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Heterocycles related to Nucleotides. VI.[1] Photochemistry of Thiopyrimidines: Photoreduction of Thiouracils and Thiouridines with Sodium Borohydride[2]

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On ultraviolet irradiation in the presence of sodium borohydride, 4-thiouracil (**1a**), 4-thiouridine (**1b**) and 2-thiouracil (**3a**) were readily reduced to 2-oxohexahydropyrimidine (**2a**), its riboside (**2b**) and 1-(3-hydroxypropyl)thiourea (**4**), respectively. By controling the energies of the ultraviolet light in the region below 300 nm the selective photoreduction of these thiopyrimidines was demonstrated, while uracil and uridine were unaffected under these conditions. Photochemical behavior of the thiopyrimidines was discussed.

A characteristic feature of t-RNA sequence is the presence of minor nucleotides. A large number of subtle structural variations may become possible by changing the distribution, number, and kind of minor components in t-RNA. 4-Thiouridine (**1b**) and 2-thiouridine (**3b**) are such examples which have been known to exist in some of purified t-RNA from *E. coli*,[4] and many procedures for the modification of 4- and 2-thiouridine and their related compounds have been developed.[5] With regards to intensive studies on the recognition site of t-RNA in protein synthesis, selective chemical modification of the minor components is still a subject of current interest.[6]

Lipsett observed that **1b** reacts on vigorous treatment with potassium borohydride liberating hydrogen sulfide.[7] Recently it has been found by Cerutti, *et al.* that **1b** is in fact reduced to N-ribosyl-2-oxohexahydropyrimidine (**2b**) with sodium borohydride, and this technique was employed for detection and determination of the minor nucleotide in t-RNA.[8a] Since it has also been known that 5,6-dihydrouridine can be reduced under analogous conditions,[8b] sodium borohydride treatment, a reducing method "in the ground state", has been utilized in an attempt to elucidate the effect of these minor bases in some t-RNA's on the enzyme recognition in protein synthesis.[9]

Earlier, Witkop, *et al.* introduced an ingenious photoreduction method into the field of nucleotide chemistry, which consists in the attack of the electronically excited pyrimidine

1) Part V: E. Sato and Y. Kanaoka, *Biochim. Biophys. Acta*, **232**, 213 (1971).
2) Photoinduced reactions. XII. For Part XI: Y. Sato, H. Nakai, H. Ogiwara, T. Mizoguchi, Y. Migita and Y. Kanaoka, *Tetrahedron Letters*, **1973**, 4565. A portion of this work has been published in a preliminary communication: E. Sato and Y. Kanaoka, *Tetrahedron Letters*, **1969**, 3547.
3) Location: *Kita-12, Nishi-6, Sapporo 060, Japan.*
4) F.G. Zachau, *Angew. Chem. Intern. Ed. Engl.*, **8**, 711 (1969).
5) *a*) See ref. 1, and papers cited therein; *b*) H. Hayatsu, *J. Am. Chem. Soc.*, **91**, 5693 (1969); *c*) M. Saneyoshi, *Tanpakushitsu-Kakusan-Koso*, **15**, 251 (1970); (in Japanese); M. Saneyoshi and K. Murao, *ibid.*, **15**, 671 (1970).
6) For recent examples, see; *a*) M.G. Pleiss and P.A. Cerutti, *Biochemistry*, **10**, 3093 (1971); *b*) L. Shugart, *Arch. Biochem. Biophys.*, **148**, 488 (1972); *c*) K.T. Walker and U.L. RajBhandary, *J. Biol. Chem.*, **247**, 4879 (1972); *d*) B.C. Pal, R. Shugart, K.R. Isham and M.P. Stulberg, *Arch. Biochem. Biophys.*, **150**, 86 (1972).
7) N.M. Lipsett, *J. Biol. Chem.*, **240**, 3975 (1965).
8) *a*) P. Cerutti, J.W. Holt and N. Miller, *J. Mol. Biol.*, **34**, 505 (1968); *b*) P. Cerutti and N. Miller, *ibid.*, **26**, 55 (1967).
9) T. Igo-Kemenes and H.G. Zachau, *Eur. J. Biochem.*, **10**, 549 (1969); *b*) L. Shugart and M.P. Stulberg, *J. Biol. Chem.*, **244**, 2806 (1969).

ring of the uridine moiety by sodium borohydride.[10] Interesting phototransformations of *E. coli* t-RNA's have recently been noted.[11] However, even at present, the known photochemistry of thiopyrimidines is limited. Irradiation of **1b** in an air-saturated *t*-butanol solution results in photoxidation.[6b] Very recently Leonard, *et al.* have described dimeric structures of photoproducts from **1a**, which were formed in the presence of cytosine.[12] Similar problem has also been studied by Yaniv, *et al.*[11a,b,13]

Ultraviolet (UV) spectra of **1a** and **1b** have strong peaks at around 3300 nm due to the π—π^* transition[12b] of the thiocarbonyl group while those of uracil and uridine have no absorption in the region of wave-length longer than 300 nm. 2-Thiouracil system also has such a longer wave-length absorption. Some of the examples are presented in Fig. 1. Inspection of Fig. 1 may readily lead to the idea that the thiopyrimidines (**1** and **3**) would be electronically excited under the conditions where the normal pyrimidines of the major bases are not. We report here in detail such a selective photoreduction of **1** and **3**. The photochemistry of nucleic acids and related compounds have been extensively studied with emphases on "photodynamic action" of irradiation and, in particular, photoreactions of the pyrimidine bases have been examined in detail.[14] Representative photochemical processes which the pyrimidines generally undergo are hydration and dimerization.[14] Therefore, to explore photoreactions of the thiopyrimidines, it seemed necessary initially to see whether or not they would undergo such fundamental reactions as the hydration and the dimerization in aqueous solutions.

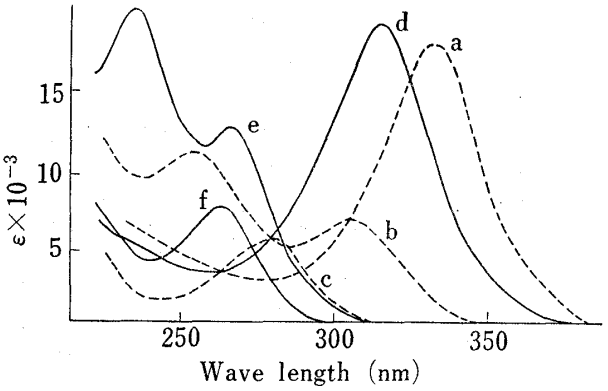

Fig. 1. Absorption Spectra of Uracil, Thiouracils and the Nucleosides at pH 11

An aqueous solution was adjusted to pH 11 with dil. NaOH. Spectra were recorded at this pH since a solution containing sodium borohydride usually has an alkaline pH.

a: 4-thiouracil d: 4-thiouridine
b: 2-thiouracil e: 2-thiouridine
c: uracil f: uridine

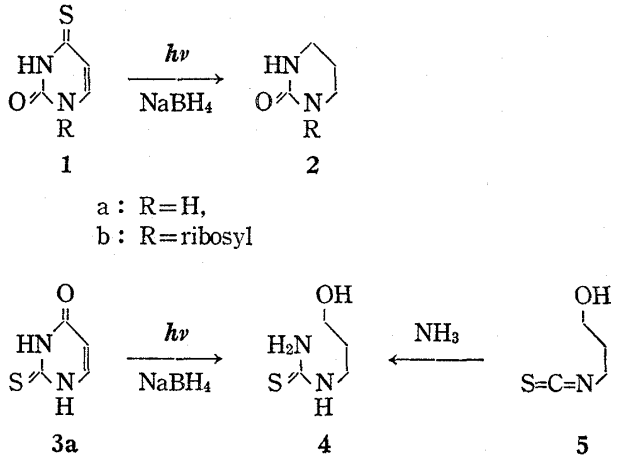

Chart 1

10) P.A. Cerutti, K. Ikeda and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2505 (1965).
11) *a*) A. Favre, M. Yaniv and A.M. Michelson, *Biochem. Biophys. Res. Comm.*, **37**, 266 (1969); *b*) M. Yaniv, A. Favre and B.G. Barrell, *Nature*, **223**, 1331 (1969); *c*) L. Chaffin, D.R. Omillianowsky and R.M. Bock, *Science*, **172**, 854 (1971).
12) *a*) D.E. Bergstrom and N.J. Leonard, *Biochemistry*, **11**, 1 (1972); *b*) *Idem*, *J. Am. Chem. Soc.*, **94**, 6178 (1972); *c*) D.E. Bergstrom, I. Inoue and N.J. Leonard, *J. Org. Chem.*, **37**, 3902 (1972).
13) *a*) J. Ninio, A. Favre and M. Yaniv, *Nature*, **223**, 1333 (1969); *b*) C. Helene, M. Yaniv and J.W. Elder, *Biochem. Biophys. Res. Comm.*, **31**, 660 (1968); *c*) C. Helene and M. Yaniv, *Eur. J. Biochem.*, **15**, 500 (1970); *d*) A. Favre and M. Yaniv, *FEBS Letters*, **17**, 236 (1971), and papers cited therein.
14) *a*) A.D. McLaren and D. Shugar, "Photochemistry of Proteins and Nucleic Acids," Pergamon Press, New York, 1964; *b*) J.G. Burr, "Advances in Photochemistry," vol. 6, ed. by W.A. Noyes, Jr., G.S. Hammond and J.N. Pitts, Jr., John Wiley and Sons, Inc., New York, 1968, p. 193; *c*) E. Fahr, *Angew. Chem. Intern. Ed. Engl.*, **8**, 578 (1969).

Aqueous solutions (1 mM) of 4- and 2-thiouracils (**1a** and **3a**) and 4- and 2-thiouridines (**1b** and **3b**) were irradiated and, after short warming in the presence of acid to warrant possible "reversible" reactions,[14c] their ultraviolet spectra were determined. As shown in Fig. 2, the characteristic peaks of the thiopyrimidines due to the sulfur-containing chromophores decreased slowly on irradiation. However, in experiments on a preparative scale, the reaction was sluggish and the extent was rather limited. These results suggest that both the hydration and the dimerization, common photoreactions for the normal hydroxy-pyrimidine system are, if any, at least not efficient processes in the thiopyrimidine system. No further attempts were made to isolate possible hydrated **6** or dimeric photoproducts (**7**). Likewise, photoreactions of frozen aqueous solutions of **1a** that would be expected to give dimers such as **7** and the isomers, seemed again very inefficient as monitored by disappearance of the absorption at 330 nm.

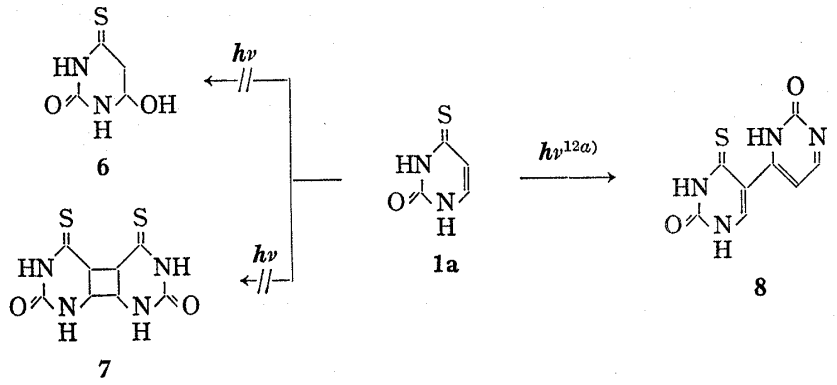

Chart 2

Thiopyrimidines (**1a**, **1b** and **3**) were next treated with sodium borohydride in water. As shown in Fig. 3, this "ground-state reduction" proceeded only very slowly.

Irradiation of them in the presence of sodium borohydride was now performed, the time courses of which are shown in Fig. 4 and 5. The absorptions of **1a** and **3a** disappeared rapidly as a function of the irradiation time, while the absorption of uracil (259 nm, pH 1) stayed unchanged.

Fig. 5 shows that **1b** is selectively reduced under similar conditions while **3b** as well as uridine is intact. Throughout these experiments on a spectroscopic scale, the reaction mixture was treated with acid under warming, before determining the UV spectrum, to see possible reversible reactions.[14c]

Preparative photoreduction of **1a** afforded colorless crystals (20%) after purification with column chromatography. The product (**2a**) showed the presence of a ureido group by the infrared (IR) bands at 1675 and 1540 cm^{-1}. The nuclear magnetic resonance (NMR) spectrum showed a triplet (3.30 ppm (J=6 Hz)) and a quintet (1.88 ppm (J=6 Hz)) in consistent with the cyclic ureido structure (**2a**). This assignment was confirmed by independent synthesis of **2a** from 1,3-diaminopropane and diphenyl carbonate. Under the similar conditions, **1b** was transformed into **2b** (35%) which was hydrolyzed to give authentic **2a**. When **3a** was reduced in a similar manner, the major product was γ-thioureidopropanol (**4**) which was obtained in 22% yield. The alcohol, mp 124—125°, had characteristic IR bands at 1645, 1550 and 1495 cm^{-1}, and in the NMR spectrum a multiplet, approximating a quintet, at 1.87 ppm (central methylene), a broad band at 3.40 ppm (methylene next to>ND), and a triplet at 3.70 ppm (methylene of primary alcohol). **4** was shown to be identical with an authentic sample prepared from ammonolysis of 3-hydroxypropylisothiocyanate (**5**).

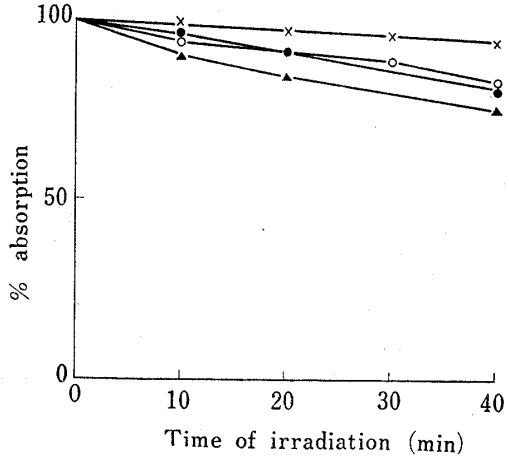

Fig. 2. Decrease of Absorption of Thiouracils and Thiouridines on Irradiation

—○—: 4-thiouridine at 333 nm
—×—: 2-thiouridine at 275 nm
—●—: 4-thiouracil at 328 nm
—▲—: 2-thiouracil at 276 nm

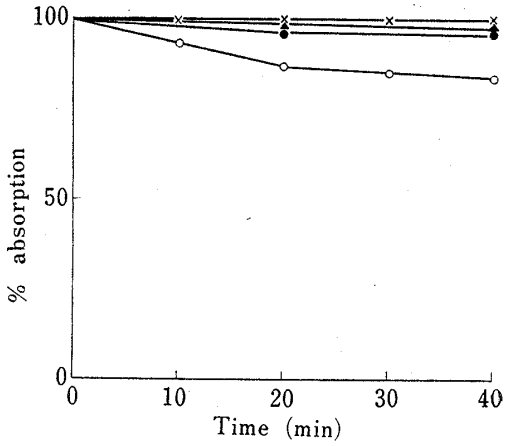

Fig. 3. Decrease of Absorption of Thiouracils and Thiouridines in the Presence of Sodium Borohydride

—○—: 4-thiouridine at 333 nm
—×—: 2-thiouridine at 275 nm
—●—: 4-thiouracil at 328 nm
—▲—: 2-thiouracil at 276 nm

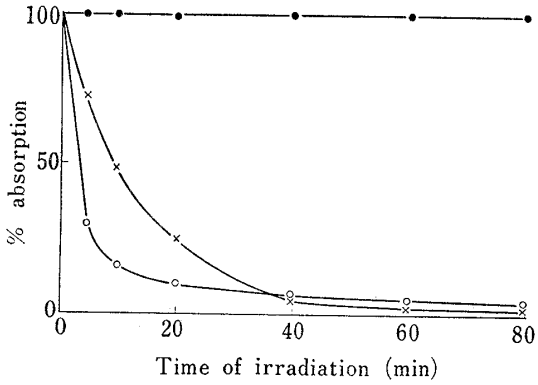

Fig. 4. Decrease of Absorption of Thiouracils and Uracil on Irradiation in the Presence of Sodium Borohydride

—○—: 4-thiouracil at 328 nm
—×—: 2-thiouracil at 276 nm
—●—: uracil at 259 nm

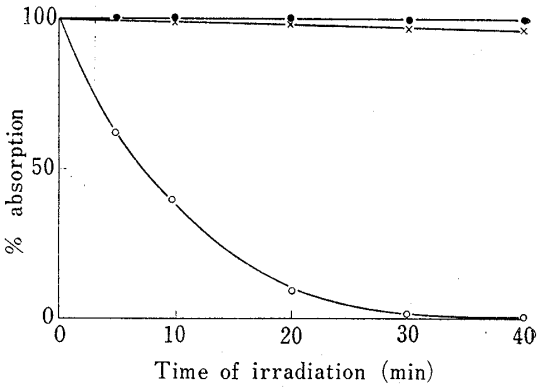

Fig. 5. Decrease of Absorption of Thiouridines and Uridine on Irradiation in the Presence of Sodium Borohydride

—○—: 4-thiouridine at 333 nm
—×—: 2-thiouridine at 275 nm
—●—: uridine at 262 nm

In Chart 3 some of possible pathways of the photoreduction are suggested. In the light of susceptibility of the dihydrouracil and dihydrothymine systems to reduction,[8b,15] the two-step mechanism proposed in the photoreduction of thymidines[15b] may be plausible. Thus the reduction of **1** may well start with the photoreduction of the 5—6 double bond of **1** followed by the "dark" reduction at the reactive thiocarbonyl moiety of **9** to lead to **2**. The similar intermediate (**10**) from **3a** may be attacked by hydride ion followed by ring-opening to the thioureido aldehyde, which is then reduced to **4**. Alternative pathways involving inital reactions at the thiocarbonyl site, however, can not be excluded.

In view of progress in photochemistry of sulfur-containg compounds, comparison of photochemical behaviors of thiocarbonyl *vs.* carbonyl group is of current interest.[16] On

15) *a*) G. Ballé, P. Cerutti and B. Witkop, *J. Am. Chem. Soc.*, **88**, 3946 (1966); *b*) Y. Kondo and B. Witkop, *ibid.*, **90**, 764 (1968); *c*) P. Cerutti, Y. Kondo, W.R. Lands and B. Witkop, *ibid.*, **90**, 771 (1968).
16) *a*) A. Ohno, Y. Ohnishi and G. Tsuchihashi, *J. Am. Chem. Soc.*, **91**, 5038 (1969); *b*) A. Ohno, T. Koizumi and Y. Ohnishi, *Bull. Chem. Soc. Japan*, **44**, 2511 (1971).

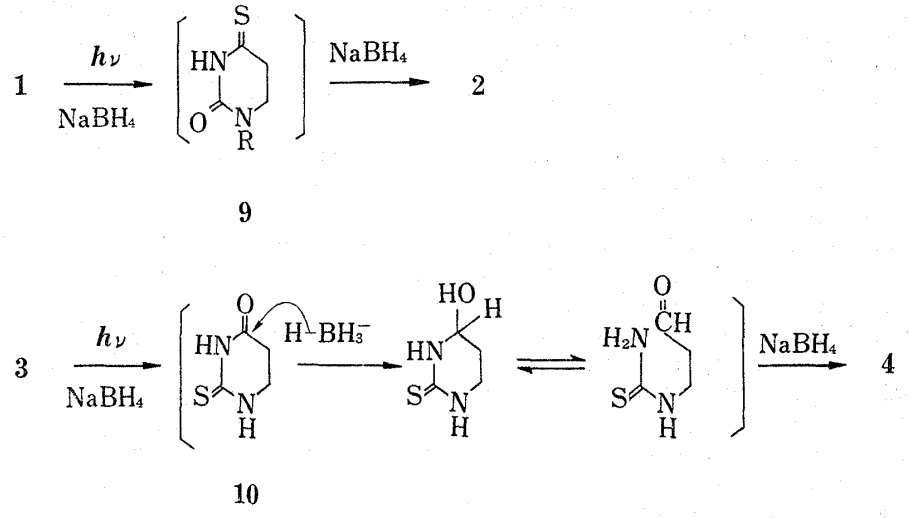

Chart 3

the other hand, to elucidate the electronic excited states of nucleic acids and related systems, emission from their excited states has recently been the subject of thorough investigation.[17] Sulfur-containing derivatives of purines and pyrimidines have spectroscopic properties quite different from their oxygenated analogs. They have very high phosphorescence quantum yields and characteristic phosphorescence lifetimes, that are much shorter than those of "normal" bases.[13b,c,18] Spin-orbit coupling induced by sulfur atoms is responsible for these properties.[13b] In fact, the results in the present paper illustrate that the thiopyrimidines behave in such a different way from that of the hydroxypyrimidines; *i. e.*, in hydration and dimerization.[19] Although the mechanistic features are not yet clear, this marked difference may well be ascribed to the variations in their excited states as above.

Photochemical modification is, not only promising in protein research,[20] expected to be an interesting approach in the field of nucleic acids.[10,11,12b,14] The present photoreduction may be employed, for example, for selective modification of the 4-thiouridine moiety in t-RNA[21] or others, which lack the reducible dihydrouridine moiety. Other chemical modification methods having the thiopyrimidines as a target, including chromogenic reporter labeling, are also currently investigated in this laboratory.

Experimental

Methods——All mp are uncorrected. The NMR spectra were recorded on a Hitachi H-60 spectrometer using TMS as internal standard. The infrared spectra were measured on a JASCO DS-301 spectrophotometer, the ultraviolet spectra on a Hitachi 3T spectrophotometer, and the mass spectra on a Hitachi RMU-5E mass spectrometer.

The irradiations both for the spectroscopic and the preparative experiments were performed with a Eikosha (Osaka) 100 W high pressure mercury lamp, PIH-100, using a water-cooled quartz immersion well, in which an aqueous solution of potassium acid phthalate (5 g/liter; 5 mm layer) was circulated as a liquid filter.[14a] Nitrogen was passed through the solution during the entire irradiation period.

Unless otherwise stated, column and thin-layer chromatography were performed using silica gel (Merck) (0.02 to 0.5 mm), and GF_{254}, respectively.

17) *a*) For leading reference see: J. Eisinger and R.G. Shulman, *Science*, **161**, 1311 (1968); *b*) M. Gueron, J. Eisinger and R.G. Shulman, *J. Chem. Phys.*, **47**, 4077 (1967).
18) F. Pochon, C. Balny, K.H. Scheit and A.M. Michelson, *Biochim. Biophys. Acta*, **228**, 49 (1971).
19) In place of **7** or the related dimers, Leonard, *et al.*[12a] isolated on the photolysis of **1a** small amount of **8** which resulted from the self-condensation of **1a** liberating hydrogen sulfide.
20) O. Yonemitsu and Y. Kanaoka, *Kagakuno-ryoiki* (Zokan) **93**, 263 (1971) (in Japanese).
21) *a*) H.M. Goodman, J. Abelson, A. Landy, S. Brenner and J.D. Smith, *Nature*, **217**, 1019 (1968); *b*) B.P. Doctor, J.E. Loebel, M.A. Sodd and D.B. Winter, *Science*, **163**, 693 (1969).

Irradiation of Thiopyrimidines in Aqueous Solutions in the Absence of Sodium Borohydride (Fig. 2)——An aqueous solution of thiopyrimidine (**1a**, **1b**, **3a** or **3b**; 1 mM, 3 ml) was adjusted to pH 11 with dil. NaOH, and irradiated in a UV-quartz cuvett using a liquid filter. An aliquot (0.5 ml) was taken at interval and the sample was mixed with 0.1N HCl (10 ml), warmed in a water-bath for 5 min and the UV spectrum was determined (pH 1).

Reduction of the Thiopyrimidines (1a, 1b, 3) without Irradiation (Fig. 3)——An aqueous solution of **1a**, **1b** or **3** (1 mM; 3 ml) was incubated with sodium borohydride (final concentration, 10 mM) at room tem., and an aliquot was determined as above.

Irradiation of the Thiopyrimidines (1a, 1b, 3a, 3b) in the Presence of Sodium Borohydride (Fig. 4 and 5)——An aqueous solution of **1a**, **1b**, **3a** or **3b** (1 mM; 3 ml) was irradiated in the presence of sodium borohydride (10 mM), and an aliquot was determined as above.

Procedures for Preparative Photoreduction——A solution of a substrate (5 mM) in water (50 ml; 10 mM) was mixed with sodium borohydride (1.9 g, 50 mM or 10 molar eq), and the solution was irradiated at room temp. until the absorption longer than 300 nm had virtually disappeared. The reaction mixture was treated with ion exchange resin (CG-120) to remove sodium ion, and evaporated to dryness *in vacuo*. The residue was dissolved in methanol and the solution was evaporated to dryness. This procedure was repeated three times to remove boric acid in the form of its volatile methyl ester. The residual product was purified by column chromatography, as appropriate.

2-Oxohexahydropyrimidine (2a)——The photoreduction of **1a** (640 mg) required 12 hr. The reaction mixture was, after working-up as above, subjected to column chromatography (EtOAc–methanol, 3: 1). Fractions were collected for every 4 ml; fractions 22—34, recovered **1a** (30 mg). Fractions 71—103 were collected and evaporated *in vacuo* and the residue was recrystallized from ethanol to give **2a** (98 mg or 20%) as colorless needles, mp 262—265° (lit.,[22] 259—260°). IR ν_{max}^{Nujol} cm^{-1}: 1675, 1540 (cyclic ureido). NMR (D_2O) ppm: 1.88 (2H, quintet, J=6 Hz, $CH_2C\underline{H}_2CH_2$), 3.30 (4H, t, J=6 Hz, $C\underline{H}_2NH$). Mass Spectrum *m/e*: 100 (M^+). *Anal.* Calcd. for $C_4H_8ON_2$: C, 47.98; H, 8.05; N, 27.98. Found: C, 47.98; H, 7.95; N, 27.83. This product was shown to be identical with the sample, prepared from 1,3-diaminopropane and diphenyl carbonate by thermal cyclization (mp 262—265°), based on mixed mp and IR-comparison.

1-(3-Hydroxypropyl)thiourea (4)——**3a** (640 mg) was irradiated for 4 hr as above and the reaction mixture was chromatographed (EtOAc; collected for every 6 ml). Fractions 88—200 were collected, evaporated *in vacuo* and the residue was recrystallized from ethanol to give **4** (149 mg or 22%) as colorless prisms, mp 124—125° (lit.,[23] 129°). UV $\lambda_{max}^{ethanol}$: 234 nm(log ε 4.1). IR ν_{max}^{Nujol} cm^{-1}: 1645, 1550, 1495 (thioureido). NMR (D_2O) ppm: 1.87 (2H, quintet, J=6 Hz, J=6 Hz, $CH_2C\underline{H}_2CH_2$), 3.40 (2H, broads, $C\underline{H}_2ND$), 3.70 (2H, triplet, J=6 Hz, $C\underline{H}_2OD$). Mass Spectrum *m/e*: 117 (M^+ −17), 116 (M^+ −18). *Anal.* Calcd. for $C_{14}H_{10}ON_2S$: C, 35.81; H, 7.51; N, 20.89; S, 23.86. Found: C, 35.67; H, 7.62; N, 20.63; S, 23.68. The reference sample was prepared by the reaction of ammonia with 3-hydroxypropylisothiocyanate, which was obtained from 3-aminopropanol according to the literature.[24] The mp of the photoreduced product **4** was unchanged on admixture with the above sample (mp 124—125°). IR spectra of them were superimposable.

N-Ribosyl-2-oxohexahydropyrimidine (2b)——**1b** (1.3 g) was irradiated for 1 hr as above and the reaction mixture was chromatographed (EtOAc–methanol, 1: 1; collected for every 8 ml). Fractions 25—28, after working up as above, gave **2b** as colorless glass; 414 mg or 35%. For confirmation of the structure **2b**, the following hydrolysis was performed: A solution of **2b** (414 mg) in 1N HCl (20 ml) was refluxed for 6 hr. The hydrolysate, obtained on evaporation of the reaction mixture, was chromatographed ($CHCl_3$–methanol, 4: 1; collected for every 4 ml). From fractions 51—120, **2a** was obtained as colorless needles of mp 262—264°; 73 mg (15% overall yield from **1b**). This product was shown to be identical with **2a** obtained by the photoreduction of **1a** by mixed mp and IR comparison.

Acknowledgement We are grateful to Professor T. Ueda and Dr. K. Muneyama for kind advice. This work was supported in part by a grant from the Naito Foundation, which is gratefully acknowledged.

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23) A. Kjær and R. BoeJensen, *Acta Chem. Scand.*, **12**, 1746 (1958).
24) J.E. Hogkins and M.G. Ettinger, *J. Org. Chem.*, **21**, 404 (1956).