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Photoinduced Reactions. IV.[1] Photoreduction of Uracil Derivatives in Formic Acid[2]

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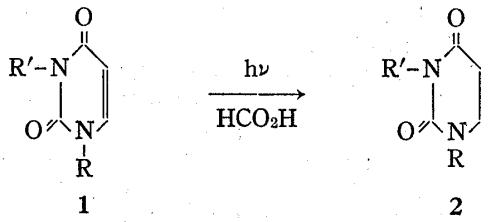
Witkop and Cerutti, *et al.* have introduced and developed an ingeneous photoreduction method in nucleic acid research,[4] which consists in selective reduction of some heterocyclic components such as uridylate with sodium borohydride under ultraviolet irradiation.[5] Meanwhile, complete elcucidation of primary structures of many tRNA molecules[6] led to reveal the presence of a number of minor nucleosides including dihydrouridine **2c**. The latter is unique being a partially saturated non-planar heterocycle component of nucleic acids from natural sources. Attention has recently been drawn to the biological function of this minor nucleotide.[7]

In the course of exploring work of photochemical modification of heterocycles related to nucleic acid, we had occasion to observe that uracil can be reduced in a medium of formic acid under ultraviolet irradiation. In this paper we describe the photochemical reduction of N,N'-dimethyluracil **1a**, uracil **1b** and uridine **1c** to corresponding dihydro derivatives **2** in the medium of formic acid in absence of any additional reducing agent. Witkop, *et al.* reported photochemical reduction of **1c** with sodium borohydride.[8] Photoreduction of **1a** and **1b** in isopropanol was also reported recently.[9]



1 **2**

a: R=R'=CH_3 **b**: R=R'=H **c**: R=ribose; R'=H

N,N'-Dimethyluracil **1a** was irradiated in a solution of formic acid. The disappearance of the starting material was followed by measuring the absorption at 266 mμ. The product was purified by column chromatography (28%) and found to be identical in every respect with a sample of authentic N,N'-dimethyldihydrouracil obtained by catalytic hydrogenation of **1a** in the presence of a palladium catalyst on carbon.[10] Uracil **1b** was reduced to 5,6-dihydrouracil **2b** in the similar manner in 20% yield. In both cases, crystalline byproducts were isolated in appreciable amount, whose structures are still unknown and are under investigation. When uridine **1c** was subjected to this photoreduction, expected dihydrouridine **2c** was obtained

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3) Location: *Kita-12, Nishi-6, Sapporo.*
4) P. Cerutti, K. Ikeda and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2505 (1965).
5) P. Cerutti, "Methods in Enzymology," Vol. 12, Part B, ed. by L. Grossman and K. Moldave, Academic Press, New York, 1968, p. 461.
6) H.G. Zachau, *Ang. Chem. Int. Edn.*, **8**, 711 (1969).
7) *a*) P. Cerutti and N. Miller, *J. Mol. Biol.*, **26**, 55 (1967); *b*) P. Cerutti, J.W. Holt and N. Miller, *ibid.*, 34, 505 (1968); *c*) P. Cerutti, *Biochem. Biophys. Res. Comm.*, **30**, 434 (1968); *d*) T. Igo-kemenes and H.G. Zachau, *European J. Biochem.*, **10**, 549 (1967).
8) P. Cerutti, Y. Kondo, W.R. Landis and B. Witkop, *J. Am. Chem. Soc.*, **90**, 771 (1968).
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10) Y. Kondo and B. Witkop, *J. Am. Chem. Soc.*, **91**, 5264 (1969).

though the product resisted to crystallization. Since labilization of the N-glycoside bond in uridine upon saturation of the 5,6-double bond was known,[8] it is noteworthy that the glycoside is stable under these conditions. In addition, reductive cleavage of dihydropyrimidine rings to ureidopropanol derivatives, which usually accompanies the photoreduction with sodium borohydride,[8,11] is not observed in the present photoreduction in the medium of formic acid.

Formic acid *per se* has been used to reduce a wide variety of organic compounds.[12] However, only very limited information is available for its photolysis.[13] Literature search revealed that formic acid has scarcely been used as a medium of photo-reaction in spite of recent accumulation of organic photochemical data. The mechanism of this novel reductive process is unknown and remains for further study. At least, however, it can be emphasized that formic acid is a good solvent for organic compounds, particularly for biopolymers such as protein and nucleic acid.[14] Preliminary experiments showed that adenine, thymine and cytosine also suffer from reductive transformation by this method, though guanine was unaltered under these conditions. Studies of the scope and application of this reaction are under way.

Experimental

Material and Methods——Melting points were uncorrected. Thin-layer chromatography was performed with alumina or silica (Merck, G-F_{254}). Column chromatography was performed with alumina unless otherwise stated. For irradiation a 100w high pressure mercury lamp (Type PIH-100, Eikōsha, Osaka) was used. A commercial 98% formic acid was dried over anhydrous cupric sulfate, distilled (bp 100—102°) and employed as a reation medium.

N,N′-Dimethyl-5,6-dihydrouracil 2a——A solution of **1a** (280 mg, 2 mmole) in HCO_2H (200 ml, 10 mmole) was irradiated in an atmosphere of N_2 at room temp for 100 min. After evaporation of HCO_2H *in vacuo*, the residue was purified through column chromatography (silica gel; EtOAc). Fractions were collected for every 5 ml: fractions 6—52, oil (115 mg); fractions 80—155, crystals, mp 234—236° (62 mg). The structure of the latter is still unknown. The oil was rechromatographed (EtOAc) to remove small amount of **1a** giving **2a** (78 mg, 28%), which was recrystallized from ether–hexane forming colorless needles of mp 54—55° (lit.,[10] mp 54.5—56°). IR ν_{max}^{Nujol} cm^{-1}: 1705 (carbonyl), 1653 (cyclic ureide). NMR ($CDCl_3$) ppm: 2.74 (2H, J=6 cps, $COCH_2CH_2N$), 3.04 (3H, NCH_3), 3.14 (3H, NCH_3) 3.40 (2H, J=6 cps, $COCH_2CH_2N$). Mass Spectrum *m/e*: 142 (M^+). *Anal.* Calcd. for $C_6H_{10}O_2N_2$: C, 50.69; H, 7.09, N, 19.71. Found: C, 50.56; H, 7.01; N, 19.75. The mp of **2a** was unchanged on admixture with the sample (mp 54—55°) prepared by the catalytic hydrogenation of **1a** with 10% Pd-C.[10] IR spectra of them were superimposable.

5,6-Dihydrouracil 2b——A solution of **1b** (450 mg) in HCO_2H (200 ml, 20 mmole) was irradiated in the same manner as above. After removal of HCO_2H *in vacuo*, the solid residue was chromatographed (MeOH: H_2O, 9:1) being monitored by TLC of alumina plate which was pretreated with 10% NaOH. Fraction was collected for every 10 ml: fraction 2—15, **2b** (90 mg); fraction 50—144, crystals, (40 mg). The structure of the crystalline product (mp 240—255°) is still unknown. Recrystallization of **2b** twice from water gave colorless prisms of mp 280—281° (lit.,[8] mp 281.5—282.5°) in 20% yield. The product was shown to be identical with the authentic sample of **2b** prepared from acrylic acid and urea[15] by mixed mp (280—281.5°) and IR comparison. IR ν_{max}^{Nujol} cm^{-1}: 3240, 3090 (NH), 1752 (carbonyl), 1690 (cyclic ureide). NMR (CF_3CO_2H–D_2O) ppm: 2.48 (2H, J=6 cps, $COCH_2CH_2NH$), 3.25 (2H, J=6 cps, $COCH_2CH_2NH$), Mass Spectrum *m/e*: 114 (M^+). *Anal.* Calcd. for $C_4H_6O_2N_2$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.27; H, 5.32; N, 24.49.

5,6-Dihydrouridine 2c——A solution of **1c** (488 mg) in HCO_2H (200 ml, 10 mmol) was irradiated for 3 hr as described above. After removal of HCO_2H *in vacuo*, the residural oil was chromatographed (MeOH: H_2O, 2:1) being monitored by TLC as above. Fraction was collected for every 10 ml: fractions 24—64 gave colorless glass (112 mg). This compound was shown to be **2c** on paper chromatography by comparison with the authentic sample which was obtained by catalytic hydrogenation of **1c** with Rh-Al.[16–18] For

11) Y. Kondo and B. Witkop, *J. Am. Chem. Soc.*, **90**, 764 (1968).
12) H.W. Gibson, *Chem. Rev.*, **69**, 678 (1969).
13) J.G. Calvert and J. N. Pitts, Jr., "Photochemistry," J. Wiley & Sons, Inc., New York, 1966, p. 430.
14) Indeed, formic acid was employed as an effective solvent for photoxidation of tryptophan residues in protein. *cf.* G. Galiazzo, G. Jori and E. Scoffone, *Biochem. Biophys. Res. Comm.*, **31**, 158 (1968).
15) E. Fischer and G. Roeder, *Ber.*, **34**, 3751 (1901).
16) W. Chon and D. Dohty, *J. Am. Chem. Soc.*, **78**, 2863 (1956).
17) M. Green and S. S. Cohen, *J. Biol. Chem.*, **225**, 397 (1957).
18) H.T. Miles, *Biochim. Biophys. Acta*, **27**, 46 (1968).

further confirmation, a solution of the product **2c** (70 mg) in 10% HCl (20 ml) was refluxed for 6 hr and the residue obtained on removal of the solvent *in vacuo* was chromatographed ($CHCl_3$: MeOH, 4:1) to give colorless crystals of mp 279° from H_2O, 20 mg or 63%. This hydrolyzed product was identical with the authentic sample of **2b** by mixed mp (279—280°).

Preliminary Experiments with Other Nucleo-bases——A solution of base in HCO_2H (4 ml, 2 mM) was irradiated as above, and the disappearance of the substrate was followed by UV measurement. The absorption of adenine, thymine and cytosine disappeared within 30 min. Guanine was stable under these conditions.

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Recherches Toxicologiques sur les Mycotoxines qui Polluent le Fourrage Artificiel du Porc

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Pour élever le porc, on emploie généralement un fourrage mixte commercial. K. Ohkubo, *et al.*,[2] ont découvert que l'on trouve souvent une cirrhose du foie chez les porcs qui sont élèves avec ce fourrage artificiel; ils se sont demandés s'il y aurait pollution des mycotoxinesau fourrage.

Un de nos collaborateurs, H. Tsunoda, a procédé aux examens toxicologiques et microbiologiques de ces fourrages.

En considérant les résultats, on a pensé que trois espèces de microbes, Aspergillus flavus, Aspergillus versicolor, et Penicillium olivino-viride pouvaient bien être responsables de cette toxicité. On sait bien que les deux premières sont des especes qui produisent des substances cancérigenes, l'aflatoxine[3] et la sterigmatocystine,[4] mais en ce qui concerne les substances mátaboliques de P. olivino-viride, il n'y avait pas encore de publication.

Nous avons donc commencé à examiner toxicologiquenmet les substances métaboliques de cette souche microbienne. Nous avons pu trouver que la souche produit une grande quantité d'acide penicillique dans le bouillon de culture de Czapek.

Il y a déjà beaucoup de bibliographies concernant la production et l'élucidation de la structure chimique de l'acide penicillique à partir des microbes, par example Penicillium puberulum Bain,[5] P. cyclopium Westl,[6] et Aspergillus ochraceus Wilhelm,[7] *etc.*

Mais, nous n'avons pas trouvé de description concernant la production de l'acide penicillique par P. olivino-viride, ainsi que son étude physico-chimique.

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5) Communication privée.
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7) Communication privée.