Co-Oligopeptides Containing Two Aromatic Residues Spaced by Glycyl Residues. X. Proton Magnetic Resonance Study of Co-Oligopeptides of Tryptophan and Glycine

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Synopsis

The ^{1}H-nmr spectra of co-oligopeptides of tryptophan and glycine with structure H-Gly-Trp-$(Gly)_n$-Trp-Gly-OH (n = 0–2) and those of several di- and tripeptides have been recorded at 360 MHz with CD_3OD solutions containing 0.1N NaOD. The assignment of resonance signals was generally possible by comparing the spectra of structurally related peptides with each other. In order to solve the remaining ambiguities in the assignment, H-(αL,βS)(α,β-d_2)Trp-OH, H-Trp-(αL,βS)(α,β-d_2)Trp-OH, and H-Trp-(δ^1,ϵ^2,ζ^2,ζ^3,η^2-d_5)Trp-OH have been prepared and their spectra compared with those of the undeuterated compounds. The distribution of rotamers around the χ_1 and (in two cases) χ_2 torsion angles of the side chains has been obtained from the vicinal coupling constants $^3J_{H\alpha H\beta}$ and from the long-range coupling constants $^4J_{H\beta H\beta'}$. These data and an analysis of the chemical shifts of the Gly-C^α protons suggest that the orientation of the aromatic side chain is influenced by the following order of decreasing interaction with the functional groups at N- and C-side: $-NH_2$ > -NHCO- > -CONH- > $-COO^-$. This rule does not hold for the second Trp residue of di- and tripeptides containing the -Trp -Trp- sequence, which has tentatively been attributed to steric effects.

INTRODUCTION

During the last few years we have investigated the spectroscopic properties (uv absorption,[1,2] CD,[1,3,4] and fluorescence[5]) of a series of peptides with the structure H-Gly-X-$(Gly)_n$-Trp-Gly-OH (X = Trp, Phe, and n = 0–2). One of the aims of this investigation was to assess whether peptides containing two aromatic residues favor specific conformations in water solution due to intramolecular interactions between the two aromatic side chains. In turn, short linear peptides having preferentially populated conformations in solution might be of interest as models of the nucleation centers proposed as primary steps in the folding of proteins into their native conformations.[6]

Our CD studies have indicated that under certain conditions (solvent,

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pH, and temperature),[1,3,4] peptides containing two adjacent aromatic residues possess preferentially populated conformations. The present ^{1}H-nmr study is aimed at identifying these preferred conformations.

Early ^{1}H-nmr studies on linear peptides containing two aromatic residues have considered the possibility of stacking between the two aromatic side chains.[7,8] However, evidence was limited to changes in the chemical shifts of the aromatic protons. An analysis of the rotamer population of the aromatic side chains in a variety of short linear peptides, based on the vicinal coupling constants of the ABX proton systems constituted by the $C^{\alpha}H$-$C^{\beta}H_2$ fragments of the aromatic residues,[9,10] has been reported by several authors.[11–22] It has been suggested that aromatic side chains preferentially orient towards the side of their amino adjacent residue.[20,21] Differences in chemical shifts observed for diastereomeric dipeptides containing a polar and an aromatic residue have been interpreted by assuming that the aromatic side chains may interact with the positive end of the neighboring polar group.[22] All these observations indicate that the aromatic side chains in linear peptides have a nonrandom orientation with respect to the rest of the molecule.

In this paper, we present an ^{1}H-nmr investigation of the co-oligopeptides of glycine and tryptophan, as well as of several di- and tripeptides in d_4-methanol solution at room temperature. CD_3OD instead of D_2O has been used as a solvent in order to extend the investigation to a low temperature study which will be discussed in a forthcoming paper.[23] Previous CD studies[3] have shown that the conformational properties of such peptides are likely to be similar in H_2O and in CH_3OH.

The present study includes an analysis of the chemical shift changes in structurally related peptides and of the rotamer populations around the χ_1 torsion angles. In favorable cases, information about the preferential value of the χ_2 torsion angle of the tryptophan side chain was obtained by measuring the long-range coupling constants $^4J_{H^{\beta}H^{\delta 1}}$. Due to exchange of NH protons in CD_3OD, no direct information about the peptide backbone conformation could be obtained.

EXPERIMENTAL

Materials and Methods

Tryptophan, diglycine, and triglycine were Fluka (Buchs, Switzerland) puriss. products. The various reagents and solvents were also Fluka products. They were always of the highest available purity and were used without further purification. The syntheses and the characterization of the undeuterated peptides used in this work have already been described.[24]

TLC was performed on silica gel HF 254 precoated plates with 0.25-mm layer thickness (Merck, FRG). Free amino acids and peptides were eluted using the mixture $CH_3OH/CHCl_3/NH_3$aq (12%) (4:4:1). For all of the other

compounds, mixtures of benzene and methanol or of chloroform and methanol were used. The spots were located with a uv lamp and I_2. Melting points were determined with a Tottoli instrument and are uncorrected. Optical rotations were measured on a digital polarimeter, Perkin Elmer model 141.

Pentadeuterotryptophan [H-(δ^1,ϵ^2,ζ^2,ζ^3,η^2-d_5)Trp-OH]

H-Trp-OH (2.04 g, 10 mmol) was shaken overnight with 20 ml D_2O. The solvent was then removed by freeze-drying and to the residue, 20 ml CF_3COOD were added slowly under nitrogen. The solution was kept 22 hr under magnetic stirring at room temperature, and finally the solvent was evaporated under reduced pressure. The residue was dissolved in D_2O containing 5% H_2SO_4 and treated with a concentrated solution of $HgSO_4$ in D_2O and 5% H_2SO_4 until no more precipitation occurred. The insoluble Hg^{++} salt of tryptophan was filtered and decomposed, and the amino acid was recovered as described by Greenstein and Winitz.[25] On recrystallization from C_2H_5OH/H_2O, 820 mg of white product were obtained (40% yield). The product showed only one spot by TLC with the same R_f as H-Trp-OH.

A ^{1}H-nmr spectrum indicated that the mean deuteration degree of the aromatic protons was 43% and showed no evidence of deuterium substitution of the $C^{\alpha}H$. This was confirmed by the high optical purity of the sample: $|\alpha|_D^{23} - 30.0$ (*c* 0.5, H_2O). Literature value for H-Trp-OH[26]: $|\alpha|_D^{20} - 31.5$ (*c* 0.5, H_2O).

(*Z*)α-Acetamido-β-Indol-3-yl-Acrylic Acid [Ac-(Dehydro)Trp-OH]

1-Acetyl-3-formyl-indole (18.7 g, 100 mmol), 11.7 g (100 mmol) Ac-Gly-OH (Ac = acetyl), and 13.8 g K_2CO_3 (100 mmol) were mixed and transferred into a reaction vessel under nitrogen atmosphere. Ac_2O (50 ml) was slowly added and the mixture was incubated at 110–115°C with magnetic stirring. Under these conditions the reaction mixture was liquid and rapidly became red-orange. After 1 hr the reaction was stopped, and the solid was suspended in 250 ml cold water. Then it was filtered and washed 3 times with 1 l. hot water. The orange solid, which is composed of (*Z*)2-methyl-4(1-acetyl-3-indolylidene)-5(4H)-oxazolinone[27] (*Z* = benzyloxycarbonyl) and part of the unreacted 1-acetyl-3-formyl-indole, was suspended in 400 ml of a 2:1 acetone/water mixture and refluxed overnight. Then the solvent was evaporated under reduced pressure, and the residue was dissolved in 250 ml water by adding K_2CO_3 until pH 10 was obtained. The solution was stirred for 20 hr and then extracted 5 times with 100 ml ethyl acetate. The aqueous phase was acidified with 5*N* HCl, and the insoluble acid was collected by filtration.

On recrystallization from 95% ethanol, 6.33 g pure Ac-(dehydro)Trp-OH

were obtained (yield 26%), mp 228–230°C, λ_{max} = 320 nm, ϵ = 16,900 cm^{-1} M^{-1} in 1*N* NaOH.

ANAL.: *Calcd.* for $C_{13}H_{12}O_3N_2 \cdot H_2O$: C, 59.54; H, 5.38; N, 10.67. *Found:* C, 59.20; H, 5.42; N, 10.61.

(αL,βS)(α,β-d_2)Tryptophan

Ac-(dehydro)Trp-OH (9.76 g, 40 mmol) was dissolved in 50 ml D_2O by adding 4 ml of 40% NaOD solution. The solution was freeze-dried, and the residue was redissolved in 100 ml D_2O. Then 1.3 g of 5% Pd on charcoal were added, and the reaction flask was connected to a D_2 source at atmospheric pressure and stirred for 3 hr. Then the reaction was stopped, and the catalyst was filtered off. A 0.5-ml sample of the filtrate was used for a 90-MHz ^{1}H-nmr spectrum, which confirmed a quantitative *cis* addition of D_2. The solution was acidified with 1*N* HCl and the racemic mixture of (αL,βS) and (αD,βR) Ac-(α,β-d_2)Trp-OH crystallized at 40°C overnight, yielding 7.95 g of white product (yield 80%). H-(αL,βS)(α,β-d_2)Trp-OH was obtained using the procedure described by Overby[28] for the resolution of L,D-tryptophan via the *N*-acetyl derivative. The white product (1.56 g), chromatographically identical with H-Trp-OH, was obtained on recrystallization from aqueous ethanol (47% yield). $|\alpha|_D^{23} - 26.1$ (*c* 0.5, H_2O). Assuming that the specific rotation of optically pure H-(αL,βS)(α,β-d_2)-Trp-OH is the same as that of L-tryptophan [$|\alpha|_D^{20} - 31.5$ (*c* 0.5, H_2O)],[26] the value measured for our sample corresponds to an optical purity of 91%.

^{1}H-NMR Spectra

^{1}H-nmr spectra were recorded with 0.02*M* solutions of the peptides in CD_3OD/NaOD (0.1*M*) (Ciba-Geigy, Switzerland) on a Bruker HXS-360 spectrometer operating in the Fourier transform mode with a digital resolution of 0.2 Hz/point and at a temperature of 25 ± 2°C. The long-range coupling constants $^4J_{H^{\delta 1}H^{\beta}}$ were measured with a digital resolution of 0.11 Hz/point. The spectra were obtained immediately after the preparation of the solutions. Computations of the spectra were done with the Nicolet ITRCAL version of the LAOCN 3 program[29] on a Nicolet B-NC 12 computer equipped with a NIC-294 disk memory. The spectral parameters derived from computations are given in Tables I–III [chemical shifts in ppm ± 0.01 (δ) relative to internal tetramethylsilane, coupling constants in Hz ± 0.2]. The root-mean-square error of computer spectra was less than 0.2 Hz, and the number of transitions used for iteration was close to the theoretical number.

RESULTS

Syntheses

H-(δ^1,ϵ^2,ζ^2,ζ^3,η^2-d_5)-Trp-OH was prepared by treating H-Trp-OH with CF_3COOD as described by Norton and Bradbury[30] and purified via the insoluble Hg^{++} salt. H-(αL,βS)(α,β-d_2)Trp-OH was prepared with a modification of the synthesis of stereoselectively labeled tryptophan reported by Kirby and Varley.[31] *N*-Acetyl-3-formyl-indole was reacted with *N*-acetyl-glycine under conditions for Erlenmeyer condensation to give the corresponding (*Z*)-oxazolinone,[27] which was hydrolized to (*Z*)-α-acetamido-β-indol-3-yl-acrylic acid [Ac-(dehydro)Trp-OH]. On catalytic reduction of the double bond with deuterium, a racemic mixture of (αL,βS) and (αD,βR)Ac-(α,β-d_2)Trp-OH was obtained. This was resolved with (−)1-phenylethylamine[28] and the (αL,βS) acid was hydrolyzed to give H-(αL,βS)(α,β-d_2)Trp-OH.

H-Trp-(αL,βS)(α,β-d_2)Trp-OH and H-Trp-(δ^1,ϵ^2,ζ^2,ζ^3,η^2-d_5)Trp-OH were prepared by coupling *Z*-Trp-ONSu with the corresponding deuterium-labeled tryptophan, followed by removal of the *Z*-protecting group with catalytic hydrogenation, as previously described for the synthesis of H-Trp-Trp-OH.[24] The two deuterium-labeled dipeptides were identical with H-Trp-Trp-OH by TLC. The CD spectrum in aqueous NaOH (0.01*N*) of H-Trp-(δ^1,ϵ^2,ζ^2,ζ^3,η^2-d_5)Trp-OH was identical to that of H-Trp-Trp-OH. In the case of H-Trp-(αL,βS)(α,β-d_2)Trp-OH, a general decrease of 15–18% in the molar ellipticity was observed with respect to the spectrum of H-Trp-Trp-OH. This was attributed to the presence of ca. 10% diastereomeric H-Trp-(αD,βR)(α,β-d_2)Trp-OH arising from incomplete resolution of the racemic mixture of Ac-(α,β-d_2)Trp-OH.

Assignment of the Resonance Signals

The chemical shifts and coupling constants of Gly-C$^\alpha$ protons, Trp-AB*X* spectra, and aromatic Trp-protons are presented in Tables I–III. The assignment of N- and C-terminal Gly-C$^\alpha$ protons is based on consideration of first-order shifts (peptide formation and titration shifts).[32] The distinction between internal and C-terminal Gly-C$^\alpha$ protons in the peptides H-Gly-Trp-(Gly)$_n$-Trp-Gly-OH (n = 1,2) comes from the observation of a decrease in the intensity of the signals attributed to the internal Gly residues on long standing (one day or more) of the solutions at room temperature. The same observation was made for the internal Gly residue in H-Gly-Gly-Gly-OH. It is attributed to the preferential deuterium exchange of these C$^\alpha$ protons via the base abstraction mechanism in the alkaline solution.[33]

The assignment of the aromatic protons of H-Trp-OH follows the accepted one.[19,24,34] Furthermore, for the peptides with two Trp residues,

TABLE I
Chemical Shifts of C^{α} Protons of Gly Residues[a]

Peptide	N-Terminal	Internal	C-Terminal
H-Gly-Gly-OH	3.29	—	3.78
H-Gly-Gly-Gly-OH	3.33	3.93	3.76
H-Gly-Trp-OH	3.13, 3.16 (16.6)	—	—
H-Trp-Gly-OH	—	—	3.70, 3.72 (17.2)
H-Gly-Trp-Gly-OH	3.17, 3.22 (16.8)	—	3.64, 3.78 (17.2)
H-Gly-Trp-Trp-OH	2.97	—	—
H-Trp-Trp-Gly-OH	—	—	3.56, 3.70 (17.2)
H-Gly-Trp-Trp-Gly-OH	3.02	—	3.53, 3.79 (17.2)
H-Gly-Trp-Gly-Trp-Gly-OH	3.17	3.57, 3.90 (16.6)	3.65, 3.77 (17.2)
H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH	3.20, 3.24 (16.8)	3.69, 3.79 (16.8) 3.71, 3.76 (16.6)	3.63, 3.79 (17.2)

[a] Shift values in ppm ± 0.01; the geminal coupling constants in Hz ± 0.2 are given in parentheses. The chemical shift of free glycine under the same conditions is 3.14 ppm.

it was possible to identify each system of four protons H_{ϵ^2}, H_{ζ^2}, H_{ζ^3}, H_{η^2} with spin-decoupling experiments, but an unequivocal assignment of the signals of each Trp residue was possible only in the case of H-Trp-Trp-OH by comparison with the spectrum of H-Trp-(δ^1,ϵ^2,ζ^2,ζ^3,η^2-d_5)Trp-OH.

Each *ABX* ($C^{\alpha}H$-$C^{\beta}H_2$) system was identified by spin-decoupling experiments. In the peptides H-Trp-Trp-OH and H-Trp-Trp-Gly-OH, the *X* protons were assigned on the basis of first-order shift considerations (see

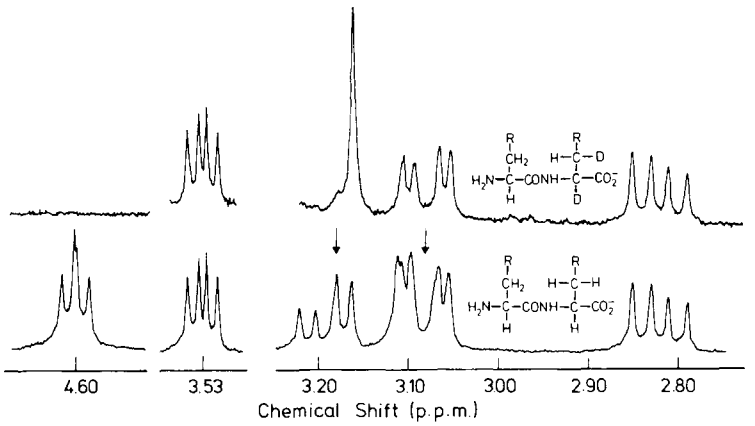


Fig. 1. The spectra of H-Trp-Trp-OH (lower trace) and of H-Trp-(αL,βS)(α,β-d_2)Trp-OH (upper trace) are compared in order to show the assignment of the α and β protons of the Trp(2) residue (*R* = indol-3-yl). These spectra were taken at 10°C in order to enhance the chemical shift difference between the two β protons. The arrows indicate the calculated chemical shifts of the β protons in the undeuterated compound. The single line in the deuterated compound is shifted upfield by ~0.02 ppm with respect to the computed value. This difference is attributed to an isotope effect.

TABLE II
Chemical Shifts and Coupling Constants of the *ABX* Spectra of the Tryptophyl Residue

Compound	δ_A, δ_B[a]	δ_X	$-J_{AB}$	J_{AX},J_{BX}[a]
H-Trp-OH	2.89, 3.30	3.55	14.3	8.7, 4.2
H-Gly-Trp-OH	3.18, 3.38	4.59	14.5	7.1, 4.8
H-Trp-Gly-OH	2.96, 3.22	3.65	14.3	7.6, 5.5
H-Gly-Trp-Gly-OH	3.16, 3.34	4.70	14.7	8.2, 5.4
H-*Trp*-Trp-OH	2.78, 3.09	3.53	14.4	7.7, 4.6
H-Trp-*Trp*-OH	3.15, 3.19	4.60	14.4	6.4, 5.5
H-Gly-*Trp*-Trp-OH[b]	2.97, 3.23	4.62	14.8	8.5, 4.7
H-Gly-Trp-*Trp*-OH[b]	3.20, 3.30	4.55	14.5	5.9, 5.1
H-*Trp*-Trp-Gly-OH	2.85, 3.02	3.56	14.3	6.8, 5.9
H-Trp-*Trp*-Gly-OH	3.04, 3.10	4.63	14.5	5.8, 7.2
H-Gly-*Trp*-Trp-Gly-OH[c]	2.98, 3.09	4.52	14.8	7.4, 6.3
H-Gly-Trp-*Trp*-Gly-OH[c]	3.08, 3.16	4.65	14.9	8.0, 5.3
H-Gly-*Trp*-Gly-Trp-Gly-OH[d]	3.15, 3.28	4.55	14.6	7.6, 6.4
H-Gly-Trp-Gly-*Trp*-Gly-OH[d]	3.15, 3.35	4.67	14.9	8.6, 5.3
H-Gly-*Trp*-$(Gly)_2$-Trp-Gly-OH[e]	3.18, 3.29	4.57	14.7	7.6, 6.4
H-Gly-Trp-$(Gly)_2$-*Trp*-Gly-OH[e]	3.17, 3.35	4.68	14.7	8.8, 5.1

[a] Proton *A* is defined as the one at higher field.

[b] Assignment is based on a comparison with the spectrum of H-Gly-Phe-Trp-OH: δ_X(Phe) = 4.55; δ_X(Trp) = 4.52.

[c] Assignment is based on a comparison with the spectrum of H-Gly-Phe-Trp-Gly-OH: δ_X(Phe) = 4.47; δ_X(Trp) = 4.70.

[d] Assignment is based on a compraison with the spectrum of H-Gly-Phe-Gly-Trp-Gly-OH: δ_X(Phe) = 4.50; δ_X(Trp) = 4.69.

[e] Assignment is based on a comparison with the spectrum of H-Gly-Phe-$(Gly)_2$-Trp-Gly-OH: δ_X(Phe) = 4.52; δ_X(Trp) = 4.68.

Table II). For H-Trp-Trp-OH the assignment was confirmed by comparison with the spectrum of H-Trp-(αL,βS)(α,β-d_2)Trp-OH (Fig. 1). For the peptides H-Gly-Trp-$(Gly)_n$-Trp-Gly-OH (n = 0–2), as well as for H-Gly-Trp-Trp-OH, the assignment is based on a comparison with the spectra of the compounds H-Gly-Phe-$(Gly)_n$-Trp-Gly-OH (n = 0–2) and H-Gly-Phe-Trp-OH (see Table II), assuming that the C^α proton signals of Trp residues in the same structural position in the two families of peptides have close resonance frequencies. The identification of Phe residues is based on the significantly higher field resonances of their *A*, *B* protons, together with a smaller geminal coupling constant J_{AB} (~14.0 Hz). In the case of H-Trp-OH, protons *A* and *B* of the *ABX* system (*A* at higher field) have been assigned to the Pro(R) and Pro(S) hydrogen atoms of the $C^\beta H_2$ group on comparison with the spectrum of H-(αR,βS)(α,β-d_2)Trp-OH (Fig. 2).

In the case of H-Trp-Trp-OH, comparison with the spectrum of H-Trp-(αL,βS)(α,β-d_2)Trp-OH (Fig. 1) demonstrated that for the Trp(2) residue, the reverse assignment [*A* = Pro(S) proton] is valid. For all the other Trp residues, the assignment of resonances *A* (at higher field) to the Pro(R) protons is suggested by a detailed temperature ^{1}H-nmr study of this series of peptides.[23]

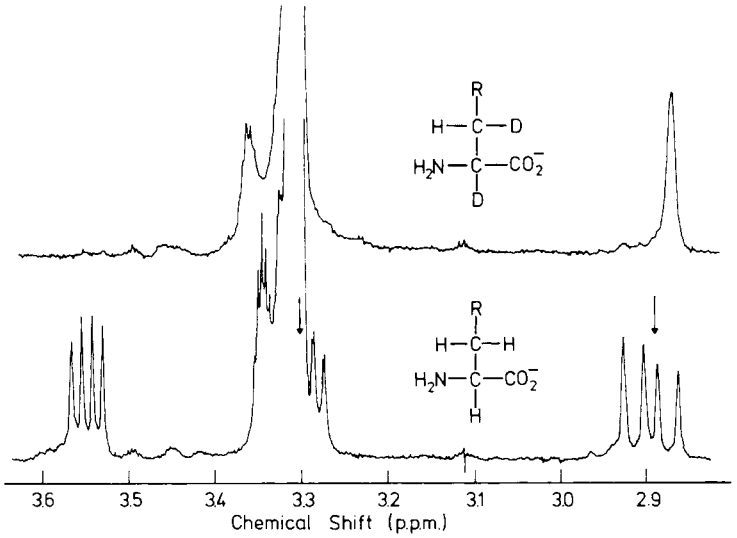


Fig. 2. The spectra of H-Trp-OH (lower trace) and of H-(αL,βS)(α,β-d_2)Trp-OH (upper trace) are compared in order to show the assignment of the β protons in H-Trp-OH (R = indol-3-yl). The arrows indicate the calculated chemical shifts of the β protons in H-Trp-OH. The upfield shift (~0.02 ppm) of the single line in H-(αL,βS)(α,β-d_2)Trp-OH with respect to the calculated value for H-Trp-OH is attributed to an isotope effect.

Chemical Shifts

For the C^α protons of N- and C-terminal Gly residues in H-Gly-Gly-OH and H-Gly-Gly-Gly-OH, chemical shifts are found within 0.04 and 0.02 ppm, respectively (see Table I). As visualized in Fig. 3, distinct upfield shifts of these protons (except for one C-terminal proton) are observed by comparing H-Gly-Gly-Gly-OH with H-Gly-Trp-Gly-OH. Furthermore, in the latter compound both the N- and C-terminal Gly-C^α protons become anisochronous ($\Delta\delta_N$ = 0.05 and $\Delta\delta_C$ = 0.14 ppm). In H-Gly-Trp-Gly-Trp-Gly-OH and H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH, the resonance frequency of these protons is very close to that of H-Gly-Trp-Gly-OH, but the anisochrony of the N-terminal Gly-C^α protons is lost in the pentapeptide. In the case of H-Gly-Trp-Trp-Gly-OH, a further upfield shift is observed for the N-terminal Gly, and the anisochrony of the C-terminal Gly-C^α protons increases to as much as 0.26 ppm by a further upfield shift of one of them. The chemical shifts of the Gly-C^α protons of H-Gly-Trp-Trp-OH and H-Trp-Trp-Gly-OH correspond to the respective ones of H-Gly-Trp-Trp-Gly-OH. In particular, the N-terminal Gly-C^α protons of H-Gly-Trp-Trp-OH and H-Gly-Trp-Trp-Gly-OH, despite the fact that they are more upfield shifted with respect to H-Gly-Gly-Gly-OH (0.36 and 0.31 ppm), remain isochronous.

The internal Gly-C^α protons in the hexapeptide H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH are shifted upfield by 0.14 and 0.24 ppm relative to those of H-Gly-Gly-Gly-OH. The anisochrony of 0.05 and 0.10 ppm increases to

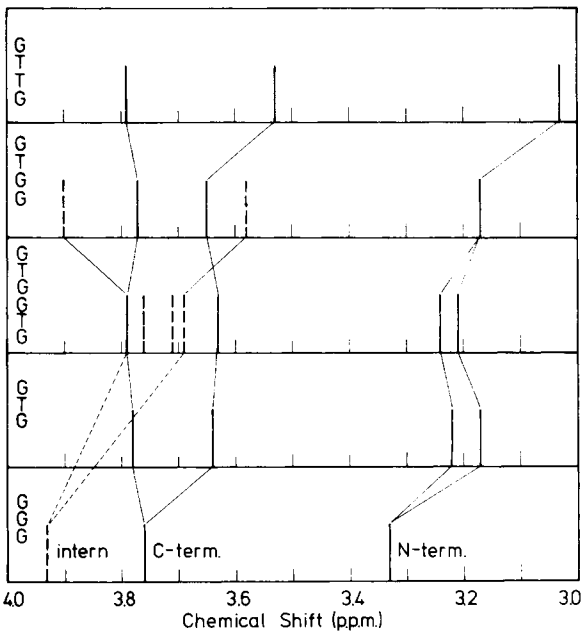


Fig. 3. Visualization of the chemical shifts of the Gly-C$^{\alpha}$ protons in the co-oligopeptides H-Gly-Trp-(Gly)$_n$-Trp-Gly-OH and in the model compounds H-Gly-Gly-Gly-OH, H-Gly-Trp-Gly-OH (G = Gly, T = Trp).

0.32 ppm on going from the hexapeptide to the pentapeptide H-Gly-Trp-Gly-Trp-Gly-OH (Fig. 3).

Table II reports the chemical shifts of the Trp-*ABX* protons in the peptides investigated here. As can be seen, the significant differences of δ_X are due exclusively to first-order shifts. Despite significant changes of δ_A and δ_B, it seems difficult to correlate them with structural changes.

The chemical shifts of the aromatic protons are reported in Table III. The chemical shifts of protons H_{η^2} and H_{ζ^2} are within 0.09 ppm of each other for all peptides. In the peptides containing two adjacent Trp residues, the signals of $H_{\delta 1}$, $H_{\epsilon 3}$, and H_{ζ^3} are clearly shifted upfield with respect to the other compounds. For the $H_{\epsilon 3}$ of the Trp (2) of H-Trp-Trp-OH, this upfield shift is as large as 0.39 ppm with respect to H-Trp-OH. Upfield shifts of the aromatic protons of H-Trp-Trp-OH with respect to H-Trp-OH have been noted by Cohen[8] and attributed to stacking. This point will be discussed in more detail in a forthcoming paper.[23]

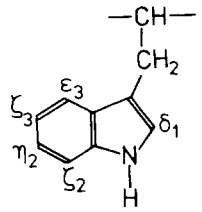
Rotamer Populations of the Aromatic Side Chains

The populations (g^+, g^-, t) of the three staggered rotamers around the torsion angle χ_1 of the tryptophan side chain have been computed from the vicinal coupling constants J_{AX}, J_{BX} (see Table II) using Feeney's approach[10] and are reported in Table IV.

TABLE III
Chemical Shifts of the Aromatic Tryptophan Protons[a]

Compound	H_{δ_1}	H_{ϵ_3}	H_{ζ_3}	H_{η_2}	H_{ζ_2}
H-Trp-OH	7.12	7.68	6.99	7.06	7.31
H-Gly-Trp-OH	7.10	7.59	6.97	7.04	7.29
H-Trp-Gly-OH	7.11	7.61	7.00	7.08	7.33
H-Gly-Trp-Gly-OH	7.12	7.59	7.00	7.07	7.31
H-*Trp*-Trp-OH	7.01	7.58	7.01	7.09	7.34
H-Trp-*Trp*-OH	6.97	7.28	6.82	7.00	7.26
H-Gly-Trp-Trp-OH	7.03	7.55	7.00	7.07	7.32
	7.02	7.41	6.86	7.02	7.28
H-Trp-Trp-Gly-OH	7.03	7.56	7.01	7.10	7.35
	6.93	7.31	6.90	7.04	7.29
H-Gly-Trp-Trp-Gly-OH	7.03	7.53	7.00	7.09	7.34
	7.01	7.33	6.95	7.07	7.31
H-Gly-Trp-Gly-Trp-Gly-OH	7.06	7.56	6.99	7.06	7.30
	7.10	7.56	7.00	7.08	7.32
H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH	7.13	7.56	7.00	7.06	7.31
	7.14	7.59	7.00	7.07	7.31

[a] The subscripts δ_1, ϵ_3, ζ_3, η_2, and ζ_2 refer to the positions indicated in the aromatic ring:

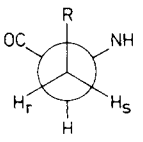
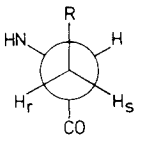
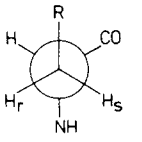


The correct calculation of rotamer populations g^- and t depends on the unequivocal assignment of resonances A and B to protons Pro(R) and Pro(S). As discussed above, this assignment has been proved for H-Trp-OH and H-Trp-*Trp*-OH by comparison with stereoselectively deuterated compounds. Furthermore, for H-Trp-OH the rotamer populations g^- and t have been verified according to the method of Hansen et al.[35] with the help of the heteronuclear coupling constants $^3J_{^{13}COH_B}$ ($^3J_{^{13}COH_A}$ + $^3J_{^{13}COH_B}$ = 4.9 Hz).

The rotamer populations calculated for H-Trp-OH, H-Gly-Trp-OH, and H-Gly-Trp-Gly-OH are in agreement with data reported for alkaline aqueous solutions.[12,16]

In the compounds containing only one Trp residue, rotamer G^- predominates. In H-Gly-Trp-OH and H-Trp-Gly-OH, the fractional population g^- is reduced with respect to H-Trp-OH, with corresponding increases of populations g^+ and t. The fractional rotamer populations of the aromatic side chains of H-Gly-Trp-$(Gly)_n$-Trp-Gly-OH (n = 1,2) closely resemble those found for H-Gly-Trp-Gly-OH. In the peptides containing

TABLE IV
Population of Rotamers Around the χ_1 Torsion Angle of the Tryptophyl Residue[a]

Compound	 G⁺ $X_1 = +60°$	 G⁻ $X_1 = -60°$	 T $X_1 = 180°$
H-Trp-OH	0.20	0.64	0.16
H-Gly-Trp-OH	0.37	0.45	0.18
H-Trp-Gly-OH	0.22	0.49	0.29
H-Gly-Trp-Gly-OH	0.14	0.56	0.30
H-*Trp*-Trp-OH	0.31	0.52	0.17
H-Trp-*Trp*-OH	0.43	0.25	0.32
H-Gly-*Trp*-Trp-OH	0.18	0.61	0.21
H-Gly-Trp-*Trp*-OH	0.51	0.32	0.17
H-*Trp*-Trp-Gly-OH	0.29	0.40	0.31
H-Trp-*Trp*-Gly-OH	0.29	0.27	0.44
H-Gly-*Trp*-Trp-Gly-OH	0.16	0.46	0.38
H-Gly-Trp-*Trp*-Gly-OH	0.18	0.54	0.28
H-Gly-*Trp*-Gly-Trp-Gly-OH	0.12	0.48	0.40
H-Gly-Trp-Gly-*Trp*-Gly-OH	0.06	0.60	0.34
H-Gly-*Trp*-$(Gly)_2$-Trp-Gly-OH	0.12	0.48	0.40
H-Gly-Trp-$(Gly)_2$-*Trp*-Gly-OH	0.09	0.63	0.28

[a] An uncertainty of ±0.2 Hz in the coupling constants J_{AX} and J_{BX} corresponds typically to an uncertainty of ±0.03 in the populations g^+, g^-, and t.

the –Trp–Trp– sequence, rotamer G^- predominates for the first Trp residue. H-Trp-Trp-OH and H-Gly-Trp-Trp-OH exhibit predominance of the G^+ rotamer for the C-terminal side chain, and H-Trp-Trp-Gly-OH has a predominance of rotamer T for Trp(2). Finally, in H-Gly-Trp-Trp-Gly-OH only subtle differences in the rotamer populations with respect to the penta- and hexapeptide H-Gly-Trp-$(Gly)_n$-Trp-Gly-OH (n = 1,2) are observed.

In the cases of H-Trp-OH and Trp(1) in H-Trp-Trp-OH, long-range coupling constants $^4J_{H_\beta H_{\delta 1}}$ for both A(Pro-R) and B(Pro-S) protons were obtained and were confirmed by spin decoupling. In both cases the coupling constant for the B proton (0.9 Hz for H-Trp-OH and 0.7 Hz for H-*Trp*-Trp-OH) is larger than for the A proton (0.6 Hz for H-Trp-OH and 0.2 Hz for H-*Trp*-Trp-OH). The coupling constants of H-Trp-OH are in close agreement with those reported by Cavanaugh[12] for the H-Trp-OH anion in aqueous solution (0.83 and 0.65 Hz). Using the angular dependence of the allylic cisoid long-range coupling constant reported by Sternhell,[36] the measured absolute values for $^4J_{H_\beta H_{\delta 1}}$ are consistent with values for χ_2 around +90° or −90°. These are in agreement with an analysis of x-ray data for tryptophyl residues in proteins[37] and conforma-

tional calculations[38] for Ac-Trp-NH_2 indicating that favorable values for χ_2 are around +90° or −90°.

DISCUSSION

The proton coupling constants of $C^{\beta}H_2$ with both $C^{\alpha}H$ and $C^{\delta 1}H$ for H-Trp-OH indicate that the preferential conformation of the aromatic side chain in methanol solution at room temperature is characterized by the torsion angles $\chi_1 = -60°$ and $\chi_2 = \pm 90°$. Conformations with χ_1 close to −60° and χ_2 close to +90° are observed for crystals of Ac-Trp-OH,[39] H-Gly-Trp-OH·$2H_2O$,[40] Ac-Trp-OCH_3,[41] and Ac-DL-Trp-$NHCH_3$.[42] As already noted, rotamer G^- is preferred in all Trp residues which have no other Trp residue adjacent. The upfield shift of the N-terminal Gly-C^{α} proton resonances observed for tryptophan-containing peptides with respect to H-Gly-Gly-Gly-OH (see Fig. 3) can be readily rationalized in terms of this preferred orientation of the aromatic side chain towards the N-terminal Gly residue, as already proposed for tyrosine-containing peptides by Wüthrich and coworkers.[20,21] Anisochrony of the Gly-C^{α} protons found for many of our tryptophan-containing peptides has also been observed in other cases and has been interpreted as an indication of the presence of preferred conformations of the peptide backbone.[43]

The differences in rotamer populations of the compounds H-Trp-OH, H-Trp-Gly-OH, H-Gly-Trp-OH, and H-Gly-Trp-Gly-OH may be understood in terms of a dipolar interaction between the Trp side chain and the functional groups $-NH_2$, –NHCO–, –CONH–, and $-COO^-$. Since in H-Trp-OH rotamer G^- predominates, we conclude that the interaction of the aromatic side chain with the amino group prevails over that with the carboxylate ($-NH_2 > -COO^-$). C-Terminal peptide formation increases ($-CONH- > -COO^-$) and N-terminal peptide formation decreases g^- ($-NH_2 >$ –NHCO–). In the case of H-Gly-Trp-Gly-OH, G^- is again the predominant rotamer (–NHCO– > –CONH–). Therefore, the following order of decreasing interaction can be derived: $-NH_2 >$ –NHCO– > –CONH– > $-COO^-$. This order is consistent with a preferential interaction of the aromatic side chain with the positive end of the backbone dipole moment[22] and will be considered in more detail in the forthcoming paper.[23] As can be seen from the comparison of the rotamer populations of the penta- and hexapeptide with those of H-Gly-Trp-Gly-OH, only minor changes in the local conformation of the aromatic residues occur with respect to the tripeptide. Thus in the longer peptides the preferential orientation of the aromatic side chains is also towards their amino end.

This rule does not hold for the second Trp residue of di- and tripeptides containing the –Trp–Trp– sequence. This can be understood in part by assuming that steric hindrance between the two aromatic side chains (mediated by a specific backbone conformation) reduces the population of rotamer G^- in the second residue. The favored rotamer is T in H-Trp-Trp-Gly-OH, but it is G^+ in H-Trp-Trp-OH and H-Gly-Trp-Trp-OH,

probably because the unfavorable interaction with the $-COO^-$ group limits the population of rotamer *T*.

On the basis of the same arguments, one would expect a similar distribution of rotamers for H-Gly-Trp-Trp-Gly-OH as is observed for H-Trp-Trp-Gly-OH; but this is not the case. We assume that this is due to the different backbone conformations. This point raises the general question of the relation between side-chain conformation and backbone conformation. In this paper we have presented no data relevant to the torsion angles ϕ and ψ of the peptide backbone. This information however is essential for a full description of the conformational equilibria of the studied peptides. Another question which has not been considered in this paper is the relationship between ^{1}H-nmr and CD data. To answer these questions we are carrying out work to obtain low-temperature ^{1}H-nmr and CD data in order to make a comparison with semiempirical calculations of the optical activity.

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References

1. Luisi, P. L., Rizzo, V., Lorenzi, G. P., Straub, B., Suter, U. & Guarnaccia, R. (1975) *Biopolymers* **14,** 2347–2362.
2. Guarnaccia, R., Rizzo, V., Gianola, P. & Luisi, P. L. (1976) *Biopolymers* **15,** 1103–1107.
3. Rizzo, V. & Luisi, P. L. (1977) *Biopolymers* **16,** 437–448.
4. Rizzo, V., Straub, B. & Luisi, P. L. (1977) *Biopolymers* **16,** 449–460.
5. Wiget, P. & Luisi, P. L. (1978) *Biopolymers* **17,** 167–180.
6. Anfinsen, C. B. & Scheraga, H. A. (1975) *Adv. Protein Chem.* **29,** 205–300.
7. Deslauriers, R. & Smith, I. C. P. (1970) *Biochem. Biophys. Res. Commun.* **40,** 179–185.
8. Cohen, J. S. (1971) *Biochim. Biophys. Acta* **229,** 603–609.
9. Pachler, K. G. R. (1964) *Spectrochim. Acta* **20,** 581–587.
10. Feeney, J. (1976) *J. Magn. Reson.* **21,** 473–478.
11. Cavanaugh, J. R. (1968) *J. Am. Chem. Soc.* **90,** 4533–4536.
12. Cavanaugh, J. R. (1970) *J. Am. Chem. Soc.* **92,** 1488–1493.
13. Bartle, K. D., Jones, D. W. & L'Amie, R. (1972) *J. Chem. Soc. Perkin Trans II,* 650–655.
14. Dale, B. J. & Jones, D. W. (1975) *Spectrochim. Acta, Part A* **31,** 83–88.
15. Dale, B. J. & Jones, D. W. (1976) *J. Chem. Soc. Perkin Trans. II,* 91–96.
16. Dale, B. J. & Jones, D. W. (1976) *J. Chem. Soc. Perkin Trans. II,* 1190–1192.
17. Kainosho, M. & Ajisaka, K. (1975) *J. Am. Chem. Soc.* **97,** 5630–5631.
18. Lala, A. K., Anteunis, M. J. O. & Lala, K. (1976) *Biochim. Biophys. Acta* **453,** 133–138.
19. Hintsche, R. & Mehlis, B. (1975) *Z. Chem.* **15,** 22–23.
20. Bundi, A., Gratwohl, C., Hochmann, J., Keller, R. M., Wagner, G. & Wüthrich, K. (1975) *J. Magn. Reson.* **18,** 191–198.
21. Wüthrich, K. & De Marco, A. (1976) *Helv. Chim. Acta* **59,** 2228–2235.
22. Deber, C. M. & Joshua, H. (1972) *Biopolymers* **11,** 2493–2503.
23. Baici, A., Rizzo, V., Skrabal, P. & Luisi, P. L. (1979) *J. Am. Chem. Soc.*, to be published.

24. Guarnaccia, R., Lorenzi, G. P., Rizzo, V. & Luisi, P. L. (1975) *Biopolymers* **14,** 2329–2346.

25. Greenstein, J. P. & Winitz, M. (1961) *Chemistry of Amino Acids,* Vol. 3, Wiley, New York, pp. 2321–2322.

26. Weast, R. C., Ed. (1972/73) *Handbook of Chemistry and Physics,* The Chemical Rubber Co., Cleveland.

27. Rao, Y. S. & Filler, R. (1975) *Synthesis,* 749–764.

28. Overby, L. R. (1958) *J. Org. Chem.* **23,** 1393–1394.

29. Castellano, S., Sun, C. & Kostelnitz, R. (1967) *J. Chem. Phys.* **46,** 327–330.

30. Norton, R. S. & Bradbury, J. H. (1976) *Mol. Cell. Biochem.* **12,** 103–111.

31. Kirby, G. W. & Varley, M. J. (1974) *J. Chem. Soc. Chem. Commun.,* 833–834.

32. Nakamura A. & Jardetzky, O. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 2212–2219.

33. Wünsch, E. (1974) in *Methoden der Organischen Chemie,* Wünsch, E., Ed., Vol. 15/1, Georg Thieme Verlag, Stuttgart, pp. 39–41.

34. Wessels, P. L., Feeney, J., Gregory, H. & Gormley, J. J. (1973) *J. Chem. Soc. Perkin Trans II,* 1691–1698.

35. Hansen, P. E., Feeney, J. & Roberts, G. C. K. (1975) *J. Magn. Reson.* **17,** 249–261.

36. Sternhell, S. (1969) *Q. Rev.* **23,** 236–270.

37. Chandrasekharan, R. & Ramachandran, G. N. (1970) *Int. J. Pept. Protein Res.* **2,** 223–233.

38. Platzer, K. E. B., Momany, F. A. & Scheraga, H. A. (1972) *Int. J. Pept. Protein Res.* **4,** 187–200.

39. Yamane, T., Andou, R. & Ashida, T. (1977) *Acta Crystallogr., Sect. B* **33,** 1650–1653.

40. Pasternak, R. A. (1956) *Acta Crystallogr.* **9,** 341–349.

41. Cotrait, M. & Barrans, Y. (1974) *Acta Crystallogr., Sect. B* **30,** 510–513.

42. Harada, Y. & Iitaka, Y. (1977) *Acta Crystallogr., Sect. B* **33,** 244–247.

43. Anteunis, M. J., Becu, C., Lala, A. K., Verhegge, G. & Narayan-Lala, K. (1977) *Bull. Soc. Chim. Belg.* **86,** 161–185.

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