

N.M.R. STUDIES ON NITROGEN-CONTAINING SUGAR DERIVATIVES SUBSTITUTED BY  $^{15}\text{N}$ .  
PART II.: THE NATURE OF THE CHELATE RING IN SUGAR FORMAZANS.

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(Received in UK 11 June 1968; accepted for publication 26 June 1968)

A cyclic hydrogen-bridged structure has been proposed for the formazans to account for their chelating ability (1) and the failure to isolate two distinct forms of unsymmetrical formazans (2). Further experimental evidence (3,4,5) supported the non-existence of two distinct forms, when R' and R'' are different (fig.I.: a and b). Two interpretations of these experimental observations were advanced. Firstly, that a and b are components of a resonance hybrid (1) or limit forms of a mesomeric structure (3,4,5). Secondly, that there is a rapid tautomeric equilibrium between these two forms with a very low energy of activation (6,7). This last concept seemed then to be confirmed by IR (8) and NMR (9) spectral data. The possibility of a fast intermolecular exchange was rejected owing to NMR considerations (9). However, to our mind, the erroneously reported absence of the NH signal in NMR spectra of formazans does not constitute an argument neither in favor of a rapid tautomerization, nor in opposition to a fast intermolecular exchange.

We have examined the NMR spectra of several sugar and non-sugar formazans. In each case the NH proton appeared as a sharp signal at very low field: e.g. at 15.4 ppm for triphenyl-formazan ( $\text{R}=\text{R}'=\text{R}'' = -\text{C}_6\text{H}_5$ ) and at 15.9 ppm for acetyl formazan ( $\text{R} = \text{CH}_3\text{CO}-$ ,  $\text{R}'=\text{R}'' = -\text{C}_6\text{H}_5$ ) in  $\text{CDCl}_3$  (TMS). In the case of penta-O-acetyl D-galactose diphenyl-formazan ( $\text{R}'=\text{R}'' = -\text{C}_6\text{H}_5$ ;  $\text{R} = -(\text{CHOAc})_4-\text{CH}_2\text{OAc}$ ) it appears at 12.70 ppm ( $\text{CDCl}_3$ ; TMS). A similar position has been reported (10) for the NH proton in D-mannose diphenyl-formazan. The down field value of the chemical shift of the NH proton is in full agreement with a chelated structure

Keeping in mind the possibility of a rapid tautomerization in the case of formazans, we have first examined the NMR spectrum of penta-O-acetyl D-galactose diphenyl-formazan substituted by one  $^{15}\text{N}$  atom in the  $\alpha_1$  position. The labelled compound was prepared according to the procedure of Mester and Messmer (11), using diazotized  $^{15}\text{N}$ -aniline (enrichment 99%). Instead of the single peak observed in the spectrum of the normal  $^{14}\text{N}$  compound (fig.I.:1), the NH signal appears as a doublet:  $J = 46.5$  Hz (fig.I.:2). The observed value of the coupling constant is quite different from the values found in the case of sugar osazones (12) and it does not fall within the range of the

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usual  $^{15}\text{N}$ -H coupling values (13). It seems probable that the observed value corresponds to an average of the coupling constants (14) through a rapid tautomerization, according to the relation:

$$J_{\text{obs}} = J_1 \cdot p_1 + J_2 \cdot p_2$$

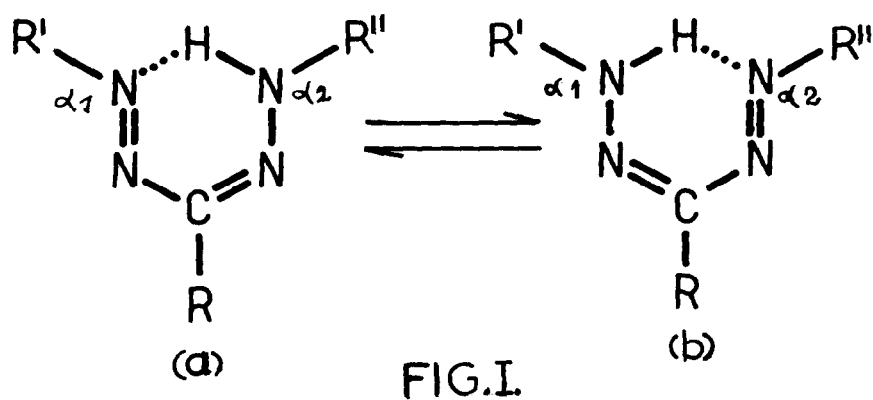
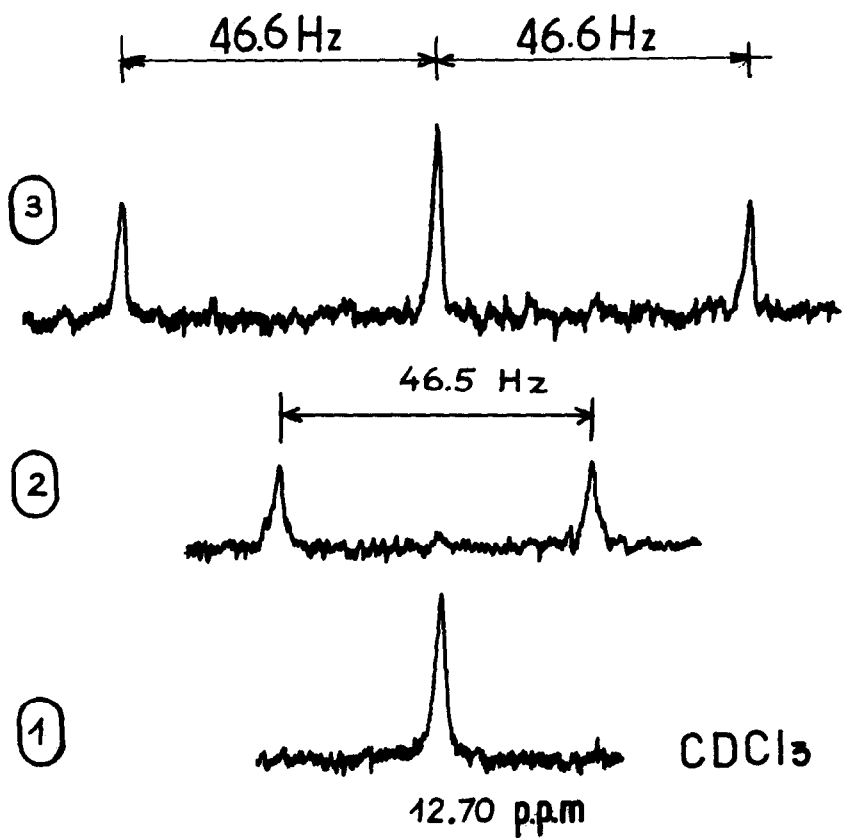
$J_1$  is the  $^{14}\text{N}$ -H coupling of the structure a,  $J_2$  is the  $^{15}\text{N}$ -H coupling constant of structure b;  $p_1$  and  $p_2$  are the percentages of the forms a and b respectively. Because of the symmetry of the molecule,  $p_1 = p_2$ , both having a value of 0.5. The  $J_{^{15}\text{N}} = 92-94$  Hz coupling constant of  $^{15}\text{N}$  substituted sugar osazones (12) gives a good value for the  $^{15}\text{N}$ -H coupling constant ( $J_2$ ) of the formazans, which are structurally very similar near the chelated NH bond ( $-\text{C}=\text{N}-\text{H}\dots\text{N}-$ ). The  $^{14}\text{N}$ -H coupling constant ( $J_1$ ) is approximately equal to zero, as illustrated by the spectrum (fig.I.:1) of the non-labelled formazan\*. Thus, according to the above relation, a rapid intramolecular exchange would lead to  $J_{\text{obs}} = 46-47$  Hz. This calculated value agrees closely with the experimental one (46.5 Hz) and supports the presence of a rapid tautomerization in the case of sugar formazans.

Furthermore, we have examined the NMR spectrum of penta-O-acetyl D-galactose formazan substituted by two  $^{15}\text{N}$  atoms in  $\alpha_1$  and  $\alpha_2$  positions.  $^{15}\text{N}$ -Aniline (enrichment 99%) and phenylhydrazine substituted by  $^{15}\text{N}$  in  $\alpha$  position (enrichment 99%) were used to prepare this compound (11). As expected, the NH signal of the formazan substituted symmetrically by two  $^{15}\text{N}$  atoms, appears as a triplet (intensities about 1:2:1) at 12.68 ppm ( $\text{CDCl}_3$ ; TMS), with an apparent coupling constant of  $46.6 \pm 0.1$  Hz for both  $^{15}\text{N}$  atoms (fig.I.:3). The results of these two experiments are incompatible with the concept of a fast intermolecular proton exchange, because in this case the collapse of the spin multiplets would be required.

The appearance of a sharp NH-signal in the spectra of the  $^{14}\text{N}$ -formazans is perfectly conceivable with a rapid tautomeric equilibrium. The possibility of a fast intermolecular proton exchange can be excluded also because the chemical shift of the NH signal is altered very little by changing the solvent ( $\text{CDCl}_3$  or  $\text{CDCl}_3 + \text{CH}_3\text{OH}$ : 12.70 ppm;  $\text{CH}_2\text{Cl}_2$ : 12.60 ppm;  $\text{C}_5\text{D}_5\text{N}$ : 12.77 ppm), or changing the temperature within the range of +30 to  $-50^\circ$ .

An alternative explanation of the experimental observations based on the symmetrical mesomeric structure of the formazans, cannot be ruled out definitely. However, according to the reported IR spectral data (6) as well as to theoretical considerations (16), this possibility appears to be remote.

\*The appearance of a sharp peak ( $\Delta\nu_{1/2} = 2$  Hz) for the NH proton of the  $^{14}\text{N}$ -formazans shows that some averaging process is taking place to destroy the multiplet structure of the signal. The lack of coupling between the proton and the nitrogen must be due to a rapid quadrupolar relaxation process involving the  $^{14}\text{N}$  atom (15).



Acknowledgement.-We would like to express our appreciation to Professor M.-M. Janot for his interest, to Mrs. M. Mester and Mrs. L. Allais for their assistance.

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L e g e n d s .

- Figure I.: N.M.R. SPECTRA IN DEUTERIOCHLOROFORM ( TMS; 60 MHz )
1. of penta-O-acetyl D-galactose diphenyl-formazan
  2. of (1) substituted by one  $^{15}\text{N}$  atom in  $\alpha_1$  position
  3. of (1) substituted by two  $^{15}\text{N}$  atoms in  $\alpha_1$  and  $\alpha_2$  positions.