Conformational Study of Some Component Peptides of Pentagastrin †

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The proton n.m.r. spectra of Asp, Phe, Asp-Phe-NH_2, Met-Asp-Phe-NH_2, Trp-Met-Asp-Phe-NH_2, and Boc-βAla-Trp-Met-Asp-Phe-NH_2 have been studied in aqueous and dimethyl sulphoxide solutions. From an analysis of the ^1H spectra of the Asp and Phe $C(\alpha)H{\cdot}C(\beta)H_2$ side-chains the vicinal coupling constants were obtained and used to calculate the rotational populations. There are no significant differences in the Phe side-chain rotamer populations between the tripeptide Met-Asp-Phe-NH_2 and gastrin pentapeptide βAla-Trp-Met-Asp-Phe-NH_2, but the Asp residue shows some small changes.

The presence of only small ring-current shielding contributions to the Phe aromatic protons from the aromatic ring in Trp can be taken to indicate that the aromatic rings are well separated (>5 Å) in the most populated conformers of gastrin pentapeptide in aqueous solution.

For dimethyl sulphoxide solutions, NH doublets could be observed for the peptides studied; the large values of the vicinal J_{NC} coupling constants for Met, Asp, and Phe in the tetrapeptide Trp-Met-Asp-Phe-NH_2 are shown to be consistent with the most populated conformations, having a ϕ angle of *ca.* −95°, from considerations of potential energy diagrams and the Bystrov-modified Karplus curve which relates dihedral angles to vicinal coupling constants.

SINCE the discovery by Gregory [1] in 1964 that the *C*-terminal tetrapeptide Trp-Met-Asp-Phe-NH_2 of gastrin has activity similar to that of gastrin itself with respect to stimulating acid secretion in the stomach, intensive studies of structure–activity relationships have been carried out on this compound. Morley and his co-workers [2] have shown that the Asp carboxy-group and one of the Phe amide protons are essential for the peptide to maintain high activity. However, since both of these groups are present in the inactive dipeptide Asp-Phe-NH_2 and tripeptide Met-Asp-Phe-NH_2 there are clearly additional structural requirements necessary to achieve activity. One possibility to explain the activity of the tetrapeptide is to assume that the Trp residue provides a means of hydrophobic bonding to the receptor as a first step in the process. Another possibility is that introduction of the Trp residue might alter the populations of the allowed conformations in solution and result in a significant increase in the population of a conformation where the active groups are favourably arranged for interaction. Of course there is no *a priori* requirement that one of the more populated conformations need be the active one, but if there is a conformational change on binding this will influence the energetics and kinetics of the hormone–receptor interaction. Thus, there is considerable interest in determining the conformational structures of such molecules in solution even though the full potential of the results will only be realised when their conformation in the bound states can be measured. Conformational studies of small linear peptides in solution are also of general interest in that, while many theoretical predictions have been made, there is very little experimental data available at the present time.

We have used high resolution n.m.r. spectroscopy to obtain conformational information in aqueous and dimethyl sulphoxide (DMSO) solutions for Asp-Phe-NH_2, Met-Asp-Phe-NH_2, Trp-Met-Asp-Phe-NH_2, and Boc-βAla-Trp-Met-Asp-Phe-NH_2.

† In this paper, the abbreviations for amino-acid residues and protecting groups are those recommended by the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature (*Biochem. J.*, 1967, **102**, 23).

EXPERIMENTAL

The samples were prepared as reported previously.[3] The ^1H n.m.r. spectra were recorded at 220 and 100 MHz using Varian HR 220 and HA 100D spectrometers. Usually the peptides were examined as solutions containing 20—30 mg in D_2O or DMSO (0·5 ml) [for Trp-Met-Asp-Phe-NH_2 a solution containing only 3 mg in D_2O (0·5 ml) was used]. Hexamethyldisiloxane (HMS) was used as an internal reference for the DMSO solutions. For the aqueous solutions an external HMS reference was used and the measured chemical shifts were converted to an internal reference [sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS)] scale.

RESULTS AND DISCUSSION

Unambiguous assignments for all the observed bands were made possible by the availability of a closely related set of peptides and by the use of spin-decoupling techniques to connect the NH, C(α)H, and $C(\beta)H_2$ absorptions in each amino-acid residue.

The ^1H n.m.r. spectra for the four peptides studied are shown in Figures 1—3, and Tables 1 and 2 present the chemical shift and coupling constant data.

Side-chain Conformations.—Conformational analysis. Information concerning the conformational situation in the $CH{\cdot}CH_2$ side-chains can be obtained by considering the measured vicinal coupling constants (see Table 2). The vicinal coupling constants were derived from an ABX analysis of the spectra of the $C(\alpha)H{\cdot}C(\beta)H_2$ fragment, the $C(\beta)H_2$ protons being non-equivalent owing to the molecular asymmetry resulting from the asymmetric α-carbon atom. The large difference between the two vicinal coupling contants suggests that hindered rotation is also a factor leading to non-equivalence of the $C(\beta)H_2$ protons.

Pachler [4] has shown how one can estimate the fractional

[1] H. J. Tracey and R. A. Gregory, *Nature*, 1964, **204**, 935.
[2] J. S. Morley, *Proc. Roy. Soc.*, 1968, *B*, **170**, 97.
[3] J. M. Davey, A. H. Laird, and J. S. Morley, *J. Chem. Soc.* (*C*), 1966, 555.
[4] K. G. R. Pachler, *Spectrochim. Acta*, 1964, **20**, 581.

TABLE 1

^{1}H Chemical shifts for pentagastrin and component peptides

Compound	Conditions	Phe C(β)H$_2$	Phe C(α)H	Asp C(β)H$_2$	Asp C(α)H	Met C(γ)H$_2$	Met C(β)H$_2$	Met C(α)H	Met SMe	Trp C(β)H$_2$	Trp C(α)H	Trp ArH	Phe ArH	Phe NH	Asp NH	Met NH	Trp ind. NH
* Asp–Phe–NH$_2$	28 °C–D$_2$O–pH>7	3·02 3·16	4·59	*ca.* 2·71	4·13								7·33				
* Met–Asp–Phe–NH$_2$	28 °C–D$_2$O–pH 7·9	2·98 3·20	4·55	*ca.* 2·55	4·76	2·39	*ca.* 1·86	4·06	2·03				7·29				
* Trp–Met–Asp–Phe–NH$_2$	28 °C–D$_2$O–pH 8·0	3·03 3·20		*ca.* 2·49			*ca.* 1·61		1·91	*ca.* 3·10			7·27				
* Boc–βAla–Trp–Met–Asp–Phe–NH$_2$	70 °C–D$_2$O–pH 9·2	*ca.* 3·2	4·55	*ca.* 2·53	4·49	2·26	*ca.* 1·81	4·26	1·98	*ca.* 3·19	4·65	7·60	7·22				
† Met–Asp–Phe–NH$_2$	28 °C–DMSO	*ca.* 2·9	4·29	*ca.* 2·58	4·50	3·76			1·96				7·17	8·14	8·77		
† Trp–Met–Asp–Phe–NH$_2$	28 °C–DMSO													7·92	8·33	8·72	10·94

* Shifts (p.p.m.) corrected to DSS (internal reference). † Measured (in p.p.m.) from HMS (internal reference).

TABLE 2

Vicinal H–H coupling constants and rotamer fractional populations for pentagastrin and component peptides

	H–H Vicinal coupling constants (Hz) *				Fractional populations					
	Asp		Phe		Asp			Phe		
Compound	J_{BX}	J_{AX}	J_{BX}	J_{AX}	$p_{(I)}$	$p_{(II)}$	$p_{(III)}$	$p_{(I)}$	$p_{(II)}$	$p_{(III)}$
Asp pH 4·8	3·3	9·2			0·06	0·60	0·33			
Asp pH 11·2	3·2	10·3			0·05	0·70	0·25			
Phe pH 2·16			4·5	8·3				0·18	0·52	0·30
Phe pH 9·84			4·9	8·4				0·21	0·44	0·35
Asp–Phe–NH$_2$ (alkaline soln.)	4·8	8·2	6·0	8·6	0·20	0·51	0·29	0·31	0·55	0·14
Met–Asp–Phe–NH$_2$ † pH 7·2	6·0	7·9	5·2	9·1	0·31	0·48	0·21	0·24	0·59	0·17
Met–Asp–Phe–NH$_2$ † pH > 7; 80°	6·0	7·9	5·2	9·1	0·31	0·48	0·21	0·24	0·59	0·17
Pentagastrin ‡ pH 9·2; 70°	7·0	7·4	5·1	9·2	0·40	0·44	0·16	0·23	0·60	0·17

* Accuracy ±0·1 Hz. † Accuracy ±0·2 Hz for Asp coupling constants. ‡ ±0·2 Hz for Phe coupling constants.

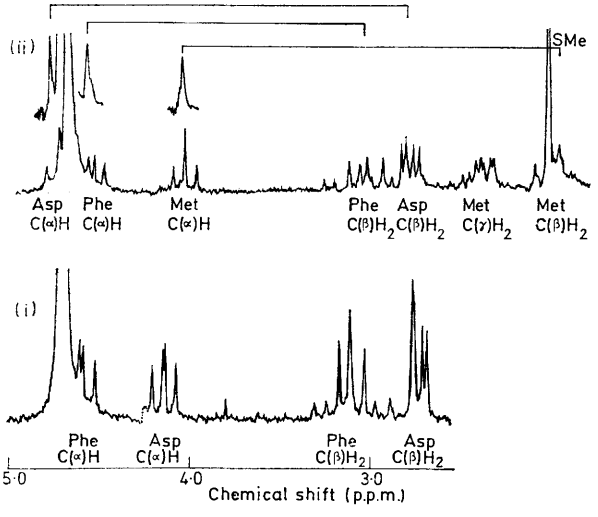


FIGURE 1 The ^{1}H n.m.r. spectrum at 100 MHz (i) of Asp–Phe–NH$_2$ in D$_2$O, and (ii) of Met–Asp–Phe–NH$_2$ in D$_2$O

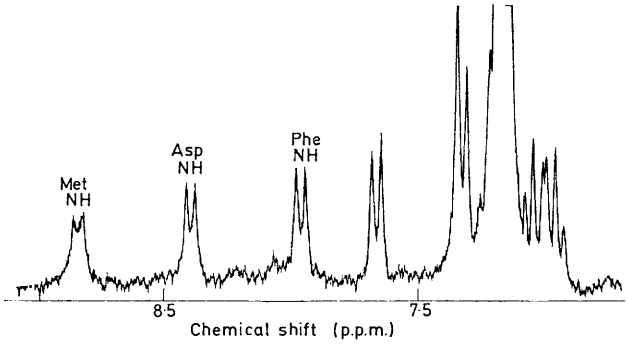


FIGURE 2 The low-field part of the ^{1}H n.m.r. spectrum of Trp–Met–Asp–Phe–NH$_2$ in DMSO at 220 MHz

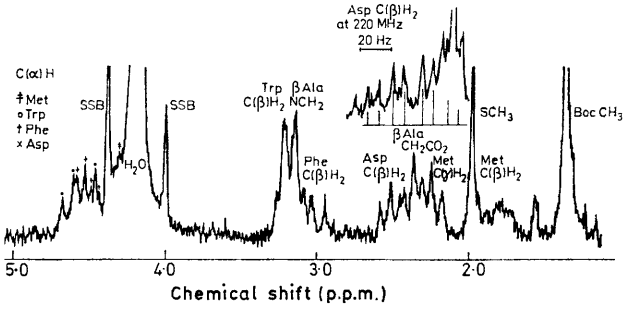
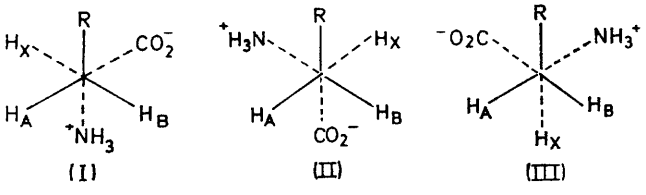


FIGURE 3 The ^{1}H n.m.r. spectrum at 100 MHz of Boc–βAla–Trp–Met–Asp–Phe–NH$_2$ in D$_2$O

populations $p_{(I)}$, $p_{(II)}$, and $p_{(III)}$ of the rotamers (I), (II), and (III) in α-amino-acids in solution by high-resolution n.m.r.



By measuring the averaged vicinal coupling constants J_{AX} and J_{BX} the fractional populations can be calculated, using values for gauche and *trans* vicinal coupling constants obtained from studies of model compounds (J_g 2·56, J_t 13·6 Hz) [5] and the equations (1)—(3)

$$p_{(I)} = (J_{BX} - J_g)/(J_t - J_g) \quad (1)$$

$$p_{(II)} = (J_{AX} - J_g)/(J_t - J_g) \quad (2)$$

$$p_{(I)} + p_{(II)} + p_{(III)} = 1 \quad (3)$$

[5] R. J. Abraham and K. A. McLauchlan, *Mol. Phys.*, 1962, **5**, 513.

This treatment assumes that the J_g and J_t values in rotamers (I), (II), and (III) are the same; although this approximation will lead to errors in the calculated fractional populations, it is possible to follow the trends in fractional populations for the different peptides studied.

In such an analysis it is not possible to distinguish between rotamers (I) and (II) unless one can assign H_A and H_B unambiguously.

Fortunately, the assignment of the $C(\beta)H_2$ protons in Asp can be made with some confidence by considering the effects of pH changes on the chemical shifts of the CH_2 protons (see later). Also a recent study [6] of stereospecifically deuterium-labelled Phe has enabled rotamers (I) and (II) to be assigned for Phe (Table 2).

Thus, by using the approach of Pachler [4] we have determined the Asp and Phe rotamer populations for the $C(\alpha)H{\cdot}C(\beta)H_2$ fragments in Asp, Phe, Asp-Phe-NH_2, Met-Asp-Phe-NH_2, and Boc β-Ala-Trp-Met-Asp-Phe-NH_2; the results are in Table 2. For the pentapeptide it is necessary to examine the spectrum at 220 MHz and at 70 °C to observe clearly the $C(\beta)H_2$ multiplets. Comparison of vicinal coupling constants in Met-Asp-Phe-NH_2 at 28 and 80 °C showed no appreciable change in the observed values (see Table 2).

For both Asp and Phe residues the $C(\beta)H_2$ proton at higher field shows the larger vicinal coupling constant in all the compounds studied; thus it seems likely that the assignments of the $C(\beta)H_2$ protons will be the same in the di-, tri-, and penta-peptide as in the Asp and Phe free amino-acids.

Similar data from the $C(\beta)H_2$ protons of the Trp, Met, and βAla side-chains could not be easily obtained because of extensive overlapping of their complex spectra.

Assignment of $C(\beta)H_2$ *protons in aspartic acid.* A consideration of the effects of pH changes on the $C(\beta)H_2$ chemical shifts allows the $C(\beta)H_2$ protons to be assigned. From such measurements the ionisation shifts at 100 MHz are found to be for $CO_2H \longrightarrow CO_2^-$ (pH 2·8—4·8) +20 H-1) and +29 Hz(H-2) and for $NH_3^+ \longrightarrow NH_2$ (pH 9·4—11·2) +14 (H-1) and +32 Hz(H-2) (shifts to higher fields as the pH increases).

In the light of these differential shifts, together with the observed coupling constants, the following points may be made. (i) In view of the small value of one of the vicinal coupling constants $J_{1,3}$ (3·3 Hz), only one of the rotamers (I) and (II) can be appreciably populated. (ii) Given this, the observation that the $C(\beta)H_2$ proton showing the larger vicinal coupling (H-2) is affected more than the other (H-1) by the $NH_3^+ \longrightarrow NH_2$ ionisation indicates that H-2 = H_A, and thus that rotamer (II) is the most populated. The large value found for $J_{2,3}$ is thus due to the large weighting from the J_t contribution. (iii) The smaller but significant differential shift on $CO_2H \longrightarrow CO_2^-$ ionisation indicates that rotamer (III) is also significantly populated.

We conclude therefore that the rotamer populations at pH 4·8 (zwitterionic form) are $p_{(I)}$ 0·06, $p_{(II)}$ 0·60, and $p_{(III)}$ 0·33: values for the rotamer populations in the anionic form are given in Table 2 and are essentially in agreement with values obtained previously for aspartic acid.[4,7] At pH values <3 the $C(\beta)H_2$ protons become equivalent and individual vicinal coupling constants cannot be extracted from the deceptively simple spectra. For such a case the relative populations of rotamers (I) and (II) cannot be obtained but the population of (III) can still be determined and at pH 2·2 the value of $p_{(III)}$ is 0·45.

Asp *and* Phe *Side-chain rotamer populations.* Table 2 shows that for aspartic acid in alkaline solution rotamer (II) has a high population (0·70), reflecting the charge repulsion of the two carboxylate anions. When the Asp side-chain in Asp-Phe-NH_2 is considered, rotamer (II) has a lower population than in aspartic acid because the formation of the peptide bond effectively neutralises the α-carboxylate group, resulting in the removal of the charge repulsion forces and an increase in the population of rotamer (I). For the Asp fragment of the tripeptide Met-Asp-Phe-NH_2, the increased bulkiness at the nitrogen atom caused by the formation of the Met-Asp bond further stabilises rotamer (I). When the pentapeptide is considered, rotamer (I) has increased its population such that rotamers (I) and (II) are almost equally populated.

In the case of the Phe side-chain, the population of rotamer (III) decreases on formation of the peptide bond in Asp-Phe-NH_2 and the other peptides, which indicates the presence of increased steric crowding in this rotamer when the α-nitrogen atom is substituted. The populations of rotamers (I) and (II) for Phe are very similar in Met-Asp-Phe-NH_2 and in the gastrin pentapeptide.

Side-chain Interactions.—In principle, the presence of the aromatic amino-acids Trp and Phe in gastrin peptides provides a means of obtaining conformational information from the presence or absence of proton shielding contributions attributable to aromatic ring-current shifts. By comparing the proton chemical shifts of gastrin pentapeptide in aqueous solution with those observed in the tripeptide and also with values calculated using the empirical chemical shift equations of Nakamura and Jardetzky [8] it was found that no large aromatic ring-current shifts were present. The Phe aromatic protons in both Met-Asp-Phe-NH_2 and βAla-Trp-Met-Asp-Phe-NH_2 are accidentally equivalent and appear as essentially a single peak, that in the pentapeptide being 0·11 p.p.m. more shielded than that in the tripeptide. Such a small ring-current shielding evenly distributed over the five protons is most likely to arise in a conformation where the rings are more or less parallel to each other, with their centres separated by at least 5 Å (from the tables of Johnson and Bovey,[9] z values of 1·3—1·5 and p values of 3·8—2·9 are seen to produce the observed shielding). One cannot rule out the possibility that the small net contribution could arise from a conformation of low population in which the aromatic rings are in much closer proximity. In this situation it would be more unlikely that the five Phe ring protons would be similarly shielded.

The absence of significant aromatic ring-current shielding effects in other parts of the gastrin pentapeptide also indicates that the aromatic rings are not in close proximity to any protons in other residues.

Backbone Conformation.—Backbone conformational studies depend on observation of the NH signals to allow measurement of the J_{NO} values and these could be measured for DMSO solutions.

The tripeptide Met-Asp-Phe-NH_2 could be examined for both aqueous and DMSO solutions; the NH doublet splittings arising from interaction with the C(α)H proton were

[6] G. W. Kirby and J. Michael, *Chem. Comm.*, 1971, 187.
[7] F. Taddei and L. Pratt, *J. Chem. Soc.*, 1964, 1553.
[8] A. Nakamura and O. Jardetzky, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **58**, 2212; *Biochemistry*, 1968, **7**, 1226.
[9] C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, 1958, **29**, 1012.

very similar for the two solvents. [For H_2O J_{NC} (Asp) 7·5 ± 0·2, J_{NC} (Phe) 8·0 ± 0·1; for DMSO J_{NC} (Asp) 7·0 ± 0·1, J_{NC} (Phe) 8·0 ± 0·1 Hz]. However, there is no reason to suppose that a similar situation will obtain for the tetra- and penta-peptides of gastrin, and their conformation in DMSO and water could well be different, as was found for oxytocin.[10,11] From the biological point of view we need to know the conformation in aqueous solution, since this is the thermodynamic reference state relevant to binding to the gastrin receptor. There is, however, some merit in examining conformations in other solvents since such studies give indications of the stability of the accessible conformational states.

Figure 2 shows the NH and aromatic region of the 220 MHz spectrum of Trp-Met-Asp-Phe-NH_2. The NH assignments were made on the basis of spin-decoupling experiments involving the NH, C(α)H, and C(β)H_2 protons. The temperature coefficients of the NH chemical shifts (0·048 p.p.m./10° C) are too large to be consistent with NH protons being involved in stable intramolecular hydrogen bonds; [12] this rules out the possibility of large populations of conformations, such as the α-helix, which are stabilised by hydrogen bonding. From the doublet splittings of the NH signals the J_{NC} values were measured as J_{NC} (Met) 8·0 ± 0·1, J_{NC} (Asp) 7·5 ± 0·1, and J_{NC} (Phe) 8·0 ± 0·1 Hz.

It is not possible to relate these J_{NC} values immediately with the appropriate dihedral angles ϕ using the Bystrov–Karplus relationship [13] because the $\cos^2\phi$ form of the equation usually allows several values of ϕ to be consistent with a measured J_{NC} value. Gibbons and his co-workers [14] have shown how this ambiguity can be reduced by taking account of the combinations of the dihedral angles ϕ and ψ [the C(α)H–CO dihedral angle] which are feasible energetically. For small peptides in solution it is to be expected that the observed J_{NC} values are weighted averages resulting from the distribution of possible conformations with different ϕ values. It is not possible from a single J_{NC} value to define this distribution of conformations. However, from potential energy calculations [15–17] one can readily predict this distribution. This can then be used to calculate the averaged J_{NC} value and by comparing this with the measured value one can establish whether a particular energy calculation adequately describes the peptide under consideration. We have carried out these calculations based on the potential energy diagrams of Scott and Scheraga [17] and Brandt and his co-workers [16] and find values of the averaged J_{NC} of 7·9 and 6·8 Hz, respectively. Tonelli and Bovey [15] have performed similar calculations based on the diagram of ref. 16 and obtained a J_{NC} value of 6·7 Hz.

Thus our results for the Met and Phe fragments could be explained by supposing that the distribution of conformers is described by the Scott and Scheraga map.[17] A feature of this map is that 97% of the allowed conformations are in the region of the map with ϕ −30 to −180°, and ψ 0 to 180° (Figure 4), with 70% of the conformers having ϕ values in the region −80 to −105°. It is of course possible to obtain a J_{NC} value of 8 Hz from other population distributions, but, bearing in mind the steric and other restrictions as expressed in the potential energy calculations, it seems likely that the predominant conformations of the Met and Phe fragments in gastrin tetrapeptide are those centred around ϕ −90 to −100° and ψ +60 to +90°.*

Thus, at the present time, measurement of J_{NC} in small linear peptides can be used to best effect in assessing the applicability of potential energy calculations.

The approach of combining information from J_{NC} values and potential energy calculations would be considerably more valuable if values of ψ could also be obtained experimentally (for example by using ^{15}N–H vicinal coupling constants).

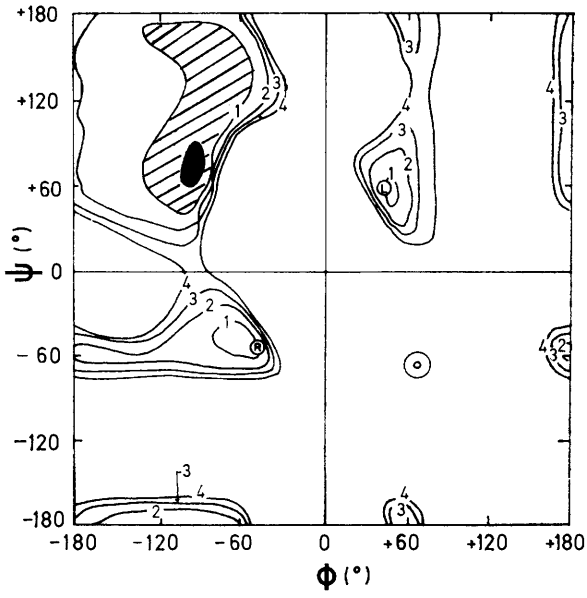


FIGURE 4 Potential energy diagram for a dipeptide as calculated by Scott and Scheraga,[17] 92% of the conformations fall in the shaded and black region and 57% in the black region of the diagram

Conclusions.—The Asp and Phe side-chain conformations in aqueous solution are found to be not very different for Met-Asp-Phe-NH_2 and pentagastrin, and it is unlikely that the relatively small differences observed could be related to the activity of pentagastrin.

The conformation of the tetrapeptide in DMSO is an extended coil with the Trp and Phe aromatic residues separated by at least 5 Å and with no CO · · · HN intramolecular hydrogen bonds. While many conformations are possible for the coil structure it seems likely that the predominant conformations for the Met and Phe fragments are those centred around ϕ −90 to −100° and ψ +60 to +90°.

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* For definition of ϕ and ψ see I.U.P.A.C.–I.U.B. Commission of Biochemical Nomenclature Report (*Biochemistry*, 1970, **9**, 3471).

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