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GLYCYL METHYLENE CHEMICAL SHIFT NON-EQUIVALENCE. A USEFUL CONFORMATIONAL PROBE.

Marc J.O. Anteunis,
Laboratory of Organic Chemistry, State University of Ghent,
Krijgslaan 271 (S4bis), GHENT - B-9000 - Belgium.

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The non-equivalence of glycine methylene protons ($\Delta\alpha$), observed in quasi-boat forms of c(Gly-X) diketopiperazines (DKP), has been introduced by Kopple and Onishi (1) as a criterion for non-planar conformations and this has been used and extended since by many others (2-4). Davies and Khaled have very recently demonstrated (4) that there exists a linear correlation between $\Delta\alpha$ and the degree of puckering β (see however 5). If we replot the literature data (figure), quoted by these authors (4, table 2, p.1242) in that we use the individual shift values ($\delta(H_S^\alpha$-Gly) and $\delta(H_R^\alpha$-Gly)) versus the degree of buckle for c(Gly-X) and c(Sar-X) [X being an aliphatic residue], it becomes clear that the mean shift remains unchanged over the whole experimental range $-30° < \beta < +30°$ (6). Therefore these mean shifts are hardly affected by the presence of either a quasi-equatorial or quasi-axial side-chain of the residue at the opposite side of Gly (or Sar) in the DKP ring, and the magnitude of $\Delta\alpha$ (with extremes of about ±0.6 p.p.m.) is only determined by the ϕ,ψ values (7). If we accept a qualitative parallelism to exist between the behaviour of $\Delta\alpha$ in cis and trans peptide bonds it is thus at first approximation only the spatial orientation of the planes of the peptide bonds encompassing the Gly fragments that are decisive for the magnitude of the non-equivalence, as long as the preceding or succeeding side chains are not aromatic.

For c(Gly-Y), where Y is an aromatic residue, the orientation of the aromatic π-frame work on the contrary plays an additional and overwhelming role. The slope of $\Delta\alpha$ versus β in this case is of reversed sign (4), and as far as data are available for DKP with a varying degree of folding, the mean shift of $\delta(H_S^\alpha)$ and $\delta(H_R^\alpha)$

also seems sensitive to the nature of the aromatic ring. It is known (8, 9) that the aromatic rings tend to face the peptidic bonds and they prefer the flagpole position in DKP-boats. Even the relative location in the ^{1}H-nmr spectrum of the methylene protons at the opposite side of the ring (e.g. $\delta(H^{ax}) > \delta(H^{eq})$) is reversed. In linear (trans) peptides a preference in orientation of aromatic side chains (towards the amino terminus) has been revealed (10). Therefore, in aromatic containing peptides, the χ-parameters that govern the side chain rotameric state have a profound influence on $\Delta\alpha$ by virtue of associated ring current effects. It is known for example that, whereas non-equivalence mostly vanishes for Gly residues that preceed a residue, this is not the case if this residue is aromatic (see also ref. 11).

We recently reported (11) that electric field gradients would be of minor importance for $\Delta\alpha$ in linear peptides but that instead conformational features connected with both the backbone and side chains (especially if aromatic) had to be chiefly involved. It has been demonstrated (cf. 12, 13) that conformational changes are related to alterations in intramolecular electrostatic interactions (14), H-bond formation, head-to-tail interactions etc... In using $\Delta\alpha$(Gly) as a probe for conformational purposes attention should in the first place be focused on the ϕ,ψ-values of the Gly residue itself and on χ-values of neighbour aromatic chains. Next to this, the nature of peptide bonds that flank the Gly moiety must be important, especially if departure from the ideal values $\omega = 0°$ (cis) or 180° (trans) may be present. Although the occurrence of cis peptide units is relatively rare, except if they preceed imino residues, it should be looked for, especially because typical ϕ,ψ-values seem to be associated with such fragments (15). A succession of repeating Gly residues in a peptide chain seems to give more flexibility to the backbone (16, 17) as revealed in Met-Enkephalin (16). The accompanying $\Delta\alpha$ changes may therefore also be used for an estimation of preferred forms within a multitude of coexistent different conformations.

The spin systems formed by the α-methylene protons of Gly residues in peptides are characterized by a relatively simple spectral appearance. Next to the use of ^{2}J-values (if non-equivalence is present) (18) $\Delta\alpha$ seems to be a promising tool to disclose local conformational features.

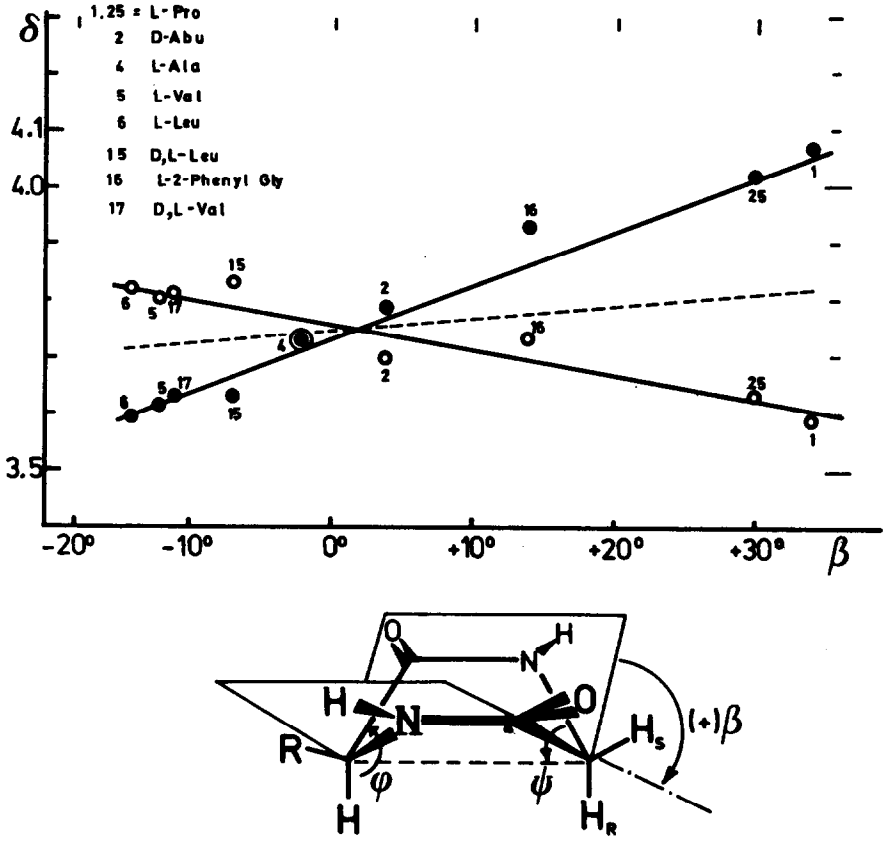

Figure: Individual shifts of H_R (•) and H_S (o) Glycyl α-protons in ppm versus degree of buckle β for c(Gly-X) in DMSO. The convention for β, it's sign and the relation with ϕ,ψ values are shown in the DKP model represented below the graph (From ref. 4).

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The author interprets the shift differences between the $^{13}C_{\beta}$ and $^{13}C_{\gamma}$-signals in proline DKP's (I.Z. Siemion, Th. Wieland and K.H. Pook; Angew. Chem., 87, 712 (1975)) in terms of a shift in the equilibrium state between planar and quasi-boat forms, rather than in terms of a continuous change in folding.

(6) In fact the straight line bisecting the angle formed by the line going through the data of $\delta(H_S^{\alpha})$ and that through $\delta(H_R^{\alpha})$ versus β has a very small slope of about 0.0022_5 p.p.m. per degree. This is about 17% of the $\Delta\alpha/\beta$ dependence.

(7) The angles ϕ,ψ are the usual torsion angles defined in a peptide unit. It has been demonstrated from X-ray data (D.B. Davies and Md.A. Khaled; J. Chem. Soc., Perkin II, 178 (1976)) that these angles are equal for cis-substituted DKP rings. Therefore $\phi = \psi = -\beta$ as follows from the definition (4) of the degree of puckering β (see inset figure).

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