Chemical Shift Nonequivalence of the Methylene Group in Certain Glycyl Dipeptides

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MAGNETIC NONEQUIVALENCE of methylene protons is caused by a variety of factors[1] and this phenomenon has been used in the structural elucidation of natural products.[2] In continuation of our studies devoted to steric effects on physical properties,[3] it was anticipated that glycyl dipeptides would exhibit similar behaviour. The n.m.r. spectra of a few glycyl compounds have been tabulated in

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several studies[4] and recently, two reports have specifically noted methylene nonequivalency.[5]

New measurements on a group of glycyl dipeptides in deuterium oxide clearly reveal patterns of both equivalency and nonequivalency (Table).

TABLE

Chemical shifts of glycyl methylene protons[a]

Compounds	Dipeptides	CH_2	J	Compounds	Dipeptides	CH_2	J
(I)	Glycyl-L-alanine	3·85s	—	(X)	L-Alanylglycine	3·83s	—
(II)	Glycyl-L-α-aminobutyric acid[b]	3·87s	—	(XI)	L-α-Aminobutyrylglycine	3·92, 3·80q	17
(III)	Glycyl-L-isoleucine	3·87s	—	(XII)	L-Isoleucylglycine	3·92, 3·72q	18
(IV)	Glycyl-L-leucine	3·84s	—	(XIII)	L-Leucylglycine	3·92, 3·74q	17
(V)	Glycyl-L-phenylalanine[b]	3·68s	—	(XIV)	L-Phenylalanylglycine	3·92, 3·64q	17
(VI)	Glycyl-L-proline	3·98s	—	(XV)	L-Prolylglycine	3·85s	—
(VII)	Glycyl-L-tryptophan[b]	3·13s	—	(XVI)	L-Tryptophanylglycine[b]	3·67	—
(VIII)	Glycyl-L-tyrosine[b]	3·23s	—	(XVII)	L-Tyrosylglycine[b]	3·72d	1
(IX)	Glycyl-L-valine	3·89s	—	(XVIII)	L-Valylglycine[b]	3·78s	—

[a] Spectra were determined on a VarianA-60 spectrometer with the centre of gravity of the chemical shift given in δ units downfield from sodium dimethylsilapentylsulphonate; s = singlet, d = doublet, q = quartet and J = Mc./sec.
[b] Drop of dilute sodium deuteroxide solution added to aid in dissolving the sample.

Dipeptides [(I)—(IX)] show only a singlet methylene signal with a fairly constant chemical shift; however, it is significantly moved downfield in (VI) and upfield in (V), (VII), and (VIII). It is believed the prolyl system interferes with the conformation of the dipeptide zwitterion, so as to produce the observed change; in the case of the aromatic residues, a shielding effect must exist with the methylene protons oriented towards the π-electron cloud.

The reversed series of peptides [(X)—(XVIII)] exhibit a typical AB quartet pattern for the methylene protons in (XI) to (XIV). This effect disappears in (X), because of the smaller α-methyl group, and is similarly absent in (XV), since a completely different conformation in solution must be forced by the planar proline ring. The glycyl methylene protons are equivalent in peptides (XVI), (XVII), and (XVIII), but this is attributed to the basicity of the solution. To test this assumption, peptides (XII) and (XIV) were re-examined under acidic and basic conditions. In (XII) the methylene protons became equivalent (4·08 and 3·80, respectively), while in (XIV) they were equivalent in acid (4·03), yet still slightly nonequivalent in base (3·71 and 3·68).

In conclusion, three factors must be satisfied before methylene nonequivalency is displayed: a peptide bond should exist at the amino-end of glycine, the second amino-acid must contain a large hydrocarbon side-chain and the solution needs to be at neutral pH. Where nonequivalence does occur, it is attributed to rigidity of conformation in the peptide zwitterion together with restricted rotation about the bond between the asymmetric carbon and the carbonyl group. This last point can be evaluated and confirmed through an application of the "rule of six" (*i.e.*, of the asymmetrical alkyl-amino-acids, only alanine has no atoms in the six position, counting from the carbonyl oxygen).[6] It should be noted that these steric effects do not necessarily apply to glycyl peptides with a third polar group, where electronic effects probably predominate.

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