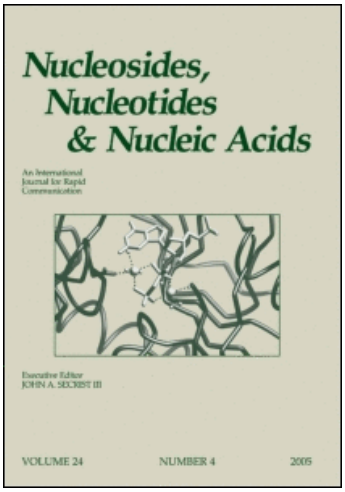

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ON THE STRUCTURE AND REACTIVITY OF Y-NUCLEOSIDE [WYOSINE]

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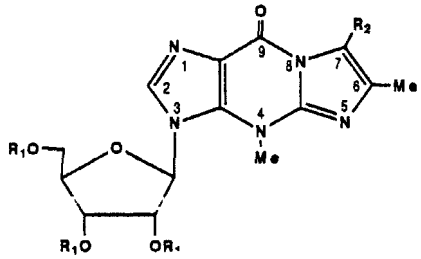
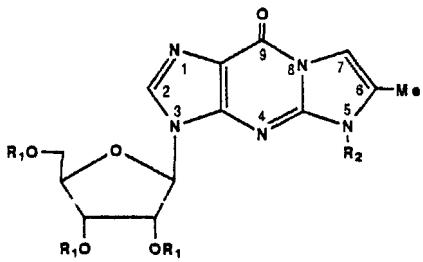
Abstract *: Wyosine-Triacetate 4 has found to undergo electrophilic reactions exclusively at the C-7 position. ^{15}N-NMR of 4 shows that the primary site of protonation is N-5 suggesting that the " right " imidazole ring is more aromatic that the " left " imidazole moiety .*

The hypermodified fluorescent Y-nucleoside 1 and its 7-substituted congeners 2 and 3 occur naturally adjacent to the 3′-end of the anticodon loop of t-RNA^{Phe}[1,2]. They are characterized by their strong fluorescence and the extreme lability of the glycosidic bond in acidic medium. The synthesis of wyosine-2′,3′,5′-O-triacetate 4 has been achieved in our laboratory recently in 74% yield[3]. This has made wyosine-triacetate 4 available in a large scale, allowing us to study its chemical reactivity and electronic properties[10].

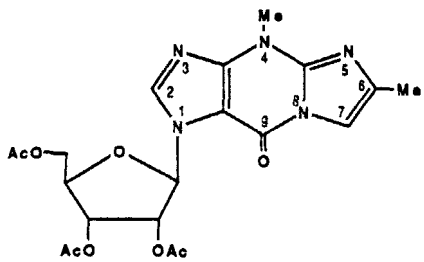
When wyosine-triacetate 4 was subjected to electrophilic substitution reactions such as Vilsmeir formylation, iodination or bromination, the reactions were found to take place exclusively at the C-7 position[10]. On the other hand, base promoted C-acetylation and C-deuteration took place exclusively at the C-2 position. These results suggest the dominating electrophilic character of the "right" imidazole ring over the "left" in wyosine-triacetate 4, the electron-deficient nature of the "left" imidazole ring is also apparent from the generation of a carbanion under the influence of a strong base. The N-5 methyl isomer 10 was found to react much more sluggishly giving a lower yield of the C-7 substituted products when submitted to the above electrophilic substitution reactions. During the investigation of the stability of wyosine-triacete 4, it has been found that the treatment of 4 with a lewis acid such as $AlCl_3$ leads to its N-1 isomer 8 in 74% yield[10].





1. $R_1 = R_2 = H$
2. $R_1 = H$, $R_2 = CH_2CH_2CH(NHCO_2Me)CO_2Me$
3. $R_1 = H$, $R_2 = CH_2CH(OH)CH(NHCO_2Me)CO_2Me$
4. $R_1 = Ac$, $R_2 = H$
5. $R_1 = H$, $R_2 = Me$
6. $R_1 = Ac$, $R_2 = Me$
7. $R_1 = Ac$, $R_2 = CHO$

9. $R_1 = Ac$, $R_2 = H$
10. $R_1 = Ac$, $R_2 = Me$



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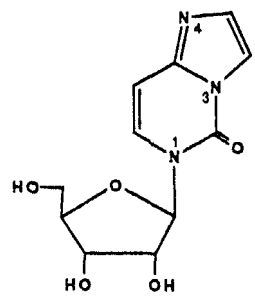

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^{15}N-NMR has been proved to be a very powerful tool to determine both the site and the magnitude of protonation[4,5] The degree of protonation of a nitrogen atom in a molecule indicates its potential reactivity to electrophiles. Although the protection of the hydroxyl function of the sugar as acetates is known to stabilize the glycosidic bond[6,10] in compounds 4 and 6, they are still considerably unstable under acidic condition due to the depurination reaction. It was then necessary to carry out the study of protonation of wyosine-triacetate 4 in a shorter time. For this purpose, a ^{1}H-decoupled INEPT pulse sequence was designed[7] which leads both to enhancement and distinction between the different nitrogens resonances, basing on the magnitude of $J_{N,H}$. The main conclusion of the protonation study is that the primary site of protonation is N-5 both in wyosine-triacetate 4 and in its C-7 methyl congener 6, while in N-5 methyl isomer 10 and N-4 desmethylwyosine 9, it is the N-1 which is exclusively protonated[10]. It should be however added that the electron-withdrawing acetates in the sugar moiety can cause a considerable change in the basicities of the imidazole ring in the purine system in compounds 1 and 5[8] Another point is that the N-4 nitrogen in 4 and 6 moves very little downfield suggesting that

the lone pair of the N-4 does not stabilize the N-5 protonated imidazole ring by delocalization. The fact that the $^2J_{N8-H7}$ in 4 is 4.3 Hz[9] as compared to $^2J_{N3-H8}$ = 3.3 Hz in planar ethenocytidine 11[4], suggests that the central pyrimidine and the "right" imidazole rings in 4 are not coplanar. We have also observed[10] a correlation between the ^{15}N-NMR shifts and the pK_a in compounds 4 and 6 respectively. A electropositive C-7 methyl group as in 6 (pK_a 2.85) increases the basicity of wyosine-triacetate 4 (pK_a 2.36), consistent with the $\Delta\delta$ of 46.3 and 39.9 ppm for N-5 of 6 and 4 . On the other hand, a electronegative C-7 formyl group as in compound 7 (pK_a -0.3) reduces the basicity of wyosine-triacetate 4.This reduction of basicity is shown by a shift of 1.0 ppm for the N-5 nitrogen. A comparison of the pK_a of Wyosino-triacetate 4(2.36) with that of its N-1 isomer 8(3.10) shows that the later is more basic. This enhancement of basicity can be attributed to the enhancement of the "right" imidazole part. This has been corroborated by the magnitude of ^{15}N shifts ($\Delta\delta$) upon protonation.

The kinetic study of the cleavage of the N-glycosidic bond in wyosine has shown that the acetylation of the hydroxyl groups in the sugar moiety retards the hydrolysis by two orders of magnitude[10]. The C-7 substituents exert only a small effect on the hydrolytic stability of wyosine-triacetate 4. Wyosine-triacetate 4 was also found to undergo acidic hydrolysis 7 times more rapidly than its N-1 isomer 8[10]. The relatively small difference in their relative rates of hydrolysis supports the absence of any steric compression[11] due to N^4-methyl group in 4.

The metal ion binding to wyosine and its analogues has also been studied due to their importance in biological interactions[12]. ^{15}N-NMR is not suitable[13] to study the complexation of nucleosides with "hard" metals ions such as Mg^{2+} and Ca^{2+},which bind preferentially to the oxygenated sites. The "soft" metal ions such as Zn^{2+} and Hg^{2+}, however, bind usually to nitrogens. The main result of this study[10] is that N-1 and N-5 nitrogens in compounds 1 and 4 move very slightly upon addition of $HgCl_2$, $Zn(NO_3)_2$ This indicates that the N-5 nitrogen is a very soft basic center and therefore does not complex with a hard metal ion such as Mg^{2+}.

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