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REACTION OF THIOURACIL AND THIOURIDINE WITH 2-HYDROXY-5-NITROBENZYL BROMIDE

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SUMMARY

4-, and 2-Thiouracils and 4-, and 2-thiouridines react with 2-hydroxy-5-nitrobenzyl bromide in neutral aqueous medium to give corresponding S-alkylated products. Most major nucleotides are unaffected under these conditions.

INTRODUCTION

Since LIPSETT[1] reported the presenc eof 4-thiouridylate in tRNA of *Escherichia coli*[1] much attention has been drawn to such sulfur-containing minor nucleotides. Although the reason for their occurrence in tRNA presents an intriguing problem, their role remains still largely unknown. Selective chemical modification of these minor nucleotide residues will provide a fruitful approach for studying the functional significance of tRNA. Thus a number of chemical reactions occurring at the 4-thioketo group of 4-thiopyrimidine nucleosides have been reported[2–5]. In connection with our studies of chemical modification of nucleic acids[6], the present communication described the selective reaction of 4- (1) and 2-thiouridine (2) with 2-hydroxy-5-nitrobenzyl bromide (3; the Koshland's reagent), a familiar protein reagent.

MATERIALS AND METHODS

General procedure of preparative reaction. A solution of (3) (0.1 mmole) in tetrahydrofuran was added to an alkaline aqueous solution of (1, 4, or 6) (0.1 mmole, pH 10, NaOH) and the mixture stood overnight at room temperature. Crystalline product which had separated was collected by filtration, washed with small amounts of water and ether, and dried.

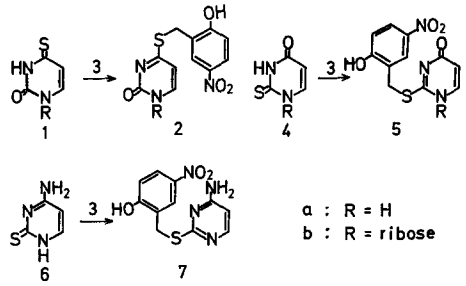
4-(2'-Hydroxy-5'-nitrobenzyl)thiouracil (2a). From (1a) (13 mg) in water (5 ml; 20 mM, pH 10) and (3) (23 mg) in tetrahydrofuran (2.5 ml); 19 mg or 68 %. Yellow needles, m.p. 239–240°. Ultraviolet absorption $\lambda_{max}^{H_2O}$ nm (log ε): pH 10.5, 304 (4.1), 410 (4.2); pH 3.0, 304 (4.2). Infrared absorption ν_{max}^{nujol} cm^{-1}: 1330, 1295(NO_2). NMR spectrum ([$^2H_6$]dimethyl sulfoxide) ppm: 4.37 (2H, singlet; $-CH_2-S$), 6.32 (1H, doublet, $J = 7$ cycles/sec; H-5), 7.00 (1H, doublet, $J = 9$; H-3'), 7.60–8.30 (3H, multiplet). Mass spectrum (*m/e*): 279(M^+). (Found: C, 47.32; H, 3.20; N, 14.99; S, 11.20. $C_{11}H_9N_3O_4S$ requires C, 47.32; H, 3.25; N, 15.05; S, 11.46 %.)

4-(2'-Hydroxy-5'-nitrobenzyl)thiouridine (2b). From (1b) (26 mg) in water (0.5 ml; 200 mM, pH 10) and (3) (23 mg) in tetrahydrofuran (1.0 ml); 30 mg or 73 %. Yellow powder, m.p. 158–161°. Ultraviolet absorption $\lambda_{max}^{H_2O}$ nm (log ε): pH 10.5, 307 (4.2), 410 (4.3); pH 3.0, 309 (4.3). Infrared absorption ν_{max}^{nujol} cm^{-1}: 1328, 1280 (NO_2). NMR ([$^2H_6$]dimethyl sulfoxide) ppm: 4.38 (2H, singlet; $-CH_2S$), 6.44 (1H, doublet, $J = 7$; H-5), 7.00 (1H, doublet, $J = 9$; H-3'), 7.94–8.30 (3H, multiplet). (Found: C, 44.90; H, 4.59; N, 9.49; S, 7.37. $C_{16}H_{17}N_3O_8S \cdot H_2O$ requires C, 44.75; H, 4.42; N, 9.79; S, 7.46 %.)

2-(2'-Hydroxy-5'-nitrobenzyl)thiouracil (5a). From (4a) (13 mg) in water (5 ml; 20 mM, pH 10) and (3) (23 mg) in tetrahydrofuran (2.5 ml); 19 mg or 68 %. Colorless needles, m.p. 217–218°. Ultraviolet absorption $\lambda_{max}^{H_2O}$ nm (log ε): pH 10.5, 218 (4.4), 279 (4.0), 411 (4.3); pH 3.0, 220 (4.3), 297 (4.1). Infrared absorption ν_{max}^{nujol} cm^{-1}: 1348, 1275(NO_2). NMR spectrum ([$^2H_6$]dimethyl sulfoxide) ppm: 4.41 (2H, singlet, CH_2S), 6.16 (1H, doublet, $J = 7$; H-5), 7.02 (1H, doublet, $J = 9$, H-3'), 7.87–8.35 (3H, multiplet). Mass spectrum (*m/e*): 279(M^+). (Found: C, 47.03; H, 3.39; N, 14.91; S, 11.45. $C_{11}H_9N_3O_4S$ requires C, 47.32; H, 3.25; N, 15.05; S, 11.46 %.)

2-(2'-Hydroxy-5'-nitrobenzyl)thiouridine (5b). From (4b) (26 mg) in water (1 ml; 100 mM, pH 10) and (23 mg) in tetrahydrofuran (0.5 ml); 33 mg or 80 %. Colorless powder, m.p. 169–171°. Ultraviolet absorption $\lambda_{max}^{H_2O}$ nm (log ε): pH 10.5, 239 (4.4), 270 (4.2), 410 (4.3); pH 3.0, 219 (4.3), 281 (4.2). Infrared absorption ν_{max}^{nujol} cm^{-1}: 1570, 1330, 1290 (NO_2). NMR spectrum ([$^2H_6$]dimethyl sulfoxide) ppm: 4.43 (2H, singlet; CH_2S), 6.03 (1H, doublet, $J = 7$; H-5) 7.02 (1H, dobulet, $J = 9$; H-3'), 7.95–8.20 (3H, multiplet). (Found: C, 46.63; H, 4.26; N, 10.18; S, 7.64. $C_{16}H_{17}N_3O_8S$ requires C, 46.72; H, 4.17; N, 10.22; S, 7.78 %.)

2-(2'-Hydroxy-5'-nitrobenzyl)thiocytosine (7). From (6) in water (2.5 ml; 40 mM, pH 10) and (3) (23 mg) in tetrahydrofuran (2.5 ml); 10 mg or 36 %. Yellow prisms, m.p. 221–223°. Ultraviolet absorption $\lambda_{max}^{H_2O}$ nm (log ε): pH 10.5, 225 (4.4), 281 (4.0), 411 (4.3): pH 3.0, 243 (4.4), 323 (3.9). Infrared absorption ν_{max}^{nujol} cm^{-1}: 1290 (NO_2). NMR spectrum ([$^2H_6$]dimethyl sulfoxide) ppm: 4.30 (2H, singlet; CH_2S), 6.19 (1H, doublet, $J = 6$; H-5), 7.00–8.45 (4H, multiplet). (Found: C, 47.33; H, 3.78; N, 19.93; S, 11.24. $C_{11}H_{10}N_4O_3S$ requires C, 47.48; H, 3.62; N, 20.14; S, 11.50 %.) Mass spectrum (*m/e*): 278 (M^+).



RESULTS AND DISCUSSION

2-Hydroxy-5-nitrobenzyl bromide (3) reacts rapidly with thiouracils and thiouridines (1 and 4) in aqueous solution at pH 10. In preparative runs, a careful set

of conditions afforded pure crystalline products (2 and 5), whose sulfur-substituted structures were confirmed by elemental analysis and the following spectral data: (i) ultraviolet absorption shows typical patterns of S-alkylated thiopyrimidines[7]; (ii) ultraviolet, infrared, nuclear magnetic resonance and mass spectra all support incorporation of the reagent group into the substrate molecules; (iii) NMR spectra invariably have proton signals of H-5 (doublet) of the pyrimidine ring. In a similar manner, 2-thiocytosine (6) also gave the S-substituted product (7).

Although the thiopyrimidine ring is an ambident substrate with heteroatoms such as nitrogen, oxygen and sulfur as well as the reactive double bond at C-5 ~ C-6, most reactions including substitution, addition and oxidation type with the ring have been known to involve the sulfur atom[2-5]. The above results establish that the reagent (3), highly reactive due to its *ortho*-hydroxyl group[8,9], also reacts selectively at the sulfur atom as a nucleophilic site.

TABLE I

REACTION OF MAJOR NUCLEOSIDES AND NUCLEOTIDES WITH 3-HYDROXY-5-NITROBENZYL BROMIDE

To 1 ml of 10 mM aqueous solution of nucleoside or nucleotide, 0.25 ml of 40 mM solution of (3) in tetrahydrofuran solution was added. After incubation for 1 min at room temperature, one drop of 14 % NH_3 was added to prevent glycoside fission, and the reaction mixture was analyzed by paper chromatography, Toyo Roshi No. 51A. Solvent system: A, isopropanol–conc. ammonia–water (7 : 1 : 2, by vol.); B, acetone–25 % trichloroacetic acid (3 : 1, by vol.)

Substrate	% *Reacted*	R_F *of the product*	*Solvent system*
4-Thiouridine	82	0.59	A
2-Thiouridine	79	0.58	A
Uridine	0	—	A
Cytidine	0	—	A
Adenosine	< 5	0.85	B
5′-UMP	0	—	A
5′-CMP	Trace	—	A
5′-GMP	0	—	B
5′-AMP	Trace	—	A

The reactivity of nucleosides and nucleotides with (3) was compared and the results are presented in Table I. Reaction of (3) with (1) and (4) takes place rapidly, while most major monomers are unaltered under specified conditions. Adenosine, 5′-CMP and 5′-AMP showed some sluggish reactivity. *p*-Nitrophenolic group confers an absorption spectrum which is sensitive to environment and, therefore, (3) can be used as a "reporter" in protein research[10,11]. Ease of preferential reaction of (3) with thiopyrimidines demonstrated in these model experiments as well as its spectroscopic characteristics may suggest that the reagent would have a potential use for chemical modification also in the nucleic acid field.

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