Tetrahedron Letters No. 6, pp 483 - 486, 1972. Pergamon Press. Printed in Great Britain.

CONFORMATIONS OF CYCLO-L-HIS-L-SER, CYCLO-L-HIS-L-ASP, AND CYCLO-L-HIS-L-HIS[1]

Ziauddin, K. D. Kopple and C. A. Bush

Department of Chemistry, Illinois Institute of Technology, Chicago, Illinois 60616

(Received in USA 16 December 1971; received in UK for publication 6 January 1972)

P.m.r. studies of solutions of the cyclic dipeptide c-Gly-L-His show that the histidine side chain prefers the $\chi_1 = 60^\circ$ rotamer, the evidence being ring current shielding of the glycine α-protons by the imidazole group[2]. This conformation, aromatic ring toward diketopiperazine ring, is favored consistently in cyclic dipeptides of phenylalanine, tyrosine, and tryptophane[2-5]. We have now examined aqueous solutions of c-L-Ser-L-His, c-L-Asp-L-His and c-L-His-L-His to see if this conformational preference of aromatic residues affects interactions between functional side chains. In these peptides, including c-L-His-L-His, the tendency of the aromatic residue to be more stable near $\chi_1 = 60^\circ$ is also apparent, and certain features of the conformation of the second side chains are noteworthy. Table I presents the data on which our conclusions are based.

c-L-Ser-L-His. The 2.6 Hz. α-β coupling constant of the serine residue in the cationic form of c-L-Ser-L-His indicate that its side chain is almost completely in the $\chi_1 = 60^\circ$ conformation, hydroxyl group toward the diketopiperazine ring (shown in I). For the cation of the O-acetyl derivative, analysis[6] of the coupling constants indicates that, in spite of the added bulk, the $\chi_1 = 60^\circ$ state of the serine side chain is still three times as densely populated as the more stable of the other two staggered forms. For the free base of c-L-Ser-L-His, the $\chi_1 = 60^\circ$ rotamer, although somewhat less favored, is again the predominant one.

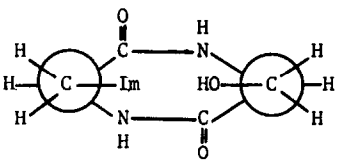
Table I. β-Proton Resonances in Histidine-Containing Cyclic Dipeptides

		Seryl or Asparty1					Histidyl			
c-L-His-L-	pH	δ	$J_{\alpha\beta}$	δ	$J_{\alpha\beta}$	$J_{\beta\beta}$	δ	$J_{\alpha\beta}$	δ	$J_{\alpha\beta}$
Ser	7-8	3.32	5.4	3.59	3.1	11.8	3.16	---	3.16	---
	3	3.53	2.6	3.93	2.6	12.2	3.20	5.0	3.46	5.9
OAc-Ser	7-8	3.53	6.0	4.08	3.5	11.5	3.14	4.8	3.25	6.1
	3	4.14	4.7	4.26	3.2	11.6	3.31	5.5	3.40	6.1
Asp	10	1.38	10.5	2.46	2.9	16.5	3.13	---	3.13	---
	5-6	2.10	8.0	2.62	3.8	16.4	3.32	---	3.32	---
	3	2.75	---	2.75	---	---	3.38	---	3.38	---
His	10						2.40	7.4	2.90	4.3
	3						3.18	---	3.18	---

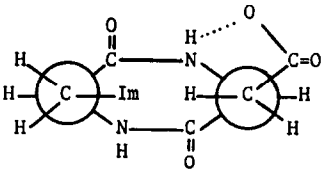
Reference: 2,2-dimethyl-2-silapentane-5-sulfonate
Ser and OAc-Ser derivatives run at 220 MHz, others at 60 MHz.

The serine β-protons in the neutral form of c-L-Ser-L-His are shifted upfield 0.6 and 0.3 ppm relative to c-L-Ser-L-Ser in water (3.91 ppm). The proton with the larger coupling constant, which spends more time trans to the α-proton, has the greater upfield shift. This is consistent with ring current shielding by the imidazole group, and indicates significant contributions from the $\chi_1 = 60^\circ$ rotamer of the histidine side chain. Analysis of the coupling constant data indicate that this is the most favored of the three staggered rotamers, although on the average only about half of the side chains are in this state.

An X-ray crystallographic analysis of the structure of c-L-Ser-L-Tyr indicates $\chi_1 = 60^\circ$ for both the side chains, with an almost planar diketopiperazine ring[7]. The nmr results presented here suggest that this (I) is probably the most favored conformation of c-L-Ser-L-His as well. However, this conformational preference does not lead to any strong interaction between the functional side chains, at least as judged by the pK_a's of the peptides. For c-Gly-L-His, c-L-Ser-L-His, and c-OAc-L-Ser-L-His the measured pK_a's at 30°, $\mu = 0.085$, are 6.3, 6.4, and 6.2, respectively. We have also determined a pH-rate profile for hydrolysis of the O-chloroacetyl derivative and find no evidence of a term for intramolecular imidazole catalysis.



I.



II.

c-L-Asp-L-His. The α-β coupling constants of the aspartic acid residue of the anionic form of c-L-Asp-L-His indicate that either $\chi_1 = 180^\circ$ or $\chi_1 = 300^\circ$ is the predominant conformation (0.7 mole fraction). There is no significant change in these coupling constants between 2 and 60°. It seems likely that the favored form is $\chi_1 = 300^\circ$, which allows a good hydrogen bond between the carboxylate and N-H of the aspartic residue (II). Again the proton closest to the imidazole ring (highest $J_{\alpha\beta}$) is markedly shifted upfield, 1.26 ppm relative to the c-Gly-Asp anion at 2.64 ppm, indicating that the $\chi_1 = 60^\circ$ conformation of the histidine residue is important. In the dipolar form of the peptide (pH 5-6), the preference for χ_1 (Asp) = 300° is diminished, possibly because of attraction between the oppositely charged side chains, although the coulombic interaction is not sufficient to affect the imidazole basicity detectably, the pKa of the imidazole being 6.3 at 30°, $\mu = 0.085$.

c-L-His-L-His. This dipeptide, in neutral form, bears a considerable resemblance to the cyclic anhydride of tyrosine[3], in that the α-β coupling constants and the upfield shifts of the β-protons indicate likelihood of a conformation in which the imidazole rings are sharing space over the diketopiperazine ring.

We have examined the ultraviolet absorption of c-L-His-L-His, c-L-His-Gly, c-L-His-L-Ser and c-L-His-L-Asp for evidence of side chain interactions. The spectra were decomposed into Gaussian bands, and a band of $\varepsilon \cong 4{,}500$ is assigned to histidine at 212 nm, at both pH 8 and in one equivalent of acid. In the case of c-L-His-L-His there is a 15% hypochromism at pH 8, outside experimental error, which does indicate some side chain interaction. Similar effects are well known in DNA and imply parallel planar arrangements of aromatic chromophores[8].

We have also examined the ultraviolet circular dichrosim of our compounds to 185 nm. The hypochromism in c-L-His-L-His led us to expect evidence of side chain interaction in the side chain CD bands, as has been observed for c-L-Tyr-L-Tyr[9]. In fact, we do not observe

evidence of side chain chromophore coupling, and it is impossible to assign unambiguously any of the CD bands to the side chain chromophore. The CD of c-L-His-Gly, c-L-His-L-Ser c-L-His-L-Asp at basic pH are similar to that of c-L-Ala-Gly[10] although we observe slightly larger negative CD, indicating that histidyl is a stronger perturber than alanyl. However, there are no CD bands which can be distinctly assigned to histidyl chromophores. The effect of protonation of the histidyl side chain on the CD curve is similar to that observed in protonation of c-L-Orn-L-Orn or c-L-Lys-L-Lys[11]. Protonation adds some positive CD in the $n-\pi^*$ region near 220 nm, but, as in the case of the basic solutions, the protonated imidazole group does not make any distinct chromophoric contribution to the CD.

Acknowledgement. This research was supported by grants from the U. S. Public Health Service, National Institute of General Medical Sciences, GM-14069 and GM-47357, and from the National Science Foundation, GP-20053.

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