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CONFORMATIONAL STUDY OF SUPER-ACTIVE ANALOGUES OF SOMATOSTATIN WITH REDUCED RING SIZE BY ^{1}H NMR

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ABSTRACTS

The conformational properties of five cyclic analogues related to somatostatin, and derived from the highly potent

D-Phe^1-Cys^2-Phe^3-D-Trp^4-Lys^5-Thr^6-Cys^7-Thr^8(ol) (SMS 201-995)

were investigated in DMSO-d6 and/or aqueous solution by ^{1}H NMR spectroscopy.

The results were compared with those previously obtained with the three closely related analogues SMS 201-995, Sandoz 204-090 and CTC. The eight compounds are active in inhibiting the secretion of growth hormone.

In water, we found the possibility of conformational equilibria involving γ turns. In DMSO, the N.M.R. results are in favour of a predominant conformation with a type II' β turn involving residues 3 to 6.

Many potent and selective analogues of the octapeptide SMS 201-995 [I] (table 1) (1-3), a very selective and long-acting somatostatin (SRIF) (4) analogue, have been synthesized (4-13) since his discovery (1).

In the analogues [II-V], [VII] and [VIII] (table 1), the Thr^8(ol) residue has been replaced by an amidated Thr (7-13), resulting in no significant change in in vitro inhibition of growth hormone secretion (7,14). In the case of SMS 201-995, this replacement (compound [II], table 1) reduces considerably the binding to brain SRIF receptors but does not affect the binding to hypophysial ones (14).

In other analogues (compounds [III] and [VI], table 1), Phe^3 has been substituted by Tyr^3, in combination (7-14) or not (5,6) with the previous modification. This substitution results in an interesting enhancement of affinity for both opiate and SRIF receptor systems. The hydroxyl group of Tyr^3 reduces the hydrophobicity of the molecules. As a consequence, in the case of the inhibition of the growth hormone release property, the potency enhancing effect of this replacement is compensated, as in the case of other

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somatostatin analogues synthesized by Veber et al. (15). To overcome this problem, the substitution of Phe^3 by Tyr^3 is coupled to the substitution of Thr^6 by Val^6 (7, 10-12, 15). The triple substitution of Phe^3 by Tyr^3, Thr^6 by Val^6 and Thr^8(ol) by Thr^8-NH_2 results in compound [IV] (table 1) (7, 10, 14). Its activity in inhibiting the growth hormone release is comparable to SMS 201-995 in vivo, but higher in vitro. Moreover, this analogue binds with very high affinity to SRIF receptors from the adenohypophyse, but does not bind to the brain ones (14). In the compound [VII] (table 1) obtained by substitution of $D\text{-}Phe^1$ by $D\text{-}Nal^1$ (D-Nal : 3-(2-naphtyl)-D-alanine) (11, 14), the growth

TABLE 1

	Code Name	Analogue
[I]	SMS 201-995	D-Phe^1-Cys^2-Phe^3-D-Trp^4 / (ol)Thr^8-Cys^7-Thr^6-Lys^5
[II]	DC-13-57	D-Phe^1-Cys^2-Phe^3-D-Trp^4 / H_2N-Thr^8-Cys^7-Thr^6-Lys^5
[III]	CTC	D-Phe^1-Cys^2-Tyr^3-D-Trp^4 / H_2N-Thr^8-Cys^7-Thr^6-Lys^5
[IV]	IM-IV-28 (DC-13-121)	D-Phe^1-Cys^2-Tyr^3-D-Trp^4 / H_2N-Thr^8-Cys^7-Val^6-Lys^5
[V]		D-Phe^1-Cys^2-Phe^3-D-Trp^4 / H_2N-Thr^8-Cys^7-Val^6-Lys^5
[VI]	Sandoz 204-090	D-Phe^1-Cys^2-Tyr^3-D-Trp^4 / (ol)Thr^8-Cys^7-Thr^6-Lys^5
[VII]	DC-13-116	D-Nal^1-Cys^2-Tyr^3-D-Trp^4 / H_2N-Thr^8-Cys^7-Val^6-Lys^5
[VIII]		D-Nal^1-Cys^2-Tyr^3-D-Trp^4 / H_2N-Thr^8-Cys^7-Abu^6-Lys^5

D-Nal : 3-(2-naphtyl)-D-alanine

Abu : α-aminobutyric acid

hormone inhibition potency is lowered, but the duration of action as compared to either analogues [I] (SMS 201-995) and [IV] (IM-IV-28) is increased. Both effects could be due in part to the increased lipophilicity (and thus slower absorption) of this compound (11).

Analogues [IV] and [VII] distinguish more clearly between pituitary and brain receptors than SMS 201-995 [I]. Compound [VII] can be radioiodinated and is stable to enzymatic degradation. It could be a very powerful ligand in the study of SRIF receptors.

N.M.R. studies of compounds [I] (16-18), [III] (19,20) and [VI] (18) have been performed recently in aqueous solution and in DMSO.

In the present study, we compare the ^{1}H N.M.R. results of compounds [II], [IV], [V] and [VII] in water and of compounds [IV] and [VII] in DMSO with the results previously obtained with the three other analogues, in order to correlate the observed conformational changes with the biological activity results.

We also present an N.M.R. study of analogue [VIII], a compound derived from [VII] and containing an Abu residue at the sixth position (Abu : α-aminobutyric acid). It retains the full biological activity of [VII] and seems to be particularly effective on inhibition of pancreatic amylase release.

MATERIALS AND METHODS

The synthesis of analogues [I-VII] has already been described in the literature (7,10). The ^{1}H N.M.R. spectra were acquired with Bruker AM 270 and AM 500 spectrometers equipped respectively with Aspect 2000 and 3000 computers.

Spectra in aqueous solution were recorded in 99.95 % $^{2}H_2O$ from the CEA at pH 4.0 (compound [IV] : 3.5 mM) and pH 2.7 (compound [II] : 2.5 mM; [V] : 1.9 mM; [VII] : 2.9 mM; [VIII] : 3.1 mM).

The amide proton parameters were obtained from 3.9 mM ([II]), 2.5 mM ([IV]), 1.9 mM ([V]), 2.4 mM ([VII]) and 2.7 mM ([VIII]) solutions containing about 10 % $^{2}H_2O$ - 90 % $^{1}H_2O$, at pH 2.7.

Samples in dimethylsulfoxyde (DMSO) were prepared by dissolving the products in $^{1}H_2O$ ([IV] : 3.0 mM; [VII] : 1.8 mM), adjusted at pH 2.6, dried in vacuo, dissolved in DMSO-d6 (99.95 %, from the CEA), evacuated and sealed.

In both solvents, the two-dimensional correlation spectroscopy (COSY) (21) and/or double-quantum filtered correlation spectroscopy (DQF-COSY) (22,23) have given the connectivities inside each amino-acid residue. To verify some assignments, we have also used the homonuclear relay experiment (RELAY) (24,25). Both types of experiments have been performed using the time-proportional phase incrementation method (TPPI) (26,27) and transformed in the phase-sensitive mode (27).

The aromatic residues were assigned with the aid of the COSY with delays (COSYLR) sequence (28,29) by observing ^4J long-range connectivities between the ß and aromatic protons (29,30). The same technique allowed the observation of some ^5J connectivities between neighbouring α protons in water (29). In DMSO, the sequencing of the molecule was achieved by using two-dimensional NOE spectrosopy (NOESY) (31,32) to obtain NOE connectivities (33) between α and amide protons of neighbouring amino acid residues (34).

Some accurate chemical shifts were given by the projection of a two-dimensional J-resolved (2D J-resolved) (35) spectrum. Sodium 2,2-dimethyl-2-silapentane (DSS) and tetramethylsilane (TMS) were choosen as external references respectively in aqueous solution and in DMSO.

All 2D data matrices were multiplied in both t_1 and t_2 dimensions by a sine-bell prior to Fourier transformation.

Due to the poor solubility of the peptides in water and in order to avoid aggregation problems (36) in DMSO, low concentrations have been used in both solvents.

RESULTS AND DISCUSSION

(1). Study in DMSO

Phase-sensitive DQF-COSY spectra at 30°C and 500 MHz yielded the connectivities within the spin systems and the direct assignment of the Lys^5, Val^6 and Thr^8-NH_2 residues (Fig. 1).

COSYLR spectra (Δ = 100 msec) provided ^4J long-range connectivities between one of the D-Phe^1 β protons and its H_2 and H_6 protons, the β and H_2 and H_6 protons of Tyr^3, and the β and H_2 protons of D-Trp^4 (Fig. 2).

No ^4J connectivities have been found between the H_1 and H_3 and β protons of D-Nal^1 (compound [VII]). At this pH, this residue was identified by default, and it doesn't exhibit any correlation between its α and NH_2 protons. Another COSYLR spectrum (Δ = 100 msec) and a second DQFCOSY spectrum, both performed at pH 5.7 (2.5 mM solution), have provided respectively a ^4J connectivity between the H_1 and one of the β proton of D-Nal^1 and correlations between the D-Nal^1 α and β protons.

Using NOESY spectra (τ_m = 120 msec) (Fig. 3), we discriminated the Cys^2 and Cys^7 systems through NOE connectivities between the NH of Cys^2 and the D-Phe^1 α proton, the NH of Tyr^3 and the Cys^2 α proton, the NH of Cys^7 and the Val^6 α proton, and the NH of Thr^8-NH_2 and the Cys^7 α proton. These last spectra confirmed the assignments of the other residues at the same

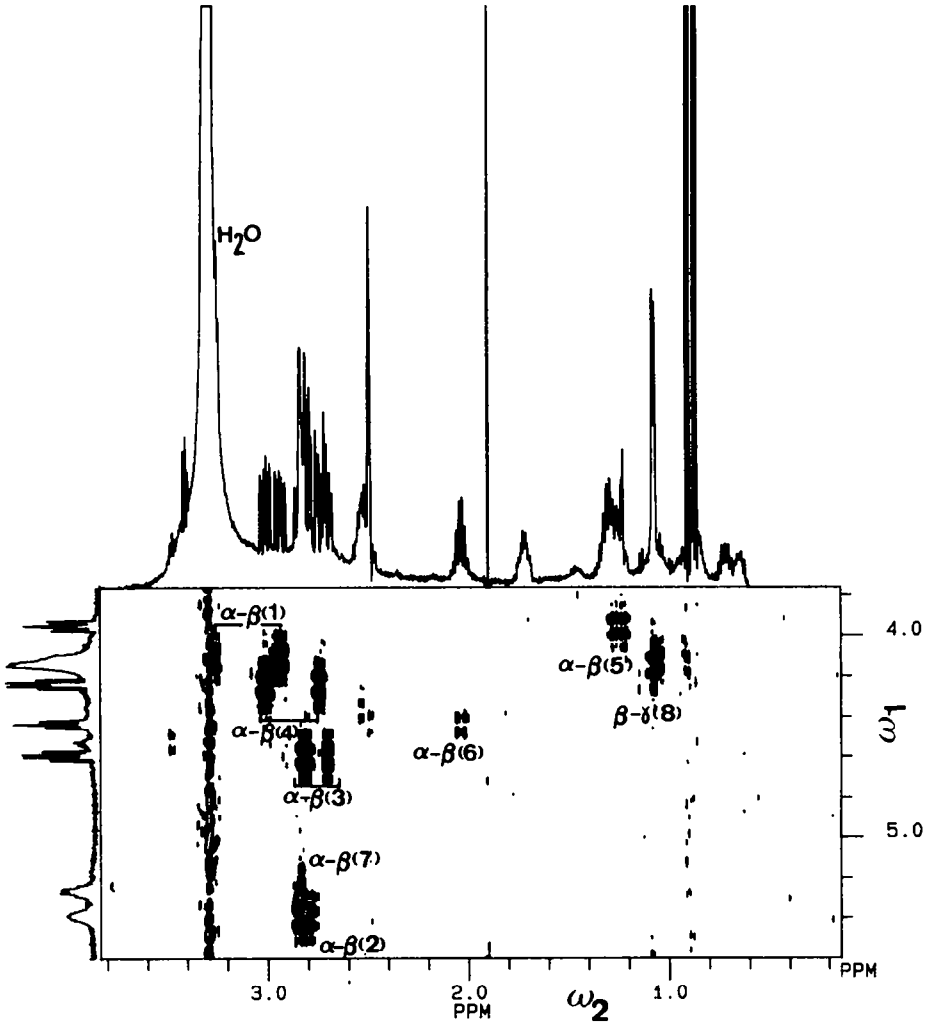

Figure 1 : 3.75-5.60 (ω_1) and 0.14-3.84 (ω_2) p.p.m. region of a ^1H phase-sensitive COSY spectrum of analogue [IV] in DMSO (3.0 mM, pH 2.6, 30°, 500 MHz).

time. An important cross-peak exists between the NH proton resonances of Lys^5 and Val^6. In the case of compound [VII], no correlation was detected between the α proton of D-Nal^1 and the Cys^2 NH proton. This is probably due to the very weak intensity of both the Cys^2 amide proton and the D-Nal^1 α proton signals.

The NH resonances were measured over 25-50°(temperature intervals = 5°). Tables 2-5 list the amide proton N.M.R. parameters and the chemical shifts of the aromatic side chain protons of both analogues [IV] and [VII].

The results of spin system assignments are given in tables 6 and 7. Some of them

TABLE 2

THE ^{1}H N.M.R. AMIDE PROTON PARAMETERS OF COMPOUND [IV] IN AQUEOUS SOLUTION (2.5 mM, pH 2.7, 25°) AND DMSO (3.0 mM, pH 2.6, 30°). The $^{3}J_{NH-C\alpha H}$ coupling constants and the $\Delta\delta/\Delta T$ values are given respectively in Hz and in p.p.b./°K.

Amino acids	In aqueous solution		In DMSO	
	$^{3}J_{NH-C\alpha H}$	$\Delta\delta/\Delta T$	$^{3}J_{NH-C\alpha H}$	$\Delta\delta/\Delta T$
D-Phe[1]	-	-	-	-
Cys[2]	8.2	0.8	8.7	-7.3
Tyr[3]	8.8	4.4	8.1	-3.7
D-Trp[4]	4.3	0.6	5.6	-6.2
Lys[5]	6.8	2.5	8.8	-3.5
Val[6]	8.0	5.7	9.5	-0.5
Cys[7]	9.2	1.8	9.5	-4.9
Thr[8]-NH_2	7.8	3.9	6.8[a]	-3.0

[a]. At pH 6.

TABLE 3

THE ^{1}H 500 MHz N.M.R. CHEMICAL SHIFTS (in p.p.m.) OF COMPOUND [IV] AROMATIC PROTONS IN $^{2}H_2O$ (2.5mM, pH 4.0, 25°) AND DMSO (3.0 mM, pH 2.6, 30°).

Amino acid		δ in $^{2}H_2O$	δ in DMSO
D-Phe[1]	o	7.394	7.334
	m	7.436[b]	7.265
	p	7.388	7.330[b]
Tyr[3]	o	7.160	6.913
	m	6.849	6.633
	OH	-	-
D-Trp[4]	H_1	10.174[a]	10.848
	H_2	7.168	7.067
	H_4	7.599	7.417
	H_5	7.177	6.974
	H_6	7.253	7.049
	H_7	7.499	7.382

[a]. Extracted from a spectrum in 90 % $^{1}H_2O$ - 10 % $^{2}H_2O$ (pH 2.7, 25°, 2.5 mM).

[b]. Obtained from a 2D-J-resolved spectrum.

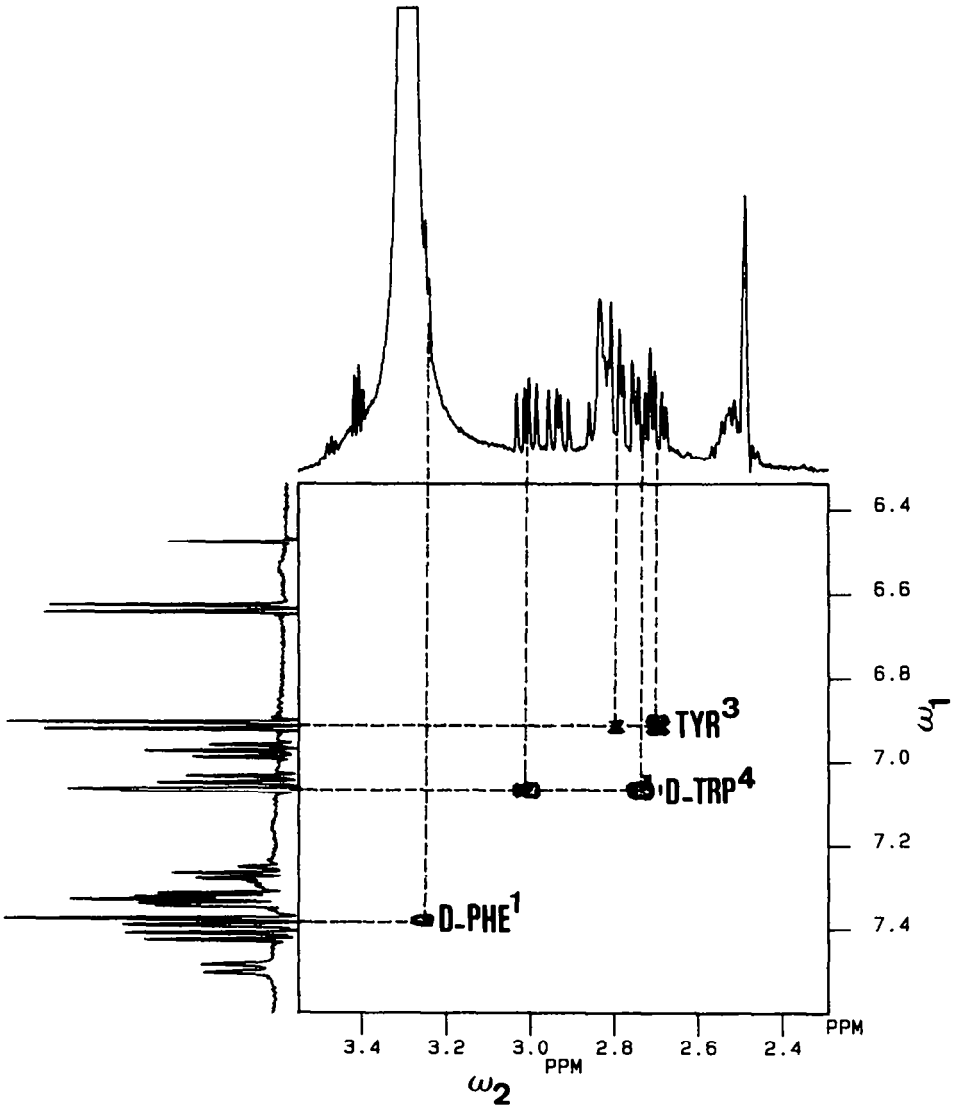

Figure 2 : 6.33-7.60 (ω_1) and 2.29-3.56 (ω_2) p.p.m. region of a ^{1}H COSY with delays spectrum (Δ = 100 msec) of compound [IV] in DMSO (pH 2.6, 3.0 mM, 30°) at 500 MHz. The dashed lines show the ^{4}J connectivities between the β and aromatic protons of the aromatic residues.

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were verified by a RELAY spectrum (delay = 25 msec; analogue [IV]).

In figures 4-6, we have represented the effects on respectively the $\Delta\delta/\Delta T$, δ_α and $^3J_{NH\text{-}C\alpha H}$ values, of the successive replacement in compound [I] of Phe^3 by Tyr^3 (compound [VI]), Thr(ol) by Thr^8-NH$_2$ (compound [III]) and Thr^6 and Val^6 (compound [IV]). The substitution of D-Phe^1 by D-Nal^1 is not represented (analogue [VII]).

The Cys^2 and Cys^7 α proton chemical shifts (Fig. 5) show important downfield shifts compared with the random coil values (37,38). They might be due to the influence of the D-Phe^1 or/and Phe^3 or Tyr^3 aromatic rings. This effect is emphasized when D-Phe^1 is replaced by D-Nal^1. From figure 5, it

TABLE 4

THE ^{1}H N.M.R. AMIDE PROTON PARAMETERS OF ANALOGUE [VII] IN DMSO (1.8 mM, pH 2.6, 30°) AND AQUEOUS SOLUTION (2.4 mM, pH 2.7, 25°). $^3J_{NH-C\alpha H}$ coupling constants : in Hz; $\Delta\delta/\Delta T$ values : in p.p.b./°K.

Amino acids	In aqueous solution		In DMSO	
	$^3J_{NH-C\alpha H}$	$\Delta\delta/\Delta T$	$^3J_{NH-C\alpha H}$	$\Delta\delta/\Delta T$
D-Nal[1]	-	-	-	-
Cys[2] .b	8.4	1.6	-.a	-5.0
Tyr[3]	8.8	4.6	8.1	-4.0
D-Trp[4]	3.8	0.9	5.4	-5.8
Lys[5]	7.3	2.8	8.7	-3.6
Val[6]	8.0	6.4	9.2	-0.2
Cys[7] .b	8.5	3.4	9.5	-5.1
Thr[8]-NH_2	8.0	5.3	8.7	-5.0

a. Strong line broadening

b. Can be reserved in aqueous solution.

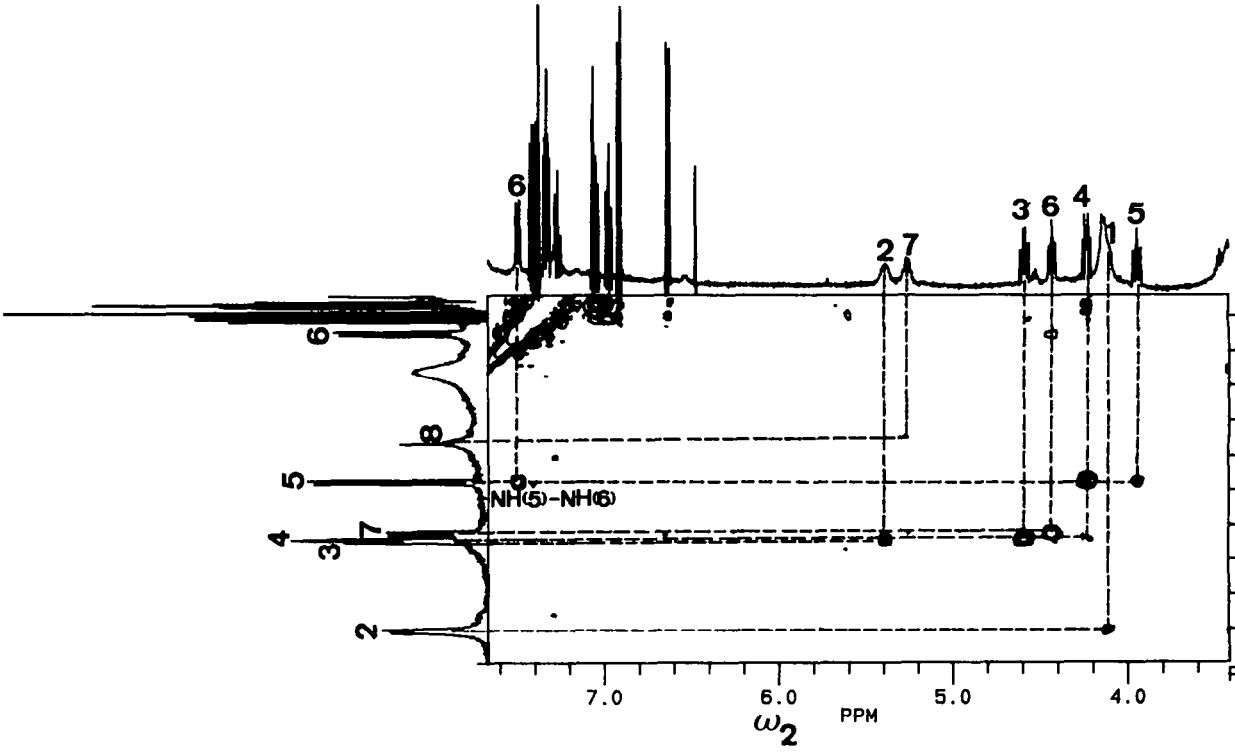


Figure 3 : 7.27-9.40 (ω_1) and 3.42-7.68 (ω_2) p.p.m. part of the 500 MHz NOESY spectrum (τ_m = 120 msec) of analogue [IV] in DMSO (3.0 mM, pH 2.6, 30°). The dashed lines indicate important NOE connectivities.

TABLE 5

THE ^{1}H 500 MHz N.M.R. CHEMICAL SHIFTS (in p.p.m.) OF ANALOGUE [VII] AROMATIC PROTONS IN $^{2}H_2O$ (2.9 mM, 25°) AND DMSO 91.8 mM, pH 2.6, 30°)

Amino acid		δ in $^{2}H_2O$	δ in DMSO
D-Nal[1]	H_1	7.798	7.875
	H_3	7.481	7.565
	H_4	7.934	7.883
	H_5	7.882·[c]	7.883
	H_6	7.56·[b]	7.49·[b]
	H_7	7.56·[b]	7.49·[b]
	H_8	7.930·[c]	7.883
Tyr[3]	o	7.129	6.913
	m	6.852	6.629
	OH	-	-
D-Trp[4]	H_1	10.158·[a]	10.804
	H_2	7.136	7.070
	H_4	7.544	7.421
	H_5	7.159	6.977
	H_6	7.239	7.052
	H_7	7.481	7.326

[a]. Obtained from a spectrum in 90 % $^{1}H_2O$ - 10 % $^{2}H_2O$ (pH 2.7, 25°, 2.4 mM).

[b]. Strong overlap.

[c]. Can be reversed.

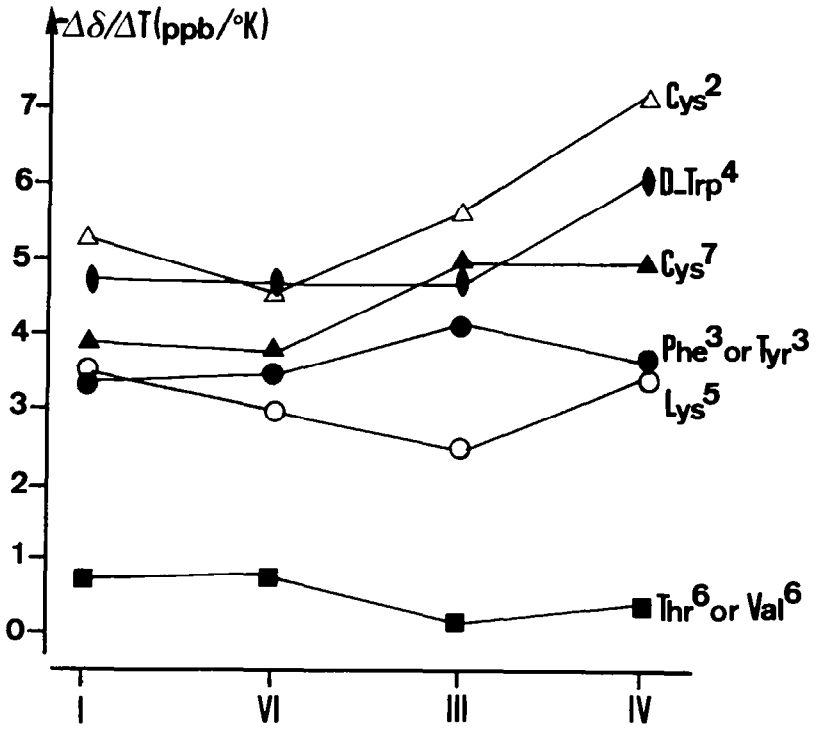


Figure 4 : comparison of the amide proton $\Delta\delta/\Delta T$ values of analogues [I], [III], [IV] and [VI] in DMSO

TABLE 6

THE ^{1}H 500 MHz N.M.R. PARAMETERS OF COMPOUND [IV] IN DMSO (3 mM, pH 2.6, 30°)
The chemical shifts δ.[a] and the coupling constants J.[a] are given respectively in p.p.m. and in Hz.

Amino acid	δ_α	δ_β	δ_γ	δ_δ	δ_ϵ	$^3J_{\alpha\beta}$	$^2J_{\beta_I\beta_{II}}$	$^3J_{\beta\gamma}$	δ_{NH}
D-Phe[1]	4.112.[b,c]	2.931 / 3.253.[b]				9.6 / 3.7.[d]	14.1		-
Cys[2]	5.384	2.783 / 2.833.[b]				10.4 / -.[c]	14.5		9.205
Tyr[3]	4.586	2.694 / 2.810				5.2 / 8.9	13.8		8.680
D-Trp[4]	4.235	2.736 / 3.008				6.0 / 9.2	14.7		8.680
Lys[5]	3.947	1.28.[c] / 1.716	0.666 / 0.723	1.28.[c]	(2.52 / 2.52).[c]	11.6 / 3.5	-		8.344
Val[6]	4.433	2.033	0.864 / 0.900			8.6		6.7 / 6.7	7.494
Cys[7]	5.260	2.828				7.6			8.637
Thr[8]-NH_2	4.138.[b,c]	4.159.[b,c]	1.072			4.1.[c,d]		6.2	8.118

[a]. If 2 β protons exist, the upper values are for the β_I protons.
[b]. Obtained from a DQF-COSY spectrum.
[c]. Strong line broadening and/or important overlap.
[d]. At pH 6.

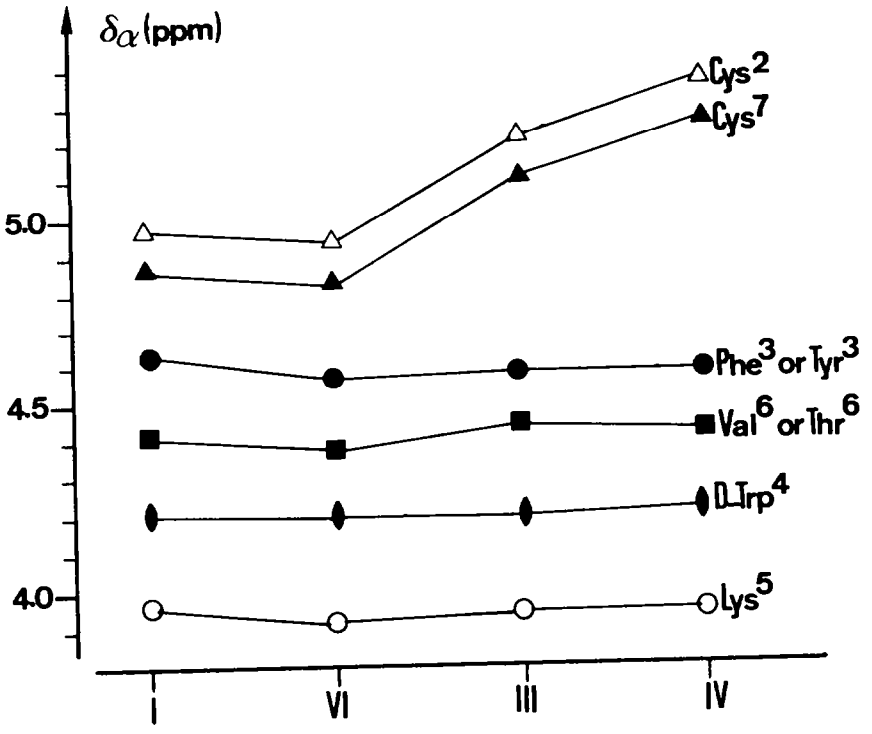


Figure 5 : comparison of the δ_α chemical shifts (endocyclic residues) of compounds [I], [III], [IV] and [VI] in DMSO.

appears clearly that the replacement of Thr^8(ol) by Thr^8-NH_2 and of Thr^6 by Val^6 both contribute to an additional increase of these downfield shifts.

The large upfield shift of Lys^5 γ proton resonances could be due to the influence of the D-Trp^4 aromatic ring on the Lys^5 γ protons (39-41).

For the five compounds, the $^3J_{NH-C\alpha H}$ values are compatible with the existence of a β turn [3,4,5,6] of type II' (42,43) and two γ turns [3,4,5] and [4,5,6] (42,43). Moreover, in the case of analogues [I] and [VI], they are also compatible with a β turn [3,4,5,6] of type I' (42,43). The small $\Delta\delta/\Delta T$ of the Thr^6 or Val^6 NH proton reveals a stabilisation of the turns by an intramolecular hydrogen bond, and excludes the [3,4,5] γ turn. The observed NOE effects (Fig. 3) are in favour of the β turn of Type II'. The very strong NOE effect between the NH of D-Trp^4 and Lys^5 α proton could be explained by the β turn of type II' which allows a good proximity of these groups. It leaves the possibility of the [4,5,6] γ turn and is a good argument against the existence of a β turn of type I'. Considering the important NOE effects existing between the NH of Phe^3 or Tyr^3 and the Cys^2 α proton, and between the NH Cys^7 and the Thr^6 or Val^6 α proton, we could assume the existence of a conformation in which the NH group of Phe^3 or Tyr^3 is oriented towards the carbonyl group of Thr^6 or Val^6.

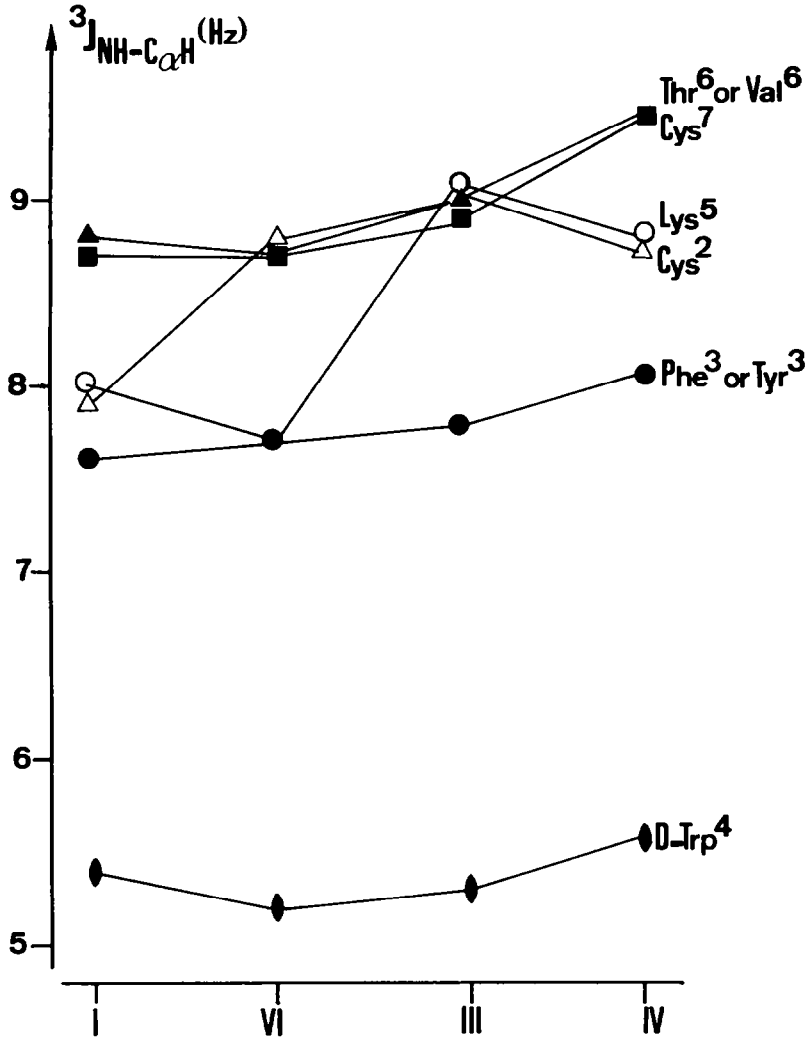


Figure 6 : comparison of the $^3J_{NH-C\alpha H}$ values of the endocyclic residues of compounds [I], [III], [IV] and [VI] in DMSO.

An important NOE effect exists between the NH's of Lys^5 and Thr^6 or Val^6 residues, yielding an additional argument in favour of the β turn of type II'. An hydrogen bond between the Thr^8(ol) or Thr^8-NH_2 NH group and the Cys^2 carbonyl group is not excluded. The Thr^8(ol) or Thr^8-NH_2 amide group exhibits a strong $\Delta\delta/\Delta T$ variation as a function of the pH. It could be due to the proximity of the D-Phe^1 or D-Nal^1 NH_2-terminal group. Compared to the native hormone SRIF (44), the D-Trp^4 and Lys^5 α resonances are upfield shifted. This is in agreement with a predominant conformation at the D-Trp^4-Lys^5 level.

To conclude, it appears that the five analogues exhibit similar backbone equilibria in DMSO, with a predominant β turn [3,4,5,6] of type II'.

If conformational differences exist, they must mostly be located at the side-chain level.

TABLE 7

THE ^{1}H 500 MHz N.M.R. PARAMETERS OF ANALOGUE [VII] IN DMSO (1.8 mM, 30°, pH 2.6).
Coupling constants J : in Hz; chemical shifts δ : in p.p.m.

Amino acids	δ_{NH}	δ_α	δ_β	δ_γ	δ_δ	δ_ϵ	$^3J_{\alpha\beta}$	$^2J_{\beta_I\beta_{II}}$	$^3J_{\beta\gamma}$
D-Nal[1]	-	3.627[a]	(2.778 / 3.236)[a]				(9.6 / 4.4)[a]	13.4[a]	
Cys[2]	9.199	5.443	2.770 / 2.834				4.0 / -[b]	14.9	
Tyr[3]	8.685	4.597	2.702 / 2.812				5.5 / 9.7	13.5	
D-Trp[4]	8.699	4.324	2.740 / 3.010				6.1 / 9.3	14.1	
Lys[5]	8.350	3.950	1.239 / 1.726	0.645 / 0.708	(1.32 / 1.32)[b]	2.530 / 2.543	11.6 / 3.3		
Val[6]	7.495	4.445	2.037	0.873 / 0.909			8.6		6.7 / 6.7
Cys[7]	8.632	5.352	2.799 / 2.85[b]				6.4 / -[b]	14.8	
Thr[8]-NH_2	8.210	4.272	4.066	1.071			3.0		6.3

[a]. At pH 5.7 (2.5 mM solution).
[b]. Important overlap and/or line broadening.

(2). Study in water

In $^{2}H_{2}O$, the connectivities within each amino acid residue were obtained from DQF-COSY spectra. Lys^{5}, Val^{6} or Thr^{6} or Abu^{6} and Thr^{8}-NH_{2} were directly assigned by inspection, but without distinction between Thr^{6} and Thr^{8}-NH_{2} (compound [II]). Phase-sensitive DQF-COSY spectra (analogues [II], [V], [VII] and [VIII]) at 500 MHz and a COSY spectrum (analogue [IV]) at 270 MHz in 90% $^{1}H_{2}O$ - 10% $^{2}H_{2}O$ yielded the correlations between the amide and α protons (see, for example, Fig. 7).

COSY with delays spectra (analogues [II], [V], [VII] and [VIII] : Δ = 100 msec; analogue [IV] : Δ = 85 msec) allowed the identification of the aromatic

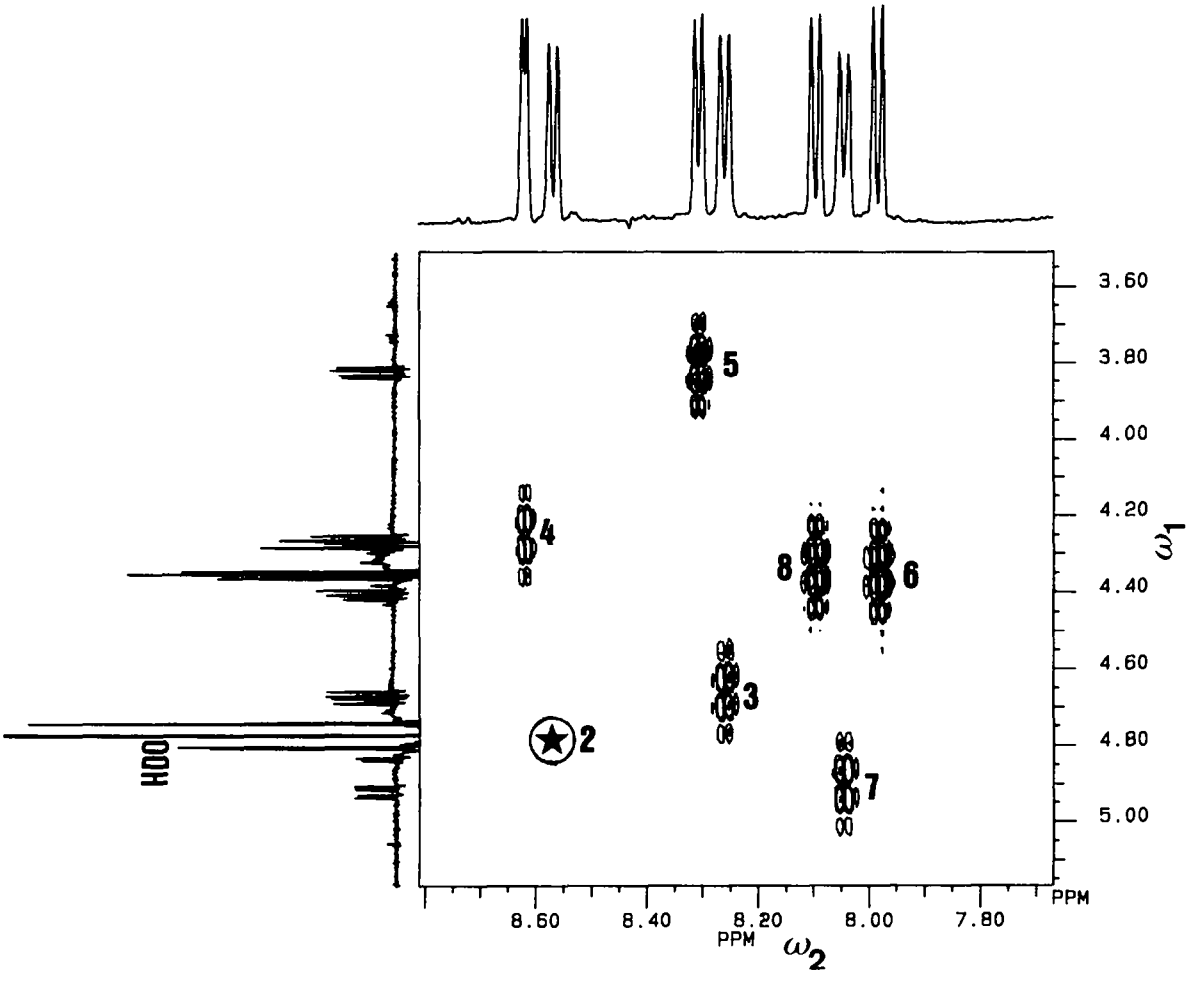
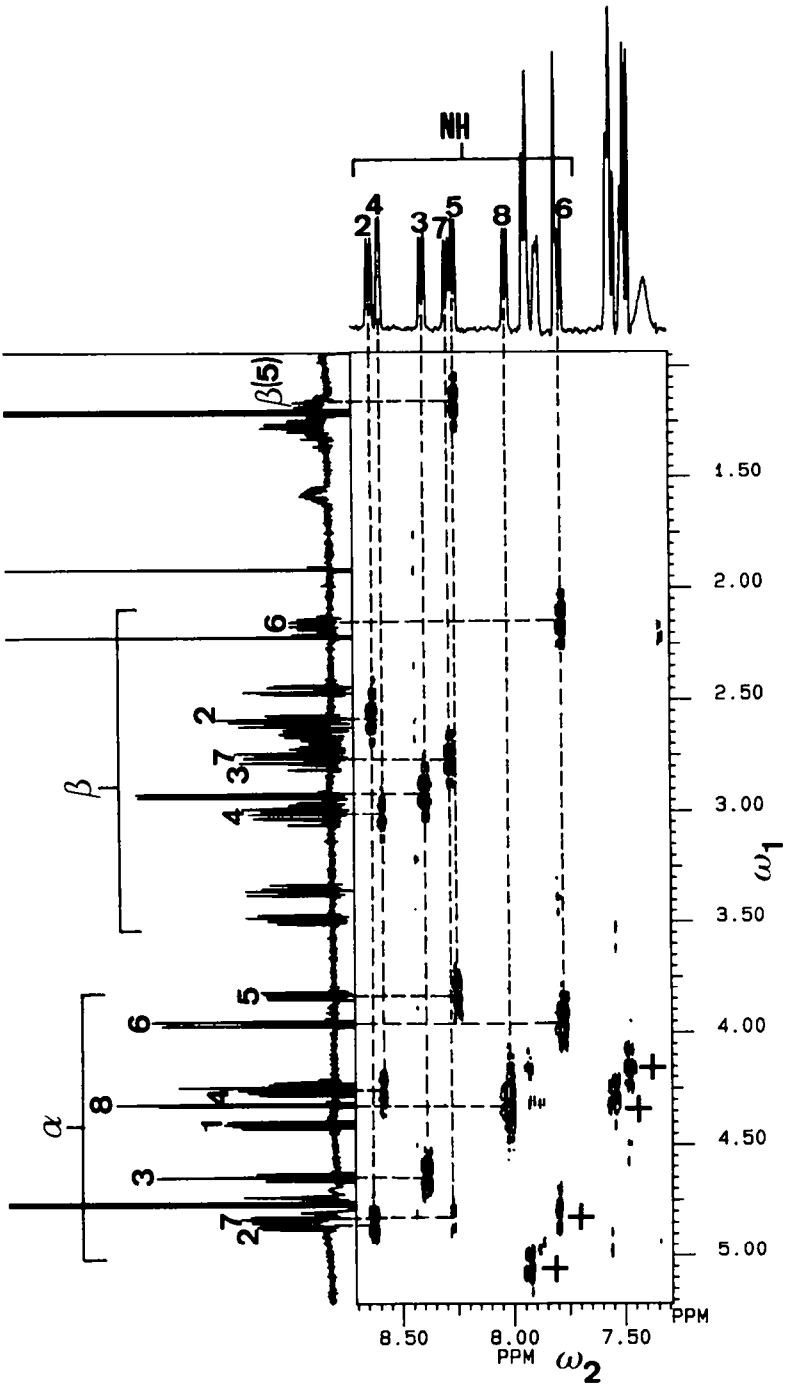


Figure 7 : 3.50-5.17 (ω_1) and 7.67-8.82 (ω_2) p.p.m. region of a ^{1}H phase-sensitive DQF-COSY spectrum of analogue [II] in 90% $^{1}H_{2}O$ - 10% $^{2}H_{2}O$ (3.9 mM, pH 2.7, 25°, 500 MHz).
★ : the correlation between the Cys^{2} NH and α protons is visible only at very low level (Cys^{2} α proton burried under the water peak).



8 : 7.28-8.72 (ω_1) and 0.91-5.22 (ω_2) p.p.m. region of a 500 MHz phase-sensitive RELAY spectrum of analogue [VII] in 90% ^1H_2O - 10% ^2H_2O (2.4 mM, pH 2.7, 500 MHz) (delay = 20 msec).
The dashed lines show the α-NH and β-NH connectivities.
+ : artefacts.

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s by obtaining ^4J connectivities between the β and H_2 protons of the β and H_2 and H_6 protons of D-Phe^1 and Tyr^3 or Phe^3, and

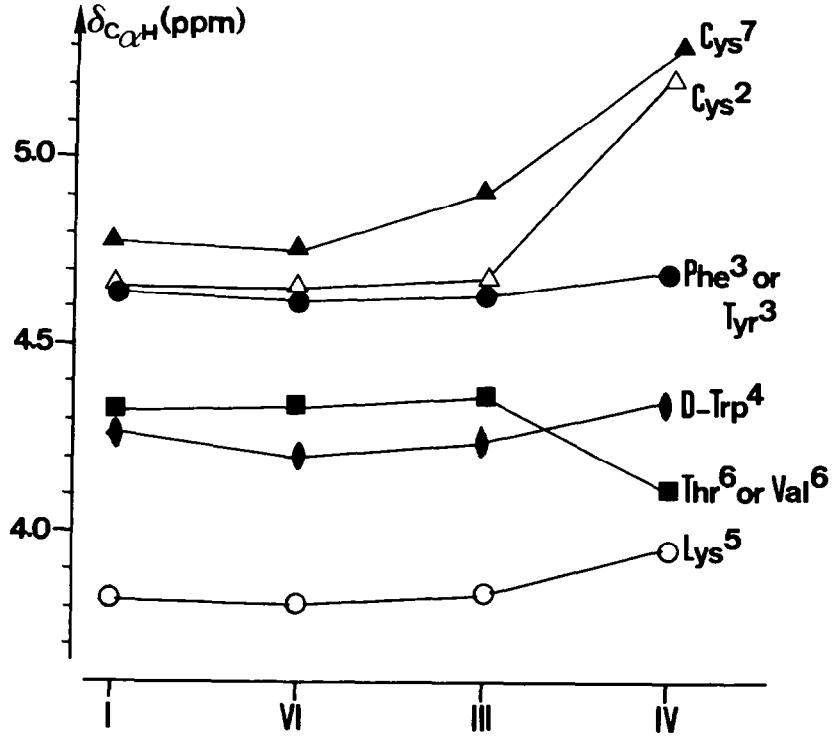

Figure 9 : comparison of the δ_α chemical shifts (endocyclic residues) of compounds [I], [III], [IV] and [VI] in ^2H_2O.

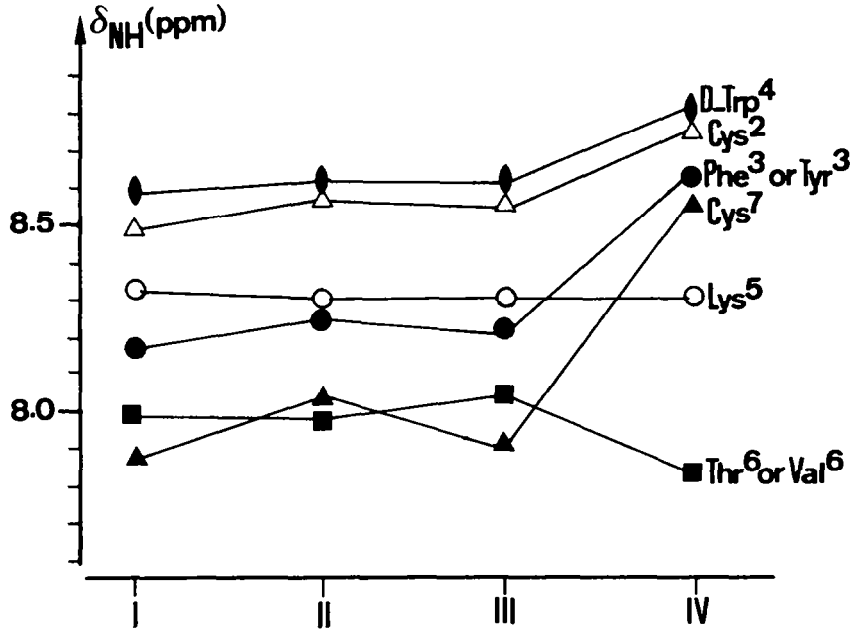

Figure 10 : comparison of the δ_{NH} values (endocyclic residues) of compounds [I-IV] in aqueous solution.

the β and H_1 and H_3 protons of D-Nal[1]. For analogue [IV], a second experiment at 40° (Δ = 50 msec) was necessary to separate the Tyr[3] ortho and D-Trp[4] H_2 proton resonances which were completely overlapping at 25°.

In the case of compound [II], the proper assignment of the Cys and Thr residues was obtained by performing a COSY with delays spectrum (Δ = 220 msec). It allowed the observation of $^5J_{\alpha-\alpha}$ connectivities between Lys[5] and Thr[6], and Thr[6] and Cys[7].

For the other analogues, attempts to discriminate the Cys[2] and Cys[7] resonances with the aid of $^5J_{\alpha-\alpha}$ connectivities were unsuccessfull. It suggests the possibility of a different conformation at least at the Val[6] (or Abu[6])-Cys[7] level of analogues [IV], [V], [VII] and [VIII] compared to [I], [II], [III] and [VI].

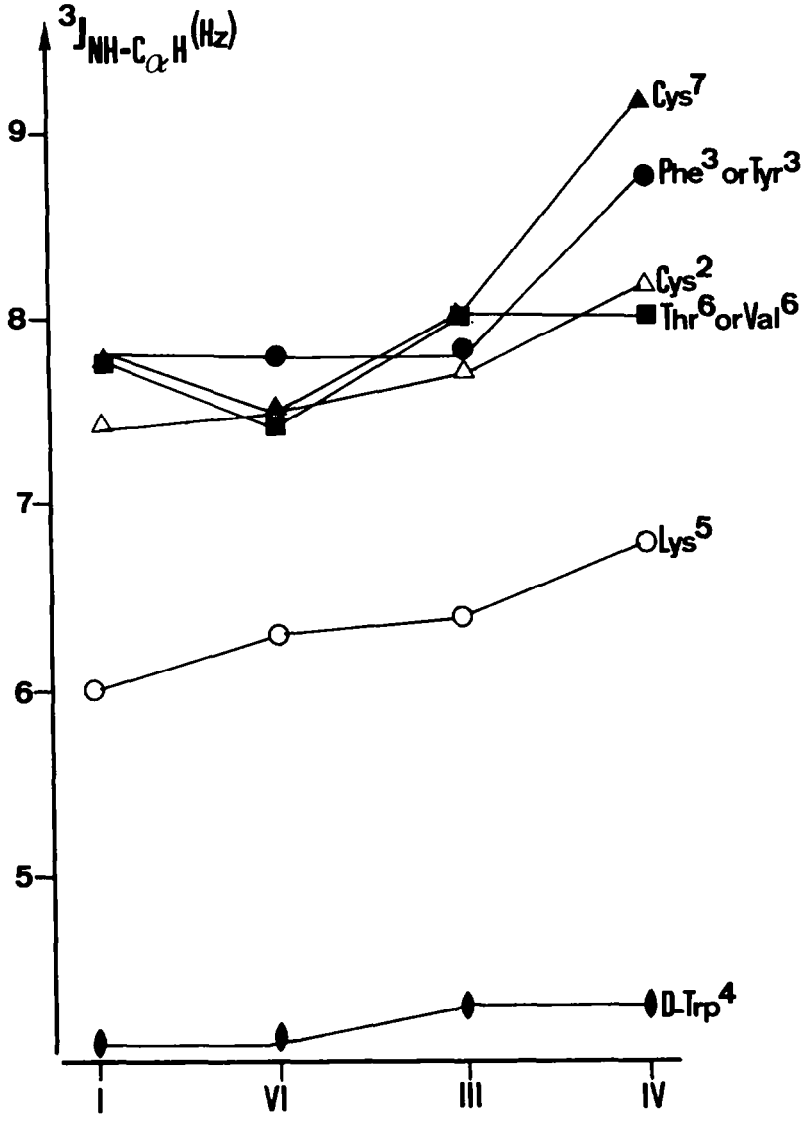

Figure 11 : comparison of the $^3J_{NH-C_\alpha H}$ coupling constants (endocyclic residues) of analogues [I], [III], [IV] and [VI] in aqueous solution.

We have also tried to obtain NOE effects with the aid of the NOESY sequence. We did not observe any NOE effect in water. This is not very surprising, considering the fact that intermediate-size peptides exhibit in general extremely weak NOE effects. The NH resonances were measured in the range 25-50° (temperature intervals = 5°). Tables 2, 4, 8 and 10 list the amide proton N.M.R. parameters of analogues [II], [IV], [V], [VII] and [VIII].

The results of the spin system assignments in H_2O are shown in tables 3, 5, 9, 11, 12-16. In the case of analogues [II], [VII] and [VIII], some assignments were verified by RELAY spectra (delays = 20 msec) (Fig. 8). For the five compounds, the accuracy of the chemical shifts was checked by performing J-resolved spectra. Let us first analyse the conformational properties of analogues [I-VII]. For the whole series, the residues in position 3 and 6 exhibit large $\Delta\delta/\Delta T$ values. A comparison between the $\Delta\delta/\Delta T$ values and the $^3J_{NH-C\alpha H}$ coupling constants of compounds [I], [II], [III] and [VI] (see ref. (18) and (20), and table 8) does not provide any significant differences between these molecules. Their $^3J_{NH-C\alpha H}$ values are compatible with a [3,4,5,6] β turn (42, 43) and two γ turns [2,3,4] and [3,4,5] (42,43). The large $\Delta\delta/\Delta T$ value of the Thr^6 residue almost exclude a major contribution of the β turn, but leaves the two other possibilities. Taking into account the $\Delta\delta/\Delta T$ values of Cys^2, D-Trp^4 and Lys^5, we found the possibility of an equilibrium between two γ turns involving residues 2, 3, 4 and 3, 4, 5.

It is possible that the Cys^2 amide proton is involved in an intramolecular hydrogen bond.

Both γ turns allow a good proximity of the D-Trp^4 and Lys^5 side chains, as confirmed by the large upfield shifts of the Lys^5 γ proton resonances (39-41).

In the case of compounds [IV], [V] and [VII], attempts to discriminate the Cys^2 and Cys^7 resonances were unsuccesfull. Nevertheless, the δ_α, $^3J_{NH-C\alpha H}$ and $\Delta\delta/\Delta T$ values are in the same order of magnitude for both residues, leading to a possible unambiguous interpretation of the results. Furthermore, if we compare the δ_{NH}, δ_α, δ_β, $\Delta\delta/\Delta T$ and $^3J_{NH-C\alpha H}$ values obtained for the Cys residues of the seven analogues (Fig. 9-13, ref. (18) and (20), and tables 2, 4, 8, 12-15), we may tentatively assign the Cys resonances by homology.

Let us compare the δ_α, δ_{NH}, $^3J_{NH-C\alpha H}$ and amide proton $\Delta\delta/\Delta T$ values of the seven analogues. In all compounds, the δ_α values of residues 3 and 6 and the δ_{NH} of residue 5 are very close to the random coil values (37,45).

The D-Trp^4 and Lys^5 α proton resonances and the Thr^6 or Val^6 amide proton resonances are appreciably upfield shifted. In the case of residues 2, 3,

TABLE 8

THE ^1H N.M.R. AMIDE PROTON PARAMETERS OF COMPOUNDS [II] (3.9 mM, pH 2.7) AND [V] (1.9 mM, pH 2.7) IN AQUEOUS SOLUTION, AT 25°.
$^3J_{NH-C\alpha H}$ coupling constants : in Hz; $\Delta\delta/\Delta T$ values : in p.p.b./°K.

Amino Acids	Analogue [II]		Analogue [V]	
	$^3J_{NH-C\alpha H}$	$\Delta\delta/\Delta T$	$^3J_{NH-C\alpha H}$	$\Delta\delta/\Delta T$
D-Phe[1]	-	-	-	-
Cys[2]	7.7	2.5	8.8	0.6
Phe[3]	8.0	5.9	8.9	4.9
D-Trp[4]	4.2	2.2	4.8	0.0
Lys[5]	6.5	2.7	8.0	3.0
Thr[6] or Val[6]	8.0	6.3	8.8	6.1
Cys[7]	8.1	6.6	9.3	1.0
Thr[8]-NH_2	8.0	4.5	8.0	3.1

TABLE 9

THE ^1H 500 MHz N.M.R. CHEMICAL SHIFTS (in p.p.m.) OF THE AROMATIC PROTONS OF ANALOGUES [II] (2.5 mM, pH 2.7) AND [V] (1.9 mM, pH 2.7) IN ^2H_2O, AT 25°.

Amino acids		Analogue [II]	Analogue [V]
D-Phe[1]	o	7.311	7.382
	m	7.428.[b]	7.432.[b]
	p	7.386.[b]	-.[c]
Phe[3]	o	7.271	7.294
	m	7.417.[b]	7.375.[b]
	p	7.362.[b]	7.321.[b]
D-Trp[4]	H_1.[a]	10.173	10.177
	H_2	7.122	7.152
	H_4	7.554	7.589
	H_5	7.192	7.166
	H_6	7.263	7.248
	H_7	7.497	7.492

[a]. Obtained from spectra in 90 % ^1H_2O - 10 % ^2H_2O (analogue [II] : pH 2.7, 3.9 mM; analogue [V] : pH 2.7, 1.9 mM).
[b]. Obtained from J-resolved spectra.
[c]. Important overlap.

TABLE 10

THE ^{1}H N.M.R. AMIDE PROTON PARAMETERS OF COMPOUND [VIII] (3.1 mM, pH 2.7, 25°) IN AQUEOUS SOLUTION

Amino acids	$^{3}J_{NH-C\alpha H}$ (in Hz)	$\Delta\delta/\Delta T$ (in p.p.b./°K)
D-Nal[1]	-	-
Cys[2] .a	8.0	4.0
Tyr[3]	9.0	4.8
D-Trp[4]	4.1	2.8
Lys[5]	6.2	2.7
Abu[6]	6.4	7.5
Cys[7] .a	9.1	8.7
Thr[8]-NH_2	7.5	6.9

a. Can be reversed

TABLE 11

THE AROMATIC PROTON ^{1}H N.M.R. PARAMETERS OF ANALOGUE [VIII] (2.7 mM, pH 2.7, 25°) IN $^{2}H_2O$

Amino acid		δ(in p.p.m.)
D-Nal[1]	H_1	7.746
	H_3	7.452
	H_4	7.957
	H_5	7.898.b
	H_6	7.583
	H_7	7.583
	H_8	7.954.b
Tyr[3]	o	7.131
	m	6.894
	OH	-
D-Trp[4]	H_1.a	10.180
	H_2	7.118
	H_4	7.501
	H_5	7.177
	H_6	7.248
	H_7	7.484

a. Obtained from a spectrum in 90 % $^{1}H_2O$ - 10 % $^{2}H_2O$ (pH 2.7, 25°, 3.1 mM).

b. Can be reversed

TABLE 12

THE ^{1}H 500 MHz N.M.R. PARAMETERS OF COMPOUND [II] IN $^{2}H_2O$ (25 mM, 25°, pH 2.7).
Chemical shifts δ [a] : in p.p.m.; coupling constants J [a] : in Hz.

Amino Acids	δ_{NH} [b]	δ_α	δ_β	δ_γ	δ_δ	δ_ϵ	$^{3}J_{\alpha\beta}$	$^{2}J_{\beta_I\beta_{II}}$	$^{3}J_{\beta\gamma}$
D-Phe[1]	-	4.312 [c]	(3.200 / 3.257) [c]				(8.1 / 6.7) [c]	14.1	
Cys[2]	8.566	4.82 [d]	2.790 / 2.827 [c]				6.4 / 2.9	14.9	
Phe[3]	8.258	4.671	2.942 / 3.087				9.1 / 6.1	13.4	
D-Trp[4]	8.618	4.266	2.858 / 2.994				6.1 / 10.5	13.9	
Lys[5]	8.305	3.824	1.264 [c] / 1.577	0.325 / 0.521	(1.310 / 1.310) [c]	(2.699 / 2.710) [c]	3.7 / 10.6	-	-
Thr[6]	7.981	4.356	4.404	1.237			4.9		6.4
Cys[7]	8.043	4.919	2.967 / 3.157				11.7 / 3.5	14.3	
Thr[8]-NH_2	8.094	4.347	4.272 [c]	1.221			3.7		6.4

[a]. If 2 β protons exist, the upper values are for the β_I protons.
[b]. Obtained from a spectrum in 10 % $^{2}H_2O$ - 90 % $^{1}H_2O$ (25°, 3.9 mM, pH 2.7)
[c]. Extracted from a J-resolved spectrum.
[d]. Burried under the residual water peak. Obtained from a phase-sensitive DQF-COSY

TABLE 13

THE ^1H 500 MHz N.M.R. PARAMETERS OF ANALOGUE [IV] IN ^2H_2O (2.5 mM, 25°, pH 4.0).
The chemical shifts δ.[a] and the coupling constants J.[a] are given respectively in p.p.m. and in Hz.

Amino acid	δ_{NH}.[b]	$\delta\alpha$	$\delta\beta$	$\delta\gamma$	$\delta\delta$	$\delta\epsilon$	$^3J_{\alpha\beta}$	$^2J_{\beta_I\beta_{II}}$	$^3J_{\beta\gamma}$
D-Phe[1]	-	4.339.[c]	3.114 3.390				8.3 6.7	14.3	
Cys[2]	8.762	5.216	2.817 2.901				4.2 10.5	14.9	
Tyr[3]	8.639	4.695	2.945 3.000				7.6 7.6	13.9	
D-Trp[4]	8.712	4.371.[d]	3.077 3.102				6.4 10.3	13.7	
Lys[5]	8.297	3.951	1.181 1.651	0.246 0.461	(1.28/1.28).[d]	2.598 2.680	3.6 11.1		
Val[6]	7.822	4.091	2.236	0.928 0.941			9.6		6.2 6.2
Cys[7]	8.575	5.270	2.980 3.060				11.5 3.6	15.0	
Thr[8]-NH_2	8.297	4.198	4.299	1.182			3.3		6.5

[a] If 2 β protons exist, the upper values are from the β_I protons.
[b]. Obtained from a spectrum in 90 % ^1H_2O - 10 % ^2H_2O (pH 2.7, 25°, 2.5 mM).
[c]. Obtained from a J-resolved spectrum.
[d]. Important overlap.

TABLE 14

THE ^{1}H MHz N.M.R. PARAMETERS OF ANALOGUE [V] IN ^2H_2O (1.9 mM, 25°, pH 2.7).
Chemical shifts δ[a] : in p.p.m.; coupling constants J[a] : in Hz.

Amino acid	δ_{NH}[b]	δ_α	δ_β	δ_γ	δ_δ	δ_ϵ	$^3J_{\alpha\beta}$	$^2J_{\beta_I\beta_{II}}$	$^3J_{\beta\gamma}$
D-Phe[1]	-	4.346	3.221 3.385				8.2 6.7	14.3	
Cys[2]	8.790	5.227	2.819 2.909				4.0 10.6	15.0	
Phe[3]	8.664	4.76[d]	3.022 3.074				8.6 5.8	13.8	
D-Trp[4]	8.715	4.367	3.065 3.090				6.1 11.7	13.8	
Lys[5]	8.292	3.940	1.193 1.642	0.231 0.452	$\left(\frac{1.257}{1.296}\right)$[d,c]	2.590 2.670	3.6 11.1		
Val[6]	7.807	4.092	2.265	0.937 0.950			9.7		4.6 4.7
Cys[7]	8.584	5.289	2.986 3.048				11.4 3.9	15.1	
Thr[8]-NH_2	8.292	4.249	4.326	1.193			3.2		6.5

[a]. If 2 β protons exist, the upper values are for the β_I protons.
[b]. Extracted from a spectrum in 90 % ^1H_2O - 10 % ^2H_2O (1.9 mM, pH 2.7).
[c]. Obtained from a J-resolved spectrum.
[d]. Obtained from a phase-sensitive DQF-COSY.

TABLE 15

THE ^{1}H 500 MHz N.M.R. PARAMETERS OF ANALOGUE [VII] IN $^{2}H_2O$ (2.9 mM, 25°, pH 2.7).
Coupling constants J : in Hz; chemical shifts δ : in p.p.m.

Amino acid	δ_{NH} [a]	δ_α	δ_β	δ_γ	δ_δ	δ_ϵ	$^{3}J_{\alpha\beta}$	$^{2}J_{\beta_I\beta_{II}}$	$^{3}J_{\beta\gamma}$
D-Nal[1]	-	4.411	3.351 3.487				9.0 5.9	13.7	
Cys[2]	8.624	4.865	2.453 2.592				4.2 11.3	15.0	
Tyr[3]	8.387	4.647	2.928 [b]				7.6		
D-Trp[4]	8.580	4.261	2.979 3.039				5.9 11.0	13.9	
Lys[5]	8.246	3.834	1.175 1.581	0.232 0.444	$\left(\frac{1.30}{1.30}\right)$ [c]	- [d]	11.0 3.8		
Val[6]	7.771	3.958	2.164	0.909 0.929			9.6		6.7 6.6
Cys[7]	8.276	4.841	2.732 2.792				4.0 11.3	14.7	
Thr[8]-NH_2	8.013	4.323	4.234	1.208			3.9		6.5

[a]. Obtained from a spectrum in 10 % $^{2}H_2O$ - 90 % $^{1}H_2O$ (pH 2.7; 2.4 mM).
[b]. Equivalent.
[c]. Important overlap.
[d]. Burried under β proton signals

4 and 7, the δ_{NH} and δ_{α} chemical shifts depend dramatically on the nature of the residues at positions 6 and 8. From figures 9-13 and the α and amide proton parameters of analogues [I-VII], it is obvious that substitution of Thr^6 by Val^6 causes important conformational perturbations, specially at the Cys^7 level.

For the three compounds containing a Val residue at the sixth position ([IV], [V] and [VII]), both Cys residues exhibit large downfield shifts compared to the random coil values. This effect is slighlty emphasized when replacing Thr^8(ol) by Thr^8-NH_2, as it is the case in DMSO.

Whereas in DMSO all analogues containing Thr^6 and Thr^8(ol) (compounds [I] and [VI]) or Thr^8-NH_2 (compound [III]) residues show important upfield shifts of both Cys α proton resonances, this is not the case in water.

We suggest the possibitity of a different interaction between the $D\text{-}Phe^1$ aromatic ring and the Cys α protons in DMSO and in aqueous solution. A good

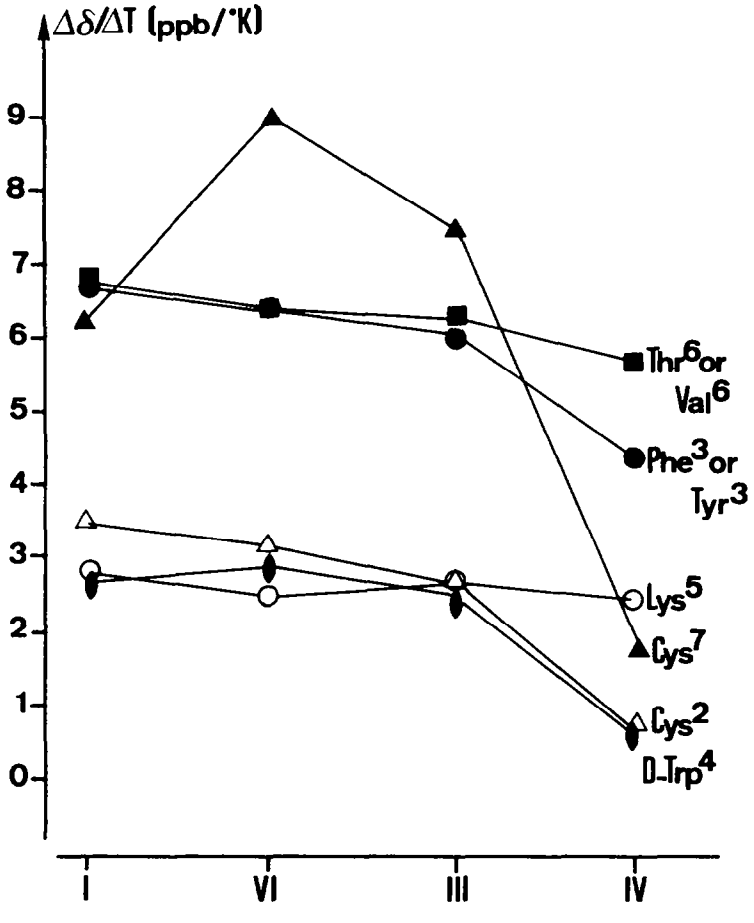

Figure 12 : comparison of the amide proton $\Delta\delta/\Delta T$ values (endocyclic residues) of analogues [I], [III], [IV] and [VI] in aqueous solution.

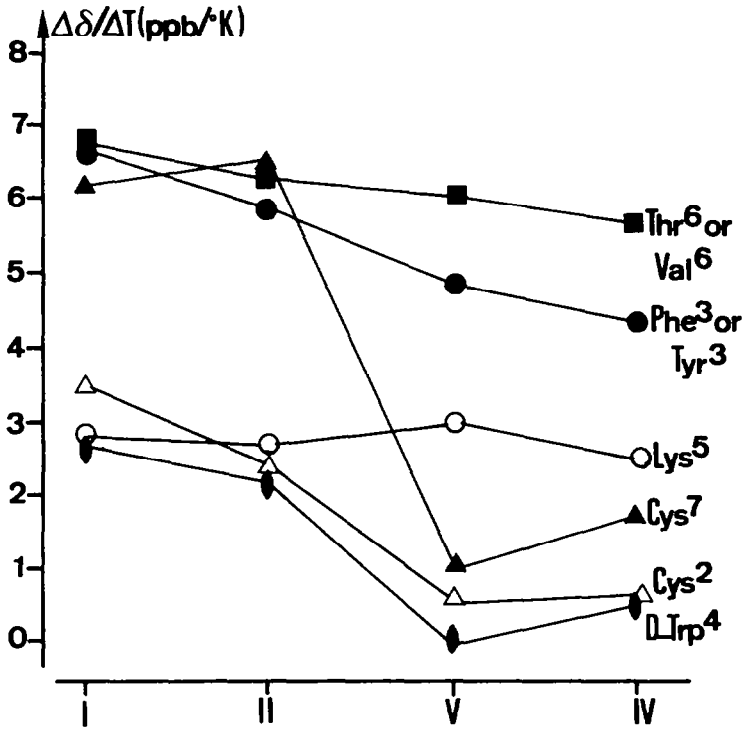

Figure 13 : comparison of the amide proton $\Delta\delta/\Delta T$ values (endocyclic residues) of analogues [I], [II], [IV] and [V] in aqueous solution.

argument in favour of this assumption is given by replacement of D-Phe^1 by D-Nal^1 (analogue [VII]). In DMSO, this substitution causes an additional downfield shift, whereas in aqueous solution the downfield shift is significantly decreased.

Compounds [IV], [V] and [VII], containing a Val^6, exhibit small amide proton $\Delta\delta/\Delta T$ values for residues 2, 4 and 7. There is certainly contribution from conformations in which the Cys^2, D-Trp^4 and Cys^7 amide protons are involved in intramolecular hydrogen bonds, specially for compounds [IV] and [V]. A participation of the Lys^5 amide proton in an hydrogen bond is not excluded, though its $\Delta\delta/\Delta T$ value is larger than the three other ones. These assumptions are completely in agreement with the important downfield shifts observed for the Cys^2, D-Trp^4 and Cys^7 amide proton resonances of these compounds.

In the case of the other analogues, only the Cys^2 and D-Trp^4 NH resonances are downfield shifted, but at a lesser degree, whereas the Cys^7 NH resonances exhibit a significant upfield shift.

The $^{3}J_{NH\text{-}C\,\alpha H}$ and the $\Delta\delta/\Delta T$ values (tables 2, 4 and 8) are compatible with the existence of γ turns [2,3,4], [3,4,5] and [5,6,7]. The very small $\Delta\delta/\Delta T$ values of Cys^{2}, $D\text{-}Trp^{4}$ and Cys^{7} are good arguments in favour of a conformational equilibrium involving a major contribution of both γ turns [2,3,4] and [5,6,7] (Fig. 14) and an intramolecular hydrogen bond between the Cys^{2} amide proton and the Cys^{7} carbonyl group. As for the other analogues, the large $\Delta\delta/\Delta T$ of the residue at position 6 argues against an important contribution of [3,4,5,6] β turn.

Let us now consider the case of analogue [VIII] (table 1). Substitution of Val^{6} (analogue [VII]) by Abu^{6} (analogue [VIII]) causes the disappearance of the large downfield shift of the Cys α proton resonances (table 16). This is in agreement with the results obtained with analogues [I-VII]. The $\Delta\delta/\Delta T$ values (table 10) are in the same order of magnitude as those obtained for analogues [I], [II], [III], and [VI] which possess a Thr residue at position 6. The $^{3}J_{NH\text{-}C\,\alpha H}$ values of both Cys and Tyr^{3} are comparable to those obtained for the compounds exhibiting a Val^{6} residue, whereas the Lys^{5} $^{3}J_{NH\text{-}C\,\alpha H}$ coupling constant is similar

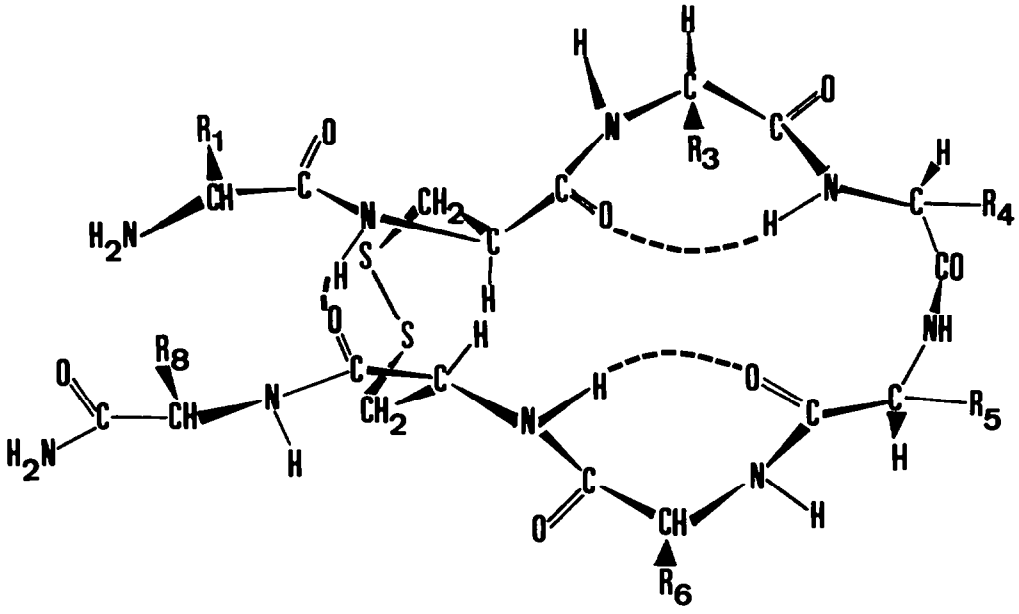

Figure 14 : the most probable conformation of analogues [IV], [V] and [VII] in aqueous solution.
R_1 : D-Phe or D-Nal; R_3 : Phe or Tyr; R_4 : D-Trp; R_5 : Lys; R_6 : Val; R_8 : Thr.

TABLE 16

THE ^{1}H 500 MHz N.M.R. PARAMETERS OF ANALOGUE [VIII] IN $^{2}H_2O$ (3.1 mM, pH 2.7, 25°).
Coupling constants J : in Hz; chemical shifts δ : in p.p.m.

Amino acids	δ_{NH} [a]	$\delta\alpha$	$\delta\beta$	$\delta\gamma$	$\delta\delta$	$\delta\epsilon$	$^{3}J_{\alpha\beta}$	$^{2}J_{\beta_I\beta_{II}}$	$^{3}J_{\beta\gamma}$
D-Nal[1]	-	4.363	3.258 3.486				9.8 5.9	13.4	
Cys[2] [c]	8.298	4.671	2.283 2.323				5.2 5.2	14.7	
Tyr[3]	8.158	4.627	2.831 2.947				9.1 6.5	14.4	
D-Trp[4]	8.425	4.105	2.810 2.962				5.6 11.5	14.1	
Lys[5]	8.199	3.690	1.192 1.531	0.267 0.478	(1.262/1.310) [b]	(2.660/2.695) [b]	10.8 3.8	-	
Abu[6]	7.790	4.108	1.799 1.883	0.953			5.8 9.1	-	7.4 7.4
Cys[7] [c]	7.742	4.555	2.773 2.886				11.6 3.5	14.5	
Thr[8]-NH_2	7.804	4.341	4.283	1.217			3.8		6.4

[a]. From a spectrum in 10 % $^{2}H_2O$ - 90 % $^{1}H_2O$ (pH 2.7, 2.7 mM).
[b]. Obtained from a J-resolved spectrum.
[c]. Can be reversed.

to the values of analogues having a Thr^6. The Abu^6 $^3J_{NH-C\alpha H}$ value is significantly smaller (6.4 Hz) than the Thr^6 or Val^6 ones ($\simeq$ 8 Hz).

A combination of the $\Delta\delta/\Delta T$ and $^3J_{NH-C\alpha H}$ values leads to the possibility of the existence of an equilibrium between two γ turns [2,3,4] and [3,4,5] (42,43). Considering the high $\Delta\delta/\Delta T$ of the Abu^6 amide proton, a participation of a β turn [3,4,5,6] in the conformational equilibrium is unprobable. The positions of the amide proton resonances are similar to those of analogues exhibiting a Thr residue at the sixth position, though the Cys^2 and $D\text{-}Trp^4$ NH signals are less downfield shifted.

It must be noted that one of the Cys residue exhibit strongly upfield shifted β proton resonances.

Finally, for compounds containing a D-Nal residue (analogues [VII] and [VIII]), we must point out that the discrimination between the H_1, H_3 and H_4 aromatic protons has been obtained by observing long-range β-aromatic and aromatic-aromatic connectivities. COSYLR spectra (Δ = 100 msec) allowed the observation of ^4J and ^5J connectivities between the H_1, H_3 and H_4 aromatic protons and the β protons, whithout discrimination between the H_3 and H_4 resonances. A small ^4J cross-peak (existing in the phase-sensitive DQF-COSY spectra) between the H_1 and H_3 proton signals allowed us to distinguish between the H_3 and H_4 protons.

(3). Discussion of the correlation with biological activity

As pointed out, the five analogues ([I-IV] and [VII]) exhibit the same predominant conformation in DMSO solution and no correlations can be found with changes in biological activity. Nevertheless, we know from previous studies (46-48) that measurements in DMSO are able to discriminate totally inactive compounds from active ones (GH inhibition) by their conformational behaviour. Drastic changes in conformations are detectable but more subtile influences seem to be hidden by a strong peptide-solvent interaction.

At the contrary, the peptide-water interaction seems to be weaker and variations in conformation appear, and can be correlated with quantitative variation in potency. We focussed our attention to the in vitro GH inhibition which is fairly well documented in this series. At this level of our investigations, in vivo results include to many parameters to be compared with structural changes.

In water, if we compare the $\delta\alpha$, $\Delta\delta/\Delta T$, $^3J_{NH-C\alpha H}$ and $^3J_{\alpha\beta}$ values of analogues [I] (16,18), [II] (tables 8 and 12), [III] (20), [IV] (tables 2 and 14), [V] (tables 8 and 14) and [VI] (18), it appears clearly that substitution of Phe^3 by Tyr^3 (analogue [VI]) or Thr^8(ol) by $Thr^8\text{-}NH_2$ (analogue [II])

TABLE 17

Analogue	Code Name	Inhibition of GH secretion[a]	
		in vivo	in vitro[c]
[I]	SMS 201-995	81[d]	5 (3)[h]
[II]	DC-13-57	45[f]	4.4
[III]	CTC	-[b]	-[b]
[IV]	IM-IV-28	79[d] (177)[f]	7.8
[V]		11[d]	4.2[d]
[VI]	Sandoz 204-090	-[g]	-[g]
[VII]	DC-13-116	10.1[e]	4.1

[a]. SRIF = 1
[b]. Not tested
[c]. From reference (14)
[d]. From reference (7)
[e]. From reference (11)
[f]. From reference (10)
[g]. Comparable to the activity of [I] (reference (5))
[h]. From reference (1)

does not introduce significant conformational changes. Accordingly, the in vitro activities of these compounds (table 17; inhibition of growth hormone secretion) are very similar.

The Val^6 side-chain introduces the most important modification, forcing the molecule to one predominant conformation (Fig. 14). The molecule where this predominancy is maximum, as shown by evolution of the NMR parameters, is compound [IV] which also shows the highest in vitro activity.

As soon as this effect is loosened as in compound [VII], the activity drops again (Table 17).

In any case, as the variations in activity between these derivaties are small, one has to be aware of the fact that other factors like the influence of lipophilicity (D-Nal^1 derivatives) or hydrophilicity (Tyr^3 derivatives) also influence the activity.

CONCLUSIONS

In aqueous solution, for compounds [I], [II], [III], [VI] and [VIII], we may assume an equilibrium between two γ turns [2,3,4] and [3,4,5]. For compounds [IV], [V] and [VII], containing a Val^6 residue, we have more probably a conformational equilibrium involving a major contribution of both the γ turns [2,3,4] and [5,6,7].

For all the analogues studied in DMSO, we found a predominant conformation with a [3,4,5,6] β turn of type II'.

In both solvents, the existence of a stabilization by an hydrogen bond involving the Cys^2 amide proton is not excluded.

It appears that in water, the molecule is more sensitive to conformational changes related to the activity whereas in DMSO the solvent effects predominate.

It appears that in DMSO conformational studies are only able to discriminate between active and non-active compounds (GH inhibition) and that quantitative variations among the active analogues are better reflected by conformational changes in water solutions. In this solvent the equilibria are less influenced by peptide-solvent interaction.

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