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STRUCTURE AND SYNTHESIS OF A FLUORESCENT Y-LIKE BASE FROM TORULOPSIS UTILIS tRNA

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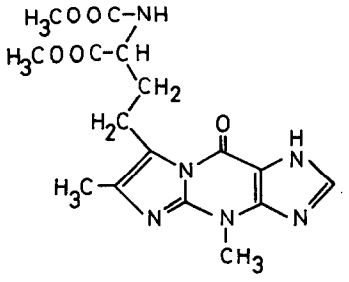
RajBhandary et al. (1) first found an unusual fluorescent nucleoside Y in baker's yeast $tRNA^{Phe}$. Similar fluorescent nucleosides were then found in wheat germ $tRNA^{Phe}$ (Yw) (2) and rat liver $tRNA^{Phe}$ (3). The nucleosides Y and Yw have been known to exist next to 3'-end of the anticodon in the respective tRNAs, and supposed to play an important role in recognition of mRNA (1,2). Owing to unusual properties of these fluorescent nucleosides, their structures had been unsolved until when quite recently Nakanishi et al. (4) proposed the structure I for the base of Y (Y base) by comparison of spectral data with model compounds.

In Torulopsis utilis $tRNA^{Phe}$ is also found a Y-like nucleoside (5) which contains a fluorescent base. The uv spectra in various pHs and the Rf values on paper chromatography of the base, Yt base, were different from those of the Y base and the Yw base. We wish to report in this communication the structure determination and the synthesis of Yt base.

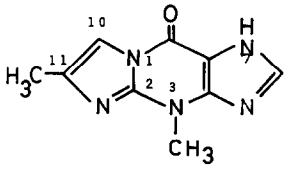
According to the method of Thiebe and Zachau (6), aqueous solution of 2.65 g tRNA from T. utilis ($tRNA^{Phe}$ rich fraction) was incubated at 37° for 4 hr at pH 2.9. After neutralization the fluorescent compound was extracted with chloroform and purified by paper chromatography (solvent: water). An intense and a faint band of fluorescence were detected on the paper. The intense band was eluted with water and evaporated to give 27.5 O.D. units (230 nm) (ca 180 μg) of the Yt base.

High-resolution mass spectrum of the Yt base gave the following peaks (7): m/e 203.0802 (M^+; calcd. for $C_9H_9N_5O$: 203.0807) relative intensity 100%; 202.0730 ($C_9H_8N_5O$: 202.0729) 70%;

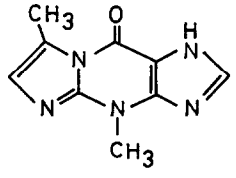
188.0590 ($C_8H_6N_5O$: 188.0572) 10%; 174.0767 ($C_8H_8N_5$: 174.0780) 10%. Thus, the base has the molecular formula $C_9H_9N_5O$ and it is suggested that this molecule has a very stable nucleus with C=O and Me groups. Shapes of uv spectra of the Yt base at acid, neutral and basic pH closely resemble those of the Y base, except that the maxima of the former are slightly blue-shifted as compared with the latter (8). PKas from the uv spectra gave the values 3.66 ± 0.05 and 8.52 ± 0.10. These data suggest that the chromophore of the Yt base is identical with that of the Y base (I). Nmr spectrum (9) in D_2O of the Yt base, which was crystallized from water, shows signals at δ 2.24 ppm (3H, d, J = 1 Hz), 3.82 ppm (3H, s), 7.32 ppm (1H, q, J = 1 Hz) and 8.07 ppm (1H, s), which can be assigned to an aromatic methyl, an N-methyl and two aromatic protons, respectively. The small coupling of about 1 Hz appeared between the 2.24 ppm methyl and the 7.32 ppm proton is attributable to a long range spin-spin coupling between methyl and proton in CH_3-C=C-H grouping. Thus, the Yt base has two methyl groups, one on C_{10} or C_{11} and the other on N_3, N_7 or N_9. Although the position of the N-methyl group was not rigorously confirmed, we carried out the synthesis of II (Me at C_{11}) and III (Me at C_{10}) having N_3-methyl group in due consideration of the structure of Y base (I).



I



II



III

By the condensation of 3-methylguanine and bromoacetone we obtained fluorescent compound A (10), whereas isomeric compound B (11) was produced by condensation of 3-methylguanine and α-bromopropionaldehyde. Nmr spectra of compounds A and B were measured in 1N DCl-D_2O. Compound A shows the C-methyl signal (2.48 ppm) at a higher magnetic field than the corresponding signal (2.72 ppm) of compound B, whereas the aromatic proton signal (7.68 ppm), which is spin-coupled with the C-methyl signal, of the former appeared at a lower field than the corresponding signal (7.26 ppm) of the latter. On the assumption that the signal of methyl and proton on C_{10} appears

at a lower magnetic field than those on C_{11} by the anisotropic effect of the carbonyl group at 6-position, the structure of compounds A and B can be assigned to II and III, respectively.

Rf values of the Yt base using several solvent systems were consistent with those of compound A (12) and the uv (Fig. 1 and 2), nmr and mass spectra, and the pKa values of the base were in full agreement with those of compound A. Hence, we conclude that the Yt base is identical with compound A having structure II.

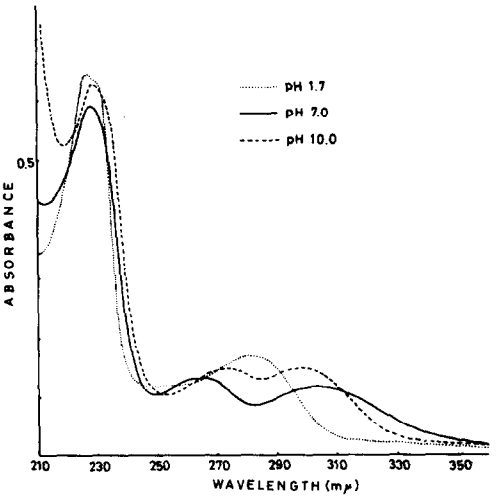


Fig. 1

Uv spectra of Yt base

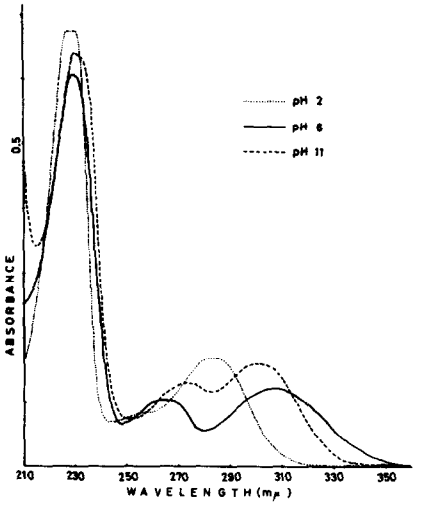


Fig. 2

Uv sprctra of compound A

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7). CEC-110-B mass spectrometer; ionization potential 70 eV; at 190°.

8). Uv spectra in 0.01M ammonium acetate at pH 7.0; Y base: λ_{max} 236, 264 and 314 nm; Yt base: λ_{max} 230, 265 and 305 nm.

9). Varian 100 MHz nmr spectrometer, 1000 scans (pulse method).

10). Compound A: Anal. Calcd. for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.47%. Found: C, 52.90; H, 4.36; N, 34.19%. $\lambda_{max}^{H_2O}$ (ε) 230 (31,000),264 (5300), 307 (5900).

11). Compound B: Anal. Calcd. for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.47%. Found: C, 53.05; H, 4.19; N, 33.90%. $\lambda_{max}^{H_2O}$ (ε) 232 (34,000), 257 (5000), 314 (5700).

12). Rf values of the Yt base and compound A.

sample	solvent a	b	c	d
Yt base	0.87	0.63	0.29	0.22
compound A	0.87	0.63	0.29	0.22

solvent a; isobutyric acid : 1M ammonia (5:3)

solvent b; isopropanol : conc. ammonia : water (7:1:2)

solvent c; water

solvent d; 4% sodium citrate