Tetrahedron Letters No.9, pp. 755-758, 1969. Pergamon Press. Printed in Great Britain.

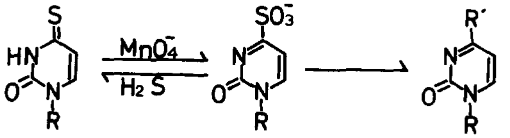
PERMANGANATE OXIDATION OF 4-THIOURACIL DERIVATIVES. ISOLATION AND PROPERTIES OF 1-SUBSTITUTED-2-PYRIMIDONE-4-SULFONATES

H. Hayatsu and M. Yano

Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan

(Received in Japan 8 January 1969; received in UK for publication 21 January 1969)

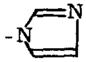
In our recent studies on the permanganate oxidation of nucleic acids, we have found that pyrimidines in nucleic acids can be rapidly and selectively oxidized with dilute aqueous potassium permanganate ($KMnO_4$) at neutral pH and 0°C (1). We report here that 4-thiouridine, a pyrimidine nucleoside now known to be present in transfer RNA as a "minor" constituent (2), is very readily oxidized with $KMnO_4$ to give corresponding 4-sulfonate. It is further shown that the sulfonate can undergo various nucleophilic substitution reactions in aqueous solution at room temperature.



I II III ~ VIII

R : $-CH_3$ (a-series), -ribofuranosyl (b-series)

R': -OH (IIIa and b), $-NH_2$ (IVa and b), $-NHCH_3$ (Va), $-N(CH_3)_2$ (VIa),

 (VIIa), $-NHCH(COOH)CH_2C_6H_5$ (VIIIb)

Results

We will first describe the experiments carried out with 1-methyl-4-thiouracil, a model compound of 4-thiouridine, and then describe the results obtained with 4-thiouridine.

Experiments with 1-Methyl-4-thiouracil (Ia)

Permanganate oxidation of Ia. Ia (3) in H_2O was oxidized by dropwise addition of 50 mM $KMnO_4$ (2 equivalent moles to Ia) at pH 7 and 0°C, the pH being maintained by addition of aq. acetic acid. The reaction was complete in 15 min. The sulfonate IIa was isolated as its potassium salt and recrystallized from H_2O-ethanol to colorless needles. Anal. Calcd for $C_5H_5N_2O_4SK$: C, 26.27; H, 2.21; N, 12.28; K, 17.13. Found: C, 26.39; H, 2.31; N, 12.31; K, 17.17. m.p. 248-252° (dec.). UV, λ mμ (ϵ): $\lambda_{max}^{pH\ 7}$ 315 (5,600), $\lambda_{min}^{pH\ 7}$ 243 (approx. 200). The yield of the sulfonate IIa was 82 %. This compound behaves as an anion in paper electrophoresis at pH 7.

Reactions of the sulfonate IIa with nucleophilic reagents. The reactions carried out are summarized in Table I. It can be seen that the 4-sulfonate group is readily replaciable in aqueous solution by a variety of nucleophiles, such as ammonia, methylamine, dimethylamine, imidazole and hydrogen sulfide. All of the reaction products were characterized by various analytical methods including elemental analysis, mixed melting test with authentic samples when the·latters were available, UV spectra, and NMR spectra.

Table I. Reactions of 1-Methyl-2-Pyrimidone-4-Sulfonate (IIa)

Experiment number	Reaction conditions[a]	Product	Yield of the crystalline product isolated (%)	m.p. (°C)
1	0.1 N HCl, 30 min	IIIa	72	235-7 (dec.)[c]
2	5.6 % NH_4OH, 30 min	IVa	93	301-2 (dec.)[d]
3	15 % aq. CH_3NH_2, 4 hr	Va	84	181-3[e]
4	18 % aq. $(CH_3)_2NH$, 4 hr	VIa	84	181-2[e]
5	Imidazole (1.5 equivalent to IIa) in H_2O, pH 8.2, 3 days	VIIa[b]	56	265-7 (dec.)
6	H_2S in H_2O, 30 min	Ia	91	195-6 (dec.)[d]

a) All of the reactions were performed at room temperature. Amount of the starting material used was: 0.50 g. in experiments No. 1 and 2; and 1.0 g. in experiments No. 3 to 6. b) UV absorption of this new pyrimidine derivative is as follows: λmμ (ϵ); $\lambda_{max}^{H_2O}$ 238, 303 (8,710, 9,260), $\lambda_{min}^{H_2O}$ 232, 268 (8,300, 4,310). c) Reported m.p.: 237-8° (4). d) Reported m.p.: 294-6° for IVa and 193-4° for Ia (3). e) Reported m.p.: 180-1° for Va and 178-9° for VIa (5).

Experiments with 4-Thiouridine (Ib)

Rate of oxidation. Under the mild reaction conditions of 0.1 mM $KMnO_4$ at pH 6.7 and 0°C, 4-thiouridine (3) was oxidized much more rapidly than thymidine or uridine. Thus, in 6 min from the start of the reaction, 4-thiouridine was completely oxidized as judged by the decrease in absorption at 330 mμ, whereas thymidine, the most susceptible to the permanganate oxidation among the "major" pyrimidine nucleosides (1), gave only 8 % decrease in absorption at 270 mμ, and uridine was virtually unaffected. It should be noted that the ease with which cytidine is oxidized has been shown to be roughly equal to that of uridine (1).

Stoichiometry of permanganate consumption. By adding varying amounts of $KMnO_4$ to a bufferized solution of 4-thiouridine and measuring the decrease in optical density at 330 mμ after allowing the reaction mixture to stand for 10 min* at pH 7 and 0°C, it was concluded that , in this reaction, 2 moles of $KMnO_4$ is utilized for the oxidation of 1 mole of 4-thiouridine. This observation is in agreement with the idea that the product is indeed the sulfonate. The following reaction scheme, therefore, is proposed for this oxidation:

$$RSH + 2\,KMnO_4 \longrightarrow RSO_3K + 2\,MnO_2 + KOH$$

Properties and reactions of the sulfonate IIb. 4-Thiouridine was treated with 2 equivalent amount of $KMnO_4$ at pH 7 (phosphate buffer) and 0°C for 15 min, and the sodium sulfonate IIb isolated as a powder. UV, $\lambda_{max}^{pH\ 7}$ 318 mμ. This material contained a small amount (ca. 5 %) of uridine as judged by paper chromatography**, but attempts at purifying this material met with little success since the sulfonate group in IIb is susceptible to hydrolysis to give uridine. Therefore the crude IIb was directly submitted to subsequent reactions.

In neutral solution, IIb is stable at least for 10 hr at room temperature, but in acid and alkali it is rapidly hydrolysed to uridine. Thus, the time required for complete hydrolysis at room temperature is as follows: at pH 2, 10 min; at pH 4, 3 hr; at pH 12, 20 min. A quantitative conversion of 4-thiouridine to uridine was actually effected by permanganate oxidation followed by acid treatment at pH 1.5 for 2 hr. When the last acid treatment was replaced by treatment with ammonia, cytidine and uridine were formed in 9/1 ratio.

Another example of reaction of the sulfonate IIb with a nucleophile is that with L-phenylalanine. Thus, after the permanganate oxidation of 4-thiouridine at pH 7, L-phenylalanine was added to the oxidation mixture and the reaction allowed to proceed for 1 hr at room temperature. Paper chromatographic analysis showed 40 % conversion of 4-thiouridine into N-(1-β-D-

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* Almost identical results were obtained when the time of standing was elongated to 30 min.
** The Rf value of IIb is zero using solvent system: n-butanol-H_2O (86:14, v/v).

ribofuranosyl-2-oxo-4-pyrimidinyl)-L-phenylalanine (VIIIb) (6).

Discussion

4-Thiouridine has been known to be readily oxidizable with such oxidizing agents as iodine (2, 3), osmium tetroxide (7), hydrogen peroxide (8) and periodate (2). Disulfide formation occurs in the iodine oxidation, but the mechanisms in the other oxidations have not been well understood. The present result, therefore, is the first demonstration of sulfonate formation in the oxidation of 4-thiouracil derivatives.

The 4-sulfonate of type II would be a useful intermediate for synthesis of various 4-substituted pyrimidine nucleosides and nucleotides. The reaction can be carried out in water at low temperature, and the yields of the products are generally high. Of special interest is that even a weak base such as imidazole (pK_a, 6.9) can undergo replacement of the sulfonate group.

It should be pointed out that the ease with which 4-thiouridine is oxidized with $KMnO_4$ could permit selective chemical modification of 4-thiouracil residues in tRNA.

Studies along these lines are in progress in our laboratory.

Acknowledgements

We thank Prof. T. Ukita for his encouragement throughout the present investigation. This work was supported by the Grant from the Ministry of Education of Japan.

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