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SYNTHESIS OF WYOSINE (NUCLEOSIDE Yt), A STRONGLY FLUORESCENT NUCLEOSIDE FOUND IN TORULOPSIS UTILIS $tRNA^{Phe}$, AND 3-METHYLGUANOSINE[1]

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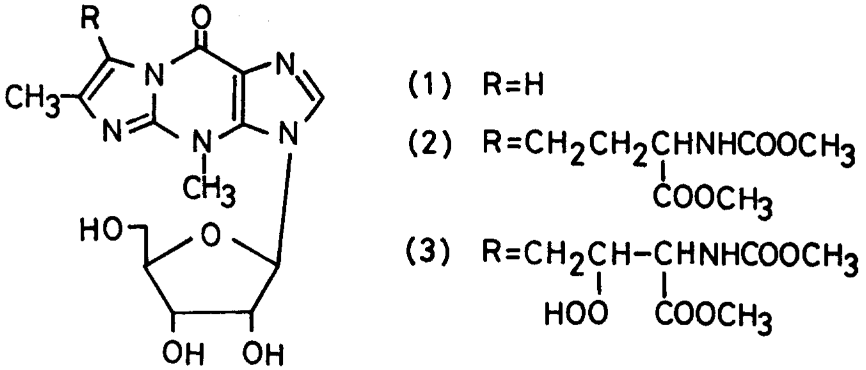
Among the modified nucleosides found in tRNA's, wyosine (1) (nucleoside Yt)[2] is one of the most unusual nucleosides from its abnormality that it gives strong fluorescence and its nucleoside bond can be hydrolyzed most easily among others in weakly acidic solutions (even at pH 4). It was found in the next position of the 3' end of the anticodon of yeast $tRNA^{Phe}$. Similar nucleosides, wybutosine (2) and wybutoxosine (3), have been found in baker's yeast $tRNA^{Phe}$,[3] and in rat liver[4] and plant Lupinus luteus,[5] respectively. The nucleoside bond is so easily hydrolyzed that these three nucleosides have been difficult to be isolated in pure form for structure analysis. Structure elucidation has been done using their bases, i.e., wye,[2] wybutine,[3] and wybutoxine,[4,5] respectively. The sugar part of wyosine was suggested as having N-1-ribosyl structure, but the abnormality of the nucleoside bond led Reese and Whithall[6] to propose 2-deoxyribosyl structure. To confirm the structure of wyosine we have carried out its total synthesis as described below.



5-Amino-4-cyano-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole (4) was treated with paraformaldehyde in THF at 50° for 3 hr followed by reduction with $NaBH_4$ in ethanol (0° to room temp) and acetylation affording in 79% yield its 5-N-methyl derivative (5) [oil, m/e 380 (M^+), $\delta_{ppm}^{CDCl_3}$ 3.15 (3H,d,J=6Hz, NCH_3)], which was hydrolyzed with aq. triethylammonium hydroxide under reflux for 5 hr, and then acetylated with acetic anhydride and triethylamine at room temp to give the 5-methylamino-4-carbamoylimidazole 6 [powder, m/e 398 (M^+), $\delta_{ppm}^{CDCl_3}$ 2.87 (3H,d, J=6Hz, NCH_3)] in 64% yield. Heating with benzylisothiocyanate in o-dichlorobenzene at 150°, 6 was converted in 96% yield to the benzyl thiourea 7 [mp 235-7°], which was methylated with CH_3I and K_2CO_3 in acetone by refluxing to give the S-methylisothiourea 8 [powder, $\delta^{CDCl_3}_{ppm}$ 2.00 (3H,s), 2.07 (3H,s), 2.14 (6H,s), 3.27 (3H,s, NCH_3)] in 63% yield. Pyrolysis of the isothiourea 8 at 160° under argon atmosphere for 1 hr gave the 3-methylguanosine derivative 9 [glass, m/e 513.1868 (M^+ calc 513.1857), $\delta^{CD_3OD}_{ppm}$ 3.79 (s, NCH_3), 4.63 (2H,m, $PhCH_2N$)] in 47% yield, which could be converted to 3-methylguanosine (11)[powder, $\lambda^{H_2O}_{max}$ 216 nm (ε 19,000), 243 (7200), 263 (7400) (Fig. 1), $\delta^{D_2O}_{ppm}$ 3.73 (s, NCH_3), 5.98 (1H,d,J=5Hz, H-1'), 7.93 (1H,s, H-8), Rf values on tlc in Table 1] through the triacetate 10 by removal of the 3-N-benzyl group by catalytic hydrogenation with H_2/Pd-C (10%) in acetic acid followed by deacetylation with saturated methanolic ammonia at room temp overnight. The yield of 11 from 9 was 68%. On acetylation with acetic anhydride and triethylamine 10 and 11 afforded the tetraacetate 12 [mp 153-4°, m/e 465.1496 (M^+ calc 465.1495), λ^{MeOH}_{max} 217 nm (ε 9500), 278 (16,500), $\delta^{CDCl_3}_{ppm}$ 2.10, 2.14, 2.16, 2.23 and 3.94 (each 3H,s), 4.30 (2H,m), 4.46 (1H,m), 5.44 (1H,m), 5.78 (1H,t,J=6Hz), 6.17 (1H, d,J=6Hz), 7.32 (1H,s), 7.76 (1H,br.s, NH)], which could also be prepared from 6 by condensation with acetylisothiocyanate followed by methylation and pyrolysis. The tetraacetate 12, however, could not be hydrolyzed to 3-methylguanosine (11) without decomposition.

Tri-O-acetyl-3-methylguanosine (10), on treatment with bromoacetone in acetone at room temp in the presence of solid K_2CO_3, gave the 1-N-acetylmethyl derivative 13 which could be transformed without isolation to wyosine triacetate (14) [λ^{MeOH}_{max} 234 nm (ε 17,300), 288 (5300), m/e 461.1555 (M^+ calc 461.1546), $\delta^{CDCl_3}_{ppm}$ 2.32 (3H,s[7], 6-Me), 4.18 (s, NCH_3), 6.23 (1H,d,J=6Hz, anomeric H), 7.40 (1H,s[7], H-7), 7.74 (1H,s, H-2)] on silica gel tlc plate. Over-all yield from 10 was 36%. Deacetylation of the triacetate 14 with saturated methanolic ammonia at room temp overnight afforded wyosine (1) [powder, $\lambda^{H_2O}_{max}$ (Fig. 1) 235 nm (ε 17,600), 294 (4200), $\delta^{DMSO\text{-}d_6}_{ppm}$ 1.21 (3H,s[7]), 3.61 (2H), 4.07 (3H,s,NCH_3), 3.9-4.2 (2H,m), 4.43 (1H,t,J=5Hz), 6.10 (1H,d,J=5Hz), 7.35 (1H,s[7]), 8.21 (1H,s)] in nearly quantitative yield.

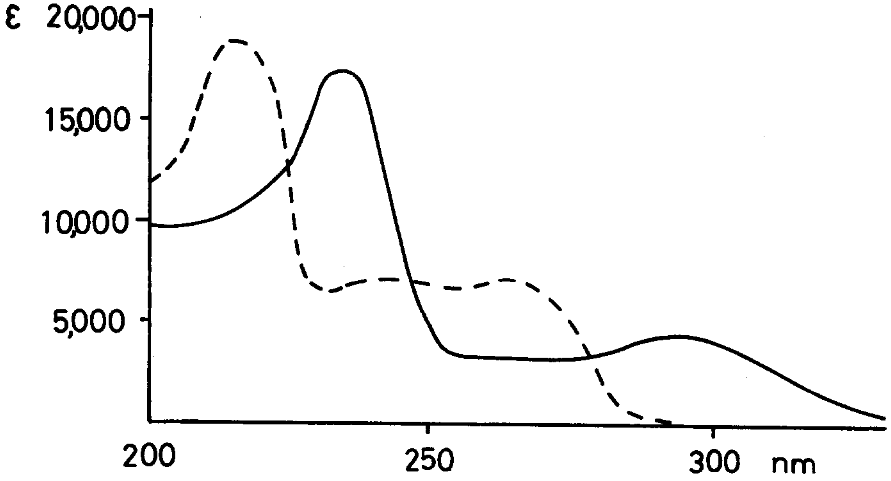
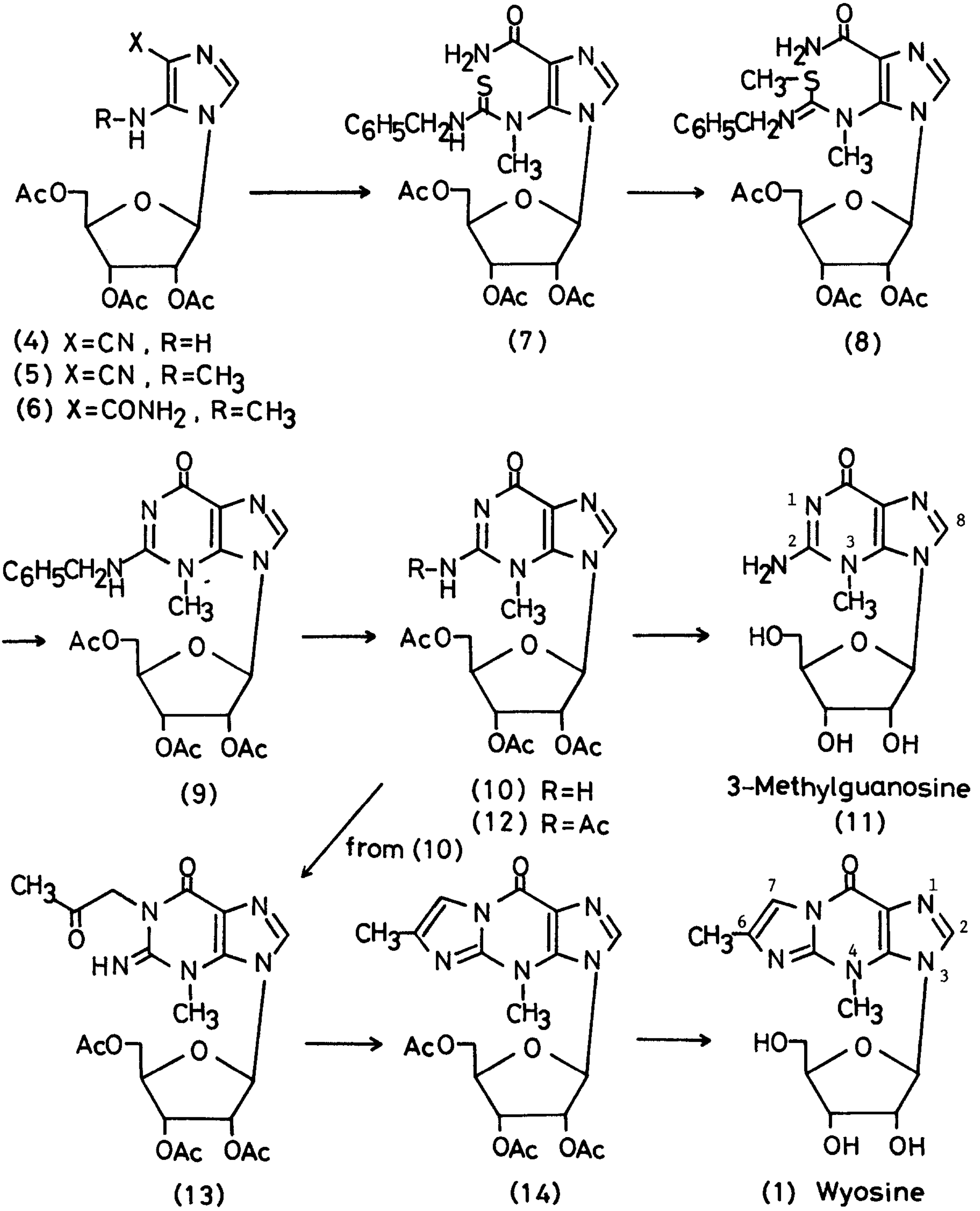


Fig. 1. Uv spectra of 3-methylguanosine (11) (---) and wyosine (1) (natural and synthetic) (——) in water


(4) X=CN, R=H
(5) X=CN, R=CH3
(6) X=CONH2, R=CH3
(7)
(8)
(9)
(10) R=H
(12) R=Ac
3-Methylguanosine
(11)
from (10)
(13)
(14)
(1) Wyosine

The synthetic wyosine was identified with natural wyosine[2,8] obtained from *Tolulopsis* $\mathrm{tRNA}^{\mathrm{Phe}}$ by comparison of their uv spectra (Fig. 1) and Rf values on Avicel tlc (Table 1). The synthetic wyosine could be converted to the triacetate (14) by acetylation with acetic anhydride and triethylamine. The nucleoside bond of wyosine and 3-methylguanosine are so weak that the nucleosides were gradually decomposed even in solid state.

Table 1. Rf values on Avicel tlc (Funakoshi, 20 cm)

	Guanosine	3-Methylguanosine	Wyosine (natural)	Wyosine (synthetic)
i-PrOH-H_2O-conc NH_4OH (7:2:1)	0.31	0.26	0.62	0.62
n-BuOH-H_2O-conc NH_4OH (86:14:5)	0.04	0.04	0.27	0.27
5% conc NH_4OH in H_2O	0.71	0.71	0.51	0.51

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7. A small long-range coupling (ca 1 Hz) was observed.
8. Natural wyosine (0.08 μmol) was isolated from *T. utilis* by S. Takemura.