NMR INVESTIGATIONS OF PROLINE AND ITS DERIVATIVES.

3- A NMR method for estimating spectroscopic and conformational changes in prolyl-compounds : the H_α PMR multiplet of proline.

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ABSTRACT

In the Proton Magnetic Resonance spectrum of proline the shape of the α proton multiplet depends on the relative position of the β_2 proton chemical shift. The system was computer simulated. The observed variation in shape of the H_α multiplet gives usefull spectroscopic and structural informations.

In the Proton Magnetic Resonance (PMR) spectrum of proline-containing peptides the α-proton multiplet of the proline residue appears with different multiplicities and shapes (i. e. relative intensities of the peaks within the multiplet) (1-9). These mainly solvent dependant variations have been related to the cis or trans configuration of the X-PRO bond, since they are already apparent in simple model systems like acetyl-proline-amide (10, 11). Although this finding has been largely used in conformational studies no explanation has yet been attempted.

We reported recently that in the PMR spectrum of proline in D_2O solution the shape of the α proton multiplet is pH-dependent and strictly related to the chemical shift of the β_2 proton (11). The pH-dependent chemical shift curves show that at basic range the $H\beta_2$ curve crosses the two parallel ones belonging to $H\gamma_1$ and $H\gamma_2$ so that small pH variations around the crossover value permutes the chemical shift sequence. As a matter of fact the $H\beta_2$ curve has a slope of 27 Hz/pHu. (at 250 MHz) whereas the near $H\gamma_1$ and $H\gamma_2$ curves have a slope of 22Hz/pHu. The more distant $H\beta_1$ curve has the same slope as the $H\gamma$ ones. $H\beta_2$, $H\gamma_1$ and $H\gamma_2$ give at high field (220, 250 MHz) the ABC part of the ABCKPQX spectrum of proline. This finding suggested us to analyse by computer

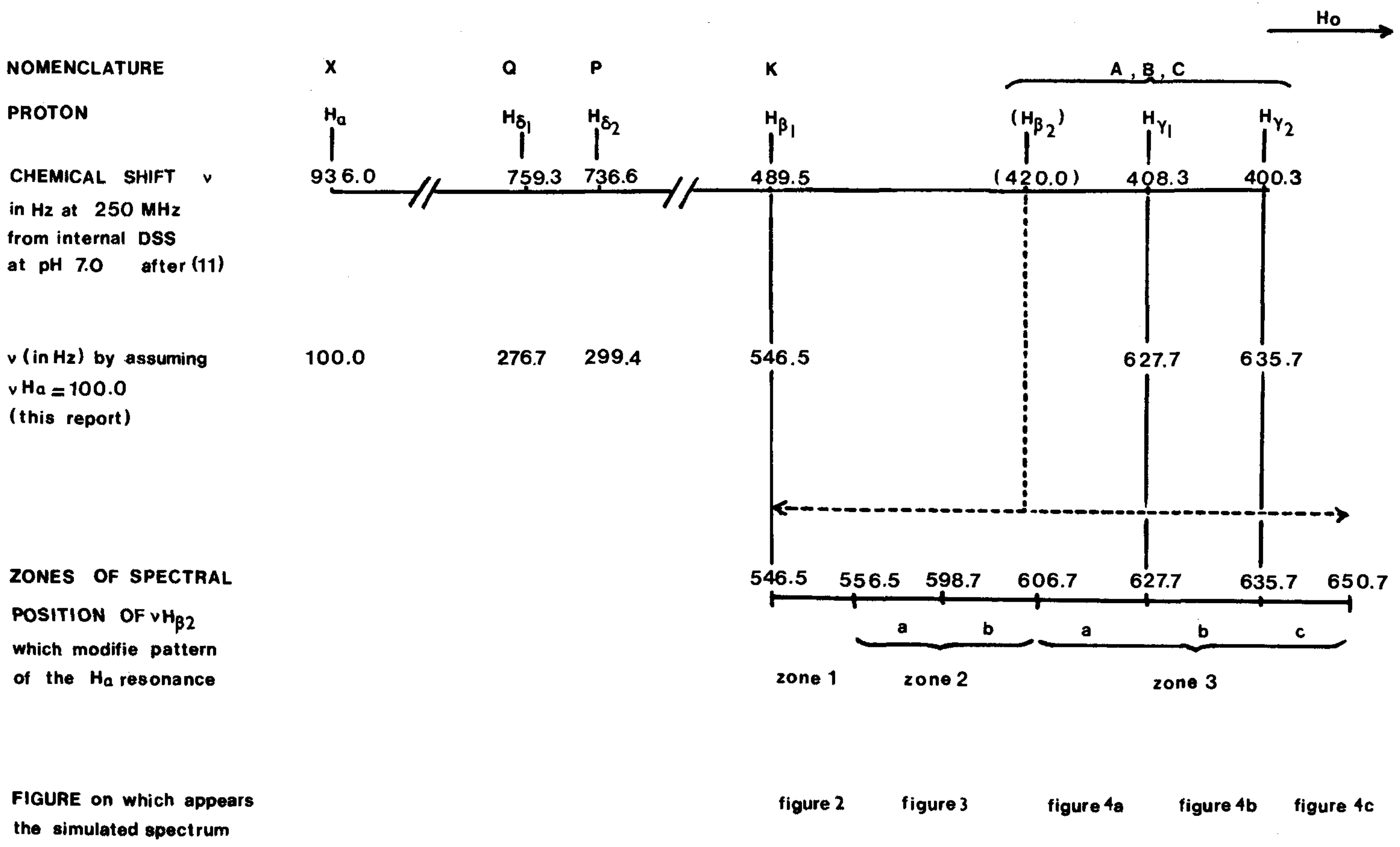

Figure 1

Variation range of the $H\beta_2$ chemical shift in the PMR spectrum of proline.

simulation the resonance of H_α (the X part of the spectrum) as a function of the relative position of H_{β_1}, H_{β_2}, H_{γ_1}, and H_{γ_2}. Since the system is linear around the crossover point it can be represented as a function of one variable. By sweeping the chemical shift value of H_{β_2} over a limited range and keeping all other elements unchanged we obtain the same relative chemical shifts for the 4 protons as by varying together all four chemical shifts with the corresponding slopes. By introducing the variable chemical shift value in the hamiltonian matrix the transition energies and intensities of the system have been computed. The pattern of the H_α multiplet results from this calculation.

CALCULATION

The calculation was carried out with the LAOCN 3 program (12) to which a curve plotting program with a Lorentzian line shape of 1.0 Hz width was added. The constant elements in the Hamiltonian matrix (all coupling constants and chemical shifts excepted the H_{β_2} one) have the values corresponding to PRO in D_2O solution at $pH^* = 7.0$ at 250 MHz. The matrix is given in (11). For the sake of clarity the H_α multiplet is assumed to be centered at the value of 100.0 Hz and the chemical shifts of the other protons are given in Hz upfield from this value. The chemical shift of H_{β_2} was varied by 2.0 Hz steps over a 104.2 Hz wide range between two extreme values : from the H_{β_1} position to 15.0 Hz up field from the H_{γ_2} position (figure 1). The resulting pattern of the H_α PMR multiplet are displayed in figures 2, 3, 4a, 4b, 4c where the highest peak of the multiplet is kept equal to 17.0 cm.

RESULTS

The appearence of the H_α multiplet is a first order quartet (eventually degenerated to a triplet) with many higher order transition lines. In the following we define its shape by the height of the four main peaks (numeration

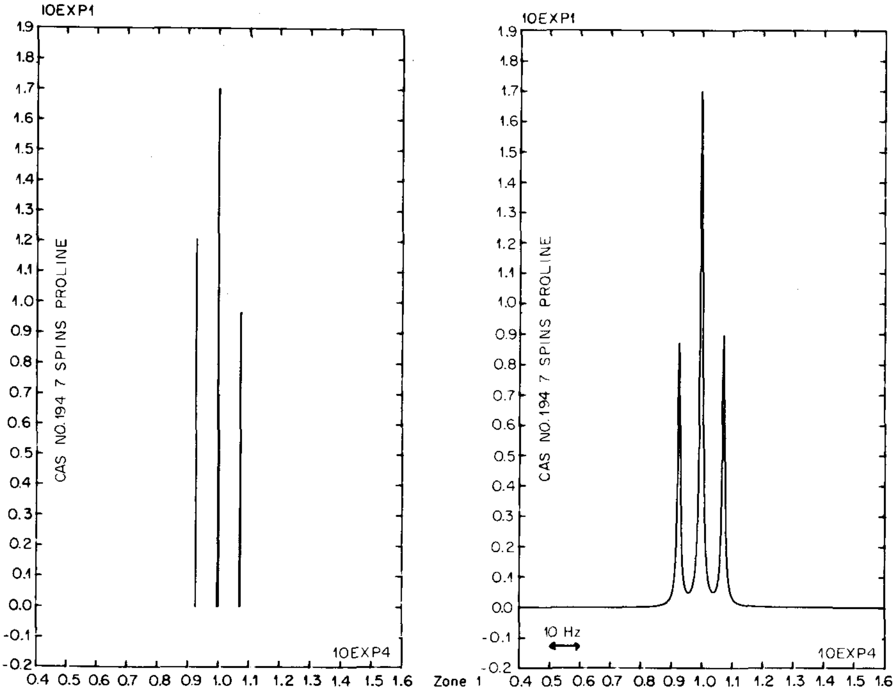

Figure 2

Shape of the H_α multiplet for zone 1 of the H_{β_2} chemical shift.

on figure 3), and its complexity by the number of NMR energy levels (NL) as given by the LAOCN3 program.

The whole variation range of the H_{β_2} relative chemical shift can be divided into three zones, the most important of which is the last one, (see figure 1). The variable chemical shift of H_{β_2} is designated by ν_v.

Zone 1 (figure 2) $\nu_{H_{\beta_1}} \leqslant \nu_v < \nu_{H_{\beta_1}} + 10.0$ Hz

The H_α quartet is degenerated to a triplet by "pseudo chemical shift equivalence" between the two β protons.

NL varies from 64 to 72.

Zone 2 (figure 3) : a) $\nu_{H_{\beta_1}} + 10.0 \text{ Hz} \leqslant \nu_v < \nu_{H_{\gamma_1}} - 29.0$ Hz

H_α give a symmetric quartet. NL goes from 72 to 78.

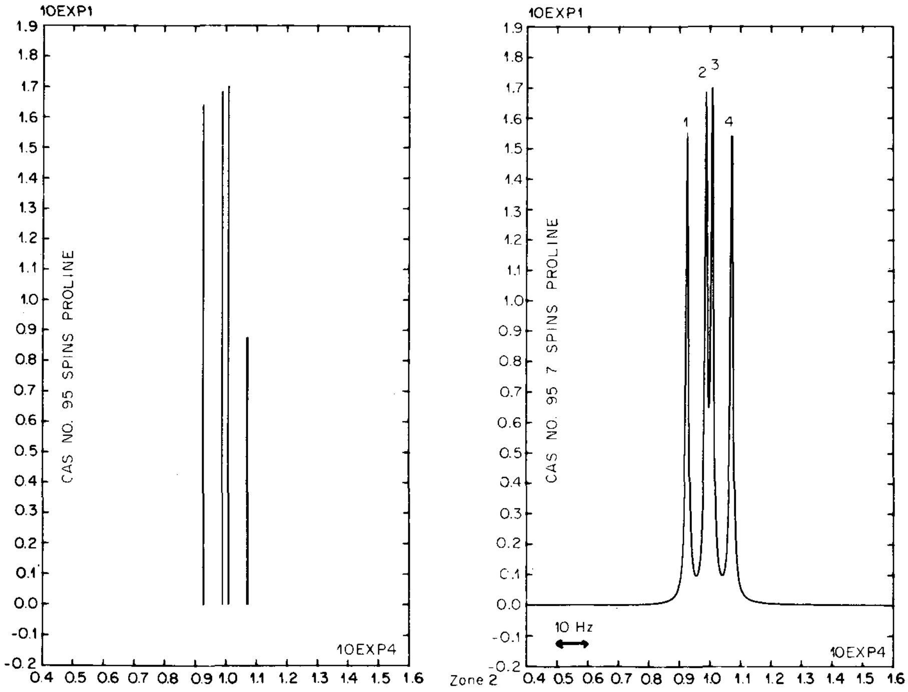

Figure 3

Shape of the H_α multiplet for zone 2 of the H_{β_2} chemical shift. Labelling of peaks 1, 2, 3, 4.

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b) $\nu_{H\gamma_1} - 29.0\ \text{Hz} \leq \nu_v < \nu_{H\gamma_1} - 17.0\ \text{Hz}$

The H_α quartet becomes assymetric. NL increases from 71 to 78.

Zone 3 (figure 4 a, b, c) $\nu_{H\gamma_1} - 17.0\ \text{Hz} \leq \nu_v < \nu_{H\gamma_2} + 15.0\ \text{Hz}$

This is the zone where the chemical shift of H_{β_2} has the greatest probability to occur (11). This is, too, the area of most dramatical change in the appearence of the H_α multiplet. Figure 5 shows the intensity of each H_α main peak as a function of ν_v, while figure 6 displays the NL vs. ν_v plot. In figure 5 three different regions become evident, called a, b, c, from left to right.

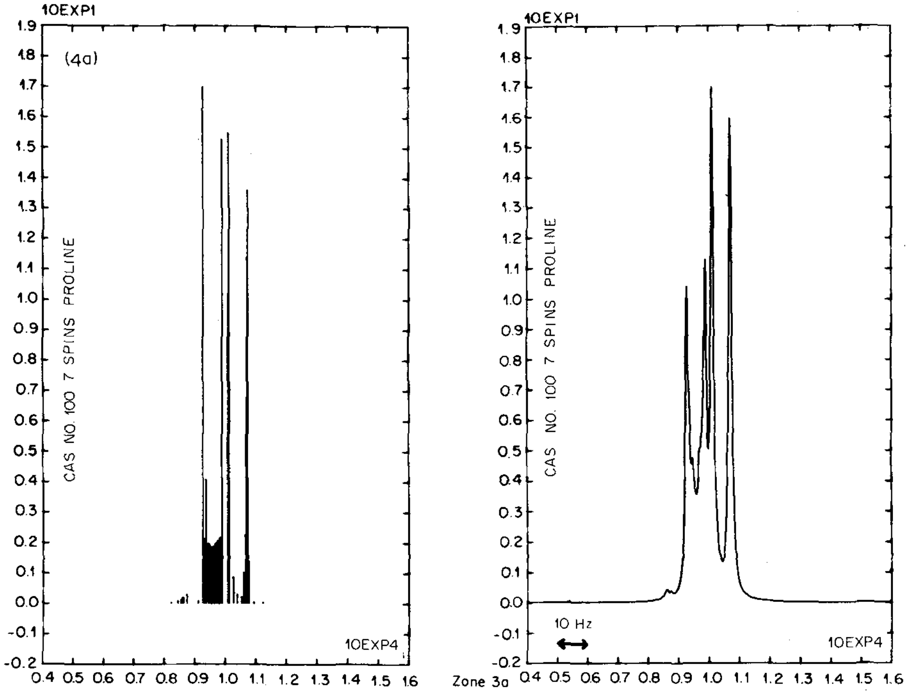


Figure 4 a

Shape of the H_α multiplet for zone 3a of the H_{β_2} chemical shift.

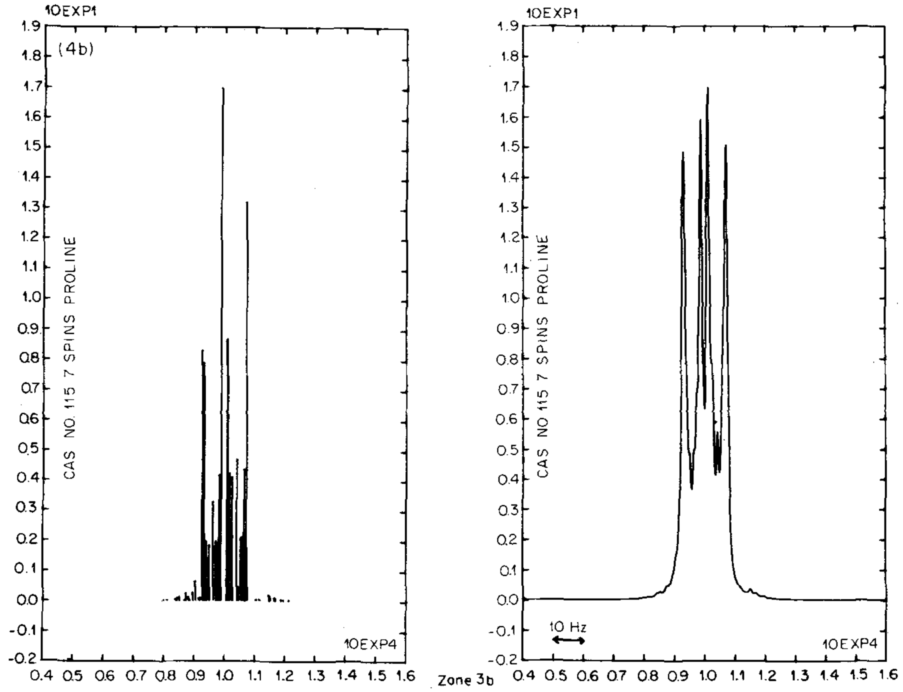


Figure 4 b

Shape of the H_α multiplet for zone 3b of the H_{β_2} chemical shift.

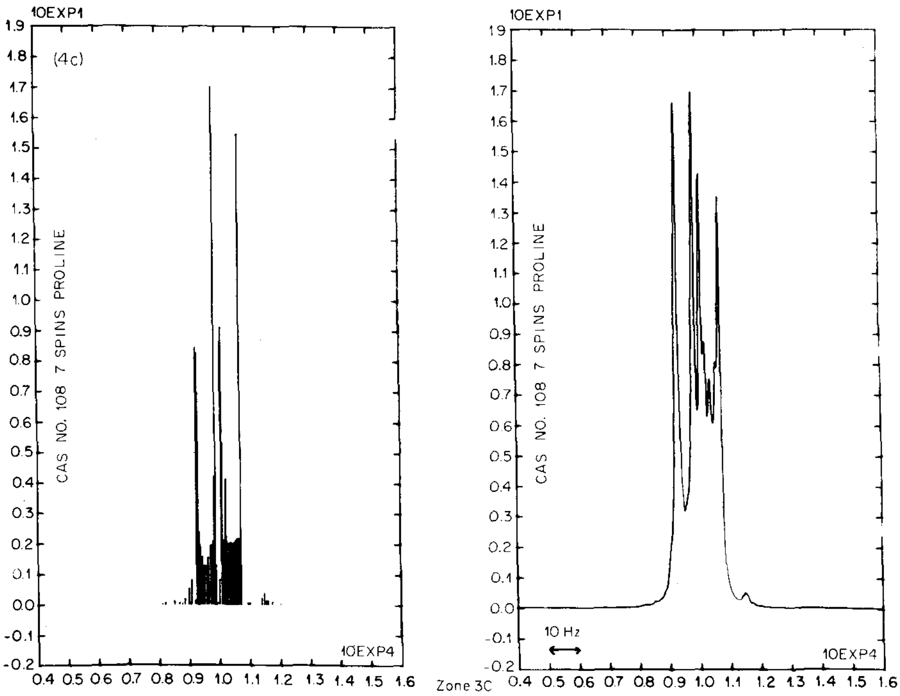


Figure 4 c

Shape of the H_α multiplet for zone 3c of the H_{β_2} chemical shift.

3a, left region (figure 4a) $\nu_{H\gamma_1} - 17.0\ \text{Hz} \leqslant \nu_v \leqslant \ _{H\gamma_1}$

The 3 and 4 peaks of H_α are more intense than 1 and 2. We have the so-called left-lowered asymmetry.

3b, central region (figure 4b) $\nu_{H\gamma_1} < \nu_v \leqslant \ _{H\gamma_2}$

The quartet is almost symmetric. In this region occurs the inversion of the H_α peaks height. 1 and 2 begin to become more intense than 3 and 4. The shape of H_α may be called central disymmetric.

3c, right region (figure 4c) $\nu_{H\gamma_2} < \nu_v < \ _{H\gamma_2} + 15.0\ \text{Hz}$

The 1 and 2 peaks are more intense than 3 and 4 ; thus the shape of the H_α curve may be called right-lowered asymmetry.

The number of energy levels of the H_α resonance in zone 3 (figure 6)

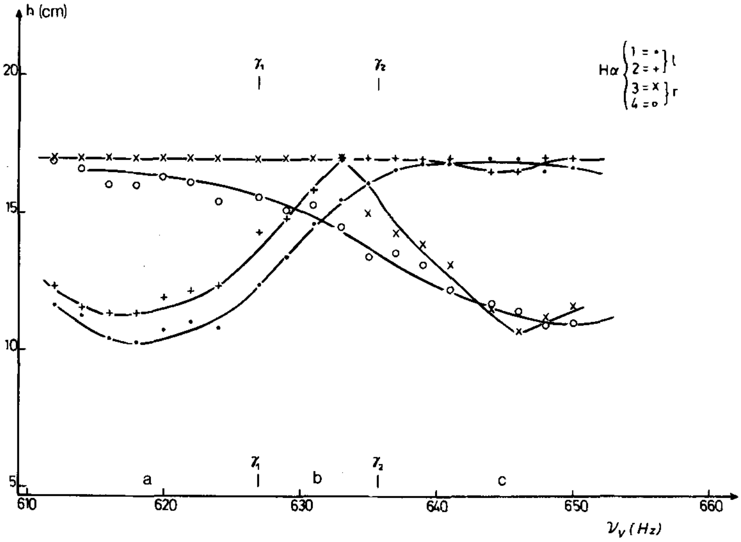


Figure 5

Intensity of the main peaks 1, 2, 3, 4 of the α proton multiplet as a function of the chemical shift (in Hz) of the β_2 proton in zone 3.

1 and 2 are left side peaks
3 and 4 are right side peaks

goes from 84 to 90 passing through a maximum of 105 within the inversion region 3b.

DISCUSSION

Our analysis shows the importance of the high order transition lines that are integrated in the experimental line width of 1.0 Hz and give the characteristical shape of the $H\alpha$ multiplet. The most meaningful changes arise in zone 3 where the vicinity of the $H\beta_2$ chemical shift to the $H\gamma_1$ and $H\gamma_2$ ones leads to a maximum of transition lines. The greater chemical shift lability of $H\beta_2$ compared to the other protons is the physical reason of this spectroscopic pattern. Two kind of implications may be related to this analysis.

Spectroscopic implications : From the shape of the $H\alpha$ protons it will

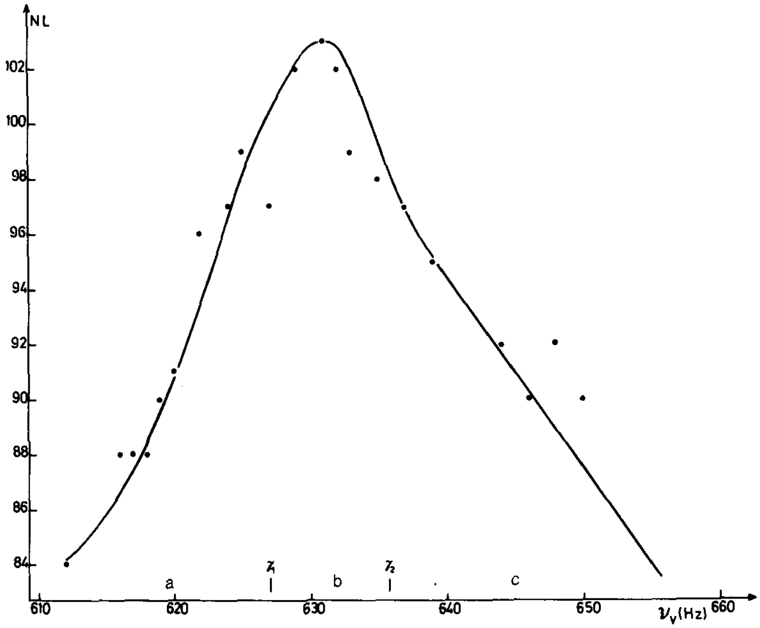


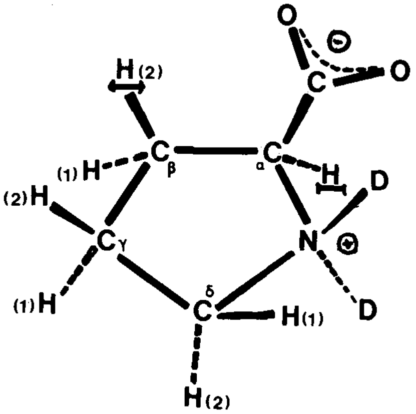
Figure 6

Number of the NMR energy levels of the α proton as a function of the chemical shift (in Hz) of the β_2 proton in zone 3.

be easier to find the approximate Hβ_2 chemical shift. This will facilitate the analysis of the PMR spectrum of prolyl-compounds.

Structural implications : As already outlined (1) the β_2 proton for its closeness to the π-plane of the carboxylic group is greatly influenced by every spatial variation relative to this plane. Perpendicularity of the COO^- π - plane to Hβ_2, shifts the Hβ_2 resonance to high field (i.e. $\nu_v > \nu_{H\gamma_2}$) and parallelism of this π-plane to the C^o - C^α - N plane, shifts Hβ_2 to low field (i.e. $\nu_v < \nu_{H\gamma_1}$). This rotation of the carboxylic plane around the C^α - C^o axis is related to the protonation of the molecule and may account for the particularities of the chemical shift vs. pH curve.

Clearly, the rotation of the carboxylic plane is the cause of the observed spectroscopic pattern. In the X-PRO compounds it may be related to the



interaction between the two peptidic planes of the prolyl residue and account for the observed differences in the H_α spectrum of the cis and trans isomer. Further investigations in this point should consider the line broadening effect of peptide solution.

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