Structure of Wyo sine, the Condensed Tricyclic Nucleoside of Torula Yeast Phenylalanine Transfer Ribonucleic Acid

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Received November 21, 2001; accepted January 11, 2002

Large-scale isolation of the minor nucleoside wyo sine of torula yeast tRNA\textsuperscript{Phe} was accomplished by a combination of enzymatic digestion and reversed-phase chromatography: the wyosine-containing nucleotide fraction, which was obtained by partial digestion of unfractionated tRNA (1 g) with nuclease P\textsubscript{1}, was concentrated by reversed-phase column chromatography followed by complete digestion with nuclease P\textsubscript{1}/alkaline phosphatase. The nucleoside mixture thus obtained was purified by reversed-phase HPLC, providing wyosine (70 μg). Comparison of this nucleoside with a chemically synthesized authentic sample has unambiguously established that the structure of wyosine is 4,6-dimethyl-3-β-D-ribofuranosyl-3,4-dihydro-9H-imidazo[1,2-a]purin-9-one (2).

Key words wyosine; minor nucleoside; torula yeast tRNA\textsuperscript{Phe}; fluorescent nucleoside; condensed tricyclic nucleoside; 1H-NMR

A fluorescent component adjacent to the 3′-end of the anticodon of torula yeast tRNA\textsuperscript{Phe} was isolated as the nucleoside wyosine,\textsuperscript{1} and the structure of its base wye has been determined to be 1.\textsuperscript{16,21} It has been proposed by comparison of the chemical properties and UV spectra of wyosine with those of model compounds that 3-β-D-ribofuranosylwye (2) is the most probable structure for this nucleoside.\textsuperscript{10} However, Reese and Whittall have claimed that wyosine is unlikely to be a ribonucleoside on the basis of the rate studies on the hydrolysis of model compounds.\textsuperscript{33} As wyosine was isolated from the tRNA in only a minute quantity (0.13 A\textsubscript{260} unit); estimated to be ca. 8 μg), precise identification of the position of glycosylation and the structure of the sugar moiety has to rest on chemical synthesis. We and other groups have already synthesized the target molecule 2.\textsuperscript{4} However, lack of a sample of wyosine has hampered its structural determination. As the exceptional susceptibility to hydrolysis of wyosine\textsuperscript{16} had been anticipated to aggravate the difficulties of its isolation, we carried out rate studies\textsuperscript{4–5} on the hydrolysis of 2, disclosing that 2 can be handled at pH 5—10 at 37 °C without suffering a heavy loss in quantity. Being encouraged by this knowledge, we started the present investigation. A preliminary communication of a part of this work has been published.\textsuperscript{9}

In order to avoid difficulties presented by the instability and the poor solubility of 2 in an organic solvent such as CDCl\textsubscript{3}, we planned to identify the nucleoside through its 1H-NMR and CD spectra. The structure of wyosine was hereby determined to be 4,6-dimethyl-3-β-D-ribofuranosyl-3,4-dihydro-9H-imidazo[1,2-a]purin-9-one (2).

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**Experimental**

**General Notes** Spectra reported herein were recorded on a JEOL JMS-SX102A mass spectrometer, a Hitachi model 320 UV spectrophotometer, a JEOL JNM-GSX-500 NMR spectrometer (measured at 25 °C with Me₄Si as an internal standard), and a JASCO J-700 polarimeter. MS measurements were performed by Dr. M. Takani and her associates at Kanazawa University. The optical rotation was measured with a JASCO DIP-181 polarimeter using a 10 cm sample tube. The HPLC system employed consisted of a Tosoh CCPD pump, an injection valve unit, a UV-8020 detector (operated at 310 nm), and a Chromatocorder 21 integrator or a Waters 6000A system using a 10 cm sample tube. The HPLC system employed consisted of a Tosoh CCPD pump, an injection valve unit, a UV-8020 detector (operated at 310 nm), and a Chromatocorder 21 integrator or a Waters 6000A system using a 10 cm sample tube. The HPLC system employed consisted of a Tosoh CCPD pump, an injection valve unit, a UV-8020 detector (operated at 310 nm), and a Chromatocorder 21 integrator or a Waters 6000A system using a 10 cm sample tube.

**References**