the resonance assignments given in Table 1 were obtained from considerations of the chemical shifts, observation of the ^{1}H-^{13}C spin-spin couplings in the undecoupled spectra, and comparison of the four different molecules. The resonance assignments for H-Gly-Gly-Gly-OH, H-Gly-Gly-L-Ala-OH, and H-Gly-L-Ala-L-Ala-OH in d-TFA were derived from mutual comparison of the three compounds. For TFA-Gly-Gly-Gly-L-Ala-OCH_3 and TFA-Gly-Gly-L-Ala-L-Ala-OCH_3, the identification of the resonances was described previously [20].

Table 1. *^{13}C-NMR. chemical shifts in deuterated trifluoroacetic acid and deuterated dimethylsulfoxide of the peptides used in the investigations on c-tetraglycyl*

Peptide	Resonance assignment		δ (ppm from TMS)	
			Solvent: TFA-d	Solvent: d_6-DMSO
c-tetraglycyl	Gly	C=O	175.6	169.8
		C^α	45.2	n.o. (DMSO)
c-diglycyl	Gly	C=O	171.8	166.0
		C^α	45.8	44.2
c-[-glycyl-L-alanyl-]	Gly	C=O	171.7	165.9
		C^α	45.9	44.2
	Ala	C=O	174.6	168.6
		C^α	53.2	49.4
		C^β	20.0	18.4
c-dialanyl	Ala	C=O	174.6	169.1
		C^α	53.3	49.5
		C^β	21.5	18.4
H-Gly(1)-Gly(2)-Gly(3)-OH	Gly(1)	C=O	169.9	
		C^α	43.8	
	Gly(2)	C=O	173.5	
		C^α	44.8	
	Gly(3)	C=O	176.8	
		C^α	43.0	

Peptide	Resonance assignment		δ (ppm from TMS)	
			Solvent: TFA-d	Solvent: d_6-DMSO
H-Gly(1)-Gly(2)-L-Ala(3)-OH	Gly(1)	C=O	169.7	
		C^α	43.7	
	Gly(2)	C=O	172.4	
		C^α	44.7	
	Ala(3)	C=O	179.9	
		C^α	51.2	
		C^β	17.8	
H-Gly(1)-L-(Ala(2)-L-Ala(3)-OH	Gly(1)	C=O	168.8	
		C^α	43.7	
	Ala(2)	C=O	176.3	
		C^α	52.6	
		C^β	18.6	
	Ala(3)	C=O	180.0	
		C^α	51.2	
		C^β	17.6	
TFA-Gly(1)-Gly(2)-Gly(3)-L--Ala(4)-OH	Gly(1)	C=O		167.5
		C^α		41.7 ± 0.3
	Gly(2)	C=O		168.6
		C^α		41.7 ± 0.3
	Gly(3)	C=O		168.8
		C^α		42.1
	Ala(4)	C=O		172.9
		C^α		47.6
		C^β		17.0
	OCH_3			51.8
TFA-Gly(1)-Gly(2)-L-Ala(3)--L-Ala(4)-OCH_3	Gly(1)	C=O		167.3
		C^α		41.9
	Gly(2)	C=O		167.9
		C^α		41.9
	Ala(3)	C=O		171.8
		C^α		48.0
		C^β		18.2
	Ala(4)	C=O		172.8
		C^α		47.7
		C^β		16.8
	OCH_3			51.8

Discussion. – Compared to the corresponding linear peptide chains the number of sterically allowed molecular conformations in cyclic peptides is greatly reduced by the ring closure conditions. In small rings, the steric restrictions can also affect the conformations of the peptide groups. It is well known that cyclo-dipeptides can only be formed by the combination of two *cis* peptide groups [21]. In the cyclo-tripeptides described so far, c-trisarcosyl [22] and c-triprolyl [23], all the three peptide bonds were found to be in a standard *cis* form, while a theoretical investigation led

to the prediction that the three *cis* peptide bonds in *c*-triprolyl would deviate appreciably from planarity [24]. The steric implications of the ring closure in cyclotetrapeptides are still such that four standard planar *trans* peptide groups [3] cannot be accomodated [25] [26], whereas rings including five or more amino acid residues can be formed with all the peptide groups in a standard *trans* form [26].

In c-tetrasarcosyl the steric requirements for the ring closure are met by a combination of *cis* and *trans* peptide groups arranged in the sequence ⌐*cis-trans-cis-trans*¬. This conformation was observed in single crystals [27] and in solution [18] [22]. Similar conformations were observed in mixed cyclotetrapeptides of sarcosine and glycine or alanine, whereby exclusively the N-methylated peptide groups were found to be in the *cis*-form [18]. The data on c-tetraglycyl presented in the foregoing section enable us now to compare the conformation of a cyclo-tetrapeptide which contains only N-unsubstituted amide groups with the earlier data on the sarcosine peptides.

The ^{1}H- and ^{13}C-NMR. spectra (Fig. 1 and 2) imply that on the NMR. time scale the four glycyl residues in c-tetraglycyl are equivalent. If this observation is combined with the results of the theoretical investigations of the molecular geometry [25] [26], one is left with two possible interpretations. First, it could be that there is a rapid equilibrium between different molecular conformations each of which would contain standard planar *cis* and *trans* peptide groups. A possible example would be the interconversion (1) between analogous species to those found in the sarcosine peptides.

$$\ulcorner cis(1)\text{-}trans(2)\text{-}cis(3)\text{-}trans(4) \urcorner \rightleftarrows \ulcorner trans(1)\text{-}cis(2)\text{-}trans(3)\text{-}cis(4) \urcorner \qquad (1)$$

In view of the generally observed high energy barrier for rotations about peptide bonds [1] [6–11] [28], it appears rather unlikely that processes of the type (1) could be rapid on the NMR. time scale. Second, the molecular species corresponding to the observed NMR. spectra could contain four non-standard, non-planar *transoid* peptide groups. To distinguish between these two different situations, the carbonyl

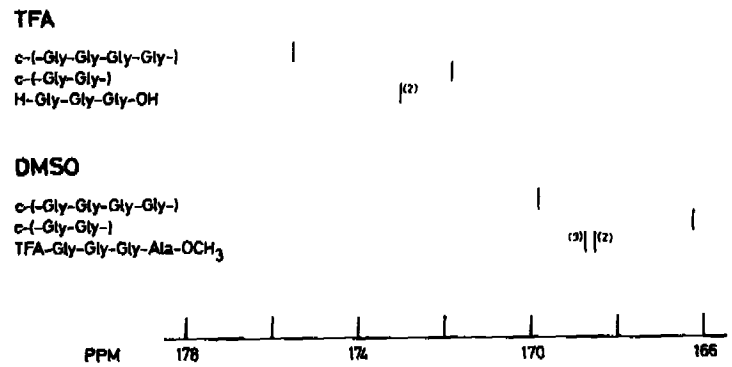

Fig. 3. *^{13}C-NMR. chemical shifts in ppm from TMS of the carbonyl carbon atoms of cyclo-tetraglycyl, cyclo-diglycyl, and the non-terminal glycyl residues in linear oligopeptides in solutions in TFA and d_6-DMSO*

carbon chemical shifts of c-tetraglycyl were compared with those of the model peptides in Table 1.

c-Diglycyl was chosen to represent the *cis* form of a glycyl residue in a structure where it is bound to adjoining glycines [21], and the non-terminal glycines in linear oligopeptides containing the structural segment -Gly-Gly-Gly- were taken to represent the corresponding *trans* form [3]. In Fig. 3 the carbonyl carbon chemical shifts of these model glycyl residues are compared with those of c-tetraglycyl. It is seen that in the two solvents used, the carbonyl carbon resonances of the *cis* peptide bonds of glycine in diketopiperazine are consistently one to two ppm to higher field than the corresponding resonance of the *trans* peptide bonds in the linear oligopeptides. A similar spectral difference occurs in aqueous solutions [1], and is also observed for the corresponding alanine peptides (see Table 1). It seems thus well established that the observed carbonyl carbon chemical shifts between corresponding amino acid residues in the linear molecules and the cyclo-dipeptides are a manifestation of the *cis-trans* isomerism about the peptide bonds. Table 1 and Fig. 3 then show that in both solvents used, the carbonyl carbon resonance of c-tetraglycyl does not coincide with either *cis* or *trans* glycyl. This is not unexpected on the basis of the theoretical investigations of this molecule [25] [26]. Furthermore, if there were rapid *cis-trans* isomerization of the peptide groups in the tetrapeptide ring, one would expect the resulting average resonance to lie in the spectral region between the lines corresponding to the *cis* and *trans* forms, and not at the extreme low field position observed in Fig. 3. An interpretation of the experimental observations described in this paper in terms of rapid interconversions of the type (1) can therefore be excluded, and we thus have evidence for a molecular conformation of c-tetraglycyl which includes four equivalent *transoid* peptide groups. This is in contrast to an earlier suggestion according to which the ⌐*cis-trans-cis-trans*¬ type conformation of c-tetrasarcosyl should also prevail in c-tetraglycyl [29].

It would appear that a quite general statement concerning peptide and protein conformation can be made on the basis of these findings. The different behaviour of the glycyl and sarcosyl residues under the steric strains imposed by the closure of the tetrapeptide ring indicates that with the exception of the X-Pro peptide bonds [28], *transoid* peptide groups, *i.e.* non standard [3] *trans* peptide groups which also deviate from planarity, should be more likely to be encountered in polypeptide chains of the common amino acids than the *cis* form of the peptide groups.

There are two different molecular conformations of c-tetraglycyl with C_4 or S_4 symmetry, respectively, which would both be consistent with the ^{13}C-NMR. data. In principle the ^{1}H-NMR. might provide a clue to differentiate between the two species. Inspection of the CPK model of c-[-Gly_4-] implies that the temperature coefficient of -0.005 ppm/deg. for the amide protons of c-[-Gly_4-], which is quite representative for solvent exposed amide protons [30], might apply to both forms, since no internally hydrogen bonded or otherwise 'buried' amide protons could be accomodated in either of the two species. For both the C_4 and the S_4 species of c-[-Gly_4-], the vicinal coupling constants should be of the order of the observed value of 6.0 Hz, and nearly equal for the two C^α-protons. It is therefore also not very useful to differentiate between the two possible molecular conformations. On

the other hand, however, the accidental degeneracy of the chemical shifts of the two C^{α} protons of glycine (Fig. 1) would be very unexpected in a c-tetraglycyl molecule with C_4 symmetry, where all the four carbonyl oxygens would be directed to the same side of the ring plane. This provides support for a S_4 conformation where the immediate environments of the two C^{α} protons would indeed be very similar, each being located near a C=O and a N–H group, so that it seems quite conceivable that the structural non-equivalence of the C^{α} protons would not be sizeably manifested in the chemical shifts.

In conclusion the NMR. data support that c-tetraglycyl in solution occurs predominantly in a S_4-symmetrical conformation with the principal symmetry axis perpendicular to the cyclopeptide ring. In this structure the amide protons are freely accessible to the solvent. The four equivalent peptide groups are in a non-standard *transoid* form. Let us emphasize at this point why the data of Fig. 3 imply that this *transoid* form of the peptide groups deviates from planarity. The above mentioned theoretical considerations [25] had shown that the closure of the tetrapeptide ring could be attained by reducing the angle τ [3] at the C atom from its standard value of 109.5° to 95°, or by making the peptide groups non-planar, with $\omega \approx 15°$, or by a combination of less extensive modifications of these two structural features. Because it seems hardly conceivable that the distortion of the τ-angle at the C^{α} atom [3] could sizeably affect the chemical shift of the carbonyl carbon atom, the observed ^{13}C-NMR. positions (Fig. 3) appear to be compatible only with the assumption that the distortion to the *transoid* form of the peptide groups includes non-planarity. This could be either by an ω-rotation about the C'–N bond [3], or by a pyramidal distortion at the C' and the N atoms [12] [13].

Financial support by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* (projects 3.374.70 and 3.423.70) is gratefully acknowledged.

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50. A Carbon-13 Study of *bis*-Phosphine Platinum Complexes

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(4. XI. 74)

Summary. The ^{13}C-NMR. spectra of a series of complexes of type $[PtCl_2(PR_3)_2]$ and $[PtCl_4(PR_3)_2]$ have been measured. The values $^1J(Pt,C)$ are shown to reflect changes in the metal hybridization while the values $[^3J(P_A,C) + {}^5J(P_B,C)]$ are found to vary only slightly. It is suggested that the phosphorus hybridization in these complexes varies only slightly.

Introduction. – Although ^{13}C-NMR. is used increasingly in organometallic chemistry [1] there are as yet no systematic studies of the effects of molecular geometry and metal hybridization state on the ^{13}C-parameters of complexes of the type $[PtCl_2(PR_3)_2]$ and $[PtCl_4(PR_3)_2]$. These molecules are of interest in that their ^{13}C-NMR. spectra offer the opportunity to investigate a) interactions within the ligand *via* observation of the values $^nJ(P, C)$, b) changes at the metal center *via* $^nJ(Pt, C)$ and c) potential long range intra ligand interactions *via* changes in the ^{13}C chemical shifts, $\delta^{13}C$. The latter parameter is recognized to be sensitive to non-bonded steric interactions [2].

Experimental. – ^{13}C-NMR. spectra were measured as deuteriochloroform solutions in spinning 10 mm tubes using a *Bruker* HX-90 spectrometer equipped with *Fourier* Transform (FT) accessories.

The deuteriated solvent served as both internal ^{13}C reference and stabilization signal (^{2}H). Chemical shifts are reported relative to tetramethylsilane and are estimated to be correct to ± 0.1 ppm. Coupling constants are $\pm$ 1.5 Hz.

The spectra were routinely measured in FT mode using pulse lengths of 4–7 μs (90° pulse is approximately 12 μs). Samples were measured in single coil mode, thus allowing the transmitter coil, normally used in crossed coil mode, to carry a second (usually phosphorus) decoupling frequency provided by a *Bruker* frequency synthesizer. Spectra were normally run under conditions of complete ^{1}H-decoupling. The compounds under consideration are all known previously