aqueous solution of the sodium salt of the *m*-sulfanilic acid was added a 10% excess of a 5 M aqueous solution of $NaNO_2$. This was followed, over a 30-min period, by the addition of 2.5 equiv of 6 N HCl. The reaction mixture was then stirred for an additional 45 min, and the precipitated diazonium salt was separated by filtration, dried under reduced pressure, and characterized.

NMR spectra of the three more potent molecules **3b**, **10b**, and **15b** were recorded. **3b**: (D_2O) δ 3.72 (2 H, s), 7.48 (1 H, s). **10b**: (D_2O) δ 7.95 (1 H, t), 8.46 (1 H, d), 8.58 (1 H, d), 8.83 (1 H, s). **15b**: (D_2O) δ 8.06 (1 H, t), 8.14 (1 H, t), 8.54 (1 H, d), 8.73 (1 H, d), 9.05 (1 H, d), 9.23 (1 H, d).

Biochemical Assays. [^{3}H]GABA Binding Assays. The compounds were investigated for their ability to displace [^{3}H]-GABA from its receptor site. Experiments were performed with plasmatic membranes prepared according to Masmoudi and Rendon.[39] Bindings assays were carried out in triplicate at 4 °C for 20 min. The reaction mixture in a final volume of 0.6 mL contained 0.1 mL of plasmatic membranes suspension (about 0.08 mg of protein), 0.3 mL of [^{3}H]GABA [γ-[2,3-^{3}H(N)]aminobutyric acid, 25–40 Ci/mmol; New England Nuclear, Boston, MA] in a final concentration of 10^{-8} M, and 0.2 mL of unlabeled drug, 1 mM GABA, or 200 mM K_2HPO_4/citrate buffer, pH 7.15. At the end of the incubation, the mixture was centrifuged at 50000*g* for 30 min. The supernatant fluid was decanted, and the pellet was rinsed twice rapidly and superficially with 0.75 mL of ice-cold buffer. The pellets were dissolved in 0.250 mL of 5% SDS (sodium dodecyl sulfate). The solution (0.225 mL) was added to 6 mL of Beckman scintillation liquid "Ready-Solv HP", and bound radioactivity was evaluated by scintillation counting (Beckman LS 9800).

(39) Masmoudi, A.; Rendon, A. *J. Neurosci. Methods* **1985**, *14*, 81.

Acknowledgment. We thank Dr. Souhail Tebib for the intercharge distance measurements of several molecules and the Centre National de la Recherche Scientifique for financial support.

Registry No. **1a**, 7720-39-0; **1a**·Na, 110295-87-9; **1b**, 50846-98-5; **2a**, 74141-18-7; **2a**·HCl, 110295-95-9; **2a** (ethyl ester), 110295-88-0; **2b**, 110295-80-2; **3a**, 73086-08-5; **3a** (methyl ester, TFA salt), 110295-91-5; **3a**·TFA, 110295-92-6; **3b**, 110295-81-3; **5a**, 110295-78-8; **5a** (ethyl ester, free base), 53266-94-7; **5b**, 110295-82-4; **6a**, 110295-79-9; **6a** (methyl ester), 110295-93-7; **6b**, 110295-83-5; **7a**, 3535-75-9; **7b**, 35332-76-4; **8a**, 106940-10-7; **9a**, 121-57-3; **9b**, 2154-66-7; **10a**·Na, 1126-34-7; **10b**, 39948-22-6; **11a**, 99-05-8; **11b**, 1743-37-9; **12a**, 123-30-8; **12b**, 932-97-8; **13a**, 2835-04-3; **13b**, 110295-84-6; **14a**, 98-37-3; **14b**, 110295-85-7; **15a**, 82-75-7; **15b**, 20653-35-4; **16a**, 86-60-2; **16b**, 110295-86-8; $BrCH_2CO_2Et$, 105-36-2; p-$HO_2CCH_2C_6H_4NH_2$, 1197-55-3; $(NH_2)_2CS$, 62-56-6; $OHCCHBrCH_2CO_2Me$, 16565-77-8; t-$BuOCO_2Bu$-t, 34619-03-9; imidazoleacetic acid, 645-65-8; methyl imidazole-4(5)-acetate hydrochloride, 51718-80-0; methyl imidazole-4(5)-acetate, 4200-46-8; methyl 2-[[(*p*-carboxymethyl)phenyl]azo]imidazole-4(5)-acetate, 110295-89-1; 3-[[*N*-[(*tert*-butyloxy)carbonyl]amino]-methyl]pyridine, 102297-41-6; 3-(aminomethyl)pyridine, 3731-52-0; 3-[[*N*-[(*tert*-butyloxy)carbonyl]amino]methyl]pyridine *N*-oxide, 110295-94-8.

N-Substituted Oxopyrimidines and Nucleosides: Structure–Activity Relationship for Hypnotic Activity as Central Nervous System Depressant

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N^3-Benzyluridine (3-(phenylmethyl)-1-β-D-ribofuranosyluracil) (**1f**) and its related compounds were synthesized and evaluated for hypnotic activity as central depressants. The primary structural modification has been carried out at the N^3 position of the pyrimidine ring in uridine. N^3-Benzyl-substituted uridine exhibited hypnotic activity as well as pentobarbital (PB) induced sleep effect on mice when administered by intracerebroventricular (icv) injection. From this result, the secondary modification was performed, namely, converting the benzyl group into a benzyl analogous group. These compounds also showed hypnotic activity, but their intensities were varied. Thirdly, changing the sugar moiety was investigated; however, it was found to be necessary for hypnotic activity. In general, introduction of benzyl analogous groups at the N^3 position of uridine increased the hypnotic activity, and modification of the sugar moiety decreased the activity. Intravenous (iv) administration failed to indicate hypnotic activity in most of the compounds tested. However, modified sugars such as 2′,3′,5′-tri-*O*-methyl or -acetyl derivatives of **1f** elicited hypnotic activity by iv injection. The majority of compounds were found to show potentiation of the PB-induced sleep, and their effects were in parallel with the hypnotic activity. The result clearly indicates that the benzyl group and β-D-ribofuranosyl, at the N^3 and N^1 positions, respectively, are necessary for hypnotic activity. The critical portion of the chemical structure for both effects appears to be the uridine moiety.

Recently, uridine has been reported to be a sleep-promoting substance that is extracted from sleep-derived rat brain stems and to have the natural sleep-promoting effect by nocturnal intracerebral infusion.[1-3] However, uridine itself does not show hypnotic activity determined by loss of righting reflex in experimental animals. In connection with this point, we found that introduction of an allyl group into the N position of barbiturates and other related compounds led to either enhancement or reduction of the sleep effect.[4-7] Furthermore, this could provide a new type of hypnotic compounds such as **1f**.[8] In the same manner, N^3-allyluridine (3-propenyl-1-β-D-ribofuranosyluracil) (**1d**) exerted central nervous system (CNS) depressant activity,

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(1) Komoda, Y.; Ishikawa, M.; Nagasaki, H.; Iriki, M.; Honda, K.; Inoue, S.; Higashi, A.; Uchizono, K. *Biomed. Res.* **1983**, *4*, 223.
(2) Honda, K.; Komoda, Y.; Nishida, S.; Nagasaki, H.; Higashi, A.; Uchizono, K.; Inoue, S. *Neurosci. Res.* **1984**, *1*, 243.
(3) Inoue, S.; Honda, K.; Komoda, Y.; Uchizono, K.; Ueno, R.; Hayaishi, O. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6240.
(4) Yamamoto, I.; Tateoka, Y.; Watanabe, K.; Nabeshima, T.; Fontenot, H. J.; Ho, I. K. *Res. Commun. Chem. Pathol. Pharmacol.* **1985**, *50*, 209.
(5) Tateoka, Y.; Kimura, T.; Watanabe, K.; Yamamoto, I.; Ho, I. K. *Chem. Pharm. Bull.* **1987**, *35*, 778.
(6) Tateoka, Y.; Yamamoto, I.; Watanabe, K.; Ho, I. K. *Res. Commun. Chem. Pathol. Pharmacol.* **1986**, *52*, 17.

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but did not show any hypnotic activity.[9] It is of interest to study the structure–activity relationship between hypnotic effect and modification of uridine or other oxopyrimidine derivatives, because there are no reports regarding hypnotics containing a sugar moiety. These hypnotic compounds include both the hydrophilic and hydrophobic portions on the same molecule. From these data, the N^3 position of uridine may be the key point for exhibiting sleep action.

This paper deals with the structure–activity relationship of N-substituted uridine and related compounds in order to establish their depressant effect on the CNS as a new type of hypnotic.

Chemicals. Uridine, uracil and cytidine were purchased from Wako Chemical Co., Ltd., Osaka. Futraful [1-(2-tetrahydrofuryl)-5-fluorouracil] was obtained from Taiho Yakuhin Co., Ltd., Tokyo. 3-Methyl-1-β-D-ribofuranosyluracil (**1a**), 3-ethyl-1-β-D-ribofuranosyluracil (**1b**), **1d**, 3-(3-methyl-2-butenyl)-1-β-D-ribofuranosyluracil (**1e**), **1f**, 3-[(4-chlorophenyl)methyl]-1-β-D-ribofuranosyluracil (**1g**), 3-[(2-methylphenyl)methyl]-1-β-D-ribofuranosyluracil (**1h**), 3-[(3-methylphenyl)methyl]-1-β-D-ribofuranosyluracil (**1i**), 3-[(4-methylphenyl)methyl]-1-β-D-ribofuranosyluracil (**1j**), and 3-(1-phenylethyl)-1-β-D-ribofuranosyluracil (**1k**) were synthesized according to the method of Sasaki et al.[10] 3-Propyl-1-β-D-ribofuranosyluracil (**1c**) was prepared from **1d** by palladium-catalyzed hydrogenation. 3-(Phenylmethyl)-1-(2,3,5-tri-*O*-methyl-β-D-ribofuranosyl)uracil (**1c**) was obtained from 1-(2,3,5-tri-*O*-methyl-β-D-ribofuranosyl)uracil (**2a**), which was prepared from cytidine by the method of Kusmierek et al.[11] 1,3-Bis(phenylmethyl)uracil (**5b**) was obtained according to the method reported by Kunieda and Witkop.[12] 3-(Phenylmethyl)-1-(2-tetrahydrofuryl)-5-fluorouracil (**4a**) and 1-(phenylmethyl)uracil (**5a**) were also synthesized by similar methods to those described above.

Target Design. Uridine, which is one of the sleep-promoting substances, provides a starting point, since it may be a likely target for a hypnotic receptor, as an agonist effector. Incorporation of a benzyl group at the N^3 position of the oxopyrimidine ring of uridine leads to hypnotic compounds (**1f**). Pharmacological effects of uridine have been reported.[13-15] Pyrimidines and their nucleosides decreased mouse spontaneous activity at high doses by icv administration.[13] Moreover, they exhibited anticonvulsant effect against Metrazol- and penicillin-induced seizures.[14,15] Recently, Inoue et al.[16] reported that uridine increased the total nocturnal sleep time of rats by the infusion technique to the third ventricule. However, uracil that is deficient only in the sugar moiety resulted in no significant change in sleep times. These references suggest that the sugar moiety of uridine plays an important role in sleep-promoting effects. Yamamoto et al.[4-7] reported that some N-substituted barbiturates exhibited both hypnotic and stimulant activity; e.g., *N,N'*-diallylpentobarbital (DAPB) prolonged PB-induced sleep time compared with the control, while DAPB antagonized barbital-induced sleep.[4,7] Furthermore, introduction of a benzyl group at the N position of bemegride altered its pharmacological activity from CNS stimulant to CNS depressant.[17] On the basis of these facts and the similarity between barbiturates and the pyrimidines, it was of interest to study the effect of substitution of uridine.

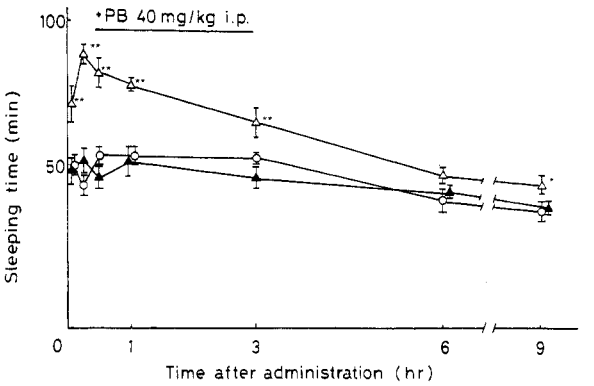


Figure 1. Time-dependent potentiation of **1f** and uridine on PB-induced sleep. Compounds tested were administered icv (2.0 μmol/mouse) at each time interval before PB challenge. Results are depicted as the mean value (n = 8), and error bars indicate the SEM. Symbols denote sleeping time with **1f** (△), uridine (▲), and 3% Tween 80/saline as control (○). * and ** indicate significant differences from the control level of $p < 0.05$ and $p < 0.01$, respectively.

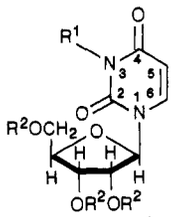
Results and Discussion

Pharmacological Results. Pharmacological effects on the CNS were examined by administering the compounds (icv or iv) to mice. The results are shown in Figure 1 and Tables I–V. The data for the parent compounds uracil and uridine are indicated for comparison.

PB-Induced Sleep Effect. The time course of the effect of **1f** and uridine are illustrated in Figure 1. The peak time of PB-induced sleep-prolonging effect was achieved 15 min after pretreatment with **1f**: uridine was significant even 9 h after treatment. The time course pattern was the same as that of **1d**, which had been examined in our laboratory.[9] In Table I, PB-induced sleep was summarized as the percent of control sleeping time. All compounds except for **5a** potentiated the PB-induced sleep. Particularly, **1f**, **1h**, **1i**, and **1j** were more synergistic than any other compounds, and their intensities of CNS depressant activity were more than threefold that of the control at a dose of 2.0 μmol/mouse, icv. In compounds **1a–d**, an increase in the number of carbons corresponds to an increase in CNS depressant activity. The effect of **1d** compared favorably with that of **1c**. It is suggested that this result is due to the difference between the saturated and unsaturated bonds in the N^3 side chain of the uridine analogous structure. With regard to **1h–k**, a methyl group at the α-position of benzyl lowered the effect. Introduction of a methyl group into the aromatic ring increased CNS depressant activity compared with introduction at the α-position of the benzyl moiety. In particular, N^3-xylyl derivatives (**1h–j**) potentiated the PB-induced sleep effect better than **1f**; however, **1k** proved less effective in comparison to **1f**. With respect to the introduction of halogen

(7) Yamamoto, I.; Tateoka, Y.; Watanabe, K.; Ho, I. K. *Life Sci.* **1987**, *40*, 1439.
(8) Yamamoto, I.; Kimura, T.; Tateoka, Y.; Watanabe, K.; Ho, I. K. *Chem. Pharm. Bull.* **1985**, *33*, 4088.
(9) Yamamoto, I.; Kimura, T.; Tateoka, Y.; Watanabe, K.; Ho, I. K. *Res. Commun. Chem. Pathol. Pharmacol.* **1986**, *52*, 321.
(10) Sasaki, T.; Minamoto, K.; Suzuki, H. *J. Org. Chem.* **1973**, *38*, 598.
(11) Kuśmierek, J. T.; Giziewicz, J.; Shugar, D. *Biochemistry* **1973**, *12*, 194.
(12) Kunieda, T.; Witkop, B. *J. Am. Chem. Soc.* **1971**, *93*, 3478.
(13) Krooth, R. S.; Hsiao, W. L.; Lam, G. F. M. *J. Pharmacol. Exp. Ther.* **1978**, *207*, 504.
(14) Wenzel, D. G. *J. Am. Pharm. Assoc.* **1955**, *44*, 550.
(15) Roberts, C. A. *Brain Res.* **1973**, *55*, 291.
(16) Honda, K.; Komoda, Y.; Inoue, S. Report of the Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, **1984**, *18*, 93.
(17) Shulman, A. Proceedings of the Royal Australian Chemical Institute, **1964**, *31*, 41.

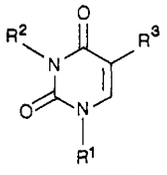
Table I. CNS Depressant Activities of Uridine Derivatives



compd	R^1	R^2	mp, °C (lit.)	mol formula[a]	crystn solvent	yield, %	PB-induced sleep effect[b] (μmol/mouse)[c]
uridine	R^1	H		$C_9H_{12}N_2O_6$			93 (1.0) 135 (2.4) 138 (3.8)
1a	CH_3	H	117–118 (115–116)[d]	$C_{10}H_{14}N_2O_6$	methanol/ethyl acetate/petroleum ether	63	117 (3.8)
1b	CH_2CH_3	H	117–118 (112–112.5)[e]	$C_{11}H_{16}N_2O_6$	methanol/ethyl acetate/petroleum ether	43	124 (3.8)
1c	$CH_2CH_2CH_3$	H	110–112	$C_{12}H_{18}N_2O_6$	ethyl acetate	60	134 (3.8)
1d	$CH_2CH{=}CH_2$	H	131–133	$C_{12}H_{16}N_2O_6$	ethyl acetate	64	173 (3.8)
1e	$CH_2CH{=}C(CH_3)_2$	H	124–126	$C_{14}H_{20}N_2O_6$	chloroform	32	228 (3.8)
1f	$CH_2C_6H_5$	H	180–182 (181–182)[f]	$C_{16}H_{18}N_2O_6$	water	60	154 (1.0) 292 (2.0) 404 (3.8)
1g	*p*-$ClC_6H_4CH_2$	H	110–112	$C_{16}H_{17}N_2O_6Cl$	ethyl acetate	60	203 (3.8)
1h	*o*-$CH_2C_6H_4CH_3$	H	170–171	$C_{17}H_{20}N_2O_6$	water/methanol	62	344 (2.0)
1i	*m*-$CH_2C_6H_4CH_3$	H	140–141	$C_{17}H_{20}N_2O_6$	methanol	69	326 (2.0)
1j	*p*-$CH_2C_6H_4CH_3$	H	150–151	$C_{17}H_{20}N_2O_6$	chloroform/benzene	75	308 (2.0)
1k	$CH(CH_3)C_6H_5$	H	160–163	$C_{17}H_{20}N_2O_6$	benzene/ligroin	45	231 (2.0) 303 (3.8)
2a	H	CH_3	oil	$C_{12}H_{18}N_2O_6$		24	144 (2.0)
2b	$CH_2C_6H_5$	CH_3	oil	$C_{19}H_{24}N_2O_6$		85	200 (2.0)
3a	H	$COCH_3$	126–127 (128–130)[g]	$C_{15}H_{18}N_2O_9$		80	178 (2.0)
3b	$CH_2C_6H_5$	$COCH_3$	oil	$C_{22}H_{24}N_2O_9$		94	186 (2.0)

[a] The microanalyses were within ±0.4% of the calculated values (C, H, and N). [b] All compounds tested were administered by icv injection, followed by PB (40 mg/kg) applied by ip injection. PB-induced sleep effect was evaluated as the percent of control sleeping time. [c] The icv injection doses of compounds tested are shown in paraentheses. [d] Reference 20. [e] Reference 22. [f] Reference 10. [g] Reference 23.

Table II. CNS Depressant Activity of Uracil Derivatives



compd	R^1	R^2	R^3	mp, °C (lit.)	mol formula[a]	crystn solvent	yield, %	PB-induced sleep effect: % of control[a] (dose)[a]
Futraful	C_4H_7O	H	F		$C_8H_9N_2O_3F$			139 (2.0)
4a	C_4H_7O	$CH_2C_6H_5$	F	73–76	$C_{15}H_{15}N_2O_3F$	water/methanol	95	262 (2.0)
uracil	H	H	H		$C_4H_4N_2O_2$			99 (1.0)
5a	$CH_2C_6H_5$	H	H	173–175 (174–176)[b]	$C_{11}H_{10}N_2O_2$	ethanol	60	86 (2.0)
5b	$CH_2C_6H_5$	$CH_2C_6H_5$	H	67–68 (75–76.5)[c]	$C_{18}H_{16}N_2O_2$	benzene/ligroin	76	112 (3.8)

[a] See Table I. [b] Reference 24. [c] Reference 12.

on benzyl, **1g** decreased the activity as compared with **1f** and **1j**. For compounds **1g–k**, the order of CNS depressant effect is **1h > 1i > 1j > 1f > 1k > 1g** at a dose of 2.0 μmol/mouse, icv. Table II shows the PB-induced sleep effect of Futraful, uracil, and their N^3-benzyl-substituted derivatives, compounds having no sugar moiety at the N^1 position of uracil. PB-induced sleep effect of these compounds is of interest in determing the requirements for the sugar moiety at the N^1 position. Compounds **5a** and **5b** have no effect on the activity. These results suggest that the N^1 and N^3 positions of uracil need to connect with the ribose of tetrahydrofuran ring and benzyl or its related groups, respectively, for the CNS depressant activity.

Hypnotic Activity. Experiments were conducted to examine the comparative potency of each compound. The icv injection method provided direct indication of CNS activity, but other administration methods, e.g., iv or ip, caused some problems: passage of the blood–brain barrier and metabolic effects. Comparative potencies of these compounds are summarized in Table III. In the 1 series, for hypnotic activity, the order of activity was **1h > 1i > 1j = 1g > 1k**. Compounds **1a–e** and uridine had no ac-

Table III. Hypnotic Activity of Compounds Tested in Mice

compd	sleeping time (min)	
	icv (dose)[a]	iv (0.5 mmol/kg)[b]
uridine	none[c] (3.8)	none
1a	none (3.8)	none
1b	none (3.8)	none
1c	none (3.8)	none
1d	none (3.8)	none
1e	none (3.8)	none
1f	36 ± 2 (2.0)	
	153 ± 11 (3.8)	none
1g	20 ± 2 (2.0)	none
1h	72 ± 3 (2.0)	none
1i	56 ± 3 (2.0)	none
1j	36 ± 2 (2.0)	none
1k	7 (2.0)	none
2a	none (2.0)	none
2b	6 ± 1 (2.0)	29 ± 4
3a	8 ± 1 (2.0)	none
3b	4 ± 1 (2.0)	5 ± 0
Futraful	none (2.0)	none
4a	3 ± 0 (2.0)	7 ± 1
uracil	none (2.0)	none
5a	none (2.0)	none
5b	none (2.0)	
	15 ± 1 (12.0)	17 ± 1

[a] Compounds tested were administered to male ddN strain mice by icv injection. Doses are shown in parentheses; μmol/mouse. [b] Compounds tested were administered through mouse tail vein as iv injection at the dose of 0.5 mmol/kg. [c] None indicates that the mouse could not fall into sleep.

Table IV. Dose–Response in Hypnotic Activity of Compounds **1f**, **1h**, and **1k**

compd[a]	sleeping time, min[b] (numbers of animals slept/n)[c]		
	1.0 μmol/mouse	2.0 μmol/mouse	3.0 μmol/mouse
1f	none[d] (0/8)	28 ± 2 (7/8)	106 ± 5 (8/8)
1h	45 ± 4 (8/8)	70 ± 5 (6/6)	129 ± 9 (4/4)
1k	none (0/8)	7 ± 0 (2/8)	27 ± 4 (5/8)

[a] Compounds tested were administered to male ddN strain mice by icv injection at the doses of 1.0, 2.0, and 3.0 μmol/mouse. [b] Results are express as the mean ± SEM. [c] Ratio of animal slept to animal tested is shown in parentheses. [d] See Table III.

Table V. Hypnotic Activity of N^3-Benzyl-Substituted Compounds and Related Compounds by Iv Administration

compd[a]	sleeping time, min:[b] mmol/kg, iv[c]			partition coefficient[d]
	0.1	0.3	0.5	
1f	none	none	none	2.16
2a	none	none	none	0.50
2b	none	6 ± 0	29 ± 4	64.45
3	none	none	none	0.44
3b	none	1 ± 0	5 ± 0	32.73
futraful	none	none	none	0.35
4a	none	2 ± 0	7 ± 1	23.94

[a] Compounds tested were administered to male ddN strain mice by iv injection at the doses 0.1, 0.3, and 0.5 mmol/kg. [b] Mouse sleeping time was expressed as the mean ± SEM. [c] See Table III. [d] Partition coefficient is expressed as the ratio of equilibrium concentration of compounds tested in the organic phase to that in an equal volume of pH 7.4 aqueous buffer. See Experimental Section for details.

tivity. It is noted that **1e** did not show hypnotic activity, although PB-induced sleep with **1e** was more potent than that with **1g**. Modification of the benzyl moiety (**1g–k**) showed that introduction of a methyl group to the benzene ring resulted in increased activity, while introduction of a methyl group at the α-position of benzyl decreased the hypnotic activity as compared with **1f**. Furthermore, other N^3-benzyl-substituted compounds, **2b**, **3b**, **4a**, and **5b**, also showed hypnotic activity, but their activities were very low. Compound **2a**, Futraful, and **5a** were devoid of activity as compared with their substituted compounds. It was interesting to note that **3a**, having no benzyl group, possessed a slight hypnotic activity (icv). Table IV summarizes the dose–response in the hypnotic activity of **1f**, **1h**, and **1k**. Another approach was the evaluation using iv injection (Table III), in which **2b**, **3b**, and **4a** show hypnotic activity. More detailed studies including partition coefficient were then carried and are summarized in Table V. In mice, **2b**, **3b**, and **4a** had potent hypnotic activity. They were effective by both icv and iv routes. This study revealed that the parallel correlation between mouse sleep time by iv injection and partition coefficient of these compounds may be due to easier passage through the blood–brain barrier.

Since some of the compounds tested have hypnotic and synergistic effects with barbiturates, these compounds may occupy the same active site as barbiturates in the CNS. The active site of barbiturate exists near the GABA receptor, which couples with the benzodiazepine receptor; therefore, uridine analogues may relate to those receptors. Whittle and Turner[18] suggested the different effects of sedative and anticonvulsant barbiturates on specific GABA binding site. Moreover, Willow and Johnston[19] described the dual action of PB on the GABA binding site. As uridine basically has the anticonvulsant and sedative effects and its structure is similar to the barbiturate structure, these facts may provide information on the interaction with certain receptors and evidence of the action mechanism of barbiturates.

In conclusion, we demonstrated that the requirement for hypnotic activity of uridine analogues is substitution with a benzyl group or benzyl analogue such as a xylyl group at the N^3 position of uridine. N^3-*o*-Xylyluridine (**1h**) was the most potent hypnotic by icv administration among the N^3-substituted compounds tested. Furthermore, an unmodified sugar moiety acts as an important factor in increasing hypnotic activity. These indications provide a possible speculation on a close relation between hydrophilic and hydrophobic sites on some sleep-related receptors in the brain. The binding studies of these compounds, tested for GABA and benzodiazepine receptors, are now in progress.

Experimental Section

Melting points were determined with a Yanaco micro melting point apparatus and are uncorrected. Microanalyses were performed with a Perkin-Elmer 240C elemental analyzer. When analyses are indicated only by symbols of elements, analytical values were within ±0.4% of theoretical values. Mass spectra were obtained with a JEOL JMS-DX 300 mass spectrometer. ^{1}H NMR spectra were obtained for all compounds and were consistent with structures and assignments. ^{1}H NMR spectra were recorded on a JEOL-MH 100 spectrometer. Purity of compounds were checked by TLC with Wako B-5 silica gel. Column chromatography was accomplished with Merck Kieselgel 60.

3-Methyl-1-β-D-ribofuranosyluracil (1a). Uridine (1.47 g, 6.0 mmol) and anhydrous K_2CO_3 (1.41 g, 10.2 mmol) were added to a solution of DMF (6 mL) and acetone (6 mL). Methyl iodide (0.37 mL, 9.0 mmol) was added dropwise to the solution, and the resulting mixture was refluxed at 50–60 °C for 4 h. The mixture was evaporated to dryness, and the residue was applied to a column of silica gel. The product was eluted with chloroform/ethyl acetate/methanol (5:4:1, v/v/v) and crystallized from methanol, ethyl acetate, and petroleum ether, giving 973 mg (62.8% yield) of **1a**: mp 117–118 °C (lit. 111–112.5 °C); ^{1}H NMR (D_2O) δ 3.16 (3 H, s, CH_3), 3.66–3.80 (2 H, m, H-5′, H-5″), 3.92–4.24 (3 H, m, H-2′, H-3′, H-4′), 5.68–5.76 (2 H, m, H-1′, H-5), 7.54–7.62

(18) Whittle, S. R.; Turner, A. J. *Biochem. Pharmacol.* **1982**, *31*, 2891.
(19) Willow, M.; Johnston, G. A. R. *J. Neurochem.* **1981**, *37*, 1291.

(1 H, d, J = 8 Hz, H-6). Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

In a similar manner, **1b** and **1d–k** were obtained by using the haloalkyl or haloaryl reagents (**1b**, ethyl bromide; **1d**, allyl bromide; **1e**, γ-methylcrotyl bromide; **1f**, benzyl bromide; **1g**, *p*-chlorobenzyl bromide; **1h**, α-bromo-*o*-xylene; **1i**, α-bromo-*m*-xylene; **1j**, α-bromo-*p*-xylene; **1k**, α-phenylethyl bromide) instead of methyl iodide. ^{1}H NMR data of new compounds are as follows. **1e**: (D_2O) δ 1.70 (3 H, s, CH_3), 1.78 (3 H, s, CH_3), 3.80–3.92 (2 H, m, H-5′, H-5″), 3.96–4.38 (3 H, m, H-2′, H-3′, H-4′), 4.48 (2 H, d, J = 8 Hz, NCH_2), 4.96–5.14 (1 H, m, C=CH), 5.86–6.00 (2 H, m, H-1′, H-5), 7.88 (1 H, d, J = 8 Hz, H-6). **1g**: (DMSO-d_6) δ 3.56–3.76 (2 H, m, H-5′, H-5″), 3.84–4.16 (3 H, m, H-2′, H-3′, H-4′), 5.02 (1 H, s, NCH_2), 5.86–5.96 (2 H, m, H-1′, H-5), 7.40–7.48 (4 H, m, C_6H_4), 8.12 (1 H, d, J = 8 Hz, H-6). **1h**: (DMSO-d_6) δ 2.28 (3 H, s, CH_3), 3.38–3.54 (2 H, m, H-5′, H-5″), 3.86–3.96 (3 H, m, H-2′, H-3′, H-4′), 4.78 (2 H, s, NCH_2), 5.56–5.64 (2 H, m, H-1′, H-5), 6.48–7.00 (4 H, m, C_6H_4), 7.76 (1 H, d, J = 8 Hz, H-1). **1i**: (DMSO-d_6) δ 2.26 (3 H, s, CH_3), 3.48–3.64 (2 H, m, H-5′, H-5″), 3.72–4.04 (3 H, m, H-2′, H-3′, H-4′), 4.88 (2 H, s, NCH_2), 5.66–5.80 (2 H, m, H-1′, H-5), 6.84–7.12 (4 H, m, C_6H_4), 7.92 (1 H, d, J = 8 Hz, H-6). **1j**: (DMSO-d_6) δ 2.28 (3 H, s, CH_3), 3.36–3.52 (2 H, m, H-5′, H-5″), 3.62–3.88 (3 H, m, H-2′, H-3′, H-4′), 4.74 (2 H, s, NCH_2), 5.48–5.60 (2 H, m, H-1′, H-5′), 6.72–7.00 (4 H, m, C_6H_4), 7.68 (1 H, d, J = 8 Hz, H-1). **1k**: (DMSO-d_6) δ 1.66–1.72 (3 H, d, J = 6 Hz, CH_3), 3.38–3.58 (2 H, m, H-5′, H-5″), 3.62–3.69 (3 H, m, H-2′, H-3′, H-4′), 5.44–5.60 (2 H, m, CHPh, H-5), 5.82–5.96 (1 H, m, H-1′), 6.92–7.12 (5 H, m, C_6H_5), 7.66 (1 H, d, J = 8 Hz, H-6).

3-Propyl-1-β-D-ribofuranosyluracil (1c). Compound **1c** was prepared from **1d**. Compound **1d** (284 mg, 1.0 mmol) and palladium on activated carbon (100 mg, 10% purity) were dissolved in 10 mL of ethanol and hydrogenated with hydrogen gas. The mixture was filtered, and ethanol was removed in vacuo. The residue was crystallized from ethyl acetate, yielding 168 mg (60% yield) of **1c**: mp 110–112 °C; ^{1}H NMR (D_2O) δ 0.96 (3 H, t, CH_3), 1.52–1.82 (2 H, m, CH_2), 3.84–4.16 (4 H, m, H-5′, H-5″, NCH_2), 4.18–4.62 (3 H, m, H-2′, H-3′, H-4′), 6.14–6.30 (2 H, m, H-1′, H-5), 8.26 (1 H, d, J = 8 Hz, H-6). Anal. ($C_{12}H_{18}N_2O_6$) C, H, N.

3-(Phenylmethyl)-1-(2,3,5-tri-*O*-methyl-β-D-ribofuranosyl)uracil (2b). In a similar manner to that for **1f**, **2b** was prepared from **2a** (oil, 85% yield): ^{1}H NMR ($CDCl_3$) δ 3.10 (3 H, s, OCH_3), 3.16 (3 H, s, OCH_3), 3.22 (3 H, s, OCH_3), 3.38–3.46 (2 H, m, H-5′, H-5″), 3.48–3.90 (3 H, m, H-2′, H-3′, H-4′), 4.70 (2 H, s, NCH_2), 5.30 (1 H, d, J = 8 Hz, H-5), 5.50 (1 H, s, H-1′), 6.64–6.94 (4 H, m, C_6H_4), 7.30 (1 H, d, J = 8 Hz, H-6); MS, m/z (relative intensity) 376 (M^+, 25), 313 (5), 275 (3), 245 (12), 202 (7). Anal. ($C_{19}H_{24}N_2O_6$) C, H, N.

3-(Phenylmethyl)-1-(2-tetrahydrofuryl)-5-fluorouracil (4a). To a solution of Futraful (0.5 g, 2.5 mmol) in DMF (30 mL) and acetone (30 mL) were added anhydrous K_2CO_3 and, dropwise, 0.03 mL (3.0 mmol) of benzyl bromide. The mixture was refluxed at 50–60 °C for 4 h. The solution was evaporated in vacuo. The residue was dissolved in water, and the solution was extracted with chloroform (2 × 100 mL). The organic phase was washed with water (2 × 50 mL) and dried over anhydrous Na_2SO_4. Chloroform was evaporated to dryness, and the residue was crystallized from aqueous methanol to yield 515 mg of **4a**: mp 73–76 °C; ^{1}H NMR ($CDCl_3$) δ 1.84–2.72 (4 H, m, H-3′, H-3″, H-4′, H-4″), 3.96–4.52 (4 H, m, C_6H_4). Anal. ($C_{15}H_{15}N_2O_3F$) C, H, N.

PB-Induced Sleep Effect. All compounds were suspended in 3% Tween 80/saline solution because of their insolubility in saline. PB was injected ip to mice (ddN strain, male, body weight 25 ± 3 g) at a dose of 40 mg/kg. In previous studies,[9] it was noted that the maximum PB-induced sleep effect, on mice pretreated with **1d**, occurred 15 min after pretreatment. Compound **1f** in the present study also exhibited the maximum effect at 15 min (Figure 1). On the basis of these data, all compounds tested were given by icv administration according to the method of Haley and McCormick[21] 15 min before the PB challenge. This icv injection method can evaluate the real CNS activity. PB-induced sleep time for mice was measured as the time between the loss and recovery of the righting reflex. Sleeping time was compared with that of the control group, and their effects were summarized as a percent of the control value in Tables I and II.

Hypnotic Activity. The sleeping time produced by hypnotic compounds in mice was measured as the time between the loss and recovery of the righting reflex as well as PB-induced sleep effect by both icv and iv administration routes. The iv injection was performed through the mouse tail vein at the doses of 0.1, 0.3, and 0.5 mmol/kg. The dose–response for the hypnotic activity of **1f** and **2a–4a** by iv injection was also shown as the sleeping time of mice. With icv injection, the dose of compounds used was indicated in parentheses on the tables.

Determination of Partition Coefficients. Partition coefficients were measured at ambient temperature by mechanically shaking mixtures of compounds (**2a**, **2b**, **3b**, and **4a**; 10 mg/5 mL of octanol). Stock solutions of these compounds were prepared in each solvent and diluted 30-fold with the same solvent as standard. After 3 h of agitation, the organic and aqueous phases were separated by centrifugation (3000 rpm), and each phase was diluted 30-fold. The concentrations of compounds tested in each phase were calculated from the ratios of their ultraviolet absorption spectra at 264 nm (274 nm for Futraful and **4a**) to those of standards. Partition coefficient were then expressed as the ratio of the concentration of each compound in the organic solvent to that in the corresponding aqueous buffer.

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Registry No. **1a**, 2140-69-4; **1b**, 27190-00-7; **1c**, 110319-06-7; **1d**, 103951-13-9; **1e**, 103993-72-2; **1f**, 14985-34-3; **1g**, 110319-07-8; **1h**, 110319-08-9; **1i**, 110319-09-0; **1j**, 110319-10-3; **1k**, 110319-11-4; **2a**, 39848-57-2; **2b**, 110319-12-5; **3a**, 4105-38-8; **3b**, 93960-47-5; **4a**, 64504-15-0; **5a**, 717-00-0; **5b**, 34001-56-4; uridine, 58-96-8; γ-methylcrotyl bromide, 870-63-3; *p*-chlorobenzyl bromide, 622-95-7; α-bromo-*o*-xylene, 89-92-9; α-bromo-*m*-xylene, 620-13-3; α-bromo-*p*-xylene, 104-81-4; α-phenylethyl bromide, 585-71-7; Futraful, 17902-23-7.

(20) Haines, J. A.; Reese, C. B.; Todd, L. *J. Chem. Soc.* **1964**, 1406.
(21) Haley, T. J.; McCormick, W. G. *Br. J. Pharmacol.* **1957**, *12*, 12.
(22) Yoshizaki, F.; Kondo, Y.; Takemoto, T. *Chem. Pharm. Bull.* **1975**, *23*, 2485.
(23) Brown, D. M.; Todd, A.; Varadarajan, S. *J. Chem. Soc.* **1956**, 2388.
(24) Chatamra, B.; Jones, A. S. *J. Chem. Soc.* **1963**, 811.