NMR Studies on the Conformation of Aromatic Cyclodipeptides with two Non-identical L-Aromatic Amino-acid Residues in Solution: Cyclo-[L-5(MeO)Trp-L-Tyr(Me)]

M. Sheinblatt
Raymond and Beverly Sackler Faculty of Exact Sciences, School of Chemistry, Tel-Aviv University, Tel-Aviv 69978, Israel

The conformation of aromatic cyclodipeptides in solution is governed mainly by the interaction between the aromatic groups and the diketopiperazine ring. As a result, the aromatic groups in these molecules are folded over the DKP ring. In the case of cyclodipeptides containing two non-identical L-aromatic amino-acid residues, only one residue can be folded over the DKP ring. Thus, it is possible to study the relative strength of the interaction existing between the DKP ring and the different aromatic groups, by determining the conformation of these molecules in solutions. ^{1}H NMR conformation studies of cyclo-[L-5(MeO)Trp-L-Tyr(Me)] (**2**), and cyclo-[L-5(MeO)Trp-Gly] (**3**) in DMSO and DMF solutions are reported here. [Compound (**3**) serves as a model compound for cyclodipeptide molecules where the indole moiety of the 5(MeO)Trp residue is folded over the DKP ring]. The conformation analysis of (**2**) could be completed only by careful analysis of the results of the chemical shifts, the spin–spin coupling constants, and the NOE measurements. It was shown that the fractional populations of the side chain of the Trp and Tyr residues of (**2**), among the folded (*F*) and extended-to-nitrogen (E_n) conformers are approximately: 0.6, 0.4 and 0.4, 0.6 respectively. This conclusion implies the existence of a fast conformational equilibria (above room temperature) for each of the two residues between the *F* and E_n conformers. The study indicates, further, the sensitivity of conformational analysis of cyclodipeptides in solutions in determining the relative strength of the interaction between aromatic moieties and the DKP ring.

The interaction between different aromatic moieties and the diketopiperazine (DKP) ring plays a dominant role in determining the conformation of aromatic cyclodipeptide molecules. It was shown that in the solid [1,2] and in solution,[3–6] the indole [cyclo-(L-Trp-Gly)],[7,8] the benzene [cyclo-(L-Phe-Gly)],[7] the [cyclo-(L-Tyr-Gly)],[9,10] and the imidazole [cyclo-(L-His-Gly)] [7] are folded over their respective DKP rings.

NMR conformational studies in solutions of a cyclodipeptide molecule containing two non-identical L-aromatic amino acid residues [cyclo-(L-Trp-L-His) (**1**)],[11] show that the Trp residue is folded over the DKP ring (conformation *F*). The His residue is removed from the space over the DKP ring into the solvent, occupying the extended-to-nitrogen (E_n) conformer. (The His H-5 proton lies over the His peptide hydrogen of the DKP ring). Accordingly, we may conclude that the interaction between the indole and the DKP ring is stronger than the corresponding interaction between the imidazole (or imidazolium) and the DKP ring. The results indicate further that there is a definite spatial relationship between the two aromatic rings. The planes defined by the two aromatic rings intersect with each other in such a way that the His H-5 and one of its β-methylene protons lie over the six- and five-membered rings of the indole respectively. This proposed conformation differs from the conformation suggested for cyclodipeptide molecules with identical L-aromatic amino-acid residues, where it is assumed that the aromatic groups occupy the space over the DKP ring in a 'face-to-face' fashion.[6,12,13]

In order to extend our knowledge of the conformation of aromatic cyclodipeptides in solutions, and, hence, learn of the possible intramolecular interactions existing among aromatic rings, aromatic moieties and the DKP rings, we undertook to continue the studies on the conformation of cyclodipeptides with two non-identical L-aromatic amino-acid residues.

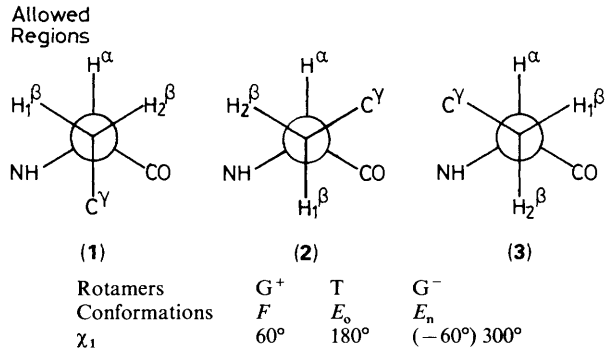


Figure 1. Newman projection of the rotational isomers about the C^{α}–C^{β} bond. $H_1{}^{\beta}$ and $H_2{}^{\beta}$ represent the *pro*-S and *pro*-R prochiral β protons of the Trp and Tyr residues respectively.

The conformation of aromatic cyclodipeptide molecules can be characterized by the geometry of the DKP ring, and by the two dihedral angles χ_1 and χ_2 describing the rotation of each of the side chains about the C^{α}–C^{β} and C^{β}–C^{γ} bonds respectively. The allowed conformations about the χ_1 angle is usually described by the three rotational isomers: G^+ (the folded conformation, *F*), T (extended to oxygen conformation, E_o), and G^- (extended to nitrogen conformation, E_n) respectively. (Newman projection of the three rotamers are shown in Figure 1). Each of the allowed rotamers is divided, as a function of χ_2, into two suballowed regions, corresponding to the perpendicular and antiperpendicular configurations respectively.

In the present paper, we present ^{1}H NMR studies of the conformation of the following cyclodipeptides in solution: cyclo-[L-5(MeO)Trp-L-Tyr(Me)] (**2**) together with the reference compounds cyclo-[L-5(MeO)Trp-Gly) (**3**).

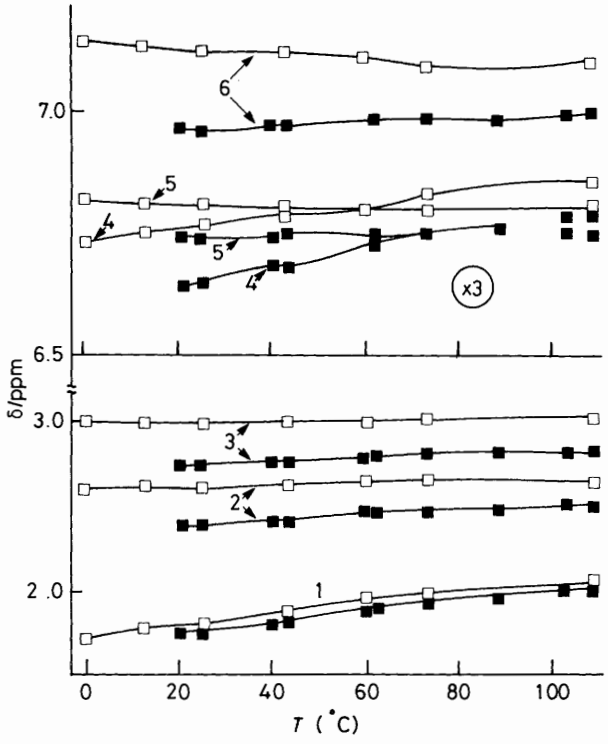


Figure 2. The chemical shift temperature dependence of several resonances of cyclo-[L-5(MeO)Trp-L-Tyr(Me)] (**2**) in DMSO (full squares) and DMF (open squares) solutions. The curves represent the following signals: 1-Tyr-$H^{\beta}(h)$, 2-Tyr $H^{\beta}(l)$, 3-Trp $H^{\beta}(l)$, 4-Tyr H^{δ}, 5-Tyr H^{ε}, 6-Trp H_2 (Note: the differences in scale of the ordinate in the upper and lower part of the figure).

Experimental

Cyclo-[L-5(MeO)Trp-L-Tyr(Me)] (**2**), and cyclo-[L-5(MeO)Trp-Gly] (**3**) were obtained from private sources (M. Wilchek). The proton NMR spectra were recorded on a Bruker AM-360 spectrometer. Chemical shifts were measured relative to internal references. The digital resolution of the spectra at room temperature was 0.15, at other temperatures 0.25 Hz per point.

The assignment of the spectra was accomplished by: successive decoupling studies and NOE measurements. Steady-state 1-D NOE and phase sensitive 2-D NOE were measured in DMSO solutions at room temperature. The results of the 2-D experiment are presented by the corresponding projection's rows which show the conductivities between the diagonal and the off-diagonal NOE signals.

Results and Discussion

The ^1H chemical shifts of (**2**) in DMSO and DMF solutions, and of (**3**) in DMSO solution at room temperature are summarized in Table 1. (The subscripts *h* and *l* of the β-methylene resonances represent the relative position of these signals in the spectra, high and low field respectively.) The temperature dependence on the chemical shifts of some of the resonances of (**2**) are shown in Figure 2. Some of the NOE results are shown in Figure 3. The measured coupling constants at room temperature of (**2**), (**3**), and (**1**) (for comparison purposes), which are important to the conformational analysis, are summarized in Table 2.

The results of (**3**) are in agreement with those reported previously for cyclo-(X-Gly) where X stands for an aromatic amino-acid residue. [Particularly there is a good agreement with the results of cyclo-(L-Trp-Gly).] The chemical-shift data, the measured spin–spin coupling constants ($^3J_{NH,H^{\alpha}}$ and $^3J_{H^{\alpha}H^{\beta}}$) and their temperature dependence, together with NOE results, suggest unequivocally that the 5(MeO)Trp residue of (**3**) occupies mainly the G^+ rotamer. It is worth mentioning that the NOE measurements indicate that the indole moiety is folded over the DKP ring occupying both the perpendicular and antiperpendicular conformations. (The last conclusion is based on the observed NOE signals of the Trp H-2 and H-4 following the selective irradiation of the Trp peptide hydrogen resonance). Further it is possible to assign the two Trp β-methylene protons from the NOE measurements.[11] Accordingly, the $H^{\beta}(h)$ and $H^{\beta}(l)$ resonances represent the prochiral $H_1{}^{\beta}$ and $H_2{}^{\beta}$ protons respectively.

The configuration of the DKP ring in (**2**) can be estimated from the measured values of the coupling constants $^3J_{NH,H^{\alpha}}$.[14] The measured values of $^3J_{NH,H^{\alpha}}$ of the two residues of (**2**) in DMSO solutions are equal. These values are a little higher than those calculated for planar DKP ring (2.1–2.5 Hz).[15–17] The above result indicates a small equal deviation from planarity of the DKP ring of (**2**) in DMSO (the H^{α} atoms being in the pseudo-equatorial position.) In DMF solutions, the results indicate that the DKP ring is almost planar.

The ^1H spectrum of the aromatic protons of the Tyr residue of (**2**) at room temperature are of an AB type, indicating the equivalence, among the two H^{δ} and two H^{ε} protons in the NMR time scale. The assignment of the H^{δ} and H^{ε} resonances of the Tyr residue at room temperature was accomplished by observing NOE signals of the H^{ε} and H^{δ} following the selective irradiation of the methyl resonance of the Tyr(Me) and the Tyr peptide hydrogen respectively.

The result, that at room temperature the H^{δ} resonates at a higher field than the H^{ε} (6.64 and 6.74 ppm respectively), differs from the measurement in random coil Tyr residues, where the Tyr H^{ε}s signals resonate at a higher field than those of the H^{δ}s (6.86 and 7.15 ppm respectively).[18] It is worth mentioning that the above observation seems to be odd even in ^1H NMR spectra of proteins, where the shifts of many resonances are influenced by the chemical-shift anisotropy effect of aromatic and other groups, *e.g.* lysozyme.[19]

Increasing the temperature results in a low-field shift of the Tyr H^{δ} resonance. At a certain range of temperature, 60–70 °C, the two signals of the Tyr H^{δ} and H^{ε} coalesce. Only at higher temperature (above 80 °C) the H^{ε} signal resonates at a higher field than the signal of H^{δ}. The calculated temperature coefficient of the chemical shifts of the NH resonances of the peptide Tyr, peptide Trp, and indole are in DMSO: -6.3×10^{-3}, -7.8×10^{-3}, and -6.3×10^{-3} ppm per °C and in DMF: -8.5×10^{-3}, -8.0×10^{-3}, -5.6×10^{-3} ppm per °C respectively. These results indicate that the NH protons of (**2**) are exposed to the solvent, more than the NH protons of (**1**).

The results for (**2**) show the following features: (*i*) High-field shifts of the Tyr resonances of one of its β-methylene protons $H^{\beta}(h)$, of H^{δ}, and of its peptide hydrogen. (*ii*) The measured values of the vicinal coupling of the Tyr residue show that $^3J_{H^{\alpha}H^{\beta}(h)} > {}^3J_{H^{\alpha}H^{\beta}(l)}$. (*iii*) Increasing the temperature results in: (*a*) a moderate low-field shift of the Tyr resonances of $H^{\beta}(h)$ and H^{δ}; (*b*) a small decrease and an increase in the values of the Tyr $^3J_{H^{\alpha}H^{\beta}(h)}$ and $^3J_{H^{\alpha}H^{\beta}(l)}$ respectively, and a small increase in the value of $^3J_{H^{\alpha}H^{\beta}(h)}$ of the Trp residue.

On the basis of previous studies,[7,9,11] it seems reasonable to conclude that the above results indicate the existence of a preferred conformation of (**2**). In this conformation the Trp residue is folded over the DKP ring and the Try residue occupies the extended conformers. Accordingly, the indole ring current of the folded Trp residue causes upfield shifts to the resonances of the Tyr H^{δ}, the Tyr peptide hydrogen, and to one of the Tyr H^{β} protons, which is *trans* with respect to the Tyr H^{α}.

However, careful comparison of the results for (**2**) and (**1**)

Table 1. ^{1}H Chemical shift (in ppm) of the Tyr(Me) and 5(MeO)Trp of (**2**) and the 5(MeO)Trp of (**3**) in DMSO (*D*) and DMF (*E*) solutions at room temperature.

Peptide/	Tyr(Me)							5(MeO)Trp									
Solvent	H^{α}	$H^{\beta}(h)$	$H^{\beta}(l)$	H^{δ}	H^{ϵ}	NH^{a}	CH_3	H^{α}	$H^{\beta}(h)$	$H^{\beta}(l)$	H-2	H-4	H-6	H-7	NH^{a}	CH_3	NH^{b}
(**2**)/*D*	3.78	1.77	2.41	6.64	6.74	7.68	3.66	3.96	2.50	2.77	6.97	7.00	6.73	7.21	7.90	3.77	10.76
(**2**)/*E*	3.93	1.81	2.63	6.76	6.81	7.65	3.73	4.13	2.69	3.04	7.12	7.17	6.79	7.29	7.87	3.84	10.85
(**3**)/*D*								4.01	2.98	3.22	7.02	7.06	6.69	7.21	8.08	3.75	10.76

[a] NH peptide. [b] NH indole.

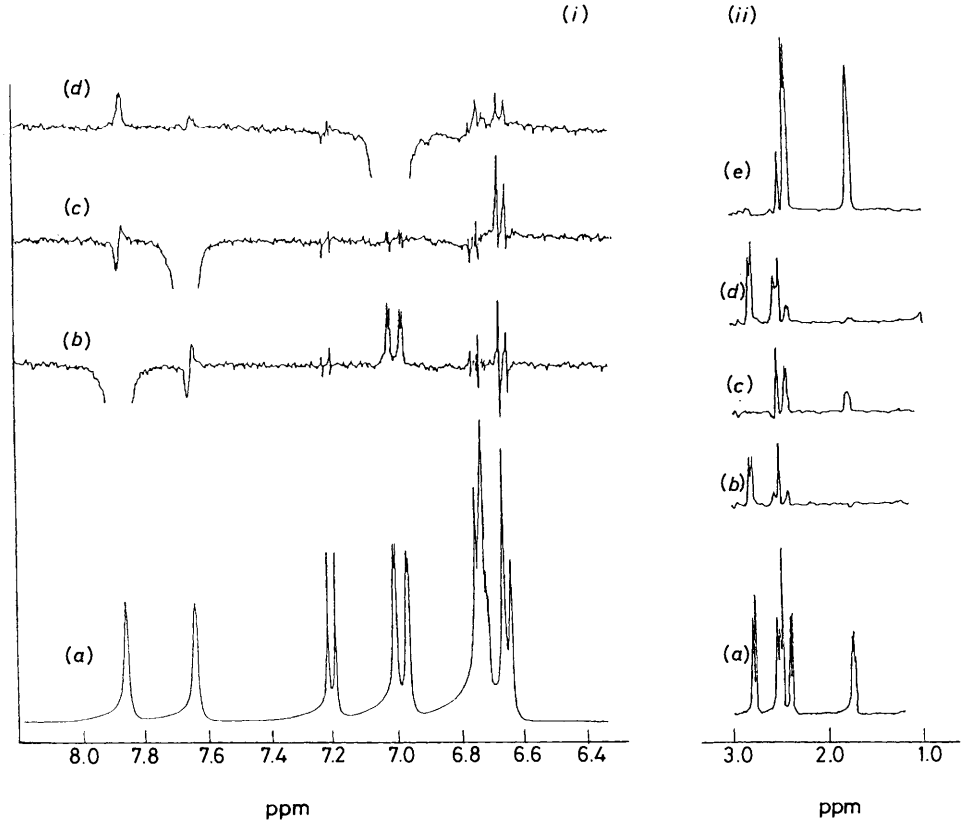


Figure 3. NOE spectra of (**2**) in DMSO solution at room temperature. Typical results of steady state 1-D NOE measurements are shown in (*i*). Sections of some of the projected rows of the phase sensitive 2-D experiments are shown in (*ii*).

(*ia*) A section of the low-field spectrum. The spectrum (from left to right) consist of the following signals. The peptide Trp NH, peptide Tyr NH, Trp H-4, Trp H-2, Tyr H^{ϵ} overlapping with Trp H-6, and Tyr H^{δ}.

(*ib*) NOE signals of the H-2 and H-4 following the selective irradiation of the Trp peptide hydrogen resonance.

(*ic*) NOE signal of the Tyr H^{δ} following the selective irradiation of the peptide Tyr NH resonance.

(*id*) NOE signals of the Tyr H^{δ}, Tyr H^{ϵ}, and Trp peptide hydrogen following the selective irradiation of the Trp (H-2) + (H-4) resonances.

(*iia*) A section of the high-field spectrum. The spectrum (from left to right) consist of the following signals: Trp $H^{\beta}(l)$, Trp $H^{\beta}(h)$, Tyr $H^{\beta}(l)$, and Tyr $H^{\beta}(h)$.

(*iib*). The NOE signal of the Trp H^{β} (*l*) which is connected with the diagonal signal of the peptide Trp NH.

(*iic*) The NOE signals of the Tyr $H^{\beta}(l)$ and $H^{\beta}(h)$ which are connected with the diagonal signal of the peptide Tyr NH.

(*iid*) The NOE signals of the Trp $H^{\beta}(h)$ and $H^{\beta}(l)$ which are connected with the diagonal Trp (H-2) + (H-4) signals.

(*iie*) The NOE signals of the Tyr $H^{\beta}(h)$ and $H^{\beta}(l)$ which are connected with the diagonal Tyr H^{δ} signal.

with their respective model compounds, show the following features: (*i*) The high-field shifts of the Tyr H^{δ} and $H^{\beta}(h)$ of (**2**) (which arises from the indole ring current effect) are smaller than the corresponding high-field shift of the His H-5 and $H^{\beta}(h)$ of (**1**) (which arise also from the indole ring current effect). (*ii*) The chemical shifts temperature dependence of the Tyr H^{δ} and $H^{\beta}(h)$ resonances of (**2**) is much smaller than that of the corresponding chemical shift temperature dependence of the His H-5 and $H^{\beta}(h)$ of (**1**). (*iii*) The vicinal coupling constant of $^3J_{H^{\alpha}H^{\beta}(h)}$ of the Tyr residue of (**2**) is smaller compared with the values of $^3J_{H^{\alpha}H^{\beta}(h)}$ ($\sim {}^3J_{trans}$) of the His residue in (**1**). (*iv*) There is a definite high-field shift of the resonances of the β-methylene group, and higher values of the $^3J_{H^{\alpha}H^{\beta}}$ of the Trp residue in (**2**) as compared to the values measured for the Trp in (**1**) and (**3**).

An explanation to the above observations would be to assume that in (**2**) the Trp and the Tyr residues occupy both the folded and one of the extended conformers. The relative decrease in the measured values of $^3J_{H^{\alpha}H^{\beta}}$ and the relative high-field shift of the Trp β-methylene resonances results from the partial occupancy of the Tyr residue of the *F* conformer. The

Table 2. Measured values of the spin–spin coupling constants (in Hz) of, $^3J_{NH,H^\alpha}$, $^3J_{H^\alpha H^\beta(h)}$, and $^3J_{H^\alpha H^\beta(l)}$ of (**2**), (**3**), and of (**1**) (for comparison purposes) at room temperature in DMSO (*D*) and DMF (*E*) solutions.

	(2)				(3)	(1)	
	Trp		Tyr		Trp	Trp	His
J/Hz	*D*	*E*	*D*	*E*	*D*	*C*	*C*
$^3J_{H^\alpha H^\beta(h)}$	5.8	6.9	7.2	7.9	4.4	4.6	9.9
$^3J_{H^\alpha H^\beta(l)}$	4.4	4.2	4.6	4.3	4.2	3.6	4.4
$^3J_{NH,H^\alpha}$	2.6	2.3	2.7	2.5	2.4	2.5	2.7

relative increase in the measured values of the Trp $^3J_{H^\alpha H^\beta}$ and relatively lower high-field shifts of the Tyr H^β and H^δ resonances arise from the partial occupancy of the Trp residue of the extended conformer.

It is possible to estimate the fractional population of the rotamer G^+ (g^+) of the two residues without knowing the exact assignment of the resonances of the β-methylene protons from the measured values of the respective $^3J_{H^\alpha H^\beta}$.[14] These calculations show that in (**2**), at room temperature, the fractional population of the Trp and Tyr residues in the G^+ rotamer are: 0.6 and 0.4 respectively, in agreement with the previous assumption.

One of the main obstacles in pursuing further the conformational analysis (*i.e.* the determination of the fractional population of each residue, between the extended E_n and E_o conformers) lies in the ambiguity in the assignment of the resonances of the diastereotopic β protons of each of the amino-acid residues.

The complete conformational analysis can be ultimately resolved, without the stereoselective deuteriation of the β-methyl groups,[20–23] only by a careful analysis of the results obtained from the chemical shifts, the spin–spin coupling constants, and the NOE measurements.

The relative spatial proximity between the peptide hydrogen (NH) and the H^β protons of a particular residue can be readily deduced from geometrical considerations (Figure 1). Thus, the distances NH–$H_1{}^\beta$ in the *F*, NH–$H_2{}^\beta$ in the E_n, and NH–$H_1{}^\beta$ in the E_o conformers are considerably shorter than the corresponding distances NH–$H_2{}^\beta$, NH–$H_1{}^\beta$, and NH–$H_2{}^\beta$ respectively. Further, the distances NH–$H_1{}^\beta$ in the *F* conformer are shorter than either NH–$H_2{}^\beta$ and NH–$H_1{}^\beta$ in the E_n and E_o conformers respectively.

The NOE measurements of the Trp residue show relatively strong NOE signals for the pair: $H^\beta(l)$ and the peptide hydrogen [Figure 3(*iib*)]. This observation can be attributed to the occupancy of the Trp residue in the folded conformer where $H_1{}^\beta$ is in spatial proximity with the peptide hydrogen. The above result implies, further, that the Trp residue occupies partially the E_n conformer out of the two extended conformers. In the E_n conformer we expect that $^3J_{H^\alpha H_2{}^\beta} > {}^3J_{H^\alpha H_1{}^\beta}$ and that signal of $H_2{}^\beta$ should shift toward higher fields, compared with the shift of the $H_1{}^\beta$ signal. (In the E_o conformer we expect that $^3J_{H^\alpha H_1{}^\beta} > {}^3J_{H^\alpha H_2{}^\beta}$ and that the $H_1{}^\beta$ signal should shift towards higher fields compared with $H_2{}^\beta$). Accordingly, we would assign the Trp $H^\beta(h)$ and $H^\beta(l)$ resonances to represent the prochiral $H_2{}^\beta$ and $H_1{}^\beta$ respectively. It is worth emphasizing that the above assignment is the opposite of that offered for the Trp residues in (**1**) and (**3**) where the Trp residues occupy mainly the *F* conformer.

The NOE measurements of the Tyr residue which indicate relatively strong NOE signals for the pairs $H^\beta(l)$—peptide hydrogen and a smaller NOE signal for the pair $H^\beta(h)$—peptide hydrogen [Figure 3(*iic*)] can be explained by assuming that the Tyr residue occupies the E_n conformer (out of the two extended conformers) as well as the *F* conformer. [The relatively strong NOE signal of the Tyr $H^\beta(l)$ arises from the partial occupancy of the Tyr residue of the *F* conformer ($g^+ = 0.4$). The relatively small NOE signal of the Tyr $H^\beta(h)$ arises from the partial occupancy of the Tyr residue of the E_n conformer ($g^- = 0.6$).]

Accordingly, the assignment of the β-methylene resonances of the Tyr residue is the following: The $H^\beta(h)$ and $H^\beta(l)$ resonances represent the prochiral $H_2{}^\beta$ and $H_1{}^\beta$ protons respectively.

The observed equal NOE signals of the Trp H-2 and H-4 following the selective irradiation of the Trp peptide NH resonance [Figure 3(*ib*)] suggest that the indole moiety in the *F* (and most probably also in the E_n conformer) occupies equally the perpendicular and antiperpendicular configurations.

The proximity between the two aromatic rings occupying the *F* and E_n conformers can be elucidated from the NOE signals of the Tyr H^δ and H^ε following the selective irradiation of the Trp H-2 and H-4 resonances [Figure 3(*id*)].

Establishing the assignments of the protons of the two β-methylene groups, it is possible to calculate the distribution of the fractional population of each of the two residues among the three rotamers using the well established procedure.[14] The exact results, however, depend on the values substituted for $^3J_{(gauche)}$ and $^3J_{(trans)}$ in the various rotamers.[24–26] We have chosen to apply two approaches, which are based essentially on the equation proposed by Kopple *et al.*[26] In the first method, we substituted for $^3J_{(gauche)}$ and $^3J_{(trans)}$ 3.25 and 12.5 Hz respectively. (These values are usually used in this type of calculation). In the second method we used a procedure identical with that described for (**1**).[11] (The averaged values of $^3J_{H^\alpha H^\beta}$, based on Kopple's equation, were calculated for the two β-methylene protons of each residue in the various allowed regions, where the actual size and geometry of the allowed regions were taken into consideration.) The calculated values of g^+, *t*, and g^- in DMSO and DMF solutions as a function of temperature, using the above two procedures, are summarized in Table 3. The calculated values obtained by the two methods have the same general features. The *T* rotamer is the less populated rotamer. However, the values obtained by the first method show a higher population of the *T* rotamer than those calculated by the second method. It is worth mentioning that geometrical considerations indicate that in aromatic cyclodipeptide molecules the two residues cannot be folded simultaneously over the DKP ring. Accordingly, the sum of g^+ of the two residues cannot be larger than 1. The results summarized in Table 3 show that this restriction is practically not violated by either of the two methods of calculations.

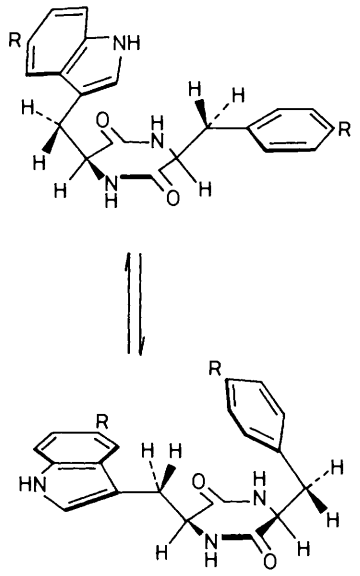
A decrease in the temperature, below *ca.* −20 °C, of the DMF solution is associated with the broadening of the Tyr $H^\beta(h)$ signal. This result indicates a decrease in the transition rate between the folded and extended conformers of the Tyr residue. The observation that the broadening of $H^\beta(h)$ resonances is more significant than other signals, can be explained by the large chemical shifts differences (> 1 ppm) of this signal in the *F* and E_n conformer, as well as by the differences in the expected values of $^3J_{H^\alpha H^\beta}$, in the above two conformers.

The NOE results in DMSO and DMF solutions are in good agreement, indicating that the conformation of (**2**) in the two solvents are essentially the same. However, the relative small differences in the chemical shifts and the coupling constants of $^3J_{H^\alpha H^\beta}$ in the two solvents (which are usually observed in various other cyclodipeptides measured in different solvents)[3,4,7] is interpreted quantitatively for (**2**) in terms of small changes in the calculated distributions of the fractional population among the rotational isomers of the two residues (Table 3). This difference arises, most probably, from the

Table 3. Measured values of the vicinal coupling constants $^3J_{H^\alpha H^\beta}$ of Tyr and Trp residues of (**2**), and the calculated distribution of the fractional populations among the three rotamers as a function of temperature in DMSO (*D*) and DMF (*E*) solutions.[a]

	Tyr		Trp		Tyr			Trp		
T/°C	$^3J_{H^\alpha H^\beta(h)}$	$^3J_{H^\alpha H^\beta(l)}$	$^3J_{H^\alpha H^\beta(h)}$	$^3J_{H^\alpha H^\beta(l)}$	g^+	g^+	t	g^+	t	g^-
Solvent *D*										
22	7.2	4.6	5.8	4.4	(0.42)	(0.15)	(0.43)	(0.60)	(0.13)	(0.28)
					0.43	0.08	0.49	0.66	—	0.34
40	7.2	4.7	5.8	4.4	(0.41)	(0.16)	(0.43)	(0.60)	(0.12)	(0.28)
					0.41	0.10	0.49	0.66	—	0.34
62	7.1	4.8	6.0	4.5	(0.42)	(0.10)	(0.42)	(0.56)	(0.14)	(0.30)
					0.43	0.10	0.47	0.61	0.03	0.36
89	7.0	4.8	6.0	4.5	(0.42)	(0.17)	(0.41)	(0.56)	(0.14)	(0.30)
					0.43	0.10	0.47	0.61	0.03	0.36
103	7.0	4.8	6.1	4.5	(0.42)	(0.17)	(0.41)	(0.55)	(0.14)	(0.31)
					0.43	0.10	0.47	0.60	0.03	0.37
Solvent *E*										
−10	8.1	4.0	6.0	4.1	(0.39)	(0.08)	(0.53)	(0.61)	(0.09)	(0.30)
					0.39	0.03	0.58	0.67	−0.03	0.36
0	8.0	4.1	6.1	4.1	(0.39)	(0.09)	(0.52)	(0.60)	(0.09)	(0.31)
					0.39	0.04	0.57	0.65	−0.02	0.35
13	7.9	4.2	6.4	4.1	(0.39)	(0.10)	(0.51)	(0.56)	(0.09)	(0.34)
					0.39	0.05	0.56	0.61	—	0.40
25	7.9	4.3	6.3	4.2	(0.38)	(0.11)	(0.51)	(0.56)	(0.10)	(0.33)
					0.37	0.07	0.56	0.61	0.61	0.39

[a] The values in parentheses were calculated substituting $^2J_{(gauche)}$ 3.25 and $^3J_{(trans)}$ 12.4 Hz.



Scheme. A schematic representation of the conformational equilibrium between the *F* and E_n conformer, of the two aromatic amino acid residues of cyclo-[L-5(MeO)Trp-L-Tyr(Me)] (**2**) in solution.

different interaction between the aromatic rings with the solvents, especially when the aromatic moieties occupy the E_n conformation.

Conclusions

The studies reported here indicate the possibility of evaluating in detail the conformation of cyclodipeptide molecules with two non-identical L-aromatic amino acid residues in solution from ^{1}H NMR measurements. The conformational analysis of cyclo-[L-5(MeO)Trp-L-Tyr(Me)] (**2**) in DMSO and DMF solutions reveals that each of the two residues occupies, to different degrees both the folded (*F*) and the extended to nitrogen (E_n) conformers. Calculations show that the fractional populations among the *F* and E_n conformer of the Trp and Tyr residues are approximately: 0.6, 0.4 and 0.4, 0.6 respectively. Accordingly, there is a fast conformational equilibrium (on the NMR time scale) of each of the two residues between *F* and E_n conformers (Scheme). The above results suggest that conformational analysis of cyclodipeptide molecules with two non-identical L-aromatic amino-acid residues in solution, enables us to determine a scale, which defines the relative strength of the interaction between different aromatic moieties and DKP rings.

This work indicates the unambiguity of applying chemical shifts data of a set of related compounds, (or of deuteriated β-methylene proton of model amino acids), for the assignment of the resonance of prochiral β-methylene protons of the corresponding amino-acid residue in peptides or proteins containing neighbouring aromatic amino acid.

Acknowledgements

The author is grateful to Professor M. Wilchek from the Weizmann Institute for supplying the two cyclodipeptides used in this work.

References

1 E. Benedetti, 1977 in 'Peptides, Proceedings of the Fifth American Peptide Symposium,' eds. M. Goodman and J. Meienhofer, pp. 257–273, Wiley, New York.
2 R. Ramani, K. Ventakeran, and R. E. Marsh, *J. Am. Chem. Soc.*, 1978, **100**, 949 and references cited therein.
3 J. J. Hruby, in 'Chemistry and Biochemistry of Amino Acids, Peptides and Proteins,' ed. B. Weinstein, Marcel Dekker, New York, 1974, pp. 1–188.
4 F. A. Bovey, in 'Peptides Polypeptides and Proteins,' eds. E. R. Blout, F. A. Bovey, M. Goodman, and N. Lotan, Wiley Interscience Publishers, New York, 1974, pp. 248–265.
5 Y. Imanishi, *Adv. Polym. Sci.*, 1976, **20**, 22.
6 M. J. O. Anteunis, *Bull. Soc. Chim. Belg.*, 1978, **87**, 627.
7 K. D. Kopple and M. Ohnishi, *J. Am. Chem. Soc.*, 1969, **91**, 962.
8 A. J. Morris, A. J. Geddes, and B. Sheldrick, *Cryst. Struct. Commun.*, 1974, **3**, 345.

9 K. D. Kopple and D. H. Marr, *J. Am. Chem. Soc.*, 1967, **89**, 6193.
10 C. F. Lim and L. E. Webb, *J. Am. Chem. Soc.*, 1973, **95**, 6803.
11 M. Sheinblatt, M. Andoren, and A. Rudi, *Int. J. Pept. Protein Res.*, 1988, **31**, 373.
12 H. Edelhoch, R. S. Bernstein, and M. Wilchek, *J. Biol. Chem.*, 1968, **243**, 5985; H. Edelhoch, R. E. Lippoldt, and M. Wilchek, *ibid.*, **243**, 4799.
13 R. Deslauriers, Z. Grzonka, K. Schaumburg, T. Shiba, and R. Walter, *J. Am. Chem. Soc.*, 1975, **97**, 5093.
14 V. F. Bystrov, in 'Progress in N.M.R. Spectroscopy,' eds. J. W. Emsley, J. Feeny, and L. H. Sutcliffe, 1976, pp. 41–81, Pergamon Press, Oxford.
15 V. F. Bystrov, S. L. Portnova, V. I. Tsetlin, V. T. Ivanov, and Y. A. Ovchinnikov, *Tetrahedron*, 1976, **25**, 493.
16 G. N. Ramachandran, R. Ramachandran, and K. D. Kopple, *Biopolymers*, 1971, **10**, 2113.
17 M. T. Cung, M. Marraud, and J. N'eet, *Macromolecules*, 1974, **7**, 606.
18 A. Bundi and K. Wuthrich, *Biopolymers*, 1979, **18**, 285.
19 C. Redfield, F. Poulsen, and C. M. Dobson, *Eur. J. Biochem.*, 1982, **128**, 527.
20 J. Kobayashi and U. Nagai, *Biopolymers*, 1978, **17**, 2265.
21 M. Kainosho and K. Ajisaka, *J. Am. Chem. Soc.*, 1979, **97**, 5630.
22 J. Kobayashi, T. Higashijima, S. Sekido, and T. Miyazawa, *Int. J. Pept. Protein Res.*, 1981, **17**, 486.
23 J. Kobayashi, T. Higashijima, and T. Miyazawa, *Int. J. Pept. Protein Res.*, 1984, **24**, 40.
24 K. Pachler, *G.R. Spectrochim. Acta*, 1964, **20**, 581.
25 M. T. Chung and M. Marraud, *Biopolymers*, 1982, **21**, 953.
26 K. D. Kopple, G. R. Wieley, and R. Taube, *Biopolymers*, 1973, **12**, 627.

Paper 9/00516A
Received 31*st January* 1989
Accepted 10*th July* 1989