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Conversion of Thiocarbonyl into Carbonyl in Uracil, Uridine, and *Escherichia coli* Transfer RNA using Hypervalent Iodine Oxidation

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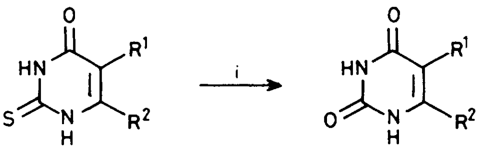
2-Thiouracil, 5-, and 6-methyl-2-thiouracil were converted into uracil, 5-, and 6-methyluracil by PhIO in acetone; the 4-thiouridine site in tRNA was transformed to uridine by $PhI(OH)OSO_2C_6H_4Me$-*p* at pH 4.0.

4-Thiouridine (S^4U) is a minor nucleoside which frequently occurs in the eighth position of *Escherichia coli* tRNA.[1] One approach used to investigate the possible structural and functional uniqueness of S^4U is to determine the effect of conversion of S^4U into U on its biochemical and physical properties in tRNA. Several methods for converting S^4U into U are known.[2] Now we report a simple procedure for the conversion of the thiocarbonyl into carbonyl group in uracil, uridine, and tRNA.

Treatment of the 2-thiouracils (**1a**—**c**) with iodosylbenzene in acetone afforded the corresponding uracils (**2a**—**c**) in good yield (58—65%).†[3]

2-Thiouracil served as a model compound for 4-thiouridine which is present as a minor base in tRNA. The course of oxidation of 4-thiouridine (1.28×10^{-5} M) with PhI(OH)OTs (3.98×10^{-5} M) (Ts = *p*-$MeC_6H_4SO_2$), in sodium acetate buffer at pH 4.0, was monitored by following changes in the absorbance spectrum. The results are shown in Figure 1. The absorbance band of 4-thiouridine (curve a) with a maximum at 330 nm slowly disappears with time (curves b—f) and a new absorbance band with a maximum at 315 nm appears. The new absorbance band may be due to formation of an intermediate, the 4-sulphonate of uridine[4] which slowly diminishes during four days and only an absorbance band at 260 nm remains which corresponds to the chromophore in uridine.[5] The reaction at pH 7.0 proceeds very slowly.

The S^4U moiety in tRNA (*E. coli* from Sigma) was also converted into U by treatment with PhI(OH)OTs. Reaction of tRNA (1.33×10^{-3}M) and PhI(OH)OTs (2.65×10^{-4} M) in buffer (sodium acetate) at pH 4.0 was monitored by the disappearance of the S^4U band at 333 nm (Figure 2).



(**1**) (**2**)

a; $R^1 = R^2 = H$
b; $R^1 = Me, R^2 = H$
c; $R^1 = H, R^2 = Me$

Conditions: PhIO, acetone, room temp.

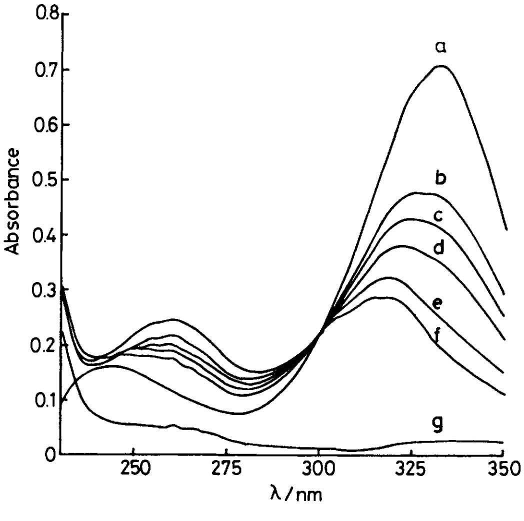


Figure 1. Absorbance spectra demonstrating the reaction of S^4U and PhI(OH)OTs, pH 4 at 22 °C: (a) S^4U before reaction; (b) reaction mixture after 1 min; (c) after 8 min; (d) after 20 min; (e) after 54 min; (f) after 131 min; (g) PhI(OH)OTs in buffer.

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† In a typical experiment a mixture of 2-thiouracil (0.01 mol) and iodosylbenzene (0.03 mol) in acetone (50 ml) was stirred at room temperature for 15 h. The clear solution obtained was evaporated to dryness. Addition of MeOH-hexane (1 : 9, v/v, 10 ml) to the residue afforded uracil which was crystallized from water.

(**2a**) 62% yield, m.p. > 332—335 °C, *m/z* 112 (M^+); (**2b**) 65% yield, m.p. > 313—316 °C, *m/z* 126 (M^+); (**2c**) 58% yield, m.p. > 315—316 °C, *m/z* 126 (M^+).

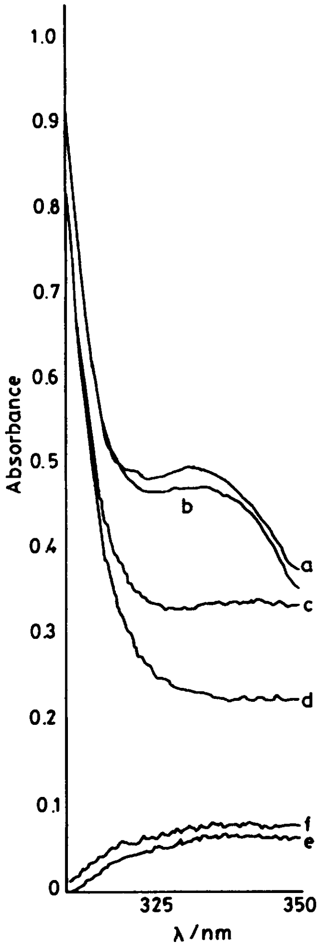

Figure 2. Absorbance spectra of the S^4U moiety of unfractionated *E. coli* tRNA showing the conversion of S^4U into U: (a) before reaction; (b) reaction mixture after 1 min; (c) after 47 min; (d) after 269 min; (e) buffer (sodium acetate); (f) PhI(OH)OTs in buffer.

This work represents a mild oxidative procedure for thiocarbonyl into carbonyl conversion in minor bases, nucleosides, and nucleotides.

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3 Uracil reacts with PhI(OH)OTs in dimethylformamide to give the 5-phenyliodonium salt (unpublished results); 5-phenyliodonium salt formation from uracil with iodosylbenzene diacetate and toluene-*p*-sulphonic acid has also been reported by B. Ya. Karele, S. V. Kalnin, I. P. Grinberga, and O. Ya. Neiland, *Khim. Gieterotsikl. Soedin.* (*Engl. Trans.*), 1973, 510.

4 Identification of an intermediate, the 4-sulphonate of UMP, then its slow conversion into UMP has been reported by Wong and Kearns[2b] and H. Hayatsu and M. Yano, *Tetrahedron Lett.*, 1969, 755.

5 Iodosobenzene diacetate cleaves 1,2-diols (R. Criegee and H. Beuker, *Liebigs Ann. Chem.*, 1939, 218). Conditions: using PhI(OH)OTs, pH 4 at 22 °C lead to cleavage of the diol system in uridine. For preparative purposes, 2′,3′,5′-tribenzoyl 4-thiouridine was used for the oxidation.