Hypnotic Action of N^3-Substituted Arabinofuranosyluracils on Mice

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Methyl (2), ethyl (3), propyl (4), butyl (5), allyl (6), benzyl (7), *o*-, *m*-, *p*-xylyl (8—10), and α-phenylethyl (11) derivatives of arabinofuranosyluracil (1) were synthesized and their pharmacological effects in mice were examined by using hypnotic activity and synergism with pentobarbital as indices for the CNS depressant effects. At a dose of 2.0 μmol/mouse by intracerebroventricular injection, the values of mean sleeping time induced by 7—11 were 144, 154, 117, 33, and 34 min, respectively, whereas the alkyl (2—6) derivatives did not cause any hypnotic activity. N^3-*o*-Xylylarabinofuranosyluracil (8) displayed the most potent hypnotic activity among the derivatives tested. Certain derivatives (6—11) significantly prolonged the pentobarbital-induced sleeping time compared to control. The present study indicated that substitution with benzyl and/or related groups on the N^3-position of arabinofuranosyluracil produced CNS depressant effects.

Key words N^3-substituted oxopyrimidine nucleoside; arabinofuranosyluracil; hypnotic activity; pentobarbital-induced sleep prolongation; CNS depressant

It is known that uridine is one of the pyrimidine nucleosides that displays a sleep-promoting effect in rats.[2)] However, uridine itself does not possess any hypnotic activity as determined by loss of the righting reflex in mice. We earlier found that N^3-benzyl substituted uridine exerts a hypnotic action on mice by intracerebroventricular (i.c.v.) administration.[3)] Subsequently, studies on the structure–activity relationships of uridine, 6-azauridine, thymidine, 2′-deoxyuridine, and 2′,3′-*O*-isopropylideneuridine for CNS depressant effects, including sedative and hypnotic activity, were carried out.[4—12)] However, so far the related pyrimidine nucleoside arabinofuranosyluracil (**1**) has not been used as a lead compound. Structurally, arabinofuranosyluracil and uridine differ in the position of the 2′ hydroxy group on the ribose moiety. We thus investigated whether the introduction of a substituent group at the N^3-position of **1** leads to CNS depressant effects or not. The present paper describes N^3-substituted arabinofuranosyluracil derivatives that possess CNS depressant activity in mice.

Experimental

Animals Male std-ddY mice weighing 22 to 28 g were obtained from Sankyo Laboratories (Toyama, Japan). The mice were kept in an air-conditioned room (24±2 °C) with controlled lighting (8:00 to 20:00 light period). They were given food and water *ad libitum.* Experiments on hypnotic activity and pentobarbital-induced prolongation effects were carried out from 10:00.

Chemicals Sodium pentobarbital and halogenated alkyls were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). β-D-Arabinofuranosyluracil was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Syntheses of N^3-Substituted 1 N^3-Substituted derivatives of arabinofuranosyluracil (1-β-D-arabinofuranosyluracil) were synthesized by the methods described previously.[4—13)] Briefly, **1** (3 mmol) dissolved in dimethylsulfoxide (3 ml) and acetone (3 ml) was reacted with halogenated alkyls (3 mmol) in the presence of a base (K_2CO_3 5 mmol). The product was purified by silica gel column chromatography with a solvent system of chloroform–ethyl acetate–methanol (5 : 4 : 1).

Analytical data of the derivatives prepared were as follows:

N^3-Methylarabinofuranosyluracil (3-methyl-1-β-D-arabinofuranosyluracil) (**2**): Recrystallization solvent, acetone and *n*-hexane, mp 171—179 °C, yield 49%, ^{1}H-NMR (DMSO-d_6) δ: 3.25 (3H, s, N–CH_3), 3.70 (3H, m, 2′-H, 3′-H, 4′-H), 3.77—4.12 (2H, m, 5′-H_2), 4.80—5.31 (1H, m, 5′-OH), 5.33—6.07 (3H, m, 2′-OH, 3′-OH, 4′-OH), 6.07 (1H, d, J=4 Hz, 1′-H), 7.77—7.90 (1H, m, 6-H). MS: m/z=258 (M^+). *Anal.* Calcd for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.43; N, 10.85. Found: C, 46.08; H, 5.39; N, 10.62.

N^3-Ethylarabinofuranosyluracil (3-ethyl-1-β-D-arabinofuranosyluracil) (**2**): Recrystallization solvent, ethanol and *n*-hexane, mp 173—174 °C, yield 76%, ^{1}H-NMR (DMSO-d_6) δ: 1.08 (3H, t, CH_3), 3.85 (2H, m, N–CH_2), 3.70 (3H, m, 2′-H, 3′-H, 4′-H), 4.00 (2H, m, 5′-H_2), 4.80—5.31 (1H, m, 5′-OH), 5.33—6.07 (3H, m, 2′-OH, 3′-OH, 5-H), 6.07 (1H, d, J=4 Hz, 1′-H), 7.80 (1H, m, 6-H). MS: m/z=272 (M^+). *Anal.* Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.88; N, 10.29. Found: C, 48.52; H, 5.91; N, 10.02.

N^3-Propylarabinofuranosyluracil (3-propyl-1-β-D-arabinofuranosyluracil) (**4**): Oil, yield 55%, ^{1}H-NMR (CD_3OD) δ: 0.76 (3H, t, CH_3), 1.13—1.76 (2H, m, –CH_2–), 3.53—4.43 (7H, m, 2′-H, 3′-H, 4′-H, N–CH_2, 5′-H_2), 5.76 (1H, d, J=8 Hz, 5-H), 6.16 (1H, d, J=4 Hz, 1′-H), 7.90 (1H, d, J=8 Hz, 6-H). MS: m/z=286 (M^+). *Anal.* Calcd for $C_{12}H_{18}N_2O_6$: C, 50.35; H, 6.29; N, 9.79. Found: C, 50.45; H, 6.18; N, 9.84.

N^3-Butylarabinofuranosyluracil (3-butyl-1-β-D-arabinofuranosyluracil) (**5**): Oil, yield 47%, ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, CH_3), 1.13—1.80 (4H, m, CH_2–CH_2–), 3.46—4.33 (7H, m, 2′-H, 3′-H, 4′-H, 5′-H_2, N–CH_2), 5.00 (1H, m, 5′-OH), 5.30—5.60 (2H, m, 2′-OH, 3′-OH), 5.67 (1H, d, J=8 Hz, 5′-H), 6.05 (1H, d, J=4 Hz, 1′-H), 7.70 (1H, d, J=8 Hz, 6-H). MS: m/z=300 (M^+). *Anal.* Calcd for $C_{13}H_{20}N_2O_6$: C, 52.00; H, 6.67; N, 9.33. Found: C, 52.00; H, 6.85; N, 8.96.

N^3-Allylarabinofuranosyluracil (3-allyl-1-β-D-arabinofuranosyluracil) (**6**): Oil, yield 33%, ^{1}H-NMR (DMSO-d_6) δ: 3.46—3.83 (3H, m, 2′-H, 3′-H, 4′-H), 3.83—4.23 (2H, m, 5′-H_2), 4.40 (2H, d, J=5 Hz, N–CH_2), 4.80—5.10 (1H, m, 5′-OH), 5.20 (2H, m, CH_2=), 5.33—5.63 (3H, m, 2′-OH, 3′-OH, CH=), 5.73 (1H, d, J=8 Hz, 5-H), 6.05 (1H, d, J=4 Hz, 1′-H), 7.73 (1H, d, J=8 Hz, 6-H). MS: m/z=284 (M^+). *Anal.* Calcd for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.63; N, 9.36. Found: C, 50.74; H, 5.86; N, 9.30.

N^3-Benzylarabinofuranosyluracil (3-benzyl-1-β-D-arabinofuranosyluracil) (**7**): Recrystallization solvent, acetone and *n*-hexane, mp 60—68 °C, yield 55%, ^{1}H-NMR (DMSO-d_6) δ: 3.43—4.23 (5H, m, 2′-H, 3′-H, 4′-H, 5′-H_2), 4.80—5.10 (3H, m, N–CH_2, 5′-OH), 5.23—5.88 (3H, d, J=4 Hz, 3′-OH, 2′-OH, 5-H), 5.90—6.13 (1H, d, J=4 Hz, 1′-H), 7.03—7.47 (5H, m, –C_6H_5), 7.63—7.85 (1H, d, J=8 Hz, 6-H). MS: m/z=334 (M^+). *Anal.* Calcd for $C_{16}H_{18}N_2O_6$: C, 57.49; H, 5.39; N, 8.38. Found: C, 57.52; H, 5.43; N, 8.24.

N^3-*o*-Xylyl-arabinofuranosyluracil (3-[(2-methylphenyl)methyl]-1-β-D-arabinofuranosyluracil) (**8**): Recrystallization solvent, methanol and ether, mp 139—141 °C, yield 52%, ^{1}H-NMR (DMSO-d_6) δ: 2.36 (3H, s, –CH_3), 3.45—3.83 (3H, m, 2′-H, 3′-H, 4′-H), 3.83—4.24 (2H, m, 5′-H_2), 4.83—5.25 (3H, m, N–CH_2, 5′-OH), 5.43 (1H, d, J=4 Hz, 3′-OH), 5.65 (1H, d, J=5 Hz, 2′-OH), 5.80 (1H, d, J=8 Hz, 5-H), 6.08 (1H, d, J=4 Hz, 1′-H), 6.73—7.37 (4H, m, –C_6H_4–), 7.72 (1H, d, J=8 Hz, 6-H). MS: m/z=348 (M^+). *Anal.* Calcd for $C_{17}H_{20}N_2O_6$: C, 58.62; H, 5.74; N, 8.05. Found: C,

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58.26; H, 5.59; N, 7.99.

N^3-*m*-Xylyl-arabinofuranosyluracil (3-[(3-methylphenyl)methyl]-1-β-D-arabinofuranosyluracil) (**9**): mp 54—57 °C, yield 64%, ^{1}H-NMR (DMSO-d_6) δ: 2.25 (3H, s, $-CH_3$), 3.39—3.78 (3H, m, 2′-H, 3′-H, 4′-H), 3.78—4.24 (2H, m, 5′-H_2), 4.85—5.26 (3H, m, N–CH_2, 5′-OH), 5.43 (1H, d, J=4 Hz, 3′-OH), 5.60 (1H, d, J=5 Hz, 2′-OH), 5.76 (1H, d, J=8 Hz, 5-H), 6.04 (1H, d, J=4 Hz, 1′-H), 6.84—7.37 (4H, m, $-C_6H_4-$), 7.72 (1H, d, J=8 Hz, 6-H). MS: m/z=348 (M^+). *Anal.* Calcd for $C_{17}H_{20}N_2O_6$: C, 58.62; H, 5.74; N, 8.05. Found: C, 58.41; H, 5.87; N, 8.05.

N^3-*p*-Xylyl-arabinofuranosyluracil (3-[(4-methylphenyl)methyl]-1-β-D-arabinofuranosyluracil) (**10**): Recrystallization solvent, methanol and H_2O, mp 61—65 °C, yield 50%, ^{1}H-NMR (DMSO-d_6) δ: 2.26 (3H, s, $-CH_3$), 3.45—3.79 (3H, m, 2′-H, 3′-H, 4′-H), 3.79—4.20 (2H, m, 5′-H_2), 4.80—5.17 (3H, m, N–CH_2, 5′-OH), 5.40 (1H, d, J=4 Hz, 3′-OH), 5.57 (1H, d, J=5 Hz, 2′-OH), 5.71 (1H, d, J=8 Hz, 5-H), 6.03 (1H, d, J=4 Hz, 1′-H), 6.97—7.30 (4H, m, $-C_6H_4-$), 7.73 (1H, d, J=8 Hz, 6-H). MS: m/z=348 (M^+). *Anal.* Calcd for $C_{17}H_{20}N_2O_6$: C, 58.62; H, 5.74; N, 8.05. Found: C, 58.27; H, 6.03; N, 7.66.

N^3-α-Phenylethylarabinofuranosyluracil (3-(1-phenylethyl)-1-β-D-arabinofuranosyluracil) (**11**) (diastereomixture): Oil, yield 15%, ^{1}H-NMR (DMSO-d_6) δ: 1.55 (3H, s, $-CH_3$), 3.32—4.20 (5H, m, 2′-H, 3′-H, 4′-H, 5′-H_2), 4.82 (1H, s, N–CH), 5.71 (1H, d, J=8 Hz, 5-H), 6.15 (1H, t, 1′-H), 7.38—7.68 (5H, m, $-C_6H_5$), 7.94 (1H, s, 6-H). MS: m/z=349 ($M+H^+$). *Anal.* Calcd for $C_{17}H_{20}N_2O_6$: C, 58.62; H, 5.74; N, 8.05. Found: C, 58.58; H, 5.79; N, 8.01.

Drug Administration N^3-Substituted arabinofuranosyluracils were suspended in saline containing 3% Tween 80 and injected intracerebroventricularly (i.c.v.).[14] Control mice were injected i.c.v. with 3% Tween 80-saline as a vehicle. Sodium pentobarbital (40 mg/kg) dissolved in saline was administered intraperitoneally (i.p.).

Hypnotic Activity Compounds were administered by i.c.v. injection. Sleeping time was measured as the period between the loss and recovery of the righting reflex.

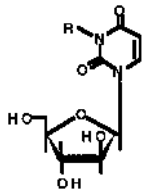
Pentobarbital-Induced Sleep Prolongation The prolongation effects of N^3-substituted arabinofuranosyluracil on pentobarbital-induced sleep were assessed by injection of sodium pentobarbital (40 mg/kg, i.p.) 15 min after administration of the test compounds. For the pentobarbital-induced sleep-prolongation effect, the statistical significance of difference between the control and test groups was analyzed by use of a one-way analysis of variance Student's *t*-test.

Results and Discussion

The CNS depressant effects of **1** and its N^3-substituted derivatives are summarized in Table 1. Compound **1**, N^3-methyl (**2**), ethyl (**3**), propyl (**4**), butyl (**5**), and allyl (**6**) derivatives did not possess any hypnotic activity in mice at a dose of 2.0 μmol/mouse by i.c.v. injection, whereas N^3-benzyl (**7**), *o*, *m*-, *p*-xylyl (**8**—**10**), and α-phenylethyl (**11**) substituted arabinofuranosyluracils possessed hypnotic activity at the same dose. Among them, N^3-*o*-xylyl derivative (**8**) exhibited the strongest hypnotic activity. The values of mean sleeping time induced by **7**—**11** were 144, 154, 117, 33 and 34 min, respectively. Since **7**—**11** exhibited hypnotic activity, the hypnotic activities of the derivatives were compared at different doses by i.c.v. injection (Fig. 1). All compounds showed no hypnotic activity at a dose of 0.5 μmol/mouse by i.c.v. injection. Compounds **7**—**10** showed 46, 40, 36, and 17 min of sleeping time even at 1.0 μmol/mouse, respectively. The hypnotic activity of **7** was 4-times stronger than that of N^3-benzyluridine at the dose of 2.0 μmol/mouse (Fig. 1), indicating an interaction between the N^3-benzyl substituted group and the 2′-hydroxy group on the hypnotic activity. Our previous work[5] demonstrated that N^3-*o*-xylyluridine had stronger activity than the benzyl derivative. However, the present study indicates that the *o*-xylyl derivative of **1** has the same potency as the benzyl derivative in the hypnotic activity test.

The effects of the derivatives on pentobarbital-induced sleep were also evaluated (Table 1). Although **1** and its N^3-alkylated derivatives, **2**—**5** (2.0 μmol/mouse, i.c.v.) did not prolong pentobarbital-induced sleeping time, the N^3-allyl substituted derivative significantly prolonged the sleeping time, suggesting that the unsaturated bond in the substituent group caused the CNS depressant activity.[6] In addition, benzyl and its related derivatives (benzyl (**7**), xylyls (**8**—**10**), and α-phenylethyl (**11**) derivatives) also significantly prolonged sleeping time at the same dose. Compounds **6**—**11** possessed 1.5, 3.9, 4.0, 4.6, 2.9, and 2.1-fold of the control sleeping time at 2.0 μmol/mouse. These results did not show the same tendency as found in N^3-substituted oxopyrimidine nucleosides as previously reported.[5] We previously reported that N^3-allyl substituted uridine and thymidine exhibited CNS de-

Table 1. CNS Activities of N^3-Substituted Arabinofuranosyluracil



No. 1—11

R	No.	Hypnotic activity[a] (min)	PB-Induced sleep[b] (% of control)
H	**1**	none	112±8
CH_3	**2**	none	104±7
CH_3CH_2	**3**	none	107±8
$CH_3CH_2CH_2$	**4**	none	128±11
$CH_3CH_2CH_2CH_2$	**5**	none	123±9
CH_2=$CHCH_2$	**6**	none	149±10*
$C_6H_5CH_2$	**7**	144±11	386±48**
o-$CH_3C_6H_4CH_2$	**8**	154±13	398±4**
m-$CH_3C_6H_4CH_2$	**9**	117±7	460±10**
p-$CH_3C_6H_4CH_2$	**10**	33±5	287±10**
$C_6H_5(CH_3)CH$	**11**	34±6	210±11**

Compounds were administered by i.c.v. injection at a dose of 2.0 μmol/mouse. *a*) Hypnotic activity is expressed as the mean sleeping time±S.E.M. (min). "none" indicates no hypnotic activity. *b*) Pentobarbital (PB)-induced sleep prolonging effect was expressed as the mean % of control sleeping time±S.E.M. (control sleeping time: 56±2 min). * and ** indicate significant difference from control at the level of $p<0.05$ and $p<0.01$, respectively.

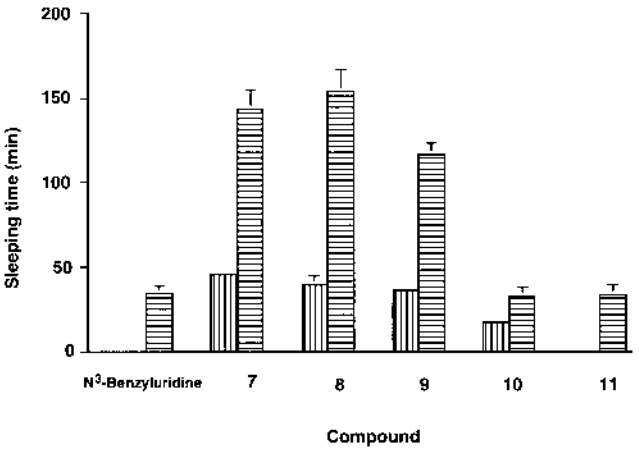


Fig. 1. Hypnotic Activities of Arabinofuranosyluracil Derivatives Substituted Benzyl Related Groups at the N^3-Position by I.c.v. Injection

▥, 1.0 μmol/mouse; ▤, 2.0 μmol/mouse; **7**, N^3-Benzylarabinofuranosyluracil; **8**, N^3-*o*-Xylylarabinofuranosyluracil; **9**, N^3-*m*-Xylylarabinofuranosyluracil; **10**, N^3-*p*-Xylylarabinofuranosyluracil; **11**, N^3-α-Phenylethylarabinofuranosyluracil.

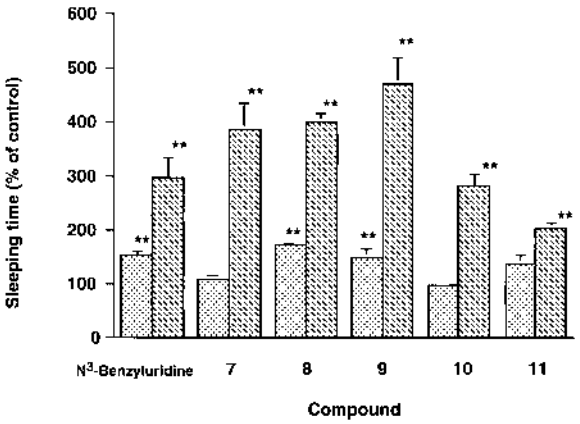


Fig. 2. Effects of Arabinofuranosyluracil Derivatives Substituted Benzyl Related Groups at the N^3-Position on Pentobarbital-Induced Sleep by I.c.v. Injection

▨, 0.5 μmol/mouse; ▩, 2.0 μmol/mouse; **7**, N^3-Benzylarabinofuranosyluracil; **8**, N^3-*o*-Xylylarabinofuranosyluracil; **9**, N^3-*m*-Xylylarabinofuranosyluracil; **10**, N^3-*p*-Xylylarabinofuranosyluracil; **11**, N^3-α-Phenethylarabinofuranosyluracil. ** indicates significant difference from control at the level of $p<0.01$.

pressant activity in mice.[4] The present results also support the conclusion that the N^3-allyl substituted derivative has CNS depressant activity.

Oxopyrimidine nucleoside **1** alone did not exhibit any hypnotic activity or potentiation of pentobarbital-induced sleep when given by i.c.v. injection. However, chemical modification of the N^3-position of **1** caused CNS depressant effects in mice. These results indicate that oxopyrimidine nucleosides basically have CNS depressant activity. Therefore, our present results are consistent with the report by Krooth *et al.*[15] that oxopyrimidine nucleosides decreased mouse locomotor activity by a CNS depressant effect. We previously reported that N^3-benzyl- or benzyl-related derivatives of uridine, 6-azauridine, thymidine, and 2′-deoxyuridine possess hypnotic activity in mice, while the derivatives of 2′,3′-*O*-isopropylideneuridine did not.[5—9,12] In the present study, N^3-benzyl- and xylyl substituted arabinofuranosyluracil derivatives also showed hypnotic activity. These results demonstrate that not only uridine derivatives, but also arabinofuranosyluracil derivatives exhibit hypnotic activity. The structure–activity relationships of arabinofuranosyluracil derivatives were different from those of the oxopyrimidines previously reported.[5] In the case of uridine derivatives, N^3-*o*-xylyluridine possessed the most potent hypnotic activity among the uridine derivatives. In the present study, **6**—**8** showed the same hypnotic activity in mice and had stronger effects as compared with oxopyrimidine nucleosides having the same N^3-substituents. This result may be due to the structural difference at the 2′-hydroxy group on ribose. Recently, we demonstrated the existence of a uridine receptor on the synaptic membrane in mammalian brain.[16] The structural specificity of arabinofuranosyl derivatives for hypnotic activity might also contribute to the conformation of the uridine receptor binding site.

In conclusion, the present study supports our previous findings that N^3-substituted oxopyrimidine nucleosides such as uridine, 6-azauridine, thymidine, and 2′-deoxyuridine possess hypnotic and sedative activities, and that the introduction of benzyl-related groups at the N^3-position is an important factor for exhibiting the CNS depressant effects of oxopyrimidine nucleoside derivatives, even though the hydroxy group on the 2′ position of ribose is different from that of uridine. Although arabinofuranosyluracil is not a natural compound, it appeared that arabinofuranosyluracil derivatives basically possess CNS depressant activity, as do uridine derivatives.

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References and Notes

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