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DETERMINATION OF THE CONFORMATION OF PEPTIDE AND N-METHYLATED PEPTIDE BONDS BY OBSERVATION OF ^5J(HH) PROTON SPIN-COUPLING USING NMDR SPECTROSCOPY

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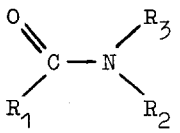
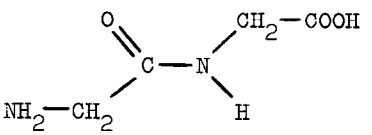
In polypeptides and proteins the peptide bond is rigid and planar and exists in the trans conformation, (I, R_2=H and II). Some linear and cyclic peptides contain N-methylated peptide bonds which can exist in either the cis or trans conformation. A necessary part of the determination of the total conformations of such molecules in solution is the ability to distinguish between the cis and trans conformations of the peptide bond.

A number of NMR phenomena have been used to distinguish between the cis and trans conformations of amide bonds[1], eg magnetic anisotropy effects, aromatic solvent induced shifts, lanthanide induced shifts, nuclear Overhauser effects and long-range spin-coupling. Only the double resonance effects are of general application for both the peptide and N-methyl peptide bonds especially for molecules dissolved in aqueous solution.

In a study of substituted amides, de Kowalewski[2] indicated that ^5J(HH) for dimethyl acetamide (I, R_1=R_2=R_3=CH_3) can be observed between the anti-periplanar methyl groups and that no long-range coupling was observed in ethyl substituted amides or in acetylated amino-acids. We have re-examined some representative examples of the compounds in which



I



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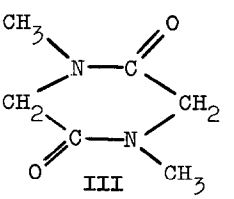

III

long-range proton spin-coupling was not observed,[2] eg a monoethyl substituted amide (N-ethyl acetamide; I, R_1=CH_3, R_2=H, R_3=CH_2), a disubstituted amide (N,N-diethyl formamide; I, R_1=H, R_2=R_3=CH_2) and an acyl amino-acid (N-acetyl glycine; I, R_1=CH_3, R_2=H, R_3=CH_2). Under NMR conditions of high resolution and small sweep width we have shown by double resonance experiments that a small long-range proton spin-coupling exists between groups anti-periplanar to each other across the amide and peptide bond, (Table I).

The value of the spin-coupling constant was estimated by measuring the difference in line-widths of coupled and decoupled signals over five successive spin-decoupling experiments. The values obtained from line-width measurements are listed in Table I. Although this procedure does not give precise values of ^5J(HH), the importance of the observation of long-range spin-coupling is in the determination of the conformations of amide and peptide bonds in solution.

We have used NMDR spectroscopy to confirm the known conformations of a linear and a cyclic peptide. In the linear dipeptide glycyl-L-alanine (II) a small long range coupling of 0.4Hz (Table 1) was observed between the glycyl methylene proton signal and that of the α-CH proton of the alanine residue. The observation of such coupling indicates that these groups are in an anti-periplanar arrangement and so confirms the trans conformation of the peptide bond. Dale and Titlestad[3] have synthesised a number of cyclic peptides using the amino-acid sarcosine (N-methyl glycine) assigning the number of peptide bonds existing in the cis or trans conformation from variable temperature NMR studies. In cyclic disarcosine (III) which can only exist in the cis conformation, the methylene group is anti-periplanar to the N-methyl group. We have observed a coupling of 0.22Hz between the methyl and methylene signals of cyclic disarcosine (III), which is expected for the molecule in the cis conformation. By an extension of these two results we have used NMDR spectroscopy to assign the two sets of methyl and methylene signals of N-acetyl sarcosine which exists as an equilibrium of the cis and trans conformational isomers in solution. In the trans isomer (I, R_1 = CH_3, R_2=CH_3, R_3=CH_2COOH), the acetyl methyl signal is anti-

periplanar to the methylene group and in the cis isomer (I, R_1=CH_3, R_2=CH_2COOH, R_3=CH_3) the acetyl methyl signal is anti planar to the N-methyl group. The upfield N-methyl signal can be assigned the cis conformation

Table I LONG RANGE PROTON SPIN COUPLING CONSTANTS

COMPOUND	SOLUTION	R_1	R_2	R_3	ISOMERS	J(±0.05)Hz R_1R_3 anti	J(±0.05)Hz R_1R_2 syn
N,N-dimethylacetamide	D_2O(20%^{V}/v)	CH_3	CH_3	CH_3	cis/trans	0.5[a]	0.1[b]
N-ethylacetamide	D_2O(20%^{V}/v)	CH_3	D	CH_2	trans	0.4[a]	-
N,N-diethylformamide	D_2O(20%^{V}/v)	H	CH_2	CH_2	cis/trans	0.18[b]	c
N-acetylglycine	D_2O(0.1M)	CH_3	D	CH_2	trans	0.15[b]	-
N-glycyl-L-alanine	D_2O(0.1M)	CH_2	D	CH	trans	0.4[b]	-
cyclic-disarcosine	D_2O(0.1M)	CH_2	CH_2	CH_3	cis	0.22[b]	-
N-acetyl sarcosine	D_2O(0.1M)	CH_3	CH_2	CH_3	cis	0.28[b]	c
		CH_3	CH_3	CH_2	trans	0.10[b]	c
Beauvericin[d]	$CDCl_3$(0.03M)	CH	CH_3	CH	trans	0.15[b]	c

[(L-MePhe-DHyIv)$_3$]

a J taken from peak separations of resolved multiplet signals.

b J derived from line-width measurements at half-height of coupled and decoupled signals

c No coupling observed within experimental error.

d Symbols L-MePhe designates N-methyl-L-phenylalanine and D-HyIv designates D-α-hydroxyisovaleric acid.

with the N-methyl group syn-coplanar to the carbonyl group[1]. NMDR spectroscopy has provided an independent confirmation of the assignment of all the signals from their spin decoupling behaviour.

As an application of this method to an unknown situation we have determined the conformation of the N-methylated peptide bond found in

the cyclic depsi-peptide antibiotic, beauvericin[4]. A small long-range coupling constant can be observed between the α-CH protons of the N-methyl phenylalanine and α-hydroxyisovaleric acid residues which indicates that these two groups exist in an anti-periplanar arrangement, ie the N-methylated peptide bond exists in the trans conformation in solution.

We have observed a five-bond long-range proton spin-coupling between groups which are anti-periplanar to each other across a peptide and N-methylated peptide bond. The observation of this coupling provides a direct determination of the cis and/or trans conformation of peptide bonds for such molecules in solution.

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