Tetrahedron Letters No.60, pp. 5283-5286, 1969. Pergamon Press. Printed in Great Britain.

CONFORMATIONAL STUDIES OF PEPTIDE SYSTEMS. NMR AND IR SPECTRA OF N-ACETYL-ALANYL-PHENYLALANINE AND N-ACETYL-PHENYLALANYL-ALANINE METHYL ESTERS.

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(Received in the UK 31 October 1969; accepted for publication 1 December, 1969)

In a previous study, the conformational states of alanyl-alanine dipeptides in solution have been elucidated.[1] The study was largely based on the dependenc of the NH-CH vicinal proton coupling constant upon the dihedral angle θ between the HNC and NCH planes:

$$^{3}J_{NHCH} = A\cos^{2}\theta - B\cos\theta + C\sin^{2}\theta .$$

Values for this dependence calculated on a IBM-360/60 computer for values the coefficients varying within the following limits (I): $8.9 \leq A \leq 9.8$ Hz, $0 \leq B \leq (A - 8.9)$ and $C = 9.8 - A$, are graphically depicted by the hatched area shown on Fig.I.

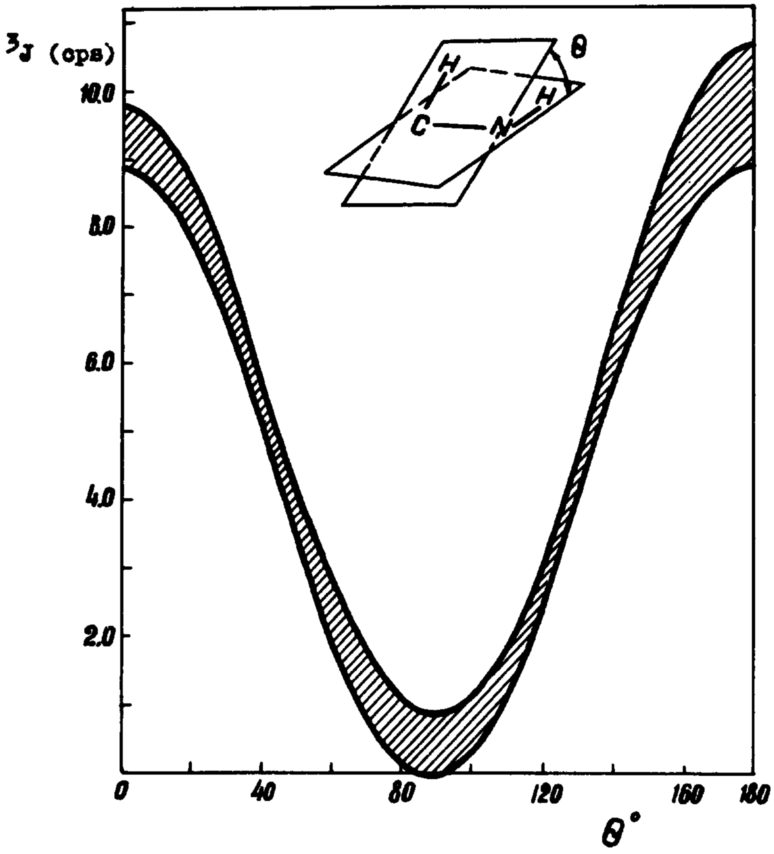
The present paper reports the results of conformational studies based on IR and NMR spectroscopy of the methyl esters of the DD- and DL-isomers of N-acetyl-alanyl-phenylalanine (I and II respectively) and N-acetyl-phenylalanyl-alanine (III and IV):

$$CH_3CON_{(6)}H-C_{(5)}R''H-CON_{(3)}H-C_{(2)}R'H-COOCH_3 .$$

In this case, besides the vicinal coupling constants, the presence of the highly magnetically anisotropic benzene ring has made possible the use for the conformational analysis also the other principal NMR parameter - the chemical shift.

The IR spectra of the dipeptides I - IV yielded data analogous to the results obtained with the alanyl-alanine dipeptides (I,2). The fraction n of the intramolecular hydrogen-bonded folded form of the dipeptides III and IV (in

Fig. 1



dilute CCl_4 solutions) in which the phenylalanine residue forms part of the folded portion, is lower (0.62 and 0.67 respectively) then for dipeptides I and II (0.76 and 0.81). Apparently the bulky R" group of the former compounds presents a certain barrier to the formation of the seven-membered hydrogen bonded ring.

Assignments of the NMR peaks were carried out by comparison with the data on the alanyl-alanine dipeptides (1) and the use of double resonance. Table 1 shows the values of the vicinal coupling constants for the $>C^{\alpha}H_X-C^{\beta}H_AH_B$ of Phe and NH-CH fragments. The coupling constants of the latter have been corrected for the effect of substituent electronegativity (1).

The IR and NMR data show that the conformation of the folded part of molecules I - IV is similar to that of the alanyl-alanine dipeptides (1). In this conformation the dihedral angle θ of the $N_{(6)}H-C_{(5)}H$ fragment is

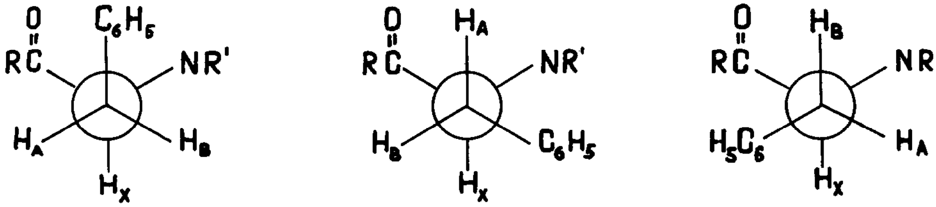
Table 1

Dipeptide	Solvent	Coupling constants (cps)					rotamer populations about C^{α}-C^{β} bond		
		AX	BX	AB	$N_{(3)}H$-$C_{(2)}H$	$N_{(6)}H$-$C_{(5)}H$	a	b	c
I	$CDCl_3$	7.09	5.50	-13.97	8.3	8.1	0.32	0.41	0.17
	DMSO-d_6	9.47	4.56	-13.92	8.1	8.3	0.18	0.64	0.18
II	$CDCl_3$	7.50	5.37	-13.97	8.3	7.9	0.30	0.45	0.25
	DMSO-d_6	10.17	5.17	-13.77	8.7	8.4	0.07	0.69	0.24
III	$CDCl_3$	deceptively simple spectrum			8.3	8.4	-	-	-
	DMSO-d_6	9.80	4.40	-13.80	7.8	9.2	0.17	0.66	0.17
IV	$CDCl_3$	8.49	5.93	-13.56	8.3	8.3	0.15	0.54	0.31
	DMSO-d_6	9.34	5.41	-13.60	7.9	9.2	0.12	0.62	0.26

within the limits 15–25° corresponding to angles $\varphi_{N-C^{\alpha}}$ in the region of 240° and $\psi_{C^{\alpha}-C'}$ in the region of 120° (3). Theoretical considerations (4) show that thisregion should exhibit energy minima on the conformational maps if account be taken of intramolecular hydrogen bonding.

Based on the data for phenylalanine (5) the assumption was made that the preferred rotamer is b with trans orientation of the C_6H_5 and C=O groups (Fig.2) Calculation of the populations of the a, b and c rotamers (see Table 1) was based on the values $J_{\underline{trans}}$=13.5 cps and $J_{\underline{gauche}}$=2.6 cps (6).

Fig.2



a (χ_1=60°) b (χ_1=300°) c (χ_1=180°)

For the $N_{(3)}H$-$C_{(2)}H$ fragment the values of the vicinal coupling constants indicate the possibility of two alternative preferable states with either cis or trans N-H and C-H bonds (φ 240° and 60° respectively). The choice between the two was made on the bases of the effect of the magnetic anisotropy of the benzene ring (7) on the screening of the alanine methyl group (as compared with

Table 2. Calculated and experimental differential chemical shifts (p.p.m.) of the alanyl CH_3 group.

Dipeptide	Calculations		experiment
	cis-NH-CH	*trans*-NH-CH	
I	-0.07	+0.0I	-0.09
II	+0.09	-0.07	-0.I7
III	-0.04	+0.I3	-0.04
IV	+0.06	-0.07	-0.I8

the corresponding alanyl-alanine stereoisomers). The calculation was carried out on the basis of the conformation determined for the folded form and rotamer populations of the side chain, assuming that the plane of the benzene ring is perpendicular to the plane, formed by the $C^{\alpha}-C^{\beta}-C^{\gamma}$ atoms. From a comparison of the experimental and calculated chemical shifts (Table 2) it is evident that for DD-dipeptides (I and III) the $N_{(3)}H-C_{(2)}H$ fragment is preferably *cis* ($\varphi \sim 240^{\circ}$) whenever for the DL-isomers (II and IV) it is preferably *trans* ($\varphi \sim 60^{\circ}$). From molecular models it follows that the preference for these rotational conformers is apparently determined by the potential energy minimum of nonbonded interactions. In the alternative conformations one may expect steric repulsion between the benzyl groups and the alanyl methyl.

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