91. Conformational Dependence of One-Bond C_α,H Spin Coupling in Cyclic Peptides[1])

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Dedicated to Prof. *Conrad Hans Eugster* on the occasion of his 60th birthday

(19.III.81)

Summary

Conformational effects on the one-bond C_α,H coupling constant have been studied in cyclic oligopeptides containing glycine, alanine, sarcosine and proline. The conformational contribution to $^1J(C_\alpha,H)$ can be described as being composed of a *positive* hyperconjugative term from the neighbouring N-p_z orbital and a *negative* one from the carbonyl π-system. For glycyl units, a quantitative relation between $^1J(C_\alpha,H)$ and the vicinal interorbital angles Φ' and Ψ' is derived with maximum values $\Delta J(\Phi') = +14.0$ Hz and $\Delta J(\Psi') = -4.9$ Hz. Applications to conformational analysis of cyclo(L-Pro-Gly)$_3$, cyclo(D-Ala-D-Ala-L-Ala-D-Ala-L-Ala-L-Ala) and cyclo(Sar)$_8$ are presented.

Introduction. – One-bond C_α,H coupling constants have recently been reported for glycine, alanine, sarcosine and related straight-chain di- and tripeptides [1]. This parameter depends upon the ionization state of the amino-acid unit, and the respective values for the cationic, zwitterionic and anionic structures were thus determined. Furthermore, additive increments (ζ) for the functional groups at the C_α-atom were derived. On the other hand, we showed that $^1J(C_\alpha,H)$ exhibits a significant conformational dependence which was utilized to study the structure of cyclic sarcosyl peptides in solution [2].

The coupling constants $^1J(C_\alpha,H)$ may be considered as being composed of a dominating and constant constitutional term, largely determined by the substituent effects mentioned above, and a variable conformational contribution depending upon the torsional angles Φ and Ψ. A quantitative treatment of this term and applications to conformational analysis of cyclic peptides are now presented.

1. Results. – The magnitude of one-bond C,H spin coupling is influenced by hyperconjugative effects originating from neighbouring orbitals with suitable stereoelectronic properties. Examples (usually considered as 'lone-pair contributions' to $^1J(C,H)$) include *N*-methylaziridine in which, for C,H-bonds *syn* and *anti* to the lone-pair, coupling constants are 171 and 160 Hz, respectively; corresponding data for the acetaldoxime stereoisomers (177 and 163 Hz) are also reported [3] [4].

[1]) ^{13}C-NMR. Spectroscopy, Part XXIX; part XXVIII see [1].

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More recently, similar effects have been observed for tricyclic orthoamides where, owing to the cumulation of three lone-pair effects, a very large difference between the *syn*-(^1J(C,H)= 184 Hz) and *anti*-arrangement (141 Hz) is observed [5]. In O-heterocycles, such as hexopyranoses [6] and pentopyranoses [7], and in 1,3,5,7-tetroxacane similar effects were found [8]. The hyperconjugative origin of the orbital effects on ^1J(C,H) was demonstrated in semi-empirical calculations by use of the *Pople-Santry* approach [9–11]. In contrast to lone-pair effects, the influence

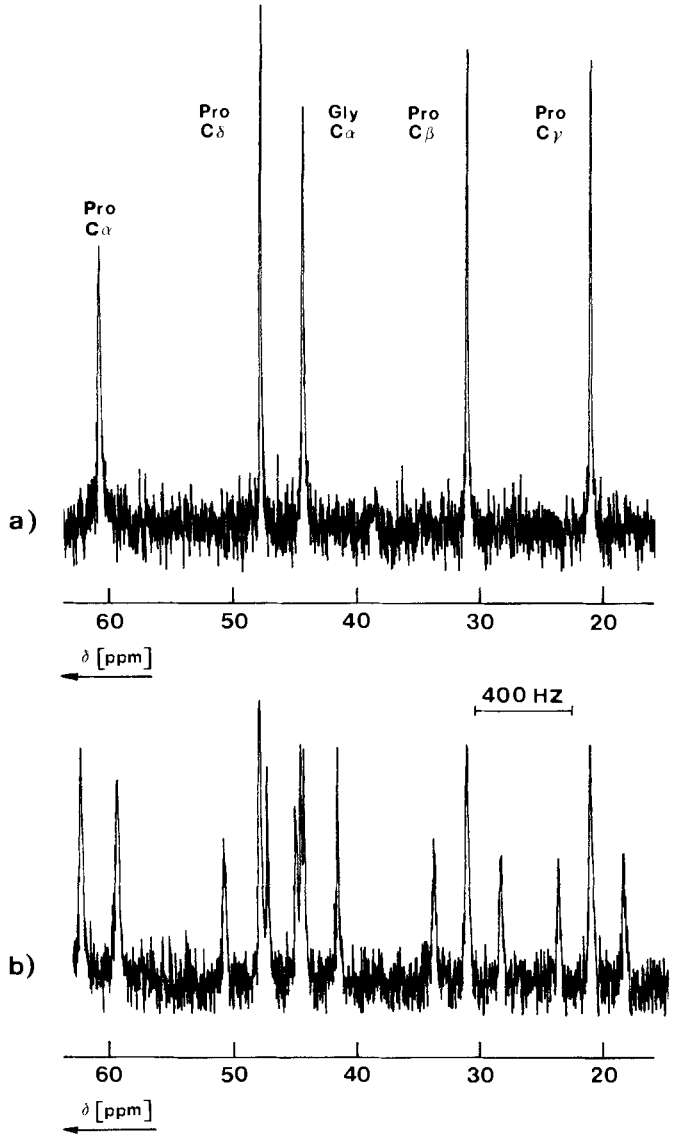


Fig. 1. *^{13}C-NMR. spectra* (low-frequency region) *of cyclo(L-Pro-Gly)$_2$* (50.3 MHz, D_2O, 80°): a) *^{1}H-noise-decoupled spectrum;* b) *^{1}H-coupled spectrum*

of neighbouring π-systems on $^1J(C,H)$ has not been investigated in detail although experimental data seem to indicate a small positive contribution in the case of C,H-bonds adjacent to aromatic systems [12]. The effect of a vacant *p*-orbital on $^1J(C,H)$ in carbenium ions was treated both experimentally [13] and by INDO/FPT-calculations [14].

For the C_α,H-bond in a peptide, two orbital effects can be envisaged originating from the two adjacent N- resp. CO-bonded amide systems. The magnitude of these effects will be controlled by the torsional angles Φ and Ψ or, more precisely, by the vicinal interorbital angles Φ' and Ψ' *(cf. Fig. 2)*. We found cyclo(Sar)$_4$ and cyclo(L-Pro-Gly)$_2$ to be excellent models for a quantitative study of the orbital effects. For cyclo(Sar)$_4$, ^{1}H-NMR. studies have proven a single and rigid centrosymmetric conformation in solution up to 150° [15]. This structure with *cis-trans-cis-trans* peptide configurations was confirmed by X-ray crystal analysis [16]. Cyclo(L-Pro-Gly)$_2$ was studied by ^{1}H- and ^{13}C-NMR. spectroscopy [17]. For the two methylene groups, molecular models suggest a local structure similar to C(2,8) in cyclo(Sar)$_4$. In contrast to cyclo(Sar)$_4$, the symmetry is reduced to C_1, but the two proline and glycine units become equivalent above +70°. The underlying process has been attributed to a rotation of the Pro-Gly amide units. The ^{1}H-coupled ^{13}C-NMR. spectrum is illustrated in *Figure 1b*. From the number of ^{13}C-resonances and an inspection of space-filling molecular models the existence of only two degenerate conformations appears reasonable. In this conformation, the C,H^o- and C,H^i-bonds (H^o,H^i, outer and inner protons [15]) in the diastereotopic C(2) and C(8) methylene groups exhibit very similar orientations with respect to the N- and CO-orbitals (rotation of the Pro-Gly amide groups by 180°). Hence, this process will not affect the C_α,H coupling constants.

From the ^{1}H-coupled ^{13}C-NMR. spectra of the two peptides, we determined the coupling constants $^1J(C_\alpha,H)$ *(Table 1)* of the sarcosine and glycine methylene groups, and of the proline methine group.

The assignment of the ^{13}C-resonances and $^1J(C_\alpha,H)$ values in cyclo(Sar)$_4$ is based on a correlation with the four proton shifts and the coupling constants $^2J(H,H)$, *via* a series of off-resonance decoupling experiments. The result is confirmed by the different values for the vicinal C,H coupling between the *N*-methyl C-atom and the diastereotopic H^i and H^o protons [2].

The very different values for $^1J(C_\alpha,H)$ of the methylene carbons C(2,8) in the two tetrapeptides (cyclo(Sar)$_4$: 150.0, 137.5 Hz; cyclo(L-Pro-Gly)$_2$: 149.2, 135.8 Hz) cannot be explained without a conformational contribution. This effect

Table 1. *^{13}C-Chemical shifts* [ppm] *and coupling constants* $^1J(C_\alpha,H)$ [Hz] *of cyclo(Sar)$_4$ and cyclo(L-Pro-Gly)$_2$*

		C(2,8)	C(5,11)
Cyclo(Sar)$_4$[a])	$\delta(C_\alpha)$	49.9	52.0
	$^1J(C_\alpha,H)$	137.5 (H^i); 150.0 (H^o)	138.2; 138.2
Cyclo(L-Pro-Gly)$_2$[b])	$\delta(C_\alpha)$	44.5	60.9
	$^1J(C_\alpha,H)$	135.8 (H^i); 149.2 (H^o)	150.8

[a]) In $CDCl_3$ containing ≤ 5% TFA, T = 30°. [b]) In D_2O, T = 80°.

can be related to the different orientations of the C_α,H-bonds in the structures of cyclo(Sar)$_4$, as obtained by X-ray analysis, and of cyclo(L-Pro-Gly)$_2$ *(Scheme 1)*. For the methylene carbons C(5,11) in cyclo(Sar)$_4$ the coupling constants with the protons H^i and H^o are the same within experimental error (138.2 Hz). In cyclo-(L-Pro-Gly)$_2$ the proline C_α-atom also exhibits an unusually large coupling of 150.8 Hz. In this case the molecular model illustrates coplanarity of the C(5,11),H-bonds with the respective nitrogen p_z-orbitals. This geometry is not affected by the dynamic process mentioned above.

On the assumption that the variations of $^1J(C_\alpha,H)$ in the tetrapeptides are determined by two different orbital effects a general approach for a quantitative treatment will be given by a *Fourier* sum of the type

$$^1J = A + \sum_{i=1}^{n} \sum_{j=1}^{2} B_{ji} \cos(i)\varphi_j + C_{ji} \sin(i)\varphi_j \qquad (1)$$

whereby A designates the constant constitutional term, the summation over the sine and cosine functions describes the conformational dependence of $^1J(C,H)$, and φ_j stands for the two vicinal interorbital angles. The simplest approximation which has proved practical in many applications, such as vicinal H,H and C,H coupling, involves a $\cos^2$ function, in the present case leading to the equation

$$^1J(C_\alpha,H) = A + B\cos^2\Phi' + C\cos^2\Psi' \qquad (2)$$

where Φ' and Ψ' describe the orientation of the C,H-bond with respect to the nitrogen p_z-orbital and the carbonyl π-system and are related to the corresponding torsional angles Φ and Ψ *(cf. Fig. 2)*. We elucidated the magnitudes of A, B and C for methylene groups in glycyl and sarcosyl units from a treatment of the experimental $^1J(C_\alpha,H)$ data in cyclo(L-Pro-Gly)$_2$, cyclo(L-Pro-Gly), and cyclo(Sar)$_4$, respectively. The information about the angles Φ' and Ψ' of the respective segments was taken from the X-ray analysis of cyclo(Sar)$_4$ and cyclo(L-Pro-Gly) [16] [18]. For the glycine segments of cyclo(L-Pro-Gly)$_2$ and cyclo(Sar)$_4$ the same local conformations have been assumed (see above).

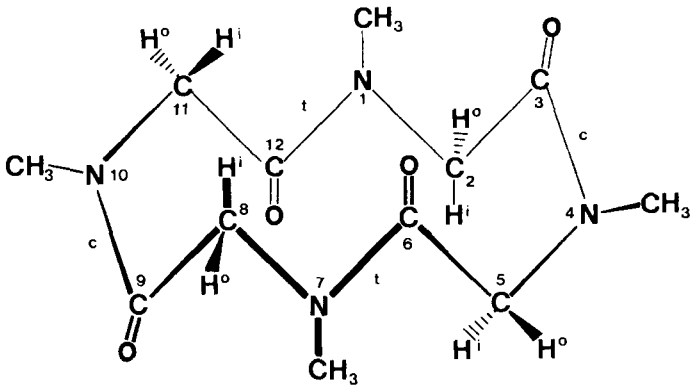
Cyclo(Sar)$_4$ yields three different experimental coupling constants and hence three simultaneous equations of type 2 can be solved for the unknown constants A, B and C. For cyclo(L-Pro-Gly)$_2$ only two coupling constants are available from the methylene groups. For this reason, data for the third equation were taken from the analysis of the ^{13}C-NMR. spectrum of cyclo(L-Pro-Gly) for which a boat-type conformation, as observed in the crystal [18], can also be assumed for the solution structure (see section 2.4). From the experimental coupling constants, 141.6 and 145.3 Hz, the larger value is assigned to the axial C,H-bond characterized by the angles $\Phi' \approx \Psi' \approx 0°$. These data were used in the third equation. The results for the L-Pro-Gly peptides and the sarcosyl peptides are given by equations 3 and 4:

$$^1J(C_\alpha,H) = (136.2 + 14.0\cos^2\Phi' - 4.9\cos^2\Psi')\ \text{Hz} \qquad (3)$$

$$^1J(C_\alpha,H) = (137.3 + 13.5\cos^2\Phi' - 3.3\cos^2\Psi')\ \text{Hz} \qquad (4)$$

Scheme 1. a) *Molecular and segmental conformations of cyclo(Sar)$_4$ and coupling constants $^1J(C_\alpha,H)$;*
b) *molecular and segmental conformations in cyclo(L-Pro-Gly)$_2$ and coupling constants $^1J(C_\alpha,H)$*

Scheme 1a

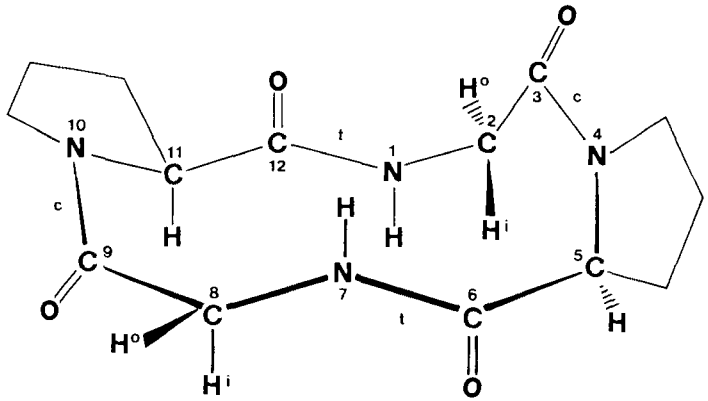


C(2,8) C(5,11)

$^1J(C_\alpha,H^o) = 150.0$ Hz
$^1J(C_\alpha,H^i) = 137.5$ Hz

$^1J(C_\alpha,H^o) = 138.2$ Hz
$^1J(C_\alpha,H^i) = 138.2$ Hz

Scheme 1b



C(2,8) C(5,11)

$^1J(C_\alpha,H^o) = 149.2$ Hz
$^1J(C_\alpha,H^i) = 135.8$ Hz

$^1J(C_\alpha,H) = 150.8$ Hz

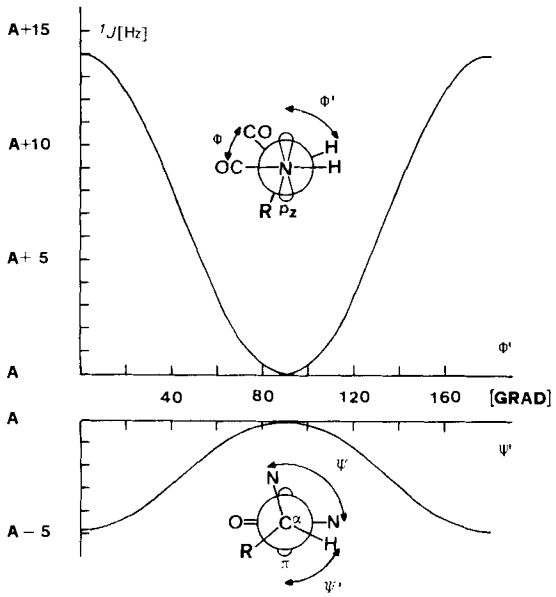


Fig. 2. *Angular dependence of* $^1J(C_\alpha, H)$ *and the definition of the torsional angles* Φ, Ψ *and the vicinal interorbital angles* Φ', Ψ'. A *denotes the constitutional term in equ. 3 and 4*

It is evident that there is a very similar conformational dependence of the C_α,H coupling constants in glycine and sarcosine units. A graphic representation for this dependence in glycyl peptides is given in *Figure 2,* which also illustrates the relationship between the torsional angles Φ, Ψ and the vicinal interorbital angles Φ', Ψ'.

The positive contribution to the conformational term can thus be attributed to the interaction of the C_α,H-bond with the nitrogen p_z-orbital ($B = +14$ Hz). The contribution from the π-orbital of the (C=O)-bond is smaller and of negative sign ($C = -4.9$ Hz). Thus, if the angles Φ (Φ'), Ψ (Ψ') can be estimated for different structural representations of a peptide (*e.g.,* α-helix, pleated sheet structures, *cf.* [19]), the total conformational contribution ΔJ can be obtained from equ. 3/ *Figure 2* and compared with experimental data provided the constitutional term A is known or can be estimated from $^1J(C_\alpha, H)$ in straight-chain peptides. For glycyl peptides the conformational contribution ΔJ in straight-chain compounds can be obtained from the experimental $^1J(C_\alpha, H)$ data *(Table 2)* and the known value for A ($\Delta J = 140.5 - 136.2 = +4.3$ Hz). A very similar value of +4.6 Hz is obtained by

Table 2. *Coupling constants* $^1J(C_\alpha, H)$ *in straight-chain glycyl peptides* (D_2O, 35°)

-*Gly*-	$^1J(C_\alpha, H)$ Hz		
	Cation	Zwitterion	Anion
Gly-*Gly*-Gly	140.8	140.6	140.0
Gly-*Gly*-Gly-Gly	141.0	141.0	140.0
Gly-Gly-*Gly*-Gly	141.0	141.0	140.0
Enkephalin (*Gly*-2)	–	141.3	–
Z-*Gly*-OEt (in $CDCl_3$)	–	141.5	–

integration of equ. 2 leading to an averaged conformational term $(B+C)/2$. For other amino acids in a straight-chain peptide, $^1J(C_\alpha, H)$ may also be calculated from additive substituent increments ζ [1] and, therefore, estimated values for the constitutional term A are generally accessible. Experimental coupling constants $^1J(C_\alpha, H)$ can thus be evaluated in terms of the conformation contribution.

2. Conformational analysis of cyclic peptides. – 2.1. *Cyclo(L-Pro-Gly)$_3$.* In order to test the applicability of equ. 3 to other cyclic peptides containing glycine and proline, we have studied the ^{1}H-coupled ^{13}C-NMR. spectrum of cyclo(L-Pro-Gly)$_3$

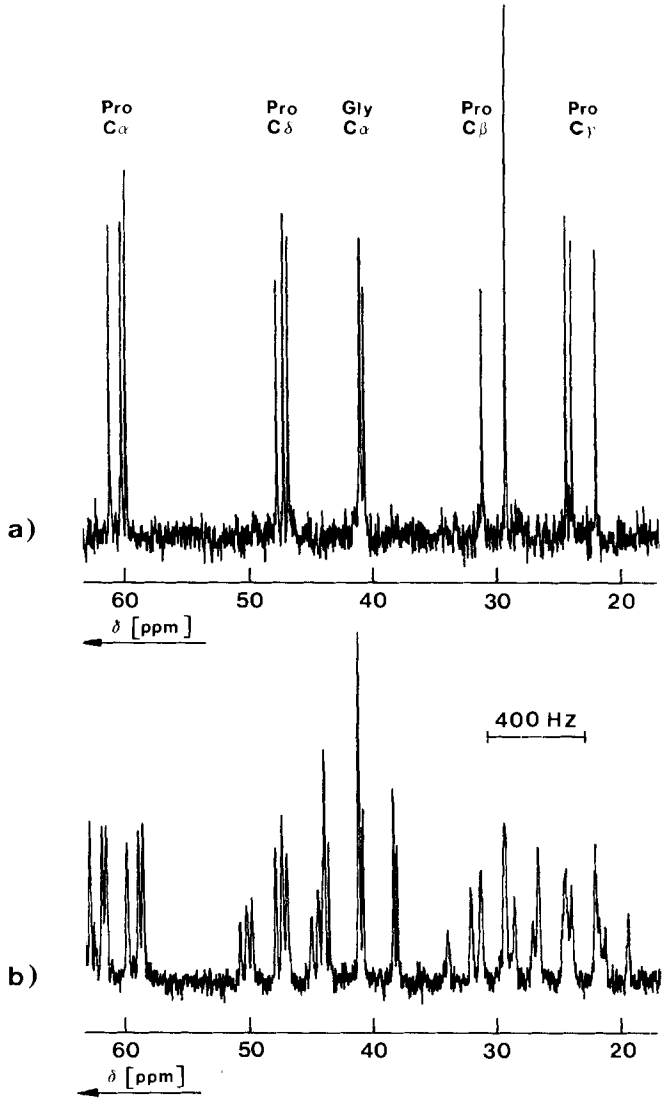


Fig. 3. *^{13}C-NMR. spectra* (low-frequency part) *of cyclo(L-Pro-Gly)$_3$* (50.3 MHz, D_2O, 45°): a) *^{1}H-noise-decoupled;* b) *^{1}H-coupled*

Table 3. *Coupling constants* $^1J(C_\alpha,H)$ *of cyclo(L-Pro-Gly)*$_3$ (D_2O) *and calculated data for two asymmetric conformations*

Amino-acid unit	Experimental data		A(*cis-cis-cis*)			A(*trans-trans-cis*)		
	$\delta(C_\alpha)$ [ppm]	$^1J(C_\alpha,H)$ [Hz]	Φ' [°]	Ψ' [°]	J(calc.) [Hz]	Φ' [°]	Ψ' [°]	J(calc.) [Hz]
Pro(C_α,1)	61.39	151.3[a])	0	90	150.0	38[c])	43[c])	142.3
Pro(C_α,3)	60.41	148.1	20	0–90	146.1[b])	38	0	140.1
Pro(C_α,5)	60.70	149.1	0	40–90	149.3[b])	38	0	140.1
Gly(C_α,2)	40.85	136.9	0–90	20	138.9[b])	75	86	137.1
		141.5	120–30	100	143.1[b])	15	34	145.9
Gly(C_α,4)	41.12	143.6	0–90	60	142.0[b])	14	5	144.5
		140.8	120–30	60	142.0[b])	74	55	135.7
Gly(C_α,6)	41.19	139.1	60	30	136.0	55	55	139.2
		139.1	60	30	136.0	65	5	133.8

[a]) Tentative assignments on the basis of the A(*cis-cis-cis*) conformation.
[b]) The calc. value corresponds to an intermediate conformation of the flexible segment.
[c]) From [20a].

which has previously been investigated by *Blout et al.* [20] using $^1H,^1H$ coupling constants, ^{13}C-chemical shifts, circular dichroism, and potential energy calculations. Both symmetric and asymmetric conformations have been proposed for the structure of this hexapeptide in solution, depending on solvent polarity. It is, therefore, tempting to compare the observed C_α,H coupling constants with values predicted for different conformations. The ^1H-decoupled ^{13}C-NMR. spectrum of the peptide in D_2O is illustrated in *Figure 3a* and closely corresponds to the spectrum reported by *Blout* [20]. From the ^1H-coupled spectrum *(Fig. 3b)*, the C_α,H coupling constants of the glycine methylene groups and of the proline methine groups can be readily determined *(Table 3)*. The number of observed chemical shifts (6 C_α resonances) and of C_α,H coupling constants (5+3 distinct values) is only compatible with an asymmetric conformation of the peptide in aqueous solution. Because of the small relative chemical shifts within the proline and glycine units, respectively, an assignment to individual C_α-atoms is difficult to obtain. However, each methylene C-atom is related to two C_α,H coupling constants and, therefore, in a comparison of experimental and calculated coupling constants a matching of three data pairs has to be achieved.

An asymmetric conformation for this peptide with *trans-trans-cis* Gly-Pro and *trans-trans-trans* Pro-Gly peptide bonds was derived from physical data of the peptide in D_2O solution and, in particular, on the basis of ^{13}C-chemical shifts of the proline units [20a]. We have used the corresponding torsional angles Φ and Ψ as given by *Blout et al.* to calculate the coupling constants $^1J(C_\alpha,H)$ according to equ. 3 *(Table 3)*. Similarly, the coupling constants were calculated for an asymmetric conformation with two *cis* Gly-Pro peptide bonds, which has also been discussed by the same author in an earlier paper [20b]. A third asymmetric conformation can be derived from the *cis-cis-cis* Gly-Pro arrangement by changes of the Φ and Ψ torsional angles only. This leads to a rather rigid structure A(*cis-cis-cis*)[2]) charac-

[2]) This notation indicates the molecular symmetry and the configurations of the three Gly-Pro peptide bonds [20].

terized by angles Φ', Ψ' and calculated $^1J(C_\alpha,H)$ coupling constants as given in *Table 3.*

The comparison of the experimental coupling constants $^1J(C_\alpha,H)$ with the calculated values for the A(*trans-trans-cis*) conformation, favoured by *Blout et al.*, gives only a very poor agreement. In particular, the three large proline coupling constants are not reproduced by this conformation. Similarly, the calculated values for the A(*trans-cis-cis*) conformation does not lead to a satisfactory agreement. On the other hand, the A(*cis-cis-cis*) conformation does lead to calculated coupling constants which can be matched with the experimental data.

From the S(*trans-trans-trans*) conformation an asymmetric structure can be derived which contains one β-turn. However, this structure is also characterized by Φ', Ψ' angles and calculated coupling constants which show no agreement with the experimental data. Thus, the C_α,H coupling constants of cyclo(L-Pro-Gly)$_3$ with its very flexible conformational behaviour prove to be a sensitive probe into peptide conformation in solution.

2.2. *Cyclo(*D-*Ala-*D-*Ala-*L-*Ala-*D-*Ala-*L-*Ala-*L-*Ala)=(Cyclo(aaAaAA)).* This hexapeptide is a representative of a class of synthetic cyclic peptides containing only L- and D-alanine and glycine, which exhibit the phenomenon of cycloenantiomerism and cyclodiastereoisomerism [21]. The structure of such peptides was studied extensively by physical methods applied in solution and in the solid state (*cf.* [22]). For this class of peptides an antiparallel pleated sheet structure, stabilized by two intramolecular H-bonds, is the accepted structural feature.

The ^{13}C-NMR. spectrum of cyclo(aaAaAA) *(Fig. 4)* exhibits six well-resolved C_α-resonances from which six different C_α,H coupling constants can be extracted *(Table 4).* This result is in agreement with the earlier report about six non-equivalent methyl resonances in the ^{1}H-NMR. spectrum [21].

Based on the antiparallel pleated sheet structure *(Scheme 2)* segmental conformations can be defined for the three pairs of carbon atoms C(1,4), C(2,5) and C(3,6). The C(2,5) resonances can be unambiguously assigned on the basis of a comparison with the chemical shifts of the L-alanyl C_α-resonances in cyclo(Gly-L-Ala-Gly)$_2$ (50.40 ppm) and cyclo(L-Ala-D-Ala-Gly)$_2$ (50.45 ppm). The experimental coupling constants of 145.5 and 144.8 Hz associated with these ^{13}C-resonances in cyclo(aaAaAA) are in good agreement with an optimum hyperconjugative interaction with both the p_z-orbital and the carbonyl π-system. For this case the expected value for $^1J(C_\alpha,H)$ is ~145 Hz (equ. 3). A similar geometry is observed for the axial C_α,H-bond in cyclo(L-Pro-Gly) (*cf.* 2.4). Since C(2) and C(5) exhibit very similar $^1J(C_\alpha,H)$ values it must be concluded that the pleated

Table 4. *^{13}C-Chemical shifts* [ppm] *and coupling constants* $^1J(C_\alpha,H)$ *in cyclo(aaAaAA)* [a=D-alanine, A=L-alanine], D_2O, 30°

	C(1,4)[a]	C(2,5)	C(3,6)
$\delta(C_\alpha)$	48.67; 49.43	50.35; 50.83	49.13; 49.69
$^1J(C_\alpha,H)$	141.6; 141.9	144.8; 145.5	143.3; 144.1

[a]) An assignment of the individual ^{13}C-resonances within the three groups has not been achieved.

sheet structure is flattened, which leads to the segmental conformations in *Scheme 2* (*cf.* [22]).

With a given conformation at the C(2,5) centers inspection of a molecular model suggests that two pairs of values for $^1J(C_\alpha, H)$ have to be expected for the C,H-bonds at the (1,4) and (3,6) centers, whereby the slightly larger coupling constants for C(3,6) *(Table 4)* are in agreement with a geometry leading to a more effective interaction of the C_α,H-bond with the p_z-orbital.

2.3. *Cyclooctasarcosyl.* The largest cyclic peptide which we have investigated is the 24-membered ring of cyclooctasarcosyl [2] [23]. The ^{1}H- and ^{13}C-NMR. spectra are consistent with a two-fold symmetry axis for the ring conformation in solution, in agreement with the results from X-ray diffraction [24], which also gave the amide configuration sequence *cis-cis-trans-trans-cis-cis-trans-trans.* In the ^{1}H-NMR.

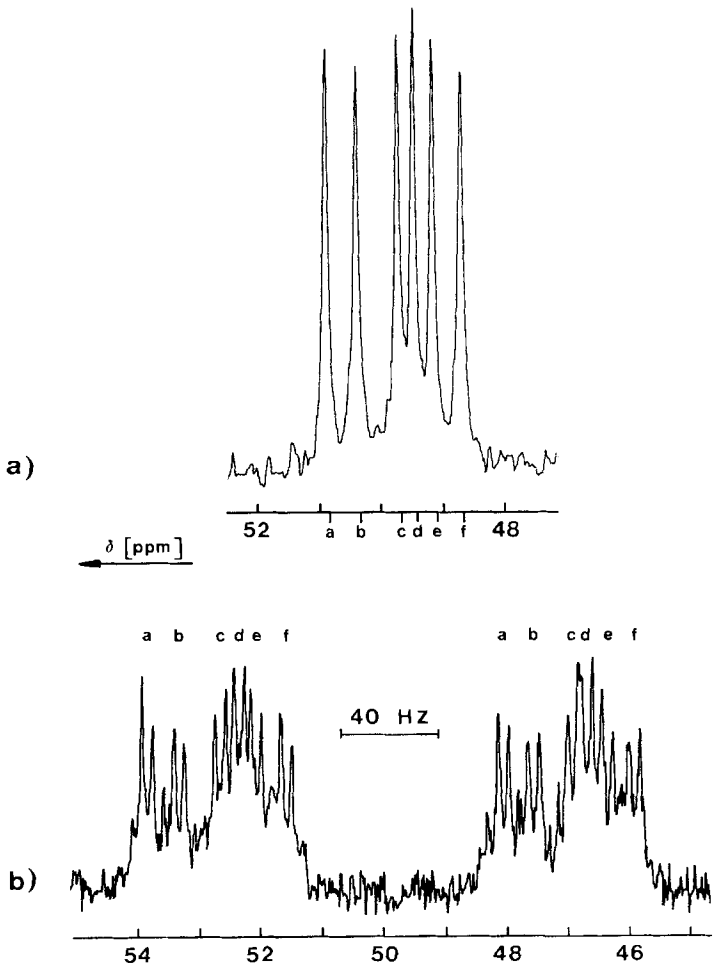
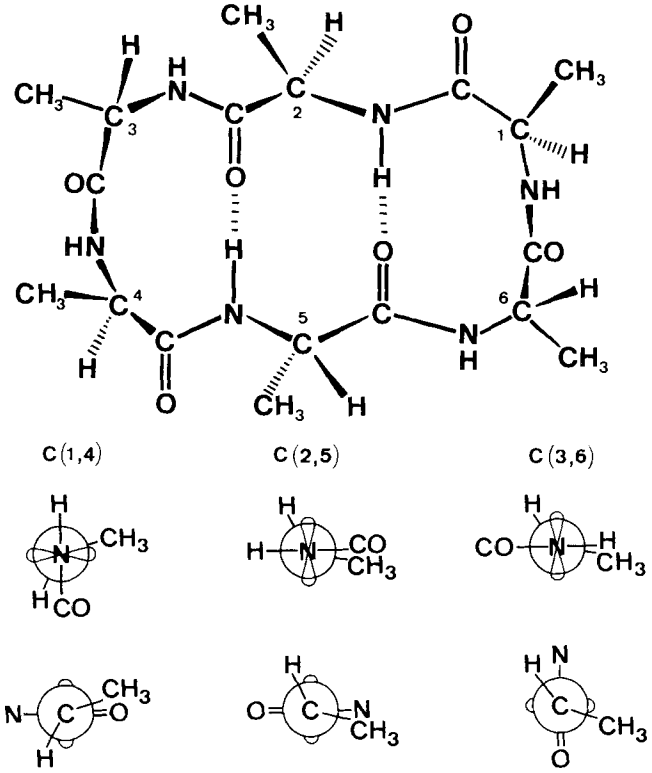


Fig. 4. *^{13}C-NMR. spectra* (C_α-region) *of cyclo(aaAaAA)* (25.14 MHz, D_2O, 30°): a) *^{1}H-noise-decoupled;* b) *^{1}H-coupled.* The letters a–f designate the individual C_α resonances in the two types of spectra.

Scheme 2. *Molecular conformation (antiparallel pleated sheet) and segmental conformations of cyclo(aaAaAA)*



spectrum, four *AB*-systems are observed for the eight methylene groups which do not show exchange broadening below 40°. The coupling constants $^1J(C_\alpha,H)$ observed indicate that certain segments of the peptide show a considerable rigidity, since the value of 137.6 Hz is too small compared with the data for conformationally averaged systems (~ 141 Hz, *Table 2*). The small C_α,H coupling constant can be discussed together with the large geminal H,H coupling constant of − 18 Hz for the diastereotopic protons of the same methylene groups. The steric situation for the torsional angles Φ and Ψ is, therefore, reminiscent of a segmental conformation already discussed for cyclo(Sar)$_4$ (*cf. Scheme 1a* and *Table 5*). The rigid part of the cyclo(Sar)$_8$ structure is in the region of the two *cis-cis* peptide bonds. On the other hand, the four remaining methylene groups in the *trans-trans* regions

Table 5. *^{13}C Chemical shifts* [ppm] *and coupling constants* [Hz] *of cyclo(Sar)$_8$* in $CDCl_3$, 30°

$\delta(C_\alpha)$	$^1J(C_\alpha,H)$[a])	$^2J(H,H)$	Conformation
50.77 (4 CH_2)	137.6	− 18	rigid
50.26 (2 CH_2)	142.5	− 17	flexible
51.59 (2 CH_2)	140.0	− 16	flexible

[a]) ± 1 Hz.

exhibit two coupling constants $^1J(C_\alpha,H)$ of 140.0 ± 1 and 142.5 ± 1 Hz which indicates more flexible structural segments in agreement with a space-filling molecular model (CPK).

2.4. *Cyclo(L-Pro-Gly).* Molecular models demonstrate that the diketopiperazine ring of this peptide is forced into a rather rigid boat-type conformation. This leads to two axial C_α,H-bonds, one in the glycine unit, the other one in the proline unit, for which optimum hyperconjugation with the nitrogen p_z-orbital and the carbonyl π-system can be expected. The experimental $^1J(C_\alpha,H)$ values (145.3 for the glycine unit and 143.5 for the proline unit) are in fact in agreement with equ. 3 and 4 and the geometry discussed above. If the coupling constants for the axial (145.3 Hz) and equatorial (141.6 Hz) C_α,H-bonds in the glycine units are used to estimate the vicinal interorbital angles the values obtained ($\Phi' \approx \Psi' \approx 5°$ and 45°, respectively) indicate a flattened boat conformation, in agreement with the crystal structure [18]. This result is compatible with a conclusion obtained from an interpretation of the ^{13}C-chemical shifts of the proline ring C-atoms [25]. The ideal boat conformation appears to be disturbed also in the proline region, since the value for the axial C_α,H-bond (143.5 Hz) is about 1.5 Hz smaller than expected. In contrast to the conclusion reached by *Siemion* [25], however, we believe that our results are in agreement with a single conformation for cyclo(L-Pro-Gly).

4. Conclusion. – Our experimental data on one-bond C_α,H coupling constants in cyclic di-, tetra-, hexa-, and octapeptides lend very strong support to the idea that the conformational term which modulates this coupling constant is in fact composed of two independent terms, a *positive* contribution from the neighbouring N-p_z-orbital and a *negative* contribution from the carbonyl π-system. A quantitative description of the conformational term derived from glycyl and sarcosyl peptides can be used to derive conformational information by a comparison of experimental $^1J(C_\alpha,H)$ values with coupling constants calculated on the basis of a proposed conformation with its particular torsional or vicinal interorbital angles Φ (Φ') and Ψ (Ψ'). Since equ. 3 and 4 contain two angular functions (Φ', Ψ'), an experimental coupling constant may be rationalized in more than one way. In order to reduce the number of possible solutions it is essential to retrieve as much information as possible about the conformations and intramolecular mobility of the peptide structure, *e.g.*, from variable temperature ^1H- and ^{13}C-NMR. spectra, circular dichroism, X-ray data and potential energy calculations. In particular, an inspection of space-filling molecular models (CPK) will reveal the specific segmental conformations in the direct neighbourhood of the C_α,H-bond. Small values of $^1J(C_\alpha,H)$ ($\leqslant 137$ Hz) and large values (about 150 Hz) are highly indicative of a particular conformation, whereas intermediate values, being close to the typical data for straight-chain peptides with extensive molecular mobility, may not permit an unambiguous interpretation. Such intermediate values may arise from a superposition of the two different angular terms or, alternatively, from motional averaging of different conformations. These two cases could be distinguished by variable temperature NMR. studies.

The authors would like to thank Profs. *E.R. Blout* (Harvard), *J. Dale* and *K. Titlestad* (Oslo), *R. Deslauriers* (Ottawa), *H. Gerlach* (Bayreuth), and *V. Prelog* (Zurich) for the very generous supply of cyclic peptides. This work was further supported by the *Swiss National Science Foundation.*

Experimental Part

The ^{13}C-NMR. spectra were measured in the FT mode at 50.3 MHz (*Varian* XL-200) or 25.14 MHz (*Varian* XL-100-12). The individual conditions for the various peptides were as follows. Cyclo(Sar)$_4$: 25.14 MHz, $CDCl_3$/TFA ($\leq$5%), 0.13M, 30°, 10 mm tube; cyclo(Sar)$_8$: 25.14 MHz, $CDCl_3$, 0.03M, 30°, 18 mm tube; cyclo(aaAaAA): 25.14 MHz, D_2O, 0.06M, 30°, 18 mm tube; cyclo(L-Pro-Gly): 50.3 MHz, D_2O, 0.9M, 25°, 10 mm tube with microcell (0.4 ml); cyclo(L-Pro-Gly)$_2$: 50.3 MHz, D_2O, 0.08M, 80°, 10 mm tube; cyclo(L-Pro-Gly)$_3$: 50.3 MHz, D_2O, 0.20M, 45°, 10 mm tube with microcell (0.4 ml, 43 mg).

The ^{1}H-coupled spectra were obtained under gated decoupling conditions, typical acquisition time 3 s (XL-100-12), 1.6 s (XL-200), typical pulse delay 10 s, spectral width 5000 Hz (XL-100-12) and 10,000 Hz (XL-200). The reproducibility of the one-bond C,H coupling constants measured under these conditions is better than $\pm$0.5 Hz.

In the analysis of the ^{1}H-coupled ^{13}C-NMR. spectra of glycyl and sarcosyl units ($H_AH_BC_X$ systems) $\Delta\nu(A,B)$ and $J(A,B)$ were obtained, whenever possible, from the ^{1}H-NMR. spectra. The spectrum of the X part was then simulated using these data and C,H coupling constants extracted from a first-order analysis of the ^{13}C-NMR. spectrum. Deviations from first-order spectra were usually within the limits of the above mentioned accuracy of the C,H coupling constants.

The cyclic peptides originate from the following laboratories, cyclo(Sar)$_4$ and cyclo(Sar)$_8$: Prof. *J. Dale*, University of Oslo; cyclo(L-Pro-Gly), cyclo(L-Pro-Gly)$_2$ and cyclo(L-Pro-Gly)$_3$: Prof. *E.R. Blout*, Harvard University; cyclo(aaAaAA) ($[\alpha]_{578} = -20.8°$): Profs. *H. Gerlach* (Bayreuth) and *V. Prelog*, ETH Zurich.

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