Carbon-13 NMR Spectra of *C*-Nucleosides. II (1). A Study on the Tautomerism of Formycin and Formycin B by the use of CMR Spectroscopy (2)

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Sir:

The natural abundance carbon-13 magnetic resonance (cmr) spectrum of formycin B (IIb), a *C*-nucleoside antibiotic, has recently been reported (4). This spectrum displayed very broad lines for all but one (C5) of the carbons of the pyrazolo[4,3-*d*]pyrimidine aglycon and only two carbon resonances of this moiety were assigned. In conjunction with our cmr investigations of *C*-nucleosides (1),

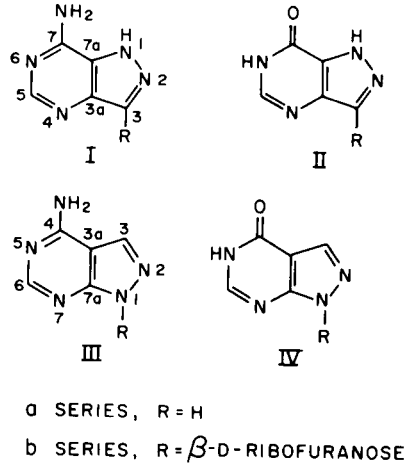

Figure 1. Numbering scheme for the molecular species studied.

this spectral work prompted us to study in detail the cmr spectra of formycin B (IIb) and formycin (Ib) (5), and on the basis of our experiments (6) we have assigned all of the resonance lines to specific carbons (Table I).

In order to interpret the cmr spectral characteristics of Ib and IIb, the chemical shifts and linewidths were studied as a function of temperature in methyl sulfoxide (DMSO) (7). Although some scatter in the chemical shift data is observed for Ib (Figure 2), it is apparent that the resonance positions for the carbons C3 and C7a of the pyrazolo-[4,3-*d*]pyrimidine aglycon are more sensitive to changes in temperature than those for the remaining carbon atoms of the nucleoside. As the temperature is increased from -14 to 145°, the signal for the bridgehead carbon C7a moves downfield whereas the signal for C3 moves upfield. These findings are consistent with a change in the proton population of N1 and N2. Energetically, the most favorable resonance structure places the proton on N1. As the temperature is increased, the population of the N2 tautomeric form is also increased, thus causing the shifts noted at C3 and C7a (8,9,10). The magnitude of the perturbation of the line positions at these two carbons are such that one can estimate an approximate 20% increase in the population of the N2 tautomer over this temperature range (160°).

Substantial variations in linewidths (Figure 3) are also observed for certain carbons in Ib. At low temperatures, all carbon signals for the ribose moiety, as well as the signal for C5, become very broad (in the range of 25 to 50 Hz) while normal linewidths of a few Hz are observed at ambient temperatures for these carbons. This behavior can be explained in terms of greatly shortened T_2's, since only carbons with directly bonded protons have a dipolar mechanism efficient enough to broaden the peaks beyond a few Hz.

The non-protonated carbons (C3, C3a, C7, C7a) experience the same relative decrease in T_2 but the lower efficiency of the dipolar term due to greater carbon-proton separation does not provide an adequate explanation for the broadening which is also observed for these carbon atoms. Instead, the peak broadening, which passes through a maximum (Figure 3) for these carbons, is attributed to tautomeric kinetic effects associated with the proton attached to either N1 and N2. The line narrowing at lower temperatures is thought to result from freezing out the more favorable tautomeric form with the hydrogen at N1. No molecules with the proton at N2, however, were observed at the lower temperatures, due presumably to a low concentration of this form. It is also possible that the line broadening is a result of kinetic effects on T_2 through the scalar coupling term. We are studying this spectral feature at the present time (11).

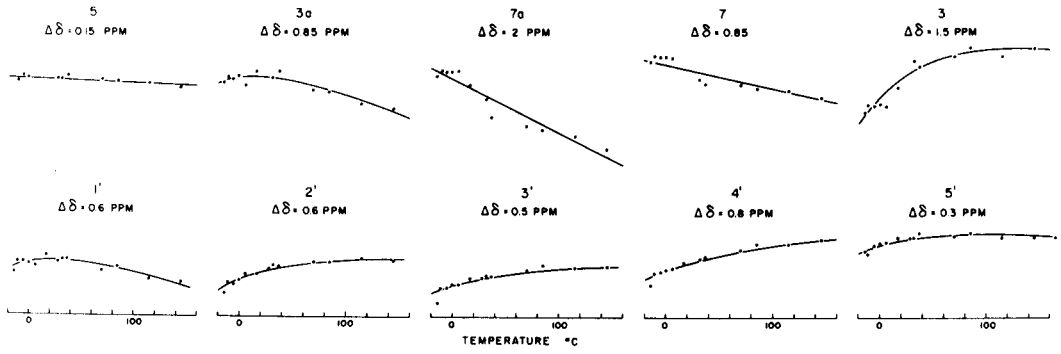

Figure 2. Chemical shifts for carbons of formycin (Ib) as a function of temperature. $\Delta\delta$'s are represented as absolute values.

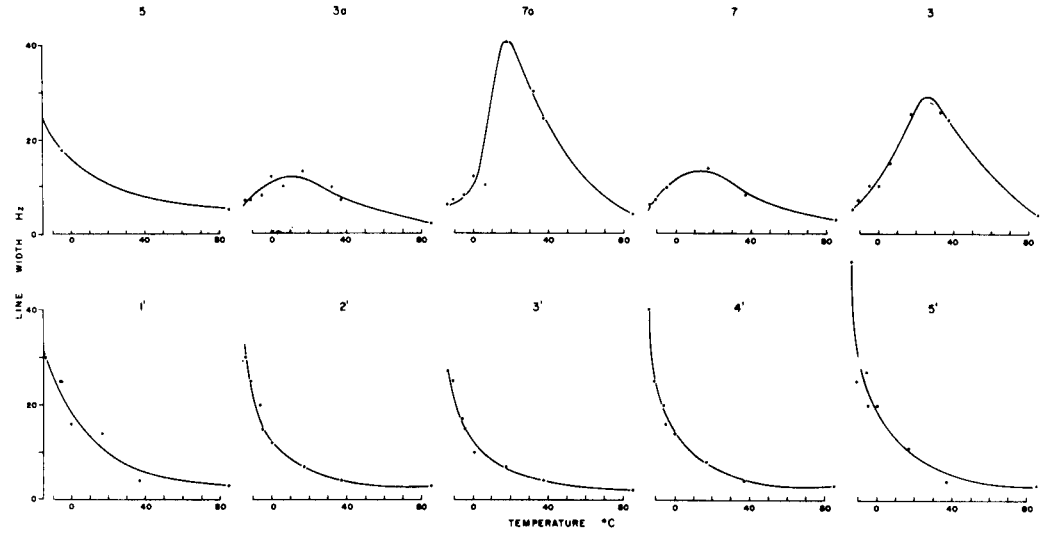

Figure 3. Linewidths for the carbons of formycin (Ib) as a function of temperature. Where data are not available in a particular temperature region, the shape of the temperature-dependence curves have been estimated.

The linewidths and chemical shifts of IIb follow the same general patterns observed for Ib, with the exception that higher temperatures (ca. 140°) are required to observe narrowing of all non-protonated carbon lines. The temperature dependence in DMSO (-2 to 145°) indicates, however, that narrowing of C3a and C7 at both ends of the temperature range proceeds at a rate in excess of that observed at C3 and C7a.

Experiments conducted on the heterocycles pyrazolo[4,3-*d*]pyrimidin-7-one (IIa) and pyrazolo[3,4-*d*]pyrimidin-4-one (IVa) provide additional evidence that the broad linewidths observed for the carbons C3 and C7a of Ib and IIb are the direct result of tautomeric averaging of the proton between N1 and N2. In both cases, the spectra of IIa and IVa exhibit broad spectral lines for their respective C3 and C7a carbons at ambient temperatures. In this respect, it is noteworthy that in the spectrum of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (IIIb, 4-APP riboside), in which tautomerism between the N1 and N2 nitrogens is eliminated, all the carbon signals are narrow at probe temperature (37°).

This study again demonstrates the importance of cmr spectroscopy for obtaining pertinent information about tautomerism in heterocyclic systems (12,13). At this time we are continuing our investigations on certain model formycin and formycin B analogs, *e.g.*, 2-methylformy-

TABLE I

Carbon-13 Chemical Shifts (a) for Formycin, Formycin B and Related Compounds

Compound	(°C) (b)	Aglycon							Ribose			
		C3	C3a	C4	C5	C6	C7	C7a	C1′	C2′ (c)	C3′ (c)	C4′	C5′
Ib	(37°)	143.17	138.31		151.41		151.57	123.36	78.18	75.32	72.56	86.08	62.56
IIb	(40°)	144.39	136.47		143.15		153.29	128.23	77.53	74.92	72.05	85.60	62.50
IIa	(40°)	133.81	139.14		143.00		153.36	126.81					
IIIb	(37°)	133.41	100.61	158.16		156.21		154.14	88.80	73.23	71.02	85.25	62.50
IVa	(37°)	134.14	105.63	157.91		147.57		154.45					

(a) All samples were dissolved in DMSO and run on a Varian XL-100/15 spectrometer equipped for Fourier transform operation. All chemical shifts were referenced to the internal dioxane (5% v/v) line and then converted to the TMS scale using the formula $\delta_{TMS} = \delta_{dioxane} - 17.5 \times 10^{-4} T(°C) - 66.32$ ppm (M. T. Chenon and D. M. Grant, manuscript in preparation). (b) Sample temperature. (c) The C2′ and C3′ carbons were assigned according to H. H. Mantsch and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **46**, 808 (1972).

cin, in order to determine the extent of tautomerism occuring in the pyrazole portion as well as in the pyrimidine portion of the heterocyclic aglycon.

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(2) This research was supported by research contract No. C 72-3710 with DCT, NCI, NIH, and research resource grant No. RR 574-02 with NIH.

(3) M. T. Chenon, visiting Research Fellow, 1971-73. Permanent address: Laboratoire de Chimie-Physique, Centre National de la Recherche Scientifique, 94-Thiais, France.

(4) L. F. Johnson and W. C. Jankowski in "Carbon-13 NMR Spectra," Wiley-Interscience, New York, 1972, Spectrum No. 375.

(5) The carbon-13 nmr spectra of formycin and formycin B were just presented by T. R. Krugh at the 13th ENC, Asilomar, California, 1972. Krugh suggested that the line broadening observed in the spectra of the formycins was caused by tautomerism of the labile proton.

(6) The assignments are based on off-resonance decoupling data and comparison with certain model compounds. A manuscript describing the experimental details and all model compounds studied is in preparation.

(7) All chemical shifts were corrected for temperature with regard to the standard employed. The method used is described in Table I.

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(10) As reported in references 8 and 9, a carbon atom adjacent to a protonated nitrogen atom is shifted upfield (α-shift) as compared to the non-protonated molecular species while carbons two atoms removed are shifted downfield (β-shift). C7a and C3 thus experience a combination of α plus β effects due to proton tautomerism. Any process which changes the population of the tautomers will likewise affect the chemical shifts at C3 and C7a in a predictable fashion as outlined by Pugmire *et al.*

(11) In an identical temperature study conducted with adenosine, the linewidths of the protonated carbons follow the pattern observed in formycin and increase with decreasing temperature whereas the linewidths of the non-protonated carbons remain narrow. Furthermore, no significant chemical shift variations are observed at any of the carbon atoms in adenosine.

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