CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS OF C-NUCLEOSIDES. THE STRUCTURE OF PYRAZOMYCIN B[1]

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Summary: The ^{13}C nmr spectra of three nucleosides and four C-nucleosides have been recorded and all carbon signals assigned. These data have been utilized for the determination of the structure and conformation of the antibiotic pyrazomycin B. Steric differences have been shown to be reflected in the chemical shift values.

Cmr spectroscopy, a powerful new method of structure analysis, has been utilized for the elucidation of the constitution of the new antibiotic pyrazomycin B[2] and in this connection for the determination of the chemical shifts of several nucleosides and C-nucleosides. This constitutes the first cmr analysis of the biologically important C-nucleosides[3].

In the light of early pmr data on pyrazomycin B[2] suggesting the antibiotic to be either a riboside or an arabinoside, the following nucleoside models adenosine (1a), 9-(β-D-arabinofuranosyl) adenine (1b), β-6-azauridine (2a) and (β-D-arabinofuranosyl)6-azauracil (2b) as well as the C-nucleosides β-pseudouridine (3a), α-pseudouridine (3b), pyrazomycin (4a) and pyrazomycin B (4b) were investigated. The data are based on an analysis of the natural abundance, noise resonance decoupled and single frequency off-resonance decoupled cmr spectra of these substances. The chemical shifts of 1a and 2a being known[4-5] permits signal assignment of the heterobase unit of 1b and the sugar moiety of 2a, while the δ values of the base portions of 2a and 2b are based on those of

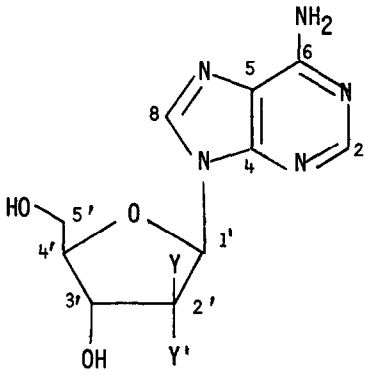
uridine[4,5] (the shift change of C_5 in the 6-aza compounds being larger than the shift difference of pyridine α-carbons and benzene carbons[7]). The shifts of the carbohydrate parts of 1b and 2b reflect those of 1-(β-D-arabinofuranosyl) uracil[5] with regard to direction and magnitude in the ribose-to-arabinose change*. The anomeric carbons are different in view of the difference of the attached bases.

The aromatic carbon shifts of the C-nucleosides 3a and 3b are nearly the same as those of uridine[4] except for C_5 being expectedly deshielded by its substituent. C_6 having the same proximity relationship in the nucleosides as in the C-nucleosides, this center remains nearly unaffected. The near-identity of the aromatic carbon shifts of pyrazomycin (4a) and pyrazomycin B(4b) show the latter to possess the trisubstituted pyrazole ring known to be part of the former[8]. The shifts match those derived from an application of aromatic substituent parameters[9] to the δ values of pyrazole[10][e.g., C_4:81-27 (OH α effect) = 54]. C-Nucleosides 3a and 4a possess the same β-ribosyl unit as nucleosides 1a and 2a and hence reveal similar sugar carbon shifts except for the C_1' signal appearing predictably at higher field**. These facts also distinguish $C_{1'}$, $C_{4'}$, and C_5' in α-pseudouridine (3b), while C_2' and C_3' cannot be differentiated. Comparison of the δ values of the carbohydrate portion of pyrazomycin B (4b) with those of pyrazomycin (4a) in the light of the contrasting glycoside shifts of β- and α-pseudouridine (3a and 3b, respectively) indicates the sugar moiety in pyrazomycin B to be an α-ribosyl unit.

Conformational analysis of the pyrazomycins on the assumed basis of their possessing a truncated chair structure with eclipsed nuclear oxygen electron pairs

*While the β-D-arabinofuranosides of cytosine and uracil show like $C_{2'}$ and $C_{3'}$ chemical shifts[5], those of the present N- and C-arabinosyl series (see Table) reveal different δ values for the two carbon sites.

**Inversion of the stereochemistry at $C_{1'}$ is expected to exert little effect on the shift of $C_{3'}$. This governs the choice of δ values for $C_{2'}$ and $C_{3'}$ in the anomers of 3a and 4a.



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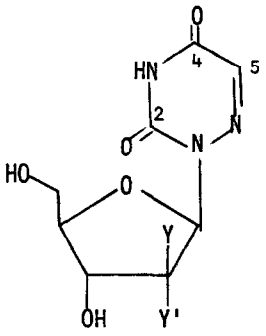

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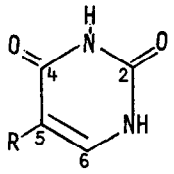
a, (β-ribo), Y=H, Y'=OH

b, (β-arabino), Y=OH, Y=H



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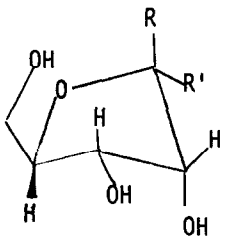
4

a, R= β-D-ribofuranosyl

b, R= α-D-ribofuranosyl



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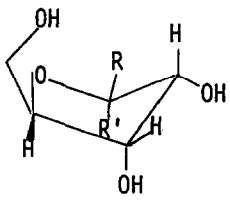

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a, R= 3-(4-hydroxy-5-carboxamido)pyrazolyl, R'=H

b, R=H, R'= 3-(4-hydroxy-5-carboxamido)pyrazolyl

TABLE

^{13}C Nmr Chemical Shifts[a]

	1a	1b	2a	2b	3a	3b	4a	4b
C_2	152.7	152.8	148.7	149.2	152.3	152.5		
C_3							132.7	130.7
C_4	149.3	149.7	157.3	157.5	164.8	164.5	139.3	139.3
C_5	119.5	118.4	136.6	136.2	110.0	109.6	127.3	127.6
C_6	156.3	154.3			141.0	140.0	163.7	164.0
C_8	140.3	140.8						
$C_{1'}$	88.4	84.1	89.5	81.8	78.6	76.6	75.4	74.9
$C_{2'}$	73.9	75.1[b]	72.7	73.4	72.9	72.5[c]	73.8	73.2
$C_{3'}$	71.1	76.0[b]	70.0	75.6	71.3	72.1[c]	70.8	71.8
$C_{4'}$	86.3	84.1	83.8	83.6	82.9	81.3	84.1	81.3
$C_{5'}$	62.1	61.0	61.4	61.9	61.1	62.0	61.1	61.1

[a]Spectra taken at 15.08 MHz on a Fourier transform spectrometer; chemical shifts in parts per million downfield from TMS; 1a and 1b in DMSO-d_6 solution all others in water solution containing dioxane as internal reference; $\delta^{TMS} = \delta^{DMSO\text{-}d_6} + 39.5 = \delta^{dioxane} + 66.3$ ppm.

[b,c]Values in any vertical column may be reversed.

and $C_{4'}$ substituents[11] requires pyrazomycin (4a) to assume conformational posture 6a and pyrazomycin B 5b***. Since the pyrazomycin conformation 6a is in accord with solution and solid state physical data[2,12], its cmr results can be utilized in interpreting the conformation of pyrazomycin B. The latter shows mild shielding of its $C_{1'}$ and $C_{3'}$ in consonance with decreased β effects and, most importantly, strong shielding of its $C_{4'}$ due to a γ effect[13]. These perturbations can be accounted for only by an axial $C_{2'}$ hydroxyl group and hence by conformation 5b for pyrazomycin B. It is noteworthy that the cmr data

***This first-order analysis is based on consideration of only non-bonded, repulsive forces.

of the pseudouridines, 3a and 3b, show the same effects. In view of the difficulty of assessing intramolecular attractive forces; e.g., hydrogen bonds among hydroxyl groups as well as between hydroxyl functions and the heterobases, and the preferred sugar-base torsion angle[14], the magnitude of the $\Delta\delta$ values of the α- and β-ribosides is hard to predict.

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