The Oxygen-Catalyzed Reaction between 4-Thiouridine and Sodium Sulfite

Sir:

In the course of our series of studies on the permanganate oxidation of nucleosides[1-3] we have discovered that 4-thiouridine, a minor nucleoside in *E. coli* transfer RNA,[4] gives uridine-4-sulfonate (1-(β-D-ribofuranosyl)-2-pyrimidone-4-sulfonate) upon treatment with dilute potassium permanganate solution for a short period.[3] Ziff and Fresco have independently obtained isopropylideneuridine-4-sulfonate by periodate oxidation of isopropylidene-4-thiouridine.[5] While following the time course of the permanganate reaction, we often observed a curious decrease in absorbance of the starting material, 4-thiouridine. We found that this was due to a reaction occurring with sodium sulfite which had been added to the reaction mixture for the termination of the oxidation. In this communication, we report the nature of this newly discovered reaction between sodium sulfite and 4-thiouridine.

A typical time course of this reaction, as followed by the decrease in characteristic absorption at 330 mμ of thiouridine, is shown in Figure 1. The reaction went

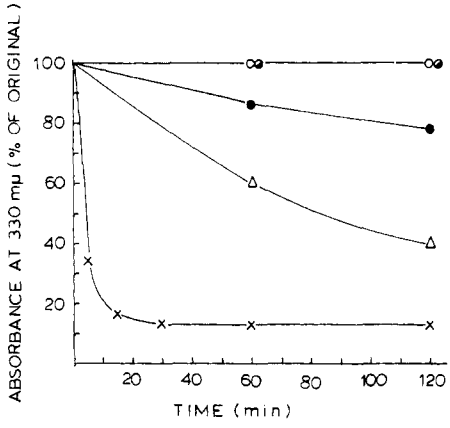


Figure 1. Air-dependent reaction of 4-thiouridine with sodium sulfite. The reaction mixture (5 ml) was 0.1 m*M* in 4-thiouridine, 10 m*M* in sodium sulfite (pH 6.9), and 20 m*M* in sodium phosphate buffer, pH 6.9. The reactions were carried out at room temperature (18–20°) with air bubbling (4 cc/sec) (×—×); with mechanical stirring in a nonstoppered 20-ml Erlenmeyer flask, but without air bubbling (Δ—Δ); in a stoppered test tube, but without preexchange of the inner air with nitrogen (•—•); in a stoppered test tube, in nitrogen atmosphere (○—○); and with air bubbling, but subtracting sodium sulfite from the above reaction mixture (◐—◐).

rapidly to completion in 10 m*M* sodium sulfite solution at room temperature and neutral pH. Oxygen was apparently required for the reaction to proceed because, whereas stirring of the reaction mixture in an open flask or air bubbling in the reaction mixture showed great accelerations, nitrogen bubbling completely inhibited the reaction and carbonate ions did not show a catalytic activity. The reaction can be carried out at any pH between 4 and 9. Studies on the effect of different concentrations of sodium sulfite on the reaction rate gave rather unexpected results: the higher the concentration of sulfite, the lower the rate. Thus, in 1 *M* sodium

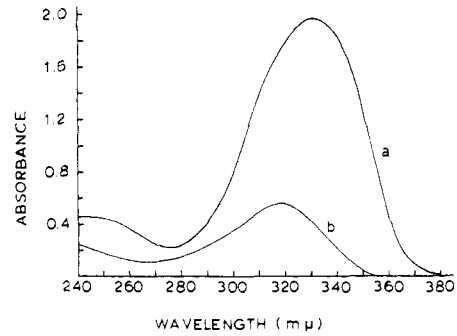
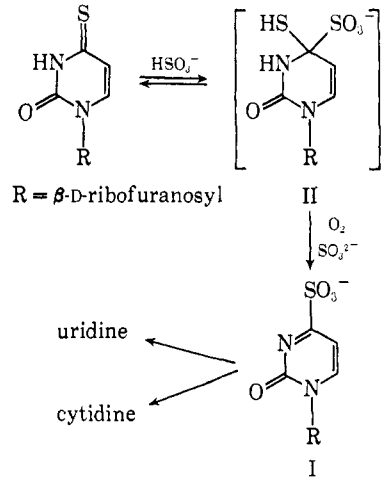


Figure 2. Spectral change in the reaction between 4-thiouridine and sodium sulfite. (a) Before reaction, 0.1 *mM* in 4-thiouridine and 20 m*M* in sodium phosphate buffer, pH 6.9; (b) after completion of the reaction, *ca.* 0.1 m*M* in nucleoside and 0.1 *M* in sodium phosphate buffer, pH 6.9. The reaction was carried out at room temperature as follows. A 2-ml aqueous solution whose concentration was 5 m*M* in 4-thiouridine and 10 m*M* in Na_2SO_3–$NaHSO_3$ (3:1, v/v, pH 6.9) was placed in a 10-ml test tube. The final pH of the solution was 7.4. Air was introduced into this solution at a rate of 4 ml/sec. Aliquots (20 μl) were withdrawn at times and added to 1 ml of 0.1 *N* HCl, and the mixture was checked for the reaction extent by recording the absorbance at 330 mμ. In order to push the reaction to completion as quickly as possible, multiple additions of 1 *M* Na_2SO_3–$NaHSO_3$ (3:1, v/v, pH 6.9) (20 μl each) were carried out at about 30-min intervals. Four such additions were made, and the reaction was complete in 2 hr. From the final solution, 200 μl (which should contain approximately 1 μmol of nucleoside) was taken up and diluted with 10 ml of 0.1 *M* sodium phosphate buffer, pH 6.9, and the spectrum recorded.

sulfite solution at pH 7, the reaction hardly proceeded, and in 0.1 *M* solution the reaction rate was intermediate between those in 1 *M* and in 10 m*M* solution.

Scheme I



Surprisingly, the reaction product was revealed to be uridine-4-sulfonate (I). The spectrum of the reaction product (λ_{max} 318 mμ ($\epsilon \approx 5 \times 10^3$)) shown in Figure 2 was identical with that of uridine-4-sulfonate.[3,5] Paper chromatographic analysis of the reaction mixture also showed the identity of these two compounds,[6] and in this analysis the yield of the sulfonate I was estimated to be 96%. That the product was indeed the sulfonate I was further demonstrated by the high reactivities of this compound toward acid and ammonia. Thus, at pH 4 and room temperature, this product was completely

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(2) H. Hayatsu and S. Iida, *Tetrahedron Lett.*, 1031 (1969).
(3) H. Hayatsu and M. Yano, *ibid.*, 755 (1969).
(4) M. N. Lipsett, *J. Biol. Chem.*, **240**, 3975 (1965).
(5) E. B. Ziff and J. R. Fresco, *J. Amer. Chem. Soc.*, **90**, 7338 (1968).

(6) The R_f values found are: in solvent 1 (1-butanol–water, 86:14, v/v), 0.0; in solvent 2 (ethanol–1 *M* ammonium acetate, pH 6.8, 7:3, v/v), 0.61. In the latter solvent, most of the material applied on the paper chromatogram traveled as the sulfonate, since the rate of ammonolysis of the sulfonate into cytidine is slow at the pH of the chromatographic solvent.

hydrolyzed into uridine in 3 hr,[3,5,7] and at pH 8.55 in 0.2 *M* ammonium chloride solution a rapid conversion of this product into cytidine took place[3,5,7] and was complete in 30 min, the half-time of the ammonolysis being 4 min, a period identical with that observed when an authentic sample of uridine-4-sulfonate[3] was subjected to the identical treatment. When these acid- or ammonia-treated samples were paper chromatographed (solvent 2[6]), a single spot of uridine or cytidine, respectively, was detectable on the chromatogram. Recovery, as estimated by extracting the spot with 0.01 *N* hydrochloric acid, was 0.88 μmole for uridine and 0.83 μmole for cytidine, starting from 1 μmole of 4-thiouridine. Considering technical losses during the work-up, the transformation of 4-thiouridine into uridine or cytidine under the above conditions appeared to be quantitative. Only speculative guesses could be made at this stage of research regarding the mechanism of this new reaction.

We found that 4-thiouridine forms a reversible complex with HSO_3^- ions. This complex formation was observable by the instantaneous decrease in the characteristic absorption at 330 mμ of 4-thiouridine, when the nucleoside was dissolved in sodium hydrosulfite solution in the nitrogen atmosphere. The complex formation was more pronounced in solutions of higher HSO_3^- concentrations,[8] and it was most extensive at pH values lower than 5. A hypothetical structure is presented in Scheme I for this complex. Structure II is analogous to that of bisulfite adducts of carbonyl compounds.[9] The reaction, 4-thiouridine ↔ complex II, was found to be reversible with respect to the change in concentrations of HSO_3^- ions.

We speculate that this complex formation is the first step of the over-all reaction. By some mechanism which is to be investigated, complex II is transformed into uridine-4-sulfonate. In this transformation, oxygen and, possibly, sulfite ions as well would be participating. In this regard it is noteworthy that 4-thiouridine was inert to molecular oxygen in the absence of sodium sulfite (see Figure 1).

A peculiar feature of the reaction is that the high concentration of sodium sulfite inhibits the over-all reaction. This also awaits explanation by future work.

Acknowledgments. The author is grateful to Mr. M. Yano of our laboratory for his technical assistance and to Professor T. Ukita for his hearty encouragement throughout this research.

(7) M. Yano and H. Hayatsu, manuscript in preparation.
(8) For example, in 1 *M* sodium hydrosulfite solution at pH 4, 4-thiouridine gave an A_{330} value 15% of that obtainable in water.
(9) J. D. Roberts and M. C. Caserio, "Basic Principles of Organic Chemistry," W. A. Benjamin, Inc., New York, N. Y., 1965, pp 441–442.

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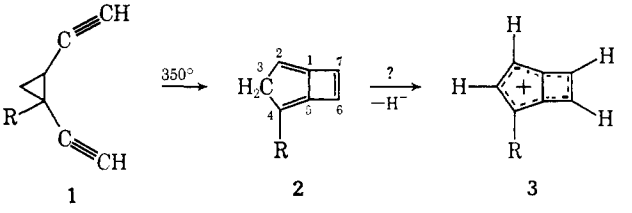
Thermal Conversion of 1-Methyl-1,2-diethynylcyclopropane to 2-Methylbicyclo[3.2.0]hepta-1,4,6-triene

Sir:

We report the synthesis and isolation of 2-methylbicyclo[3.2.0]hepta-1,4,6-triene (**2**, R = CH_3). This material is formed on flow pyrolysis of 1-methyl-1,2-diethynylcyclopropane (**1**, R = CH_3) in a nitrogen stream at 350° and is of interest due to its possible high degree of strain and potential use as a precursor[1] of cyclically conjugated[2] bicycloheptatrienyl systems (*e.g.*, **3**). The facility of the **1** → **2** rearrangement[3] and the remarkable stability of triene **2** prompt us to communicate our preliminary observations at this time.



1 **2** **3**

Direct irradiation of diazopropyne[4] (λ >3000 nm) in diethyl ether solution in the presence of 2-methyl-but-1-en-3-yne[5] is complete in 20 min. Analytical vapor chromatographic (vpc) analysis of the concentrated solution reveals the presence of two new hydrocarbons in the ratio 2:1. The minor product decomposes upon preparative vapor chromatography of the mixture, but the major isomer[6] can be collected (*ca.* 20% yield based on diazopropyne) and shown to have gross structure **1** (R = CH_3) by virtue of its pmr, ir, and mass spectra (pmr: τ 8.18 (1 H, doublet, J = 2 Hz), 8.23 (1 H, singlet), 8.30 (1 H, multiplet), 8.58 (3 H, singlet), 8.73 (1 H, doublet of doublets, J = 7, 4 Hz), and 9.25 (1 H, triplet, J = 4 Hz); ir: 3300, 3080, 3010, 2125, 1440, 1385, 1250, 945, and 930 cm^{-1}; mass spectrum: principal peaks (70 eV) at 104, 103, and 78).

Pyrolysis of **1** (R = CH_3) at 350° in a flow apparatus (nitrogen carrier gas) gives rise to a single product (30–40% yield) which exhibits significant absorption in the ultraviolet [λ_{max} 204 (ε 1.4 × 10^4), 277 nm (ε 2.1 × 10^3)]. Its mass spectrum shows that it is an isomer of starting material (parent peak at *m/e* 104), but the material exhibits no acetylenic carbon–carbon or carbon–hydrogen absorption in the ir. It can be purified by preparative vpc (6 ft × 0.25 in. 20% SE-30 on Chromosorb P, 100°); that its structure is indeed **2** (R = CH_3) is confirmed by the pmr, which exhibits signals for the cyclobutene hydrogens at τ 3.21 (1 H) and 3.33 (1 H), the one additional vinyl hydrogen at τ 4.97 (1 H), the allylic methylene at τ 6.72 (2 H), and the methyl group at τ

(1) (a) R. Breslow, W. Washburn, and R. G. Bergman, *J. Am. Chem. Soc.*, **91**, 196 (1969); (b) N. L. Bauld, C. E. Dahl, and Y. S. Rim, *ibid.*, **91**, 2787 (1969); (c) M. P. Cava, K. Narasimham, W. Zieger, L. J. Radonovich, and M. D. Glick, *ibid.*, **91**, 2379 (1969).
(2) (a) A. Streitwieser, Jr., and J. I. Brauman, "Supplemental Tables of Molecular Orbital Calculations," Pergamon Press, New York, N. Y., 1965; (b) J. O. Halford, *J. Am. Chem. Soc.*, **89**, 5338 (1967).
(3) There is precedent in acyclic systems for this rearrangement; *cf.* W. D. Huntsman and H. J. Wristers, *J. Am. Chem. Soc.*, **89**, 342 (1967).
(4) (a) P. S. Skell and J. Klebe, *ibid.*, **82**, 247 (1960); (b) J. V. Gramas, Ph.D. Dissertation, Pennsylvania State University, 1965; (c) diazopropyne was routinely generated and used in solution. In neat form, it decomposes explosively.
(5) Aldrich Chemical Co.
(6) We believe that isolated diyne **1** has both acetylenic functions *trans*, and that the unstable minor product of the irradiation is the corresponding *cis* isomer. We are attempting to isolate *cis*-**1** in order to compare its thermal reactivity with *trans*-**1** and the closely related *cis*-divinylcyclopropane.[7]
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