NUCLEOSIDES. CVI

SYNTHESES OF 1-*N*-METHYL-5-(β-D-RIBOFURANOSYL)URACIL (1-*N*-METHYL-Ψ-URIDINE) AND ITS IDENTITY WITH A METABOLITE ELABORATED BY *STREPTOMYCES PLATENSIS* VAR. *CLARENSIS**

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Very recently, 1-*N*-methyl-ψ-uridine was isolated from the culture filtrate of *Streptomyces platensis* var. *clarensis* along with an antibacterial and antiviral antibiotic, U-44590. We achieved chemical syntheses of 1-*N*-methyl-ψ-uridine by selective methylation of ψ-uridine in two different routes and established the identity of the synthetic nucleoside with the natural product.

A recent publication by ARGOUDELIS and MIZSAK[1] on the isolation of 1-*N*-methyl-pseudouridine (**4**, 1-*N*-methyl-ψ-uridine) from the culture filtrate of *Streptomyces platensis* var. *clarensis* which produces an antibacterial and antiviral antibiotic U-44590 prompted us to report the synthesis of **4** and its identity with the natural product.

Ψ-Uridine (**1**) was trimethylsilylated with hexamethyldisilazane in the presence of a catalytic amount of ammonium sulfate. The syrupy product **2** was, without purification, treated with methyl iodide in acetonitrile resulting intermediate **3**. After hydrolytic removal of the trimethylsilyl groups, 1-*N*-methyl-ψ-uridine (**4**) was isolated in crystalline form in ~80 % yield. Conversion of **2** into **4** apparently took place by a mechanism closely related to the HILBERT-JOHNSON reaction[2] which is known to occur preferentially at *N*1 of 2,4-dialkoxy (or bistrimethylsilyloxy)-pyrimidines. Thus, reaction of **2** with methyl iodide gave a quaternary salt **3** from which 2-*O*-trimethylsilyl group was eliminated by nucleophilic attack of iodide ion on the silicon center as shown in Chart 1. The near identity of the UV spectrum of **4** to that of 1-methyluracil[3] and its dissimilarity with that of 3-methyluracil established the 1-methyl-ψ-uridine structure.

The same product **4** was obtained from 4,5′-anhydro-2′,3′-*O*-isopropylidene-ψ-uridine (**5**) by methylation followed by hydrolysis. Compound **5**[4] was heated gently in dimethylformamide dimethyl acetal. The methylated crystalline product was obtained in good yield. Hydrolytic cleavage of the 4,5′-anhydro linkage and simultaneous removal of the isopropylidene group was accomplished by treatment of the product with formic acid. Compound **4** was isolated in crystalline form. Methylation of **5** occurred almost exclusively on *N*1 to give **6**, since no 3-methyl isomer was detected in the mother liquor.

The IR spectrum of synthetic **4** was identical with that of the natural product (Fig. 1). Other

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Chart 1.

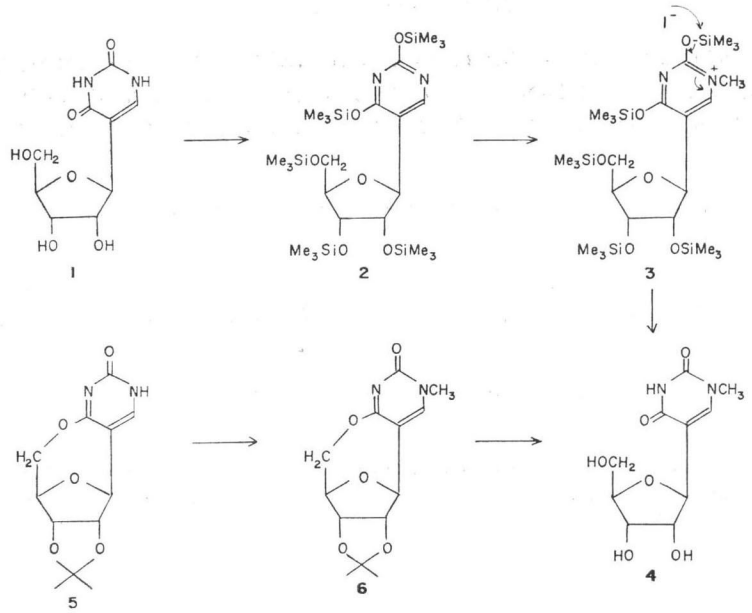
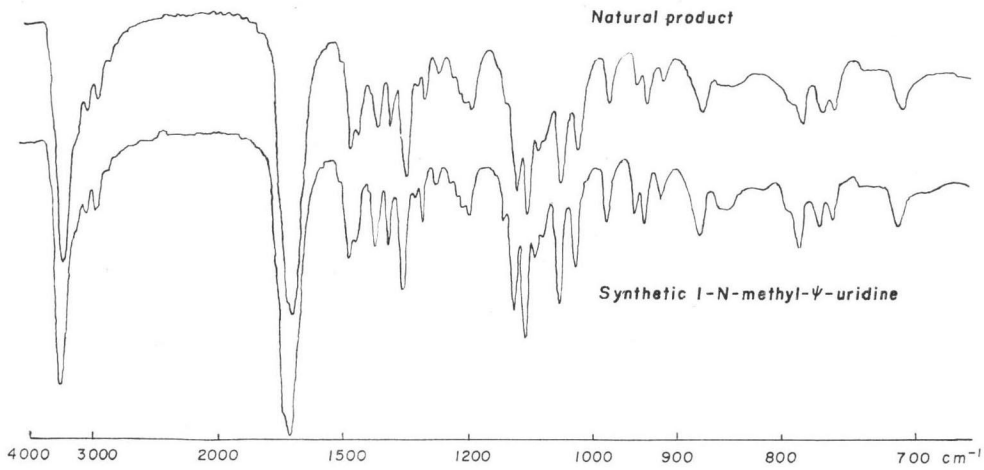


Fig. 1. IR spectra of natural and synthetic 1-*N*- methyl-ψ-uridine



physical characteristics (m.p., UV and PMR) were also identical to those reported for the natural product.

Experimental

Melting points were determined on a Thomas-Hoover capillary apparatus and are corrected. PMR spectra were obtained on a J.E.O.L.-JIM-PET-100 spectrometer with TMS as reference. Chemical shifts are reported in ppm (δ) and signals are described as s (singlet), d (doublet), and t (triplet). Values given for coupling constants are first order. IR spectra were recorded on a Perkin-Elmer Infracord using pressed KBr pellets. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

1-*N*-Methyl-5-(β-D-ribofuranosyl)uracil (**4**, 1-Methyl-ψ-uridine) from ψ-Uridine (**1**)

A mixture of ψ-uridine (0.7 g, 0.0027 mol) and ammonium sulfate (*ca.* 3 mg) in hexamethyldisilazane (17 ml) was stirred and heated to reflux. When the mixture became clear (45 minutes), it was allowed to cool to room temperature. The excess hexamethyldisilazane was removed by evaporation in high vacuum. The syrupy residue (**2**) was dissolved in dry acetonitrile (35 ml) and methyl iodide (5 ml) was added. The mixture was stirred at room temperature for 36 hours. The solvent was removed *in vacuo* below 35 °C, and the residue was treated with saturated methanolic ammonia (15 ml). The mixture was filtered through a Celite bed and the Celite was washed with methanol (30 ml). The combined filtrate and washings were concentrated to a small volume *in vacuo* whereupon crystals (500 mg) precipitated. After filtration of the crystals, an additional amount (120 mg) of crystalline product was obtained from the mother liquor. The combined crops (620 mg) were recrystallized from ethanol to give pure 1-methyl-ψ-uridine (**4**, 580 mg), m.p. 181~182 °C which was not depressed upon admixture with an authentic sample of natural product.

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.84.
Found: C, 46.41; H, 5.54; N, 10.70.

4,5′-Anhydro-2′,3′-*O*-isopropylidene-1-methylpseudouridine (**6**)

A mixture of 4,5′-anhydro-2′,3′-*O*-isopropylidenepseudouridine[4] (**5**, 200 mg) and dimethylformamide dimethyl acetal (5 ml) was gently refluxed for 1 hour. The mixture was allowed to cool to room temperature and the crystals precipitated were filtered and recrystallized from ethanol to give **6** (130 mg), m.p.>300 °C. PMR (DMSO-d_6), δ=1.27 (s, 3H, isopropyl CH_3), 1.42 (3H, s, isopropyl CH_3), 3.35 (s, 3H, NCH_3), 3.95 (d, 1H, H-5′, $J_{5',5''}\cong$12.8 Hz), 4.40 (s, 1H, H-4′), 4.54 (d, 1H, H-5″), 4.57 (d, 1H, H-3′ or H-2′, $J_{2',3'}\cong$5.8 Hz), 4.76 (s, 1H, H-1′), 4.94 (d, 1H, H-2′ or H-3′), 9.29 (s, 1H, H-6).

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.75; N, 10.00.
Found: C, 55.63; H, 5.84; N, 9.81.

1-Methyl-ψ-uridine (**4**) from **6**.

Compound **6** (30 mg) was dissolved in 88 % formic acid (3 ml) and the solution was heated at 60 °C for 2.5 hours. The solution was concentrated to dryness and traces of formic acid were removed by several coevaporations with ethanol. The residue was dissolved in methanolic ammonia (1 ml), evaporated to dryness, and recrystallized from ethanol to give **4** (17 mg), m.p. 180~181 °C, identical in all respects with the natural product.

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