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# DETERMINATION OF THE TAUTOMERIC POPULATIONS OF THE Y-BASE

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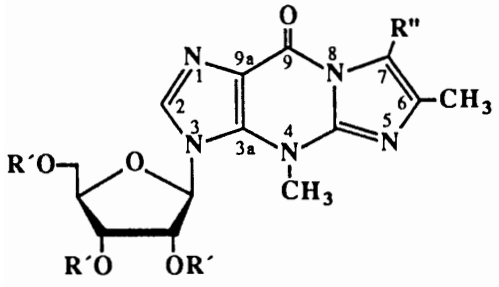
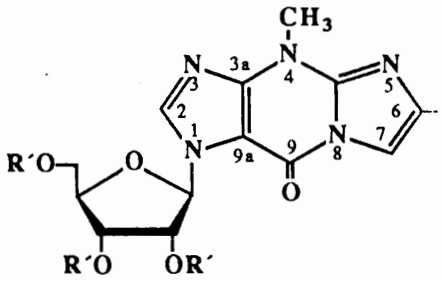
*Abstract:* *Determination of tautomeric population of the Y-base in 3 by $^{13}$C-NMR spectroscopy has shown that its most thermodynamically preferred tautomeric form is the $N^1$-H tautomer ( ~ 95 %) which explains its chemical reactivity in glycosylation reaction and also in the facile, lewis acid promoted isomerization of wyosine-2′,3′,5′-O-triacetate 5 to its $N^1$- isomer 4. It is likely that the consequence of occurrence of natural wyosine in the thermodynamically unfavoured $N^3$-form is its unusual lability across the glycosyl bond under mild acidic conditions.*

Y-nucleoside (wyosine) **1** and its 7-substituted congeners **2a - c** occur naturally, adjacent to the 3′-anticodon loop of yeast phenylalanine tRNA [$tRNA^{Phe}$][1,2]. Their glycosyl bonds are extremely labile[3] and the loss of Y-base **3** from $tRNA^{Phe}$ by mild acidic treatment causes it to loose its codon recognition property[3]. Following two chemical observations have prompted us to study the tautomerism of Y-base **3**: (1) the glycosylation of the Y-base by 1′,2′,3′,5′-tetra-O-acetyl-β-D-pentofuranose under acidic conditions gave exclusively the $N^1$-isomer of wyosine **4**; no trace of isomeric Y-nucleoside **5** was detectable[4,5]; and (2) a treatment of wyosine-triacetate **5**[12] with anhydrous $AlCl_3$ in dry $CH_2Cl_2$ gave exclusively the $N^1$-isomer **4**, while the $N^1$-isomer **4** was found to be completely stable under the latter condition[6]. We reasoned that these specific chemical behaviours might be possibly understood in terms of the thermodynamic stabilities of the preferred tautomer of the Y-base[13] in **3**. Our attempts to study this prototropic tautomerism of the imidazole moiety of Y-base in **3** by $^{15}$N-NMR spectroscopy have been unsuccessful primarily because of the fact that the expected NMR time-weighted average absorptions of $N^1$ and/or $N^3$ could not be observed due to $N^1$-H $\rightleftarrows$ $N^3$-H prototropism of Y-base in **3**. This prompted us to examine this problem by measurement of $^{13}$C chemical shifts of C3a and C9a, and the vicinal $^3J_{C9a,H2}$ and $^3J_{C3a,H2}$ coupling

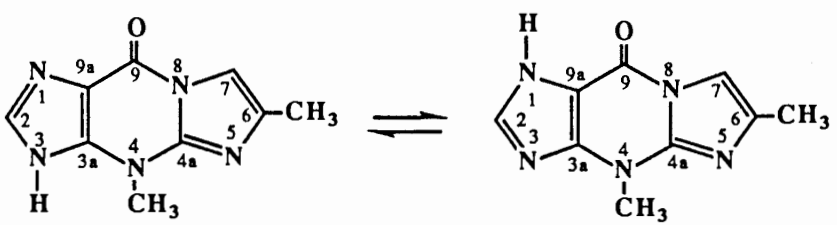
constants of Y-base in solution and determine the population of predominant tautomeric forms in **3**. At ambient temperature, the rate of tautomeric proton exchange in **3** is faster than the NMR time scale and, therefore, the weighted average of the contributing tautomeric structures in **3** leads to single chemical shift for each carbon. The calculation of tautomeric populations, hence, requires[7,9,10] the determination of the $^{13}C$ chemical shifts C3a and C9a, and the vicinal $^{3}J_{C9a,H2}$ & $^{3}J_{C3a,H2}$ coupling constants of each tautomeric form blocked specifically in the $N^1$- and $N^3$- such as pairs **1** & **6**, and their triacetates **4** & **5**[12,14].





**4** : R′ = Acetyl
**6** : R′ = H

**1** : R′ = H, R" = H
**2a** : R′ = H, R" = $CH_2CH_2CH(NHCO_2Me)CO_2Me$
**2b** : R′ = H, R" = $CH_2CH(O_2H)CH(NHCO_2Me)CO_2Me$
**2c** : R′ = H, R" = $CH_2CH(OH)CH(NHCO_2Me)CO_2Me$
**5** : R′ = Acetyl, R" = H



**3**

In the first method, the tautomeric population is determined by the difference of the $^{13}C$ chemical shift of the C3a and C9a between compounds **1** & **6**, and **4** & **5**[12,14]. In the second method, the decrease of the vicinal three bond $^{3}J_{C9a,H2}$ and $^{3}J_{C3a,H2}$ coupling constants through a pyridine- *versus* a pyrrole-type nitrogen atom is used[12,14]. The following equations were used to calculate the % $N^1$-H tautomer from either C3a or C9a chemical shifts[7]:

$$[\%\ N^1\text{-}H]_{C3a} = \frac{(\delta C3a)_{Y\text{-}base} - [(\delta C3a)_{N3} - \alpha]}{(\delta C3a)_{N1} - \beta] - [(\delta C3a)_{N3} - \alpha]} \quad \text{....} \quad \text{eqn. 1}$$

Table 1: Calculation of % of $N^1$-H tautomer using the $^{13}C$ chemical shifts of C3a & C9a and [$^3J_{C3a,H2}$] and $^3J_{C9a,H2}$] in compounds **1** & **3-6**.

| Compound | δ(C3a)* [eqn. 1] | *%N¹-H*** | [$^3J_{C3a,H2}$]# [eqn. 3] | *% N¹-H*§ | δ(C9a) [eqn. 2] | *%N¹-H* | [$^3J_{C9a,H2}$]# [eqn. 4] | *%N¹-H*§ |
|---|---|---|---|---|---|---|---|---|
| Y -base **3** | 141.8 | | 7.2 | | 105.2 | | 4.3 | |
| $N^3$-isomer **1** | 140.0 | 100% | 4.2 | 97% | 115.8 | 99% | 12.1 | 98% |
| $N^1$-isomer **6** | 142.6 | | 7.7 | | 104.8 | | 4.1 | |
| $N^1$-isomer **4** | 142.6 | 100% | + | + | 104.3 | 94% | + | + |
| $N^3$-isomer **5** | 140.1 | | + | + | 115.7 | | + | + |

\+ Coupling constants could not be obtained with sufficient accuracy.
* 0.1 Hz resolution.
** Error limit of ~10% is expected due to approximation made in the corrective factor (see refs. 7, 8 & 9).
\# 0.05 Hz resolution.
§ error limit of ~4% is expected (see ref. 9).

$$[\%\ N^1\text{-}H]_{C9a} = \frac{(\delta C9a)_{Y\text{-}base} - [(\delta C9a)_{N3} - \beta]}{(\delta C9a)_{N1} - \alpha] - [(\delta C3a)_{N3} - \beta]} \quad \ldots\ \text{eqn. 2}$$

The correction of the chemical shifts of the bridgehead carbons, C3a & C9a, by factors $\alpha$ & $\beta$ (-0.3 and -0.8 ppm, respectively) due to the replacement of the proton in the Y-base **3** by the $\beta$-D-ribofuranosyl groups in **1** & **6**, and **4** & **5** have been defined[8]. The calculation of the % $N^1$-H tautomer has been achieved using the three bond vicinal $[^3J_{C3a,H2}]$ & $[^3J_{C9a,H2}]$ of the Y-base and **1** & **6**, and **4** & **5** [ eqns. 3 &4][9,10]:

$$[^3J_{C3a,H2}]_{Y\text{-}base} = \chi\,[(J_{C3a,H2})_{N1} - \Delta] + (1-\chi)[(J_{C3a,H2})_{N3}] \quad \ldots\ \text{eqn. 3}$$

$$[^3J_{C9a,H2}]_{Y\text{-}base} = (J_{C9a,H2})_{N1} + (1-\chi)[(J_{C9a,H2})_{N3} - \Delta] \quad \ldots\ \text{eqn. 4}$$

where $\chi$ denotes population of the $N^1$–H tautomer and $\Delta$ is the corrective term (0.4 Hz) representing the $\alpha$ effect of the bridgehead carbon. The results of these studies are presented in table 1[7,9,10].

This $^{13}$C-NMR study is also supported by our recent fluorescence measurements on wyosine and its derivatives which show that the spectral emission of the Y-base and the $N^1$-blocked derivative **4** have predominant amplitude of fluorescence decay times of 7.01 ns (100%) & 10.12 ns (96%), respectively, while the wyosine-triacetate **5** show the predominant amplitude of fluorescence decay time of 0.53 ns (95%) which clearly show the *electronic similarity* of the Y-base with that of the $N^1$-blocked derivative **4**[11].

These studies together confirm that the Y-base in **3** mainly exists (~ 95 %) in the thermodynamically preferred $N^1$-H tautomeric form. The consequences of this preferred form of Y-base are its regioselective glycosylation at $N^1$, facile isomerization of **5** → **4** and the unusual instability of the glycosyl bond of **5** under mild acidic conditions.

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13. Compound **3** was obtained by depurination of wyosine **1** in 80% acetic acid at room temperature.

14. $^{13}$C-NMR spectra were recorded in dimethylsulfoxide-$d_6$ solution (0.06 M) at ~30 $^{0}$C on a Jeol GX 270 spectrometer at 67.8 MHz. The $^{13}$C chemical shifts were measured using an inverse gate proton noise decoupled mode. The [$^{3}J_{C3a,H2}$], [$^{3}J_{C9a,H2}$] coupling constants were determined using a $^{1}$H selective decoupling method.

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