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STUDIES ON NUCLEOSIDES AND NUCLEOTIDES. X.[1] DESULFURIZATION OF 2',3'-O-ISOPROPYLIDENE-2-THIOURIDINES BY DIBENZOYLDIAZENE[2]

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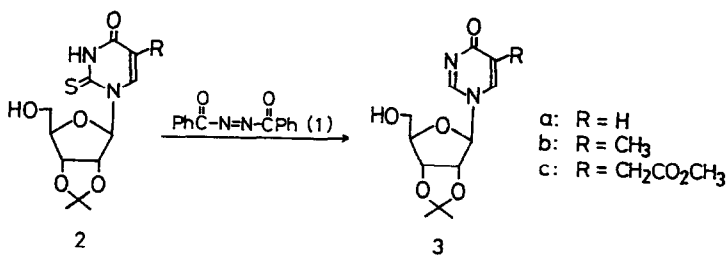
Abstract: The reaction of dibenzoyldiazene with 2',3'-O-isopropylidene derivatives of 2-thiouridine, 5-methyl-2-thiouridine, and 5-methoxycarbonylmethyl-2-thiouridine afforded the corresponding desulfurized products, 3a-3c.

Chemical modification of nucleosides in tRNA affords an important strategy for the elucidation of their functions.[3] We wish to report herein a selective desulfurization of 2-thiouridine derivatives by use of dibenzoyldiazene (1).

When 2',3'-O-isopropylidene-2-thiouridine (2a)[4] was allowed to react with six fold molar excess of 1 in tetrahydrofuran (THF)-H_2O (buffered at pH 9) at room temperature, 1-(2,3-O-isopropylidene-β-D-ribofuranosyl)-4(1H)-pyrimidinone (3a)[5] was isolated in 65% yield with 17% recovery of the starting nucleoside.



2 3

The reaction of 2',3'-O-isopropylidene-5-methyl-2-thiouridine (2b)[6] with 1 was carried out under a variety of conditions at room temperature as summarized in Table 1. When 2b was treated with an equimolar amount of 1 at pH 8 (Table 1, entry 1), orange color of 1 disappeared within 3 h. However, only 28% of 3b[7] was formed with 68% recovery of 2b. This result indicates that 1 is unstable under the conditions used.[8] The molar ratio of 2b to 1 was therefore varied from 1 : 1 to 1 : 3 (Table 1, entry 2), where the yield of 3b was increased to 75%. With

1 : 4 ratio (Table 1, entry 3), 95% of the thiouridine was consumed, while the yield of 3b was unchanged. At pH 9, the yield of 3b and the amount of unchanged 2b were markedly decreased. Although no product other than 3b could be isolated at the present stage of the investigation, these results suggest that 3b decomposes to some extent under the reaction conditions. As indicated in Table 1, a 1 : 3 ratio at pH 8 appeares preferable for the formation of 3b.

Besides 3b, small amount of a side product having $\lambda_{max}(H_2O)$ 267 nm, λ_{min} 237 nm was formed except for the reaction using 1 : 1 molar ratio (Table 1, entry 1). Although the product could not be isolated in sufficient amount to be elucidated, the structure 2',3'-O-isopropylidene-5-methyluridine was tentatively assigned by comparison with absorption maximum of an authentic sample.[9]

Table 1. Reaction of 2b with 1 in dioxane-H_2O

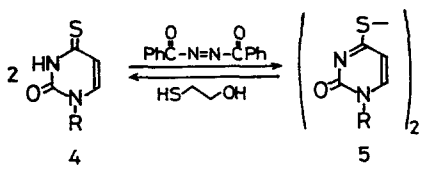
Entry	Molar Ratio 1/2b	pH (±0.3)	Yield of 3b %	Recovery of 2b %
1	1	8*	28	68
2	3	8*	75	16
3	4	8*	75	5
4	3	7*	52	38
5	3	9**	32	15

* KH_2PO_4-NaOH-H_2O buffer was used.

** $NaHCO_3$-Na_2CO_3-H_2O buffer was used.

The reaction of 2',3'-O-isopropylidene-5-methoxycarbonylmethyl-2-thiouridine (2c)[10] with 3 molar amount of 1 in dioxane-H_2O (pH 8) at room temperature resulted in the formation of the corresponding desulfurized product (3c)[11] in 47% yield (26% isolated yield).

Contrary to the case of 2-thiouridine, 4-thiouridine (4) instantaneously reacted with 1 to afford the corresponding disulfide (5).[12] When the resulting solution of 5 was successively treated with 2-mercaptoethanol, 4 was regenerated.[13] The oxidation-reduction process proceeded nearly quantitatively.



4 5

R = β-D-Ribofuranosyl

It is noteworthy that common nucleosides in tRNA, namely uridine, cytidine, adenosine, guanosine, and dihydrouridine were not affected by 1 under the conditions used in the present experiment.

The following procedures are representative. Reaction of 2a with 1: A solution of 1 (715 mg, 3 mmol) in THF (3 ml) was added to a solution of 2a (300 mg, 1 mmol) in H_2O (pH 9; 16 ml) at room temperature. After the mixture had been stirred overnight, 1 (715 mg; 3 mmol) was added and stirred overnight. Precipitate was filtered off and chloroform was added to the filtrate. Aqueous layer was evaporated and residue was applied on Avicel-cellulose preparative layer plates. The plates were developed with n-BuOH-AcOH-H_2O = 5 : 2 : 3 giving 3a in 65% yield. From organic layer, 17% of 2a was recovered by means of silica gel preparative layer chromatography (AcOEt).

Reaction of 2b with 1: To 2b (62.9 mg, 0.2 mmol) suspended in 5 ml of KH_2PO_4-NaOH buffer (pH 8) was added three 0.8-ml (0.2 mmol) portions of 0.25 M dioxane solution of 1 at one-hour interval. The pH was maintained at 8±0.3 by periodic addition of NaOH solution. After the addition of 1, the mixture was stirred for additional 18 h and precipitated N,N'-dibenzoylhydrazine was filtered off. The filtrate was concentrated to small volume in vacuo below room temperature, made up to 5 ml, and chromatographed on Toyo Roshi 51A paper. Solvent system A (n-BuOH-NH_4OH = 2 : 1) and B (saturated $(NH_4)_2SO_4$-H_2O-iPrOH = 79 : 19 : 2) were used for the determination of the amounts of 3b and unchanged 2b, respectively.

We are continuing our work to elucidate the mechanism and to deriniate the scope of the reaction.

References and Notes

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2. Presented in part at 37th Meeting of Japan Chemical Society, April 1978; "Abstracts of Papers", 3R12.
3. For reviews, see for example a) K. Miura, in "Progress in Nucleic Acid Research and Molecular Biology", J. N. Davidson and W. E. Cohen, Eds., Academic Press, 1967, Vol. 6, p 39. b) E. Moldave and L. Grossman, Eds., "Methods in Enzymology", Academic Press, New York, 1971, Vol. 20, pp 129-199. c) M. Saneyoshi and H. Hayatsu, in "Seikagaku Jikken Koza", T. Yamakawa, et al., Eds., Tokyo Kagaku Dojin, 1977, Vol. 2-III, pp 255-298. d) J. A. McClosky and S. Nishimura, Acc. Chem. Res., 10, 403 (1977). e) P. R. Schimmel, D. Söll, and J. N. Abelson, Eds., "Transfer RNA: Structure, Properties, and Recognition", Cold Spring Harbor Laboratory, 1977.
4. T. Ueda and S. Shibuya, Chem. Pharm. Bull., 22, 930 (1974).

5. For 3a, mp 131-133 °C (from EtOH); $\lambda_{max}(H_2O)$ 244 nm (ε = 17000); M^+ = 268; ^{1}H-NMR (DMSO-d^6, 60 MHz) δ 5.72 (d, H-1', $J_{1',2'}$ = 2.3 Hz), 6.1 (d, H-5, $J_{5,6}$ = 7.6 Hz), 7.95 (dd, H-6, $J_{2,6}$ = 3 Hz), 8.6 (d, H-2); Anal. Calcd for $C_{12}H_{16}N_2O_5$: C, 53.73; H, 5.97; N, 10.45. Found: C, 53.47; H, 6.29; N, 9.95. 1-(β-D-Ribofuranosyl)-4(1H)-pyrimidinone has been prepared by Raney nickel desulfrization of 2-thiouridine; H.-J. Lee and P. W. Wigler, Biochemistry, 7, 1527 (1968). See also N. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3668 (1974).

6. Compouned 2b [mp 210-211 °C; λ_{max}(MeOH) 223 nm (ε = 13600), 280 nm (ε = 14400); M^+ = 314] was prepared by the reaction of 2',3'-O-isopropylidene-O^2, 5'-dimethyluridine with H_2S in pyridine (1 : 1) at room temperature for 4 days. For a related paper, see, J. H. Hunter and I. H. Skulnick, U. S. 3975374; Chem. Abstr., 85, 177895b (1976).

7. For 3b, mp 184-185 °C (from THF); $\lambda_{max}(H_2O)$ 250 nm (ε = 14000); M^+ = 282; ^{1}H-NMR (DMSO-d^6, 60 MHz) δ 5.70 (d, H-1', $J_{1',2'}$ = 2.3 Hz), 7.9 (H-6, br 1 line, width at half height = 6-7 Hz), 8.5 (d, H-2, $J_{2,6}$ = 3.2 Hz); Anal. Calcd for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92. Found: C, 55.28; H, 6.39; N, 9.81.

8. S. G. Cohen and J. Nicholson, J. Org. Chem., 30, 1162 (1965), and references cited therein.

9. 2',3'-O-Isopropylidene-5-methyluridine exhibits λ_{max}(MeOH) 266 nm, λ_{min} 235 nm. K. H. Scheit, Tetrahedron Lett., 1971, 2145.

10. Compound 2c [mp 77-78 °C; λ_{max}(MeOH) 221.5 nm (ε = 14200), 280 nm (ε = 14600)] was prepared by the isopropylidenation of 5-methoxycarbonylmethyl-2-thiouridine. H. Vorbrüggen and P. Strehlke, Chem. Ber., 106, 3039 (1973).

11. For 3c, sirup; λ_{max}(MeOH) 247 nm (ε = 12700); M^+ = 340; ^{1}H-NMR ($CDCl_3$, 60 MHz) δ 5.55 (d, H-1', $J_{1',2'}$ = 2.3 Hz), 8.0 (H-6, br 1 line, width at half height = 6 Hz), 8.7 (H-2, br 1 line, width at half height = 7-8 Hz).

12. The reaction was followed by UV spectrophotometry.

13. See for example, R. T. Walker, Tetrahedron Lett., 1971, 2145.

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