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AMINO ACID DERIVATIVES THAT STABILIZE SECONDARY STRUCTURES OF POLYPEPTIDES
II. THE MOST STABLE CONFORMATION OF PEPTIDES CONTAINING 3-AMINO-2-PIPERIDONE-6-CARBOXYLIC ACID (Acp)

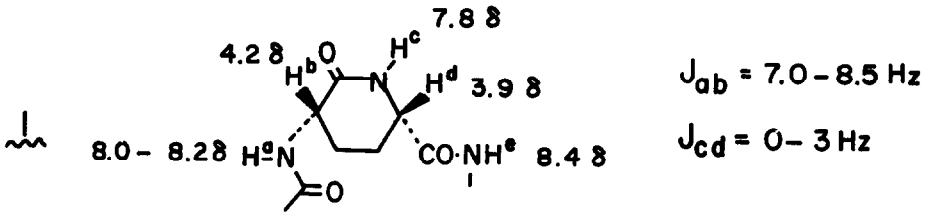
D.S. Kemp* and Paul McNamara

Department of Chemistry

Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Abstract: From ^{1}H NMR evidence, the conformation of the piperidone ring of 3-amino-6-carboxy-2-piperidone (Acp) in the derivatives Ac-Acp-NHEt. Cbz-Gly-Acp-OEt, Cbz-Gly-Acp-Gly-OEt, Boc-L-Phe-LL-Acp-L-Ala-OCH_3, and Boc-L-Phe-DD-Acp-L-Ala-OCH_3 is shown to be a chair with the RCONH function equatorially and the $CONHR^1$ function axially disposed. An internal hydrogen bond of the β-turn type is proposed for these amide derivatives.

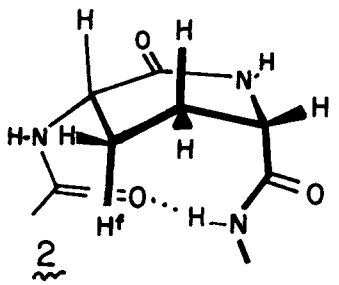
In the accompanying communication we have reported the synthesis of LL-3-amino-6-carboxy-2-piperidone (LL-Acp), a novel amino acid which appears from models to be likely to appear at a β-turn conformation if incorporated into a polypeptide sequence. In this communication we report features of a 250 MHz proton NMR study of Ac-Acp-NHEt(1.); Cbz-Gly-Acp-OR, R = OH(3.), ONa(4.), OEt(2.); Cbz-Gly-Acp-Gly-OEt(5.); Boc-L-Phe-LL-Acp-L-Ala-OCH_3(6.), and Boc-L-Phe-DD-Acp-L-Ala-OCH_3(7.).[1]



Several spectroscopic features of the Acp function appear consistently in ^{1}H NMR spectra of 1. through 7., as seen in Table I. For example the methine resonances of peptide-linked Acp functions appear at 4.15-4.20 δ (2. through 7.) and 3.90-3.95 δ (5. through 7.). Since the latter is shifted by 0.4 δ upon ionization of the acid function of 3., it belongs to H^d of 1.[2]. From the observed H^a-H^b and H^c-H^d coupling constants and Karplus curves for peptide

derivatives[3] the dihedral angles at these sites can be estimated as 0-10° or 140-150° for the former and 60-90° for the latter.

The Acp piperidone provides an ^1H spin system too complex to permit multiplet spacings to be assigned as J values. However, a conformation can be assiged the Acp residue from peak widths at half height (W_H), which have useful indicators of the orientations of cyclohexane protons.[4] Irradiation of the 8.21δ NH resonance of 1. results in a four-line H^b resonance with W_H of 17 Hz (peak sep: 6.5,4.0 Hz). This resonance must be assigned [4] to a proton with an essentially axial orientation. That of H^d (W_H = 10-11 Hz) is consistent only with a proton with an equatorial orientation. Thus the chair 2 best describes the conformation of the Acp residues of 1. through 7.[5]



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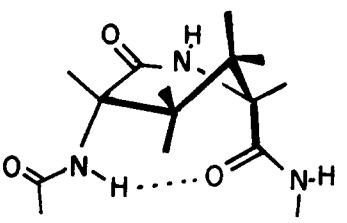

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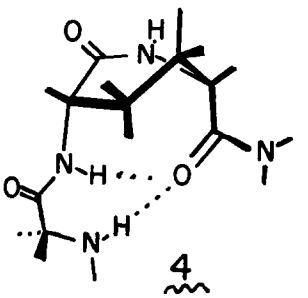

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The pattern of methylene resonances of 2.,3.,4., and 6. provides independent confirmation of this assignment. For 2., for example, a complex three-proton multiplet appears at 1.8-2.2 δ, while the remaining methylene resonance appears as a pattern of four broad lines (sep: 12 Hz) at 1.48-1.55 δ with a width between outer lines of 36 Hz. Irradiation of H^b results in a collapse to a three-line resonance (sep: 12.5 Hz) with a width of 25 Hz. This resonance must be assigned to a geminally coupled proton which is axial both to H^b and to a methylene hydrogen, conditions which are met uniquely by H^f of the chair conformation 2.[6]

This demonstration that three of the six hydrogens of the Acp function have J values expected for axial orientations excludes alternative boat-like conformations such as 3 and 4 as major contributors to the conformational equilibria for Acp derivatives. It should be noted that a dihedral angle of 60° at H^c-N-C-H^d is consistent with structure 2, and that angles of either 140-150° or 0-10° for H^a-N-C-H^b minimize nonbonded interactions. The latter range of angles permits an intramolecular hydrogen bond, as shown in 2, and this is probably the more stable orientation of the two amido groups.

Temperatures dependences in the range of 0 to $-3x10^{-3}$ ppm/°C. are characteristic of the proton resonances of amide NHs which are internally hydrogen bonded in β-turns; dependences which lie below $-6x10^{-3}$ppm° C. characterize amide NH functions that are hydrogen bonded with solvent.[7] Values between -3 and -6 $x10^{-3}$ are usually rationalized as belonging to amide protons that equilibrate between several stable conformations.[8] As can be seen from Table I, the temperature dependence of the 7.9 δ NH resonance of 1. and the 8.4 δ resonance of 5., 6., and 7. lie in the range of -3 to -4 $x10^{-3}$ ppm/°C., and are thus not inconsistent with

internally hydrogen-bonded structures but cannot be used to establish them. Rapid equilibration between 2 and the less stable conformations 3 and 4 (which are not available to the more normal type I or II β-turns formed from s-trans amides[9]) is the most plausible explanation for these findings.

These results have been obtained in DMSO solution, but similar results in other solvents[5] suggest that a single chair conformation is a reliable feature of the piperidone of an Acp residue, even when flanked by the turn-breaking[11] amino acids Phe and Ala. Regardless of the details of hydrogen bonding, the chair conformation with H^b axial and H^d equatorial enforces a turn conformation on peptides containing Acp residues.

Table 1 Chemical Shifts of ^1H NMR Resonances
25°C, 250MHz, $(CD_3)_2SO$

Substance	NH Chemical Shift δvalue, multiplicity, J value (Hz)			NH Temperature Dependence[a] per °C x(-10^9)	CH chemical shift(δ)	Width at half height (Hz)
1.	8.21	d	8.0	5.8	4.00	26
	7.92	t	5.5	4.0	3.79	11
	7.70	d	2.5	4.6		
2.	8.10	d	7.5	---	4.24	27
	7.78	bs		---	4.04	12
	7.40	t	6.0	---		
3.	8.12	d	7.5	---	4.21	26
	7.71	bs		---	3.94	11
	7.46	t	5.5	---		
4.	8.03	d	7.5	---	4.17	26
	7.47	t	6.0	---	ca3.5[b]	
	7.09	s		---		
5.	8.42	t	5.5	3.4		
	8.02	d	8.0	3.6	4.15[b]	
	7.77	d	3.0	3.5	3.92[b]	
	7.47	t	5.7	4.0		
6.	8.39	d	7.0	4.4	4.20[b]	
	8.09	d	8.5	4.0	3.95	10
	7.67	bs		3.8		
	6.84	d	8.8	6.5		
7.	8.45	d	7.5	3.8	4.20	26
	8.27	d	7.1	3.1	3.90	11
	7.81	d	3.0	5.3		
	6.85	d	8.8	4.3		

[a] Measured over the temperature range of 297° to 343°K.
[b] Peak overlaps other resonances.

Acknowledgement

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References

1. Species 1. - 7. were prepared from racemic H-Acp-OH, formed by the method of H. Newman (J. Het. Chem., 1974, 11, 449); 6. and 7. were separated by preparative HPLC, and 6. was identified by independent synthesis from H-LL-Acp-OH, described in the accompanying paper. Satisfactory elemental analyses were observed for new substances.
2. Irradiation of H^b causes collapse of the 8.0-8.2 δ doublet; for 6, irradiation of the alanine methyl identifies its methine proton, which upon irradiation is established as vicinal to the NH that has resonance at 8.39 δ.
3. Demarco, A., Llinas, M., and Wuthrich, K., Biopolymers, 1978, 17, 637; Ramachandran, G., Chandrasakaran, R., and Kopple, K., Biopolymers, 1971, 10, 2113; Bystrov, V., Portnova, S., Belashova, T., Kozmin, S., Gavrilov, Y., and Afanasev, V., Pure Appl. Chem., 1973, 36, 19.
4. For vicinal coupling, $J_{axial,\ axial}$ lies in the range of 8 - 13 Hz and $J_{axial,}$ equatorial, of 2-6 Hz; as a result, half-height width is > 15 Hz for axial and < 12 Hz for equatorial protons of chair cyclohexanes. Jackman, L.M., and Sternhell, S., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed. Pergamon, 1969, pp. 133, 288.
5. Similar peak widths are seen for methine resonances of Ac-Acp-NHEt in $CDCl_3$ and in D_2O CD_3OD.
6. We offer the tentative correlation that the separation of the chemical shift of H^f occurs in those Acp derivatives in which a phenyl group can approach the piperidone ring; this suggestion explains the difference between the diastereomers 6. and 7., since only the former exhibits this δ-difference.
7. Kopple, K., Ohnishi, M., and Go, A., J. Am. Chem. Soc., 1969, 91, 4264; Kopple, K., Schamper, T., in "Chemistry and Biology of Peptides", J. Meienhofer, ed., Ann Arbor, 1972, p.75; Hruby, V., in "Chemistry and Biochemistry of Peptides", Vol. 3, Weinstein,B. ed., Dekker, 1974, p.1.
8. Kopple, K., Go, A., and Schamper, T., J. Am. Chem. Soc., 1978, 100, 4289.
9. The distance between C^α sites of Acp derivatives is ca 0.29nm, which may be compared with 0.38nm for the C_2^α and C_3^α sites of a type I or type II β-turn. It should be noted that the chemical shifts of NHs reported in Table I for Acp derivatives do not fit the pattern reported for β-turns composed of s-trans amide groups. We attribute part of the normal chemical shift of the hydrogen-bonded NH of 7.3-8.0 δ[10] to a magnetic anisotropy of the neighboring s-trans amide. The amide function of an Acp residue is not proximate to the hydrogen bond of 1, and the models of Portnova et al. are thus inapplicable to Acp derivatives.
10. Portnova, S., Shilin, V., Balashova, T., Biernat, J., Bystrov, V., Ivanov, V., and Ovchinnikov, Y., Tetrahedron Lett., 1971, 3085.
11. Chou, P. and Fasman, G., J. Mol. Biol., 1977, 115, 135.

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