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Received March 16, 1977

A facile chemical synthesis of 1-methylpseudouridine has been accomplished by direct methylation of pseudouridine.

J. Heterocyclic Chem., 14, 699 (1977).

Sir:

The isolation and characterization of pseudouridine (**1**, Ψ) as the first naturally occurring nucleoside possessing a carbon-carbon linkage between the carbohydrate and heterocyclic moieties (1,2) created considerable interest in this area of C-nucleosides. Some recent fermentation studies (3) using *Streptomyces platensis* var. *clarensis* has revealed at least two C-nucleosides in the fermentation broth in addition to the s-triazine nucleoside antibiotic U-44590. These two nucleosides were isolated and characterized (3) as Ψ (**1**) and 1-methylpseudouridine (**3**). This was of considerable interest since **3** had already been reported (1,2) in some previous studies on the structural elucidation of Ψ . However, a perusal of the literature has revealed that in these studies, **3** was never isolated and the characterization of **3** in these early reports (1,2) was limited to only chromatographic and ultraviolet spectral data. This was of some interest to us, since we have been recently involved in a systematic study on the chemistry of Ψ which has included some N-alkylation studies (4). We now wish to report a facile chemical synthesis of **3**. This synthesis provides a 63% yield of crystalline **3** by a procedure which requires no chromatography.

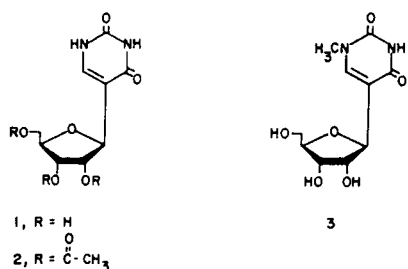
Pseudouridine (**1**) was acetylated (5) to afford a good yield of 2',3',5'-tri-O-acetylpsudouridine (**2**). Bis-trimethylsilylacetamide (BSA) (2.08 ml., 8.7 mmoles) was added to a stirred solution of **2** (4.33 mmoles) in methylene chloride. After one hour, methyl iodide (5 mmoles) was added (6) to the solution, and the solution was then heated at reflux temperature for 4 days at which time silica gel tlc [chloroform-acetone-methanol; 15:4:1 (v/v/v)] revealed the disappearance of all starting material ($R_f = 0.45$) and the appearance of a new uv absorbing spot ($R_f = 0.50$). The reaction mixture (light brown solution) was concentrated *in vacuo* to a syrup. The syrup was dissolved in ethylacetate and washed in succession with water, 1% aqueous hydrochloric acid, saturated sodium

chloride solution, 5% sodium thiosulfate solution and again with a saturated sodium chloride solution. After drying (sodium sulfate), concentration of the ethyl acetate solution yielded a stiff foam (1.1 g.) which was dissolved in methanolic ammonia (40 ml.) (saturated with ammonia at 0°) and then stored at ambient temperature for 18 hours. The crystalline solid obtained from evaporation of this solution *in vacuo* was triturated with 2-propanol to give 0.68 g. (57.2%) of N1-methylpseudouridine, **3**, m.p. 184-185°. An additional 60 mg. (5.4%), m.p. 180-184°, of **3** was obtained on partial concentration of the 2-propanol filtrate. For analysis (7), a sample of **3** was recrystallized from ethanol to give small white needles, m.p. 185-186°. [Lit. (3) m.p. 181-184°]; ¹H nmr (DMSO-d₆): δ 3.18 (s, 3H, N-CH₃); 3.60-3.76 (M, 3H, H_{4'},_{5'}); 3.98 (m, 2H, H_{2'},_{3'}); 4.50 (d, 1H, H_{1'}, J_{1',2'} = 4.0 Hz); 7.76 (s, 1H, H6); 11.35 (s, 1H, N-H). $[\alpha]_{D}^{25} -6.75^{\circ}$ (c 1.0 in water) [Lit. (3) $[\alpha]_{D} -25^{\circ}$ (c 1, in 50% aqueous ethanol)]. Uv: λ max (pH 1), 270.8 nm (ϵ , 9,550); λ min (pH 1), 236.7 (ϵ , 2,370); λ max (methanol), 270.5 (ϵ , 9,170); λ min (methanol), 234.5 (ϵ , 1,030); λ max (pH 11), 267.5 (ϵ , 7,230), 226.3 (ϵ , 7,490); λ min (pH 11), 243.0 (ϵ , 3,950).

The appearance of a 3-proton singlet, in the pmr spectrum of **3**, at δ 3.18 confirmed that N-methylation had occurred. The fact that the principal absorption in the uv spectrum occurred at ca. 270.5 nm (pH 1 and methanol spectrum) and that a slight hypsochromic shift (3 nm) was noted for this band when the spectrum was recorded at pH 11, was indicative that the methyl group resided on N1. The possibility of the methyl group residing on N3 is ruled out since it has been reported (2) that the N3-methyl derivative of uracil shows a large bathochromic shift (ca. 24 nm) in the uv spectrum on going from an acidic medium to a basic medium. Therefore, the monomethylated product obtained from the direct methylation of Ψ can be unequivocally assigned structure **3**.

This straightforward method for the N1 methylation of pseudouridine is being studied in our laboratory as a general method for the preparation of other N1 substituted derivatives of pseudouridine.

Acknowledgment.



This work was supported by Research Grant CA-11147 awarded by the National Cancer Institute, Department of Health, Education and Welfare; the American Cancer Society, Research Grant CI-89 and the Mrs. L. R. Ure Cancer Research Memorial fund.

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- (4) A preliminary account of this work was presented [D. S. Wise, R. A. Earl and L. B. Townsend, 172nd National Meeting of the American Chemical Society, San Francisco, CA, Aug. 1976, Carbohydrate Division, Paper No. 33.] before reference 3 was published.
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- (6) It is noteworthy that excess BSA was not removed prior to the addition of the alkyl halide as is the common practice for reactions of this type. Indeed, we have observed in several other instances, that BSA and other byproducts of the silylation reaction do not seem to interfere with the reaction of silylated heterocycles with alkylating agents. Thus, in some instances, the alkylation of certain heterocycles may be carried out "in one pot," *i.e.*, the heterocycle is suspended in some appropriate aprotic solvent, excess BSA is added and after the heterocycle has gone into solution, the alkylating agent is then added. This procedure avoids the necessity of isolating the silyl derivative from the silylating reagents and the associated risk of introducing moisture into the reaction mixture which would result in partial hydrolysis of the silyl derivative.
- (7) *Anal. Calcd.* for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. *Found*: C, 46.58; H, 5.52; N, 10.80.