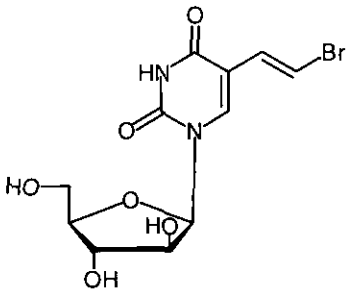
REDUCTION OF URACIL DERIVATIVES WITH AN NADH MODEL, 1-BENZYL-1,4-DIHYDRONICOTINAMIDE

Kosaku Hirota,* Keiko Kubo, Yukio Kitade, and Hironao Sajiki

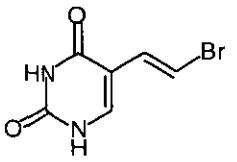
Laboratory of Medicinal Chemistry, Gifu Pharmaceutical University
Mitahora-higashi, Gifu 502–8585, Japan

Abstract – Among various C(5)-, N(1)-, and N(3)-substituted uracils, 1-substituted 5-nitrouracil derivatives were reduced by an NADH model, 1-benzyl-1,4-dihydronicotinamide, to give 5,6-dihydro-5-nitrouracil derivatives, the formation of which was accelerated to a large extent by the use of Mg^{2+} as a catalyst.

Extensive studies on the reduction of organic compounds with NAD(P)H models such as 1-benzyl-1,4-dihydronicotinamide (BNAH) and the Hantzsch ester have been made from the viewpoint of bioorganic chemistry.[1] Nevertheless, employment of BNAH for the reduction of heterocyclic compounds has been limited to a few cases.[2] It has been well known that uracil derivatives such as uracil, thymine and 5-fluorouracil (5-FU) are reduced to the corresponding 5,6-dihydrouracils by an NAD(P)H-dependent enzyme, dihydropyridine dehydrogenase (E.C.1.3.1.2), *in vivo*.[3] When an antiviral drug, sorivudine (**1**) for herpes zoster was co-administrated with 5-FU or its prodrug, the lethal toxicity was exerted by the marked accumulation of 5-FU.[4] The accumulation is due to the inhibition



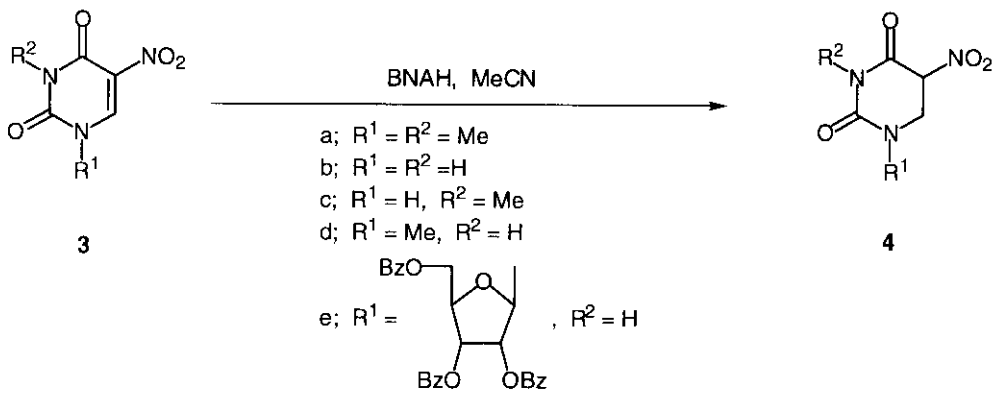
1



2

of dihydropyrimidine dehydrogenase by (*E*)-5-(2-bromovinyl)uracil (**2**) metabolized from **1**.[5] In the connection with this drug interaction, we have focused our attention on non-enzymatic reduction of several 5-substituted uracil derivatives by BNAH. This paper describes that the reduction of uracils to dihydrouracils by BNAH largely depends upon the substituent at the C(5)- and N(1)-positions, and 1-substituted 5-nitrouracils were reduced by BNAH to give 5,6-dihydro-5-nitrouracils, the formation of which was accelerated to a large extent in the presence of Mg^{2+}.

Treatment of 1,3-dimethyl-5-nitrouracil (**3a**) with BNAH (1.5 equiv) in acetonitrile at room temperature under argon atmosphere for 25 h gave 5,6-dihydro-1,3-dimethyl-5-nitrouracil (**4a**) in 79% yield. The structure of **4a** was confirmed by direct comparison with an authentic sample prepared from the reduction of **3a** with sodium borohydride.[6]



Scheme 1

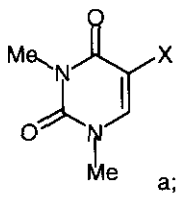
Analogous treatment of other 5-nitrouracil derivatives (**3b-e**) was carried out. As shown in Table 1, the reduction of 5-nitrouracil derivatives was significantly affected by the presence of a substituent at the N(1)-position: although no reduction occurred in the case of 1-unsubstituted derivatives (**3b**) and (**3c**), 1-substituted uracils (**3d**) and (**3e**) were reduced in 72% and 12% yields, respectively, by BNAH at room temperature. Purification of **4e** was difficult and resulted in the low yield. The monitoring of the reduction mixture by means of TLC and HPLC indicated quantitative conversion of **3a**, **3d** and **3e** to dihydrouracils (**4a**), (**4d**), and (**4e**).

Table 1. Formation of 5,6-dihydro-5-nitrouracils (4)

Entry	Substrate	Reaction time [h][a]	Product	mp [℃]	Yield [%][b]
1	**3a**	25 (6[c])	**4a**	oil	79
2	**3b**	24	**4b**	—	0
3	**3c**	24	**4c**	—	0
4	**3d**	22 (5[c])	**4d**	158–160[d]	72
5	**3e**	48 (3[c])	**4e**	186–187[d]	12

[a]The number in parenthesis indicates time required with the Mg^{2+} ion. [b]Isolated yield. [c]The 5,6-dihydro-5-nitrouracil (**4a**, **b** or **e**) was obtained quantitatively by the HPLC or TLC analysis. [d]Decomposition.

In parallel with the involvement of zinc ions in the dehydrogenase systems, metal ions, notably those of zinc and magnesium, have been observed to catalyze the reduction of a number of substrates by BNAH.[7] In the present case, addition of a catalytic amount of Mg^{2+} [$Mg(ClO_4)_2$] caused acceleration of the reduction of 1-substituted 5-nitrouracils (**3a**) and (**3d**) by BNAH. According to the TLC and HPLC analyses, the reaction time in the presence of Mg^{2+} was shortened for the reduction of **3a**, **3d** and **3e** and the reductions completed within 3–6 h (22–48 h without Mg^{2+}) at room temperature. When other 5-unsubstituted or 5-substituted compounds (**5a-c**) were analogously treated with BNAH, the corresponding reduced products were not produced even if Mg^{2+} was employed as a catalyst.



5 a; X = H
b; X = Me
c: X = CN

These facts clearly show that a nitro group, highly electron-withdrawing substituent, is requisite for the reduction with BNAH under the conditions employed.

EXPERIMENTAL

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All column chromatography was carried out with silica gel (Wakogel, C-300). All reactions were monitored by TLC performed on glass-backed silica gel 60 F254, 0.2 mm plates (MERCK), and the

compounds were visualized under UV light (254 nm). Melting points were determined on a Yanagimoto micro hot-stage apparatus and are uncorrected. IR spectra were measured using a Perkin-Elmer 1640 FT-IR spectrometer. ^{1}H NMR spectra were determined with a JEOL GX-270 or Hitachi Perkin-Elmer R-20B spectrometer using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in DMSO-d_6 as an internal standard. Coupling constants (J) are reported in hertz (Hz). UV spectra were obtained from EtOH solutions on a Shimadzu UV-260 spectrophotometer. MS were obtained in a JEOL JMS-D 300 machine operating at 70 eV. Microanalyses were carried out at the Microanalytical Laboratory of our university.

5,6-Dihydro-1,3-dimethyl-5-nitrouracil (4a) A mixture of 1-methyl-5-nitrouracil (**3a**, 500 mg, 2.7 mmol) and 1-benzylnicotinamide (690 mg, 3.2 mmol) in dry acetonitrile (10 mL) was stirred at rt under argon atmosphere for 25 h, after which it was concentrated *in vacuo*. The residue was partitioned between $CHCl_3$ (20 mL) and 10% hydrochloric acid (20 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The residue was subjected to silica gel column chromatography ($CHCl_3$: MeOH = 100 : 1) to obtain **4a** (400 mg, 79%) as an oil, which was identical with an authentic sample.[6]

5,6-Dihydro-1-methyl-5-nitrouracil (4d) was prepared by a procedure similar to that described above using 1-methyl-5-nitrouracil (**3d**, 340 mg, 2 mmol) and 1-benzylnicotinamide (640 mg, 3 mmol) in dry acetonitrile (20 mL) (250 mg, 72%): mp 158—160 ℃ (MeOH); λ_{max} (EtOH) 330 nm (ε 14100); IR (KBr) 3060, 2960, 1730, 1670, 1550, 1390, 1318, 1243, 860 and 760 cm^{-1}; ^{1}H NMR δ 2.94 (s, 3H, NMe), 4.08 (br d, 2H, J = 6.0 Hz, C_6-H), 5.91 (t, 1H, J = 6.0 Hz, C_5-H), 10.04 (br s, 1H, NH, deuterium exchangeable); MS (EI) *m/z* 174 (M^++1), 126, 83. *Anal.* Calcd for $C_5H_7N_3O_4$: C, 34.69; H, 4.08; N, 24.27. Found: C, 34.90; H, 4.05; N, 24.25.

2',3',5'-Tri-*O*-benzoyl-5,6-dihydro-5-nitrouridine (4e) was prepared by a procedure similar to that described above using 1-methyl-5-nitrouracil (**3e**, 1.21 g, 2 mmol) and 1-benzylnicotinamide (640 mg, 3 mmol) in dry acetonitrile (120 mL) (150 mg, 12%): mp 186—187 ℃; λ_{max} (EtOH) 330 nm (ε 14800); IR (KBr) 3260, 1730, 1575, 1460, 1270, 1130, 1105 and 715 cm^{-1}; ^{1}H NMR δ 4.10 (br d, 2H, J = 5.5 Hz, C_6-H), 4.31-4.94 (m, 3H, 4' and 5'-H), 5.07-5.43 (m, 1H, 3'-H), 5.66-5.94 (m, 2H, 1' and 2'-H), 6.22 (br t, 1H, J = 5.5 Hz, C_5-H), 7.07-7.70 (m, 10H, PhCO), 7.70-8.27 (m, 5H, PhCO), 10.66 (br, 1H, NH, deuterium exchangeable); MS (EI) *m/z* 445, 312, 294, 277, 228, 201, 185. *Anal.* Calcd for $C_{30}H_{25}N_3O_{11}$: C, 59.70; H, 4.17; N, 6.96. Found: C, 59.71; H, 4.24; N, 6.95.

General Procedure for the Formation of 5,6-dihydro-5-nitrouracils (4a, d and e) by the Addition of $Mg(ClO_4)_2$ A mixture of the 5-nitrouracil (**3a**, **d** or **e**, 1 equiv), 1-benzylnicotinamide (1.5 equiv) and magnesium perchlorate (1.5 equiv) in dry acetonitrile (10 mL/mmol for **3a** and **3d** and 60 mL/mmol for **3e**) was stirred at rt under argon atmosphere for the appropriate time (see Table 1). 5,6-Dihydro-5-nitrouracils (**4a**, **d** and **e**) were obtained quantitatively by the HPLC or TLC analysis.

REFERENCES AND NOTES

(1) For review, see: (a) R. J. Kill and D. A. Widdowson, *"Bioorganic Chemistry"*, Vol. IV, ed. by E. E. van Tamelen, Academic Press, Inc., New York, 1978, p. 239. (b) G. Dupas, J. Duflos, J. Bourguignon, and G. Queguiner, *"Trends in Heterocyclic Chemistry"*, Vol. 2, Research Trends, Poojapura, 1991, p. 127.

(2) (a) E. A. Braude, J. Hannah, and R. P. Linstead, *J. Chem. Soc.*, 1960, 3268. (b) S. Shinkai and T. Kunitake, *Chem. Lett.*, 1974, 1113. (c) T. Endo and M. Okawara, *J. Org. Chem.*, 1980, **45**, 2663. (d) M. Sako, K. Hirota, and Y. Maki, *Tetrahedron*, 1983, **39**, 3931.

(3) (a) E. S. Canellakis, *J. Biol. Chem*, 1956, **221**, 315. (b) L. L. Campbell, Jr., *ibid.*, 1957, **222**, 693. (c) T. Shiotani and G. Weber, *ibid.*, 1981, **256**, 219 and references cited therein.

(4) Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare: A report on investigation of side effects of sorivudine: deaths caused by interactions between sorivudine and 5-FU prodrugs (in Japanese). June, 1994.

(5) (a) H. Okuda, T. Nishiyama, Y. Ogura, S. Nagayama, K. Ikeda, S. Yamaguchi, Y. Nakamura, K. Kawaguchi, and T. Watabe, *Drug Metabolism and Disposition*, 1997, **25**, 270. (b) K. Ogura, T. Nishiyama, H. Takubo, A. Kato, H. Okuda, K. Arakawa, M. Fukushima, S. Nagayama, Y. Kawaguchi, and T. Watabe, *Cancer Letters*, 1998, **122**, 107.

(6) R. A. Long, T. R. Matthews, and R. K. Robins, *J. Med. Chem.*, 1976, **19**, 1072.

(7) (a) Y. Ohnishi and M. Kagami, *Tetrahedron Lett.*, 1975, 2437. (b) R. A. Gase, G. Boxhoon, and U. K. Pandit, *ibid.*, 1976, 2889. (c) S. Shinkai and T. C. Bruice, *J. Am. Chem. Soc.*, 1972, **94**, 8258.

Received, 30th June, 1998