The Effect of Chemical Modification on the Solution Structure of Derivatives of Substance P: Nuclear Magnetic Resonance Study of Substituted and Retro-inverso Analogues

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Four derivatives of the bioactive fragment 6—11 of Substance P were characterized by means of one- and two-dimensional ^{1}H n.m.r. spectroscopy. Indirect evidence of backbone folding for these hexapeptides was given by temperature coefficients and paramagnetic relaxation rate enhancements of the amide protons. Chemical modifications were found to modulate the solution structure. Retro-inversion caused some conformational changes and a correlation between these structural variations and the relative biological activities was attempted.

The relationship between the solution structure and the activity of biomolecules is extensively investigated in order to elucidate biochemical pathways and to design new synthetic drugs with low toxicity and high specificity. In this respect, structural studies of naturally occurring neuropeptides are of primary interest due to their physiological and pharmacological properties. Among these neuropeptides, Substance P has been thoroughly studied[1] since its recent characterization.[2] At least some of the correlations between the conformation and the biological activity of Substance P are well known.[3,4]

The occurrence of multiple subreceptors implies, for maximum activity of the neuropeptide, definite molecular shapes, which involve the backbone as well as the side chains.[1] In order to improve the biological activity of peptides a series of backbone modifications has been introduced.[5] Among these the retro-inversion of the peptide bond has been exploited.[6] These modifications involve the reversal of the direction of one or more peptide bonds in the backbone, leaving unaffected the side-chain topology, at least in the extended conformation.[6] The modification is achieved by substituting the naturally occurring amino acid residues with *gem*-diaminoalkyl [–NH–CH(SC)–NH–] and 2-substituted malonyl [–CO–CH(SC)–CO–] residues, where SC is the side chain of a substituted amino acid. Metabolic stabilization has been demonstrated for these classes of analogues[7] and their structural–functional characteristics are therefore of prime interest. In the present report, unmodified and retro-inverso analogues of the *C*-terminal hexapeptide of Substance P (SP_{6-11}) are investigated by ^{1}H n.m.r. spectroscopy to correlate their biological activities with structural features.

Experimental

^{1}H N.m.r. measurements were carried out on 20mM samples of the four peptides dissolved in [$^2H_6$]DMSO (C. Erba) at 300 ± 1 K. N.m.r. spectra were obtained using Varian XL 200 and Bruker CXP 300 instruments. The solvent isotopic impurity resonance, set at 2.5 p.p.m., was used as a reference. One-dimensional (1D) and two-dimensional (2D) experiments generated the parameters reported in Tables 1—4. The typical size of data matrices for homonuclear correlated 2D spectra, COSY,[8] and 2D nuclear Overhauser spectroscopy, NOESY,[9] acquired at 200 MHz, was 512 × 512 (digital resolution 4 Hz per point), enough to obtain qualitative information on scalar and dipolar connectivities. 1 024 × 128 data matrices (digital resolution 2 and 0.4 Hz per point in F2 and F1, respectively) were used to generated 2D *J*-correlated spectra.[10] Recycling delays of 1 s were used in all experiments, while the mixing time for NOESY experiments was fixed at 300 ms. The standard resolution enhancement routines of the Varian software were always used prior to 2D Fourier transformation of the data. The chemical shifts were successively refined by inspection of highly resolved 200 and 300 MHz 1D spectra (digital resolution 0.001 p.p.m. per point). Spectral simulations were performed using the program PANIC from the Bruker software package.

The paramagnetic relaxation rate enhancement given in Tables 1—4 is defined as $R_{1p} = R_{10} - R_{1b}$, where R_{10} is the observed spin–lattice relaxation rate of a proton in the presence of 10mM of the soluble spin-label 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) (Syva) and R_{1b} is the spin–lattice relaxation rate measured for the same proton in a diamagnetic solution.

Results and Discussion

Retro-inverso Analogues.—The smallest active *C*-terminal fragment of Substance P, SP_{6-11}[11] is an hexapeptide with the amino acid sequence: H-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2 (**1**).[1] This compound was chemically modified as follows:

[Glp^6, gPhe^8, mGly^9]SP_{6-11} (**2**),[12]
[Boc-Ala^6]SP_{6-11} (**3**),*
[Boc-Ala^6, gPhe^7, D-Phe^8, mGly^9]SP_{6-11} (**4**)[13]

where gPhe and mGly are the *gem*-diaminoalkyl and malonyl analogues of Phe and Gly, respectively (see Introduction), and Boc is t-butoxycarbonyl.

The rationale for the modifications outlined above stems from consideration of the metabolic inactivation mechanism of Substance P. It has been suggested that the peptide bonds undergoing cleavage by cytosolic enzymes are probably [Gln^6-Phe^7], [Phe^8-Gly^9], and [Gly^9-Leu^{10}].[14,15] Increased activity has been reported both for N$^{\alpha}$-Boc protected analogues of Substance P and its active fragments[16] and the [Glp^6]SP_{6-11} derivative.[17]

Although conflicting data on the extent of such activity enhancement are found in literature,[16,18] it is possible to interpret the experimental results in terms of increased metabolic stability and, therefore, to apply the retro-inverso modification to metabolically relevant locations.

* Peptides (**1**) and (**3**) were synthesized by repeated mixed anhydride condensations except for the glutamine residue which was introduced as active ester.

Table 1. ^{1}H N.m.r. relevant parameters[a] for SP_{6-11} (**1**)

		δ (p.p.m.)	J/Hz	R_{1p}/s^{-1}	$-\Delta\delta/\Delta T$ (p.p.b. K^{-1})
Gln^6	NH_3^+	8.160		4.0	0.80
	α	3.93			
	β_1,β_2	1.90 1.86			
	γ_1,γ_2	2.24 2.20			
	εNH_2	7.007 (*syn*)		5.1	3.65
		7.443 (*anti*)		6.1	4.30
Phe^{7b}	NH	8.708	8.02	2.6	2.15
	α	4.547			
	β_1	3.024	$(\beta_1\alpha)$ 4.38		
	β_2	2.846	$(\beta_2\alpha)$ 9.42		
			$(\beta_1\beta_2)$ −13.93		
	Ph	7.26—7.18			
Phe^{8b}	NH	8.434	8.00	4.0	5.10
	α	4.537			
	β_1	3.040	$(\beta_1\alpha)$ 4.47		
	β_2	2.749	$(\beta_2\alpha)$ 9.50		
			$(\beta_1\beta_2)$ −14.04		
	Ph	7.26—7.18			
Gly^9	NH	8.179		4.0	5.30
	α_1,α_2	3.73			
Leu^{10}	NH	8.022	7.9	4.3	4.05
	α	4.31			
	β_1,β_2	1.49 1.47			
	γ	1.60			
	δ_1,δ_2	0.883 0.848			
Met^{11}	NH	8.007	8.2	4.3	4.82
	α	4.24			
	β_1,β_2	1.92 1.84			
	γ_1,γ_2	2.44 2.39			
	S-CH_3	2.013			
C-terminal	NH_2	7.044 (*syn*)		5.9	4.35
		(*anti*)[c]			

[a] Chemical shifts and coupling constants measured at 300 K; $\Delta\delta \pm 0.001$ p.p.m.; $\Delta J \pm 0.2$ Hz; temperature coefficients obtained in the range 298—318 K. [b] Chemical shifts and coupling constants from simulations. [c] Obscured by aromatics.

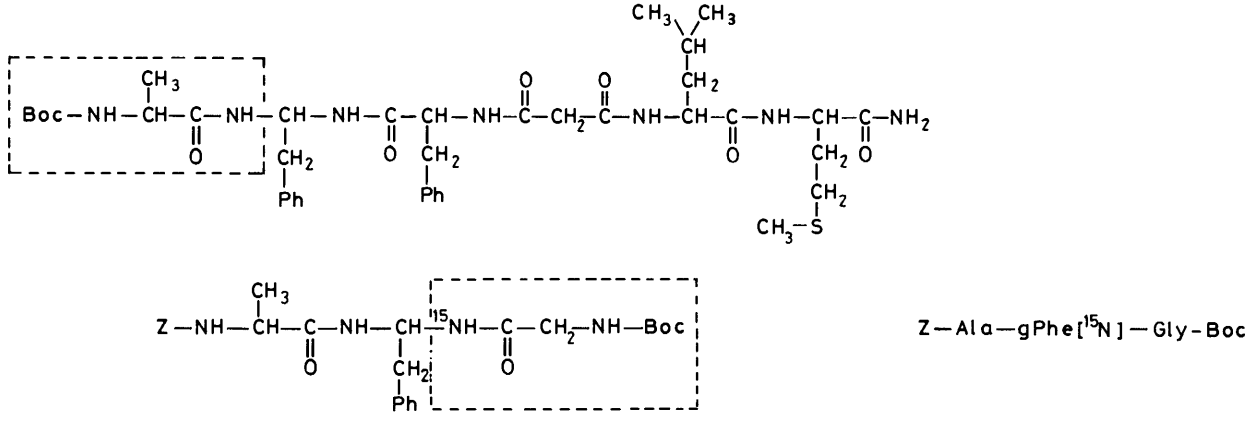


Figure 1. Analogy (boxed regions) between peptide (**4**) and ^{15}N-labelled model compound used for the assignment of NH proximal and distal resonances in [gPhe^7] (see text)

Assignments.—Tables 1—4 list the assignments and other relevant n.m.r. parameters for the compounds examined. ^{1}H 2D COSY connectivities allowed straightforward identification of the unique spin-system patterns occurring in each peptide. The strongly coupled doublets, always at δ *ca.* 3.11 in (**2**) and (**4**), were assigned to the α-CH_2 of mGly. In the case of compound (**1**) the distinction between NH of [Phe^7] and [Phe^8] is consistent with previously reported data.[19] A reverse order of the relative chemical shifts for [Phe^7] and [Phe^8] NH has also been proposed for $[Glp^6]SP_{6-11}$.[20] We note, however, that our assignment of NH[Phe^7] in (**2**) (unambiguous, because of the presence of a *gem*-diaminoalkyl residue at position 8), does not agree with the previous proposal.[20] The NH groups of the two consecutive Phe residues in (**3**) were attributed by analogy with the shift observed in the Boc-Ala-Phe-NH_2 fragment [δ_{NH}(Phe) 7.695]. The geminal of NH of gPhe in (**4**) were identified from evidence from model compounds. In Z-Ala-gPhe[^{15}N]-Gly-Boc (^{15}N labelled at the gPhe-Gly peptide bond) (Figure 1), the NH groups of the *gem*-diaminoalkyl residue are found at δ 8.133 and 8.077. Further splitting due to

Table 2. ^{1}H N.m.r. relevant parameters[a] for [Glp^6,gPhe^8,mGly^9]SP$_{6-11}$ (**2**)

		δ (p.p.m.)	*J*/Hz	R_{1p}/s^{-1}	$-\Delta\delta/\Delta T$ (p.p.b. K^{-1})
Glp^6	NH	7.688	0	3.6	4.25
	α	3.93			
	β_1,β_2	2.14 1.65			
	γ_1,γ_2	1.91 1.60			
Phe$^{7\,b}$	NH	7.955	8.51	2.5	5.15
	α	4.481			
	β_1	2.924	$(\beta_1\alpha)$ 4.56		
	β_2	2.745	$(\beta_2\alpha)$ 9.57		
			$(\beta_1\beta_2)$ −14.00		
	Ph	7.23—7.17			
gPhe$^{8\,b}$	$NH_{proximal}$[c]	8.513	7.85	3.0	4.70
	NH_{distal}[c]	8.454	7.87	3.7	6.40
	α	5.514			
	β_1	2.944	$(\beta_1\alpha)$ 8.39		
	β_2	2.936	$(\beta_2\alpha)$ 6.64		
			$(\beta_1\beta_2)$ −13.67		
	Ph	7.23—7.17			
mGly^9	α_1,α_2	3.11			
Leu^{10}	NH	8.315	8.0	4.3	4.37
	α	4.27			
	β_1,β_2	1.49			
	γ	1.60			
	δ_1,δ_2	0.875 0.836			
Met^{11}	NH	8.171	8.4	2.5	4.85
	α	4.24			
	β_1,β_2	2.01			
	γ_1,γ_2	2.46			
	S-CH_3	1.987			
C-terminal	NH_2	7.109[d] (*syn*)		1.4	2.00
		7.109[d] (*anti*)		1.4	3.95

[a] See Table 1. [b] Chemical shifts and coupling constants from simulations. [c] *N*-terminus proximal and *N*-terminus distal. [d] Two resonances are observed at higher temperatures; their assignment is tentative.

Table 3. ^{1}H N.m.r. relevant parameters[a] for [Boc-Ala^6]SP$_{6-11}$ (**3**)

		δ (p.p.m.)	*J*/Hz	R_{1p}/s^{-1}	$-\Delta\delta/\Delta T$ (p.p.b. K^{-1})
Boc		1.351			
Ala^6	NH	6.855	7.6	5.1	6.25
	α	3.89			
	β	1.036			
Phe$^{7\,b}$	NH	7.724	8.24	2.8	2.40
	α	4.515			
	β_1	3.031	$(\beta_1\alpha)$ 4.91		
	β_2	2.821	$(\beta_2\alpha)$ 9.07		
			$(\beta_1\beta_2)$ −14.07		
	Ph	7.22—7.15			
Phe$^{8\,b}$	NH	8.147	9.43	3.6	5.10
	α	4.487			
	β_1	2.967	$(\beta_1\alpha)$ 4.92		
	β_2	2.758	$(\beta_2\alpha)$ 8.84		
			$(\beta_1\beta_2)$ −13.96		
	Ph	7.22—7.15			
Gly^9	NH	8.131		3.6	5.70
	α_1,α_2	3.73			
Leu^{10}	NH	7.963	8.0	3.0	4.75
	α	4.31			
	β_1,β_2	1.49 1.48			
	γ	1.61			
	δ_1,δ_2	0.886 0.851			
Met^{11}	NH	7.929	8.2	4.0	5.12
	α	4.24			
	β_1,β_2	1.92 1.81			
	γ_1,γ_2	2.44 2.40			
	S-CH_3	2.014			
C-terminal	NH_2	7.036 (*syn*)		6.0	3.90
		(*anti*)[c]			

[a] See Table 1. [b] Chemical shifts and coupling constants from simulations. [c] Obscured by aromatics.

Table 4. ^{1}H N.m.r. relevant parameters[a] for [Boc-Ala^6,gPhe^7,mGly^9]SP$_{6-11}$ (**4**)

		δ (p.p.m.)		J/Hz	R_{1p}/s^{-1}	$-\Delta\delta/\Delta T$ (p.p.b. K^{-1})
Boc		1.364				
Ala^6	NH	6.818		7.8	5.1	6.75
	α	3.89				
	β	1.081				
gPhe^7[b]	$NH_{proximal}$[c]	8.008		7.74	3.2	5.10
	NH_{distal}[c]	8.484		8.11	4.0	6.10
	α	5.521				
	β_1	2.951		($\beta_1\alpha$) 7.97		
	β_2	2.898		($\beta_2\alpha$) 6.54		
				($\beta_1\beta_2$) −13.73		
	Ph	7.22—7.16				
D-Phe^8[b]	NH	8.291		8.37	4.4	4.10
	α	4.469				
	β_1	2.936		($\beta_1\alpha$) 4.56		
	β_2	2.697		($\beta_2\alpha$) 8.73		
				($\beta_1\beta_2$) −13.86		
	Ph	7.22—7.16				
mGly^9	α_1,α_2	3.10				
Leu^{10}	NH	8.269		7.2	4.4	3.80
	α	4.25				
	β_1,β_2	1.49	1.44			
	γ	1.61				
	δ_1,δ_2	0.871	0.834			
Met^{11}	NH	8.066		8.1	2.5	4.75
	α	4.21				
	β_1,β_2	1.93	1.83			
	γ_1,γ_2	2.45	2.41			
	S-CH_3	2.011				
C-terminal	NH_2	7.010 (*syn*)			2.9	4.50
		7.055 (*anti*)			5.2	5.65

[a] See Table 1. [b] Chemical shifts and coupling constants from simulations. [c] *N*-terminus proximal and distal.

(^{15}N-H) spin–spin coupling allows the unambiguous identification of the resonance at δ 8.077 as that arising from the Gly proximal NH of gPhe.

In view of the similarity between the Boc-Gly moiety of Z-Ala-gPhe[^{15}N]-Gly-Boc and the Boc-Ala moiety in (**4**) (Figure 1), it seems plausible to assign the proximal *N*-terminus NH of [gPhe^7] in (**4**) to the resonance at δ 8.008 (Table 4). Similar considerations cannot be applied to the NH of [gPhe^8] in (**2**). Therefore, the corresponding assignments, given in Table 2, are tentative.

The β proton spin systems of Phe in (**1**) and (**3**) were attributed after simulation of the experimental pattern both at 200 and 300 MHz (Figure 2). Simulations were also performed for the identification of the β protons of Phe and gPhe in (**2**) and (**4**), which also exhibited extensive overlap.

Finally, the amide protons of [Gln^6] side chain and *C*-terminal carboxamide group in (**1**) were distinguished by comparison with [Glp^6]SP$_{6-11}$ chemical shifts.[20]

Peptide–Solvent Interaction Studies.—It has been shown that study of the temperature dependence of amide protons[21] and of paramagnetic perturbations induced by soluble nitroxides in the spin–lattice relaxation rates, R_{1p},[22] can yield valuable information on the dynamics of solute–solvent interactions. The different ($\Delta\delta/\Delta T$) and R_{1p} values of the amide protons arise from specific conformational arrangement(s) of the peptide. Hydrogen bonding and/or other conformational features may, in fact, reduce the pure or spin-labelled solvent accessibility. As shown in Tables 1—4, temperature coefficients and paramagnetic relaxation rate enhancements were measured for all the NH protons of the four investigated derivatives of Substance P. For each peptide significantly different amide ($\Delta\delta/\Delta T$) and R_{1p} values were observed. Hence these paramaters can be analysed

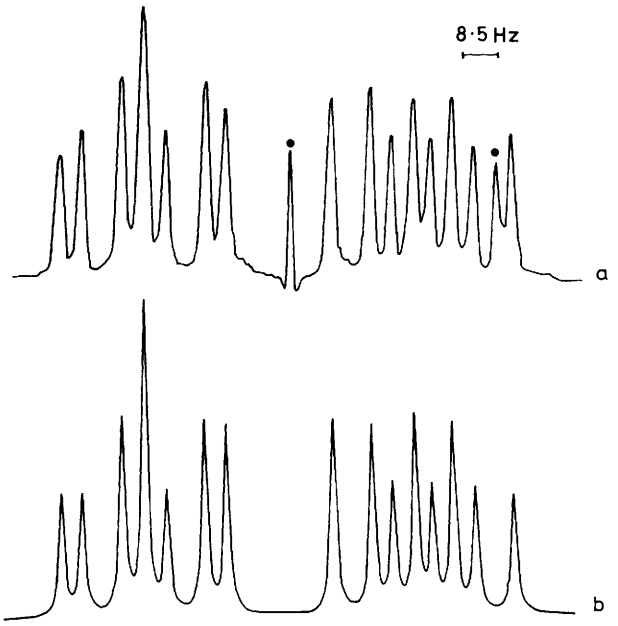
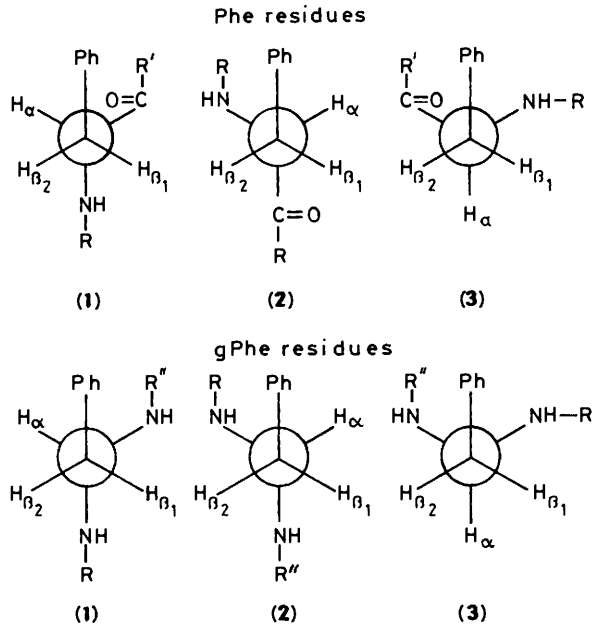


Figure 2. Pattern of βCH_2 spin systems for [Phe^7] and [Phe^8] in peptide (**3**). (a) Experimental; (b) simulated. Dots indicate impurities

in structural terms, even if averaging occurs to some extent due to the presence of conformational equilibria in solution. It is worth noting that NH[Phe^7] exhibits very low and almost identical $\Delta\delta/\Delta T$ and R_{1p} in peptides (**1**) and (**3**), which suggests that this molecular site is little affected by the Boc-Ala/Gln

Table 5.



Compound	Residue	P_1[a]	P_2[a]	P_3[a]
SP_{6-11}	Phe[7]	8.0	56.7	35.3
	Phe[8]	8.9	57.5	33.6
[Glp[6],gPhe[8],mGly[9]]SP_{6-11}	Phe[7]	9.7	58.1	32.2
	gPhe[8]	49.7	31.6	18.7
[BocAla[6]]SP_{6-11}	Phe[7]	13.1	53.4	33.5
	Phe[8]	13.2	51.1	35.7
[BocAla[6],gPhe[7],D-Phe[8],mGly[9]] SP_{6-11}	gPhe[7]	45.4	30.6	24.0
	D-Phe[8] [b]	9.8	50.0	40.2

[a] Populations were computed from $J_{\alpha\beta}$ coupling constants (Table 1—4), using the Pachler method.[31] [b] The chirality of the corresponding Newman projection should be reversed.

replacement. A similar R_{1p} value is observed for the corresponding proton in peptide (**2**), while its $\Delta\delta/\Delta T$ increases. This finding could be explained in terms of structural modifications induced by the [Phe[8]-Gly[9]]/[gPhe[8]-mGly[9]] substitution, which increases the solvent exposure of the NH[Phe[7]] in compound (**2**) (breakdown of a hydrogen bond), without complete opening of the folded structure (difficult accessibility of the nitroxide). In the same molecule partial solvent exposure can be suggested for the NH of [Met[11]], whose behaviour is similar to that of the NH of [Phe[7]]. Strong solvent shielding, at variance, can be inferred from the small R_{1p} and $\Delta\delta/\Delta T$ of the *C*-terminal carboxamide. The different temperature coefficients, measured for the *syn* and *anti* members of the pair, once their chemical shift degeneracy is removed by increasing the temperature, indicate that one amide proton is involved in a hydrogen bond.

This constraint, unequivocally found only in peptide (**2**), could replace that present in peptides (**1**) and (**3**), to maintain a folded species in solution.

In peptide (**4**) some degree of solvent shielding can be deduced from the low R_{1p} exhibited by NH[Met[11]] and the *syn* proton of the *C*-terminal carboxamide group.

The extremely low temperature coefficient (0.8 p.p.b. K^{-1}) observed for the NH_3^+ resonance of [Gln[6]] in peptide (**1**) can be ascribed either to the effect of the accompanying counterion or to hydrogen bonding of one or more hydrogens of the amino group. In the latter case, the presence of free hydrogens could account for the relatively high R_{1p} obtained for these nuclei. In summary, the chemical modifications leading to peptides (**2**) and (**4**) seem to reduce the constraints involving the NH[Phe[7]], while a different role is played by the *C*-terminal moiety.

Structural Information from N.m.r. Scalar and Dipolar Connectivities.—$J_{NH\alpha}$ Coupling constants give valuable information on the backbone conformation, due to their dependence on the dihedral angle φ.[23,24] The complete description of the backbone geometry needs, however, additional information about the other relevant backbone dihedral angle, ψ. This can be obtained, in principle, from proton homonuclear *J* couplings only for glycines.[25] In other cases inter-residue nuclear Overhauser effects (n.O.e.) are used, due to the occurrence of favourably short internuclear distances in regular secondary structures.[26,27]

The NOESY spectra did not provide any significant information, since no inter-residue cross-peaks could be observed for all the four peptides examined. This result can be explained in two different ways. (i) The overall molecular tumbling is such that the lifetime of each rotational state τ_c, is very close to ω_o^{-1}, where ω_o is the proton Larmor frequency. (ii) An equilibrium is present in solution between two or more conformers in fast exchange (on the n.m.r. time scale), which drastically reduces the intrinsic intensity of the cross-peaks arising from closely spaced nuclei.

The phase-sensitive NOESY spectrum* of [Glp[6]]SP_{6-11} (obtained in DMSO at 293 K on a Bruker AC200 spectrometer), showed two weak negative cross-peaks between the α-CH and NH resonances of consecutive residues, *i.e.* [Glp[6]-Phe[7]] and [Leu[10]-Met[11]]. This agrees with previous reports,[1,3,4,19] which proposed an extended structure in the two strands branching from the bend around [Phe[8]] and [Gly[9]]. Moreover, the occurrence of weak negative cross-peaks indicates that $\omega_o\tau_c < 1$ for [Glp[6]]SP_{6-11} for the above experimental conditions.[29] In view of the similarity between [Glp[6]]SP_{6-11} and the peptides (**1**)—(**4**), the latter observation may suggest that the absence of inter-residue NOESY cross-peaks for the four peptides under examination could be due to unfavourable τ_c, *i.e.* $\omega_o\tau_c$ *ca.* 1.12,[29] although the existence of fast conformational equilibria in solution cannot be excluded *a priori*.

Side-chain Conformation.—Coupling constants may be used to describe the side-chain conformations,[24] since the experimental $^3J\chi_n$ values reflect the local conformational distribution. In veiw of the possible relevance of the side-chain conformation of the Phe residues in Substance P,[1-4,19] an analysis of the $J_{\alpha\beta}$ coupling constants was carried out (Tables 1—4). The conformational conclusions inferred are dependent on the stereospecific assignment of the β protons. For Phe residues, the classic assignment was assumed (*i.e. pro-R* absolute configuration for the upfield β proton resonance[30]). For gPhe residues the same assignment was achieved on the basis of model compounds containing ^{15}N labelled gPhe. The rotamer populations were obtained using the method proposed by Pachler,[31] with limiting values for J_{trans} and J_{gauche} of 13.90 and 3.55 Hz, respectively.[32]

The set of *J* limiting values for *gem*-diaminoalkyl residues was first evaluated by including the appropriate electronegativity corrections in the general Karplus relationship proposed by Haasnoot *et al.*,[33] and then scaling the effect on the values previously employed for unmodified residues. As a result we obtained J'_t 13.24 and J'_g 3.59 Hz. Table 5 summarizes the rotamer distributions obtained from the analysis. The most abundant rotamers are invariably types 2 and 3 for 'normal

* Quadrature detection in F_1 dimension was achieved using the TPPI method.[28]

residues' (Table 5), whereas a different distribution is obtained for *gem*-diaminoalkyl analogues, namely the predominant rotamers now become types 1 and 2 (Table 5).

It has been observed that the lack of activity of SP_{7-11} and SP_{8-11} may be related to a different rotamer distribution at [Phe^7] and [Phe^8], respectively.[1,19] From our data, it seems possible that the change in conformational distributions observed for the gPhe residues in peptides (**2**) and (**4**) does not play a determining role in their biological activity. It appears much more probable that the chemical modification of the latter peptides maintains some conformational accessibility for any rotamers, the specific biologically active form being selected at the receptor site.

Conclusions.—Many *C*-terminal fragments of Substance P maintain the biological activity of the parent peptide.[1] The activity is suddenly lost in SP_{n-11} with $n \geqslant 7$.[1,34,35] This behaviour has been related to the loss of the three-dimensional arrangement of the three binding loci, *i.e.* [Phe^7], [Phe^8], and [Met^{11}]$CONH_2$.[1] It has been proposed, in fact, that the *C*-terminal carbonyl group binds the [Gln^6] amide proton, either at the backbone or at the side chain.[1,19] In any case, a folded conformation of the *C*-terminal hexapeptide is necessary.

In addition, the *C*-terminal carboxamide also provides the essential NH_2 binding site, as clearly demonstrated by the reduced activity of [deamido^{11}]SP derivative.[1]

Previous n.m.r. evidence showed that the [Gln^6] backbone NH invariably exhibits a low temperature coefficient in any active fragment.[19] In SP_{6-11}, however, unambiguous conclusions cannot be drawn from the temperature coefficients of the [Gln^6]*N*-terminus, because of its different chemical nature (amine instead of amide)[18] (Table 1). Irrespective of the specific model adopted to describe the solution dynamics accounting for our experimental results, *i.e.* a predominant conformation with $\omega_o\tau_c$ *ca.*1 or a fast conformational equilibrium, the temperature coefficients as well as the R_{1p} data reflect some structural features supporting the presence of a folded structure in solution.

The small $\Delta\delta/\Delta T$ and R_{1p} values of NH[Phe^7] in peptides (**1**) and (**3**) may suggest the involvement of the latter proton in intramolecular hydrogen bonding. In the case of peptide (**2**) our data indicate that the donor role in a possible intramolecular hydrogen bond could be played by one of the [Met^{11}] carboxamide protons. No definite conclusions can be drawn for the corresponding data of peptide (**4**), though some degree of solvent shielding still exists at its *C*-terminal moiety.

The biological activities of peptides (**1**)—(**4**) were tested by guinea pig ileum contraction assay.[36] Therefore a correlation between the above structural considerations and activity data may be attempted. Two of the examined peptides (**1**) and (**3**) exhibit full biological activity, while the retro-inverso analogues (**2**) and (**4**) are still active, although at a five-fold lower level. This limited activity decrease might be due to the presence in solution of conformation(s) where the accessibility of [Met^{11}]-$CONH_2$ is reduced. However, selection of active structure may still take place at the receptor site.

Acknowledgements

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