HIGH FIELD ^{1}H NMR STUDIES. INFLUENCE OF THE CIS/TRANS ISOMERISM ON THE N-ACETYL 4-HYDROXY PROLINE RING CONFORMATION.

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SUMMARY:

Complete analysis of the high resolution NMR spectra of N-acetyl 4-hydroxy Proline (AcOH-PRO) has been made and it is shown that conformational cyclic changes may be explained in terms of cis/trans isomerism of the N-Acetyl group. In extension it is suggested that the greater stabilities of the trans forms of poly(PRO) and poly(OH-PRO) as well as many proline-containing peptides and polymers may be explained in terms of restricted orientation of the C-4 carbon atoms.

Because of its cyclic nature, proline occupies a unique place among the naturally occuring amino-acids. In fact proline seems to play a key-role not only in most of the hormonal peptides like vasopressin (1), oxytocin (2), luteinizing hormone releasing factor (3), thyrotrophin releasing hormone (TRH) (4) and angiotensin (5), but in many cyclic ion carrier peptides such as gramicidin S (6). Last, both proline (PRO) and hydroxy-proline (OH-PRO) occur to a significant extent in collagen (7).

Two important steps for the elucidation of the mode of action of proline-containing peptides are:

i) to determine the cis/trans isomerism around the N-PRO amide bond (both cis and trans conformations have been established in TRH (9) and angiotensin (5). And ii) to define the overall conformational shape of the molecules.

As a result of their biological importance, these peptides have been extensively studied by means of ^{1}H and ^{13}C NMR spectroscopy (8-12), and the overall conformations of PRO and OH-PRO have been resolved at different pD's by ABRAHAM (12), TORCHIA (13), and ELLENBERGER(10). On the other hand, no known attempt has been made to evaluate the cis/trans isomerism influence on the cyclic conformations. Knowledge of the preferential conformations in the

PRO ring would provide some information regarding the general structure of both PRO and OH-PRO-containing peptides.

As the differences in chemical shifts between similar hydrogen atoms in both isomers are so weak as prohibit their use, it was expedient to use model compounds one of the most attractive of which was N-Acetyl 4-hydroxy-proline (Ac-OH-PRO).

MATERIAL and METHOD:

Ac-OH-PRO was prepared by the direct acetylation of OH-PRO in a 1:1 mixture of boiling acetic acid and acetic anhydride followed by direct evaporation to dryness *in vacuo* (mp= 133°C, EtOH/Et_2O). NMR spectra were recorded on a VARIAN HR-300 spectrometer in D_2O at 30°C. and the theoretical simulations have been run on a BENSON plotter connected to a UNIVAC-1108 computer, giving a line Lorentzian profile.

RESULTS and DISCUSSION:

The experimental spectrum (fig.1-upper) clearly exhibits the cis/trans equilibrium in a 17:83 ratio (*via* integration). Resolution is good enough to set up all the chemical shifts (δ) and coupling constants (J) for both isomers. These trial parameters have been optimized by the DECHAMIT program and are detailed in Tables I and II. The resultant theoretical curve is given (fig.1-lower.)

From the data in Table I, it can be seen that the ^3J couplings are different in the two rotamers. As vicinal couplings are dihedral angle dependant (14) it is clear that slight modifications of the cyclic conformations occur under the influence of the N-Acetyl group rotation. The spatial geometries can be described using the modified KARPLUS relation (13): $^3J = a.\cos^2(\alpha)+b$, where b= 1.4 Hz, a= 8.5 Hz ($0<\alpha<90°$) or a= 10.5 Hz ($90<\alpha<180°$), and α the dihedral angle of the two C-H bonds involved in the coupling.

The configurations in fig.2 are those giving the lowest value for the agreement factor $\varepsilon = \sqrt{\Sigma(\overline{^3J_{calc}} - \overline{^3J_{obs}})^2}$ when applied to the six vicinal couplings.

In D_2O, Ac-OH-PRO can be considered as a weaker acid than the free OH-PRO (pK= 3.4 and 1.9 respectively) and thus their conformations can only be compared in strong acid media.

Both rotamers of Ac-OH-PRO exist predominantly in one conformation in solution. If the molecules were flipping between two conformations, the observed couplings would be a weighted mean of the couplings of each conformer and the values obtained would not show such large variations. The great differences observed between the couplings H_4 and H_{3a-b} on one hand, and H_{5a-b} on the other exclude the possibility of a rapid *endo/exo* equilibrium on C-4. The KARPLUS equation indicates that the proline ring bends on C-4 so that the hydroxyl group takes up a pseudo-axial position. These results are in

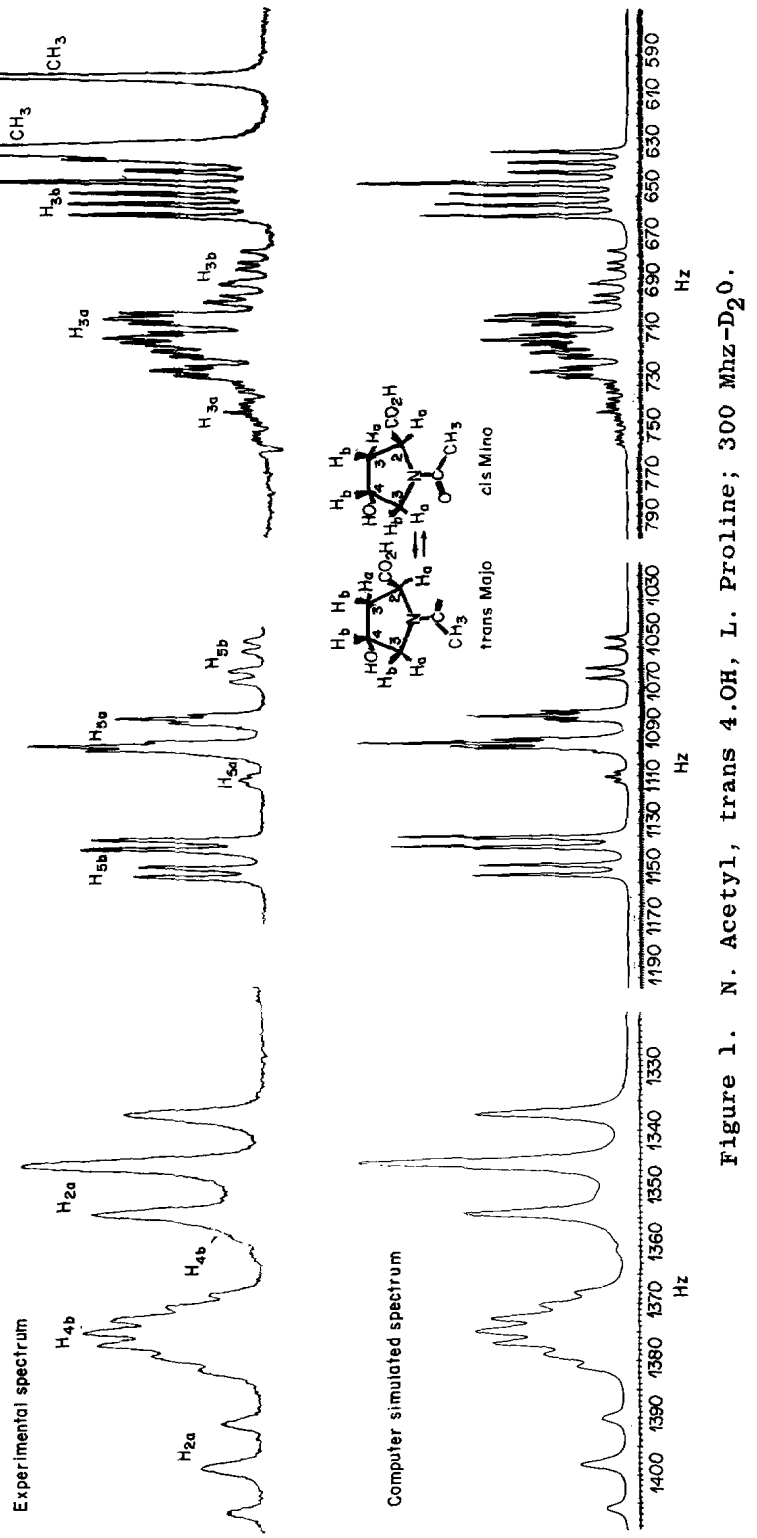

Figure 1. N. Acetyl, trans 4.OH, L. Proline; 300 Mhz-D_2O.

TABLE I - Chemical shifts of N-Acetyl 4-hydroxy Proline:

δ(ppm)*	H_2	H_{3a}	H_{3b}	H_4	H_{5a}	H_{5b}
Trans AcOH-PRO	4.48	2.39	2.17	4.58	3.64	3.81
cisAcOH-PRO	4.66	2.48	2.31	4.51	3.68	3.54

* Downfield from DSS.

Table II - J couplings in N-Acetyl 4-hydroxy Proline :

^3J (Hz) (vicinal)	J_{2-3a}	J_{2-3b}	J_{3a-4}	J_{3b-4}	J_{4-5a}	J_{4-5b}
Trans AcOH-PRO	8.21	8.84	2.45	4.55	1.82	4.17
cis AcOH-PRO	8.17	7.80	3.39	4.98	2.13	4.40

^2J (Hz)* (geminal)	J_{3a-3b}	J_{5a-5b}	^4J (Hz) (long range)		J_{2-4} †	J_{3a-5a}
Trans AcOH-PRO	13.81	11.70			≃ 0.5	1.79
Cis Ac OH-PRO	13.69	12.66			≃ 0.5	2.03

* Usually negative.

† A -0.7 Hz value is reported in the case of OH-PRO (10) ; however the line broadening did not permit accurate measurement of both H_2 and H_4 in AcOHPRO.

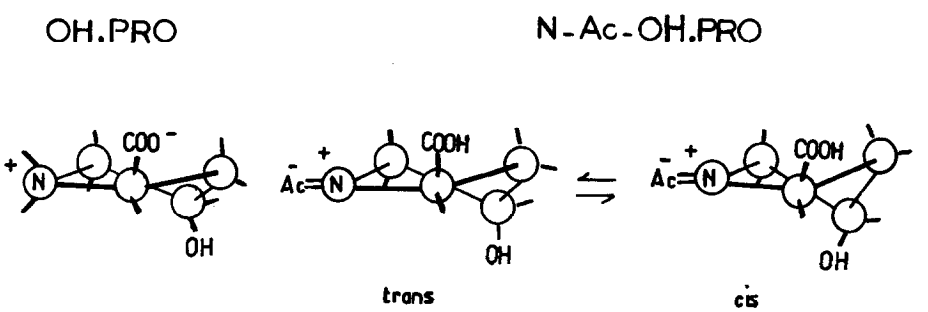

Figure 2

accordance with those reported for OH-PRO in aqueous acid (12) and also as the solid crystalline (15).

The ^{3}J coupling values are indicative of the fact that the ring is more puckered in Ac-OH-PRO than in OH-PRO and it would seem that this is even more pronounced in the case of the cis-isomer. The value of the long range coupling $^{4}J_{3a-5a}$ is known to be very sensitive to the planarity of the hydrogen atoms concerned and such would confirm the assumption that H_{3a} and H_{5a} are in what could be described as more quasi-equatorial positions.

This paper will discuss certain relationships between the monomer units PRO and OH-PRO, and the corresponding polymers or peptides.

Poly(PRO) and poly(OH-PRO) are known to exist in two well-defined forms - both in the solid state and in solution - they are designated I and II. The cis-peptide form (I) is barely crystalline and converts easily to the trans-peptide form (II) on dissolution in a hydroxylic solvent. In the solid state, II is characterized by a triple helical structure (16) bridged by hydrogen bonds.

The explanation presently offered for the lower stability of the cis-I form suggests that the primary contribution is a steric one. However the possibility of a secondary effect must be considered for, as the C-4 hydroxyl group bends it becomes more *endo* than even in the trans rotamer. Thus the formation of hydrogen bonds between the chains becomes progressively more difficult. Due to the considerable similarities between poly(PRO) and poly(OH-PRO) it is reasonable to expect similar ring conformations being induced in Ac-OH-PRO and Ac-PRO. This is borne out by the fact that SMITH and al. (9) recently reported that angiotensin II contained a proline distribution characterized by 80% trans / 20% cis isomers.

However the results of the ^{13}C T_1 measurements on TRH and melanocyte stimulating hormonal releasing factor (MSH-R-IF) suggest an even greater mobility for the proline C-4 carbon atom in these two compounds.

Thus it is believed that the effects of N-PRO isomerism on the C-4 carbon atom possibly explain the stability of these polymers and peptides, and in particular, satisfies certain of the observed behaviours of such as collagen(7)

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