[Chem. Pharm. Bull. 35(12)4928—4934(1987)]

Potentiating Effects of N^1,N^3-Diallyluracil, N^1,N^3-Diallylthymine and N^1,N^3-Diallyl-6-methyluracil on Pentobarbital-Induced Sleep and Diazepam-Induced Motor Incoordination

YUJI TATEOKA,[a] TOSHIYUKI KIMURA,[a] KAZUHITO WATANABE,[a] IKUO YAMAMOTO*,[a] and ING KANG HO[b]

Department of Hygienic Chemistry, School of Pharmacy, Hokuriku University,[a] 3, Ho Kanagawa-machi, Kanazawa 920–11, Japan and Department of Pharmacology and Toxicology, The University of Mississippi Medical Center,[b] 2500 North State Street, Jackson, Mississippi 39216, U.S.A.

(Received April 13, 1987)

N-Allyl derivatives of uracil (U), thymine (T) and 6-methyluracil (6-MU) were prepared, and their pharmacological activities (hypnotic activity and anticonvulsant activity against pentylenetetrazol (PTZ)-induced seizures) and interactions with three sedative-hypnotics [pentobarbital (PB), barbital (B) and diazepam (DZ)] were investigated in mice. N^1,N^3-Diallyluracil (DAU) alone exhibited hypnotic and anticonvulsant activities. None of the other allyl derivatives showed both pharmacological activities. As regards interactions, most of the compounds tested prolonged PB-induced sleep at either 80 or 160 mg/kg, i.p. Further, U, T, and 6-MU (160 mg/kg, i.p.) also prolonged the PB-induced sleeping time. DAU showed a prolonging effect on PB-induced sleep when given by intracerebroventricular (i.c.v.) injection. DAU, N^1,N^3-diallylthymine (DAT) and N^1-monoallyluracil (N^1-MAU) significantly prolonged the B-induced sleeping time at a dose of 160 mg/kg, i.p. Further, DAU and DAT (40 mg/kg, i.p.) enhanced DZ-induced motor incoordination. These results indicate that U and related compounds possess central nervous system (CNS)-depressant effects and DAU is the most potent among the *N*-allyl derivatives tested.

Keywords——N^1,N^3-diallyluracil; N^1,N^3-diallylthymine; N^1,N^3-diallyl-6-methyluracil; hypnotic activity; anticonvulsant activity; barbiturate-induced sleep; diazepam-induced motor incoordination

In previous studies,[1–6] we reported that allyl-substituted derivatives of barbiturates and related compounds exhibited various pharmacological activities in mice. *N*-Monoallylbarbital had more potent hypnotic activity than barbital (B).[2] Further, these allyl derivatives showed a potent synergistic effect on sedative hypnotic-induced narcosis. In contrast, *N,N'*-diallylpentobarbital apparently showed an antagonistic effect on B-induced sleep.[1,6] These results indicate that allyl-substituted barbiturates have multiple pharmacological activities.

It has been reported that uracil (U) and related oxypyrimidines exhibit some central depressant properties.[7–9] U shows a prolonging effect on hexobarbital-induced sleep and anticonvulsant activity against maximal electroshock seizures.[7,8] Krooth *et al.* have reported that pyrimidine bases increase the spontaneous activity at lower doses and decrease the activity at higher ones.[9] On the other hand, there is a report that U dose not affect the duration or frequency of wakefulness, slow wave sleep or paradoxical sleep at any dose in rats.[10] Since U and thymine (T) have an oxypyrimidine moiety, like the barbiturate, the structure–activity relationships of both compound groups are of interest. However, there has been no study on the pharmacological activity of *N*-allyl-substituted derivatives of U, T and 6-methyluracil (6-MU), although syntheses of some *N*-allyl compounds have been reported.[11–15] In order to reexamine the above findings and confirm the effect of *N*-allyl

substitution, we prepared these *N*-allyl compounds, and then systematically investigated their pharmacological activities.

Experimental

Animals——Male ddN mice weighing 22—28 g were used. Animals were housed under a normal light—dark cycle (7:00—19:00) at ambient temperature (23±1 °C). Food and water were given *ad lib.*

Chemicals and Preparation of *N*-Allyl Compounds——U, T and sodium B (Wako Pure Chemical Ind.), 6-MU (Nakarai Chemicals, Ltd.), sodium pentobarbital (PB, Tokyo Kasei Kogyo Co., Ltd.), diazepam (DZ, Yamanouchi Seiyaku Co., Ltd.) and pentylenetetrazol (PTZ, K & K Laboratories) were used. Preparation of *N*-allyl-substituted derivatives of U, T and 6-MU was carried out according to the reported method,[3] that is, each pyrimidine base was reacted with allyl bromide in acetone: 1 N NaOH (1 : 1, v/v). The physical and spectral data of the *N*-allyl compounds prepared are listed in Table I. Separation of N^1-monoallyl and N^3-monoallyl derivatives was performed by silica gel column chromatography with chloroform: isopropanol = 97.5 : 2.5. Identification of N^1- and N^3-monoallyl derivatives was performed based on the shift (or lack of it) in the ultraviolet (UV) absorption maximum in basic solution and by comparing the values with those in the literature.[11—15]

Animal Experiments——All allyl compounds, U, T, 6-MU and DZ were suspended in 1% Tween 80–saline solution and the other drugs were dissolved in saline.

Hypnotic Activity [50% Hypnotic Dose (HD_{50})]: Allyl compounds were administered to each group of 6—10 mice at a dose of 500 or 640 mg/kg, i.p. and then the behavior of the treated mice was observed till 3 h after administration. Sleeping time was measured as the interval between loss and recovery of an effective righting reflex (considered to be recovery from a side position within 1 min[16]). The number of mice that lost the righting reflex was recorded for each dose, 400, 430, 460 and 500 mg/kg, i.p. of DAU, and the dose required to induce the effect in 50% of the animals (HD_{50} with 95% confidence limits) was determined.

Anticovulsant Activity [50% Effective Dose against PTZ-Induced Seizures (PTZ-ED_{50})]: The anticonvulsant activity was evaluated in terms of the protection against PTZ-induced seizures, using a modification of the method described by Andrews *et al.*[16] Allyl compounds (250 mg/kg, i.p.) were administered to each group of 8 mice 20 min prior to the subcutaneous injection of PTZ, 120 mg/kg. The blocking of tonic-extensor convulsion was considered to be evidence of activity.

Acute Toxicity [50% Lethal Dose (LD_{50})]: The LD_{50} value for each compound tested was also determined. N^1,N^3-Diallyl-substituted compounds were administered at four different dosage levels. Mortality was observed for 3 d.

Effects on PB- and B-Induced Sleeping Time: The effects of allyl derivatives on PB-induced sleep were tested by two routes of administration [i.v. and intracerebroventricular (i.c.v.)]. All allyl derivatives and parent compounds (80 and 160 mg/kg = 0.1 ml/10 g of body weight, i.p. and 200 μg/mouse = 25 μl, i.c.v.) were injected 15 min prior to the administration of PB (40 mg/kg, i.p.). In the same way, allyl compounds were administered at a dose of 160 mg/kg, i.p. 15 min prior to the 300 mg/kg, i.p. injection of B. Barbiturate-induced sleeping time was measured. Control mice were injected with the 1% Tween 80–saline solution instead of test compounds. The i.c.v. administration was performed by the method of Haley and McCormick.[17]

Effects of N^1,N^3-Diallyluracil (DAU) and N^1,N^3-Diallylthymine (DAT) on DZ-Induced Motor Incoordination: Motor incoordination was measured according to the method previously reported.[1] DAU and DAT, 40 mg/kg, i.p. were administered to groups of ten mice 15 min prior to the 5 mg/kg, i.p. injection of DZ. The percentage of mice which fell off the rods within 30 seconds was recorded.

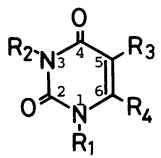
Statistical Analysis——HD_{50}, PTZ-ED_{50}, LD_{50} and their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.[18] Statistical significance was analyzed by using Student's *t*-test.

Results

Pharmacological Activity

Table II summarizes the pharmacological activities of each of the *N*-allyl-substituted derivatives alone. DAU showed some hypnotic and anticonvulsant activities. The HD_{50} and PTZ-ED_{50} of DAU were 433 (406—462) mg/kg, i.p. and 259 (215—312) mg/kg, i.p., respectively. The onset time and duration of loss of righting reflex in the DAU-treated group were 5±1 min and 60±5 min ($N = 7/10$), respectively, at a dose of 460 mg/kg, i.p. The PTZ-ED_{50} for DAU was about 60% of its ED_{50} value. On the other hand, allyl compounds except for DAU exhibited neither loss of righting reflex nor anticonvulsant activity. The acute toxicity (LD_{50}, mg/kg, i.p.) of DAU, 560 (526—596), was lower than that of DAT, 375

TABLE I. Physical and Spectral Data for *N*-Allyl-Substituted Derivatives of U, T and 6-MU



Compd.	R_1	R_2	R_3	R_4	Yield (%)	mp (°C) (Lit.)	Recryst. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	UV λ_{max} nm (log ε) EtOH	pH 12	^{1}H-NMR δ (in $CDCl_3$)
N^1-MAU	$-C_3H_5$	H	H	H	36	100—103 (105—108)[11]	C_6H_6	$C_7H_8N_2O_2$	55.26 (55.64	5.30 5.29	18.41 18.35)	267 (4.00)	265 (4.14)	4.42 (2H, d, J=5 Hz, N^1-CH_2–), 5.37—5.58 (2H, m, =CH_2), 5.81 (1H, d, J=8 Hz, 5-H), 7.27 (1H, d, J=8 Hz, 6-H)
N^3-MAU	H	$-C_3H_5$	H	H	2	135—137 (133—134)[12]	C_6H_6	$C_7H_8N_2O_2$	55.26 (55.06	5.30 5.23	18.41 18.35)	261 (3.96)	286 (4.12)	4.53 (2H, d, J=5 Hz, N^3-CH_2–), 5.08—5.36 (2H, m, =CH_2), 7.09—7.18 (1H, m, 6-H), 10.48 (1H, br s, N^1H)
DAU	$-C_3H_5$	$-C_3H_5$	H	H	14	Oil[a]		$C_{10}H_{12}N_2O_2$	62.49 (62.32	6.29 6.27	14.57 13.97)	267 (3.93) 266[13] (3.97)		4.48 (2H, d, J=5 Hz, N^1-CH_2–), 4.63 (2H, d, J=5 Hz, N^3-CH_2–), 5.19—5.55 (4H, m, (=CH_2)$_2$), 5.86 (1H, d, J=6 Hz, 5-H), 7.37 (1H, d, J=6 Hz, 6-H)
N^1-MAT	$-C_3H_5$	H	$-CH_3$	H	28	97—99 (96—97)[14]	C_6H_6	$C_8H_{10}N_2O_2$	57.82 (57.53	6.07 6.00	16.86 16.84)	272 (4.01)	271 (3.88)	1.95 (3H, s, 5-CH_3), 4.42 (2H, d, J=6 Hz, N^1-CH_2–), 5.04—5.51 (2H, m, =CH_2), 5.68—6.20 (1H, m, –CH=), 7.12 (1H, s, 6-H)

N^3-MAT	H	$-C_3H_5$	$-CH_3$	H	6	174—175 (175—177)[12]	C_6H_6	$C_8H_{10}N_2O_2$	57.82 (57.70	6.07 6.08	16.86 16.83)	267 (4.09)	293 (4.24)	1.93 (3H, s, 5-CH_3), 4.63 (2H, d, J=7 Hz, N^3-CH_2–), 5.19—5.46 (2H, m, =CH_2), 5.79—6.19 (1H, m, –CH=), 7.17 (1H, d, J=5 Hz, 6-H), 10.53 (1H, br s, N^1H)
DAT	$-C_3H_5$	$-C_3H_5$	$-CH_3$	H	14	Oil[a]		$C_{11}H_{14}N_2O_2$	64.06 (63.48	6.84 6.82	13.58 13.86)	272 (4.03)		1.95 (3H, s, 5-CH_3), 4.42 (2H, d, J=6 Hz, N^1-CH_2–), 4.65 (2H, d, J=5 Hz, N^3-CH_2–), 5.02—5.52 (4H, m, (=CH_2)$_2$), 5.68—6.20 (2H, m, (–CH=)$_2$), 7.07 (1H, br s, 6-H)
N^1-MA-6-MU	$-C_3H_5$	H	H	$-CH_3$	10	174—175	$CHCl_3$–MeOH (9:1)	$C_8H_{10}N_2O_2$	57.82 (57.64	6.07 6.13	16.86 16.98)	265 (3.94)	267 (4.20)	2.25 (3H, s, 6-CH_3), 4.48 (2H, d, J=6 Hz, N^1-CH_2–), 4.99—5.35 (2H, m, =CH_2), 5.57 (1H, s, 5-H), 5.67—6.09 (1H, m, –CH=), 9.57 (1H, br s, N^3H)
N^3-MA-6-MU	H	$-C_3H_5$	H	$-CH_3$	10	175—179 (184)[15]	C_6H_6	$C_8H_{10}N_2O_2$	57.82 (57.61	6.07 6.06	16.86 16.77)	262 (4.09)	282 (4.20)	2.15 (3H, s, 6-CH_3), 4.51 (2H, d, J=6 Hz, N^3-CH_2–), 5.10—5.34 (2H, m, =CH_2), 5.59 (1H, s, 5-H), 5.66—6.10 (1H, m, –CH=), 10.64 (1H, br s, N^1H)
DA-6-MU	$-C_3H_5$	$-C_3H_5$	H	$-CH_3$	6	58—59	C_6H_{12}	$C_{11}H_{14}N_2O_2$	64.06 (64.12	6.84 6.95	13.58 13.74)	269 (4.27)		2.25 (3H, s, 6-CH_3), 4.45—4.64 (4H, m, (–CH_2–)$_2$), 4.99—5.37 (4H, m, (=CH_2)$_2$), 5.60 (1H, s, 5-H), 5.67—6.11 (1H, m, –CH=)

Abbreviations used are: MAU, monoallyluracil; DAU, N^1,N^3-diallyluracil; MAT, monoallylthymine; DAT, N^1,N^3-diallylthymine; MA-6-MU, monoallyl-6-methyluracil; DA-6-MU, N^1,N^3-diallyl-6-methyluracil; $-C_3H_5$, $-CH_2CH=CH_2$. *a*) Oily compounds were purified by column chromatography on silica gel.

TABLE II. Pharmacological Activities of *N*-Allyl-Substituted Derivatives of U, T and 6-MU

Compd.	HD_{50} (mg/kg, i.p.)	PTZ-ED_{50} (mg/kg, i.p.)	LD_{50} (mg/kg, i.p.)
U	None (640)[a]	>250	>640
N^1-MAU	None (640)	>250	>640
N^3-MAU	None (500)	>250	>500
DAU	433 (406—462)[b]	259 (215—312)	560 (526—596)
T	None (640)	>250	>640
N^1-MAT	None (640)	>250	>640
N^3-MAT	None (500)	>250	>500
DAT	None (550)	>250	375 (347—406)
6-MU	None (640)	>250	>640
N^1-MA-6-MU	None (640)	>250	>640
N^3-MA-6-MU	None (640)	>250	>640
DA-6-MU	None (480)	>250	425 (389—464)

a) The word "None" means that there was no loss of righting reflex even at the dose indicated in parentheses. *b*) The 95% confidence limits are shown in parentheses.

TABLE III. Effects of *N*-Allyl-Substituted Derivatives of U, T and 6-MU on PB- and B-Induced Sleep

Compd.	Sleeping time (min)			
	PB (40 mg/kg, i.p.)			B (300 mg/kg, i.p.)
	80 mg/kg, i.p.	160 mg/kg, i.p.	200 μg/mouse i.c.v.	160 mg/kg, i.p.
Control	21± 2 (30)		67± 5 (30)	106±20 (30)
U	33± 4[a] (10)	36± 5[b] (10)	64± 7 (10)	108±28 (20)
N^1-MAU	39± 6[b] (10)	70± 6[b] (10)	88±17 (10)	204±45[a] (12)
N^3-MAU	27± 3 (10)	29± 4 (10)	63± 5 (10)	81±19 (10)
DAU	113±10[b] (10)	297±26[b] (10)	112± 9[b] (10)	177±12[a] (12)
T	33± 4[a] (10)	47± 7[b] (10)	58± 7 (10)	103±21 (10)
N^1-MAT	66±12[b] (10)	107±12[b] (10)	79±15 (10)	121±34 (10)
N^3-MAT	33± 3[a] (10)	68± 4[b] (10)	71± 7 (10)	138±28 (10)
DAT	101±11[b] (10)	211±12[b] (10)	82±10 (10)	284±30[b] (10)
6-MU	18± 2 (10)	35± 5[a] (10)	57± 6 (10)	87± 9 (10)
N^1-MA-6-MU	27± 5 (10)	69±11[b] (10)	65± 6 (10)	93±19 (10)
N^3-MA-6-MU	38± 6[b] (10)	67± 5[b] (10)	67± 5 (10)	74± 5 (10)
DA-6-MU	94±15[b] (10)	107± 8[b] (10)	88±12 (10)	97±13 (10)

Compounds tested were administered 15 min prior to the injection of PB or B. Data are expressed as the mean ± S.E. Numbers in parentheses represent numbers of animals used. *a*) Significantly different from the control ($p<0.05$). *b*) Significantly different from the control ($p<0.01$).

(347—406), or N^1,N^3-diallyl-6-methyluracil (DA-6-MU), 425 (389—464). The LD_{50} values of the other allyl compounds were larger than 500 or 640 mg/kg, i.p.

Potentiation of PB-, B- or DZ-Induced Depressant Activity

Table III shows the effects of *N*-allyl derivatives of U, T and 6-MU on PB- and B-induced sleep. DAU and DAT markedly prolonged the PB-induced sleeping time. The duration of sleeping time prolonged by DAU pretreatment was 14-fold greater than the control. DAU

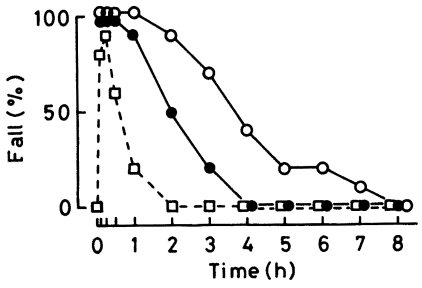


Fig. 1. Effects of DAU and DAT on DZ-Induced Motor Incoordination

DAU and DAT were administered i.p. 15 min prior to the i.p. injection of DZ. The control group was pretreated with 1% Tween 80–saline (vehicle). Ten mice were used for each group.
□---□, control (1% Tween 80–saline + DZ 5 mg/kg); ○—○, DAU 40 mg/kg + DZ 5 mg/kg; ●—●, DAT 40 mg/kg + DZ 5 mg/kg.

was the most potent compound as regards PB-induced sleep prolongation among the compounds tested. The other *N*-allyl-substituted compounds also prolonged PB-induced sleep at a dose of 160 mg/kg, i.p., except for N^3-monoallyluracil (N^3-MAU). The parent compounds, even, U, T and 6-MU (160 mg/kg, i.p.), significantly prolonged the sleeping time induced by PB. As a preliminary experiment, the time course (1, 15 and 30 min) of the effect of pretreatment with N^1-monoallyluracil (N^1-MAU) or DAU (200 μg/mouse, i.c.v.) on the PB (40 mg/kg, i.p.)-induced sleep was examined. Since the sleeping time of the DAU pretreated group was within the range from 110 to 120 min at all times examined (data not shown), the time of peak effect could not be determined. Thus administration of PB was set at 15 min after injection of *N*-allyl compounds. DAU only significantly prolonged the PB-induced sleeping time when given by i.c.v. injection. The other compounds did not show a prolonging effect on PB-induced sleep when given by i.c.v. injection, whereas DAT, N^1-MAU and DAU significantly prolonged the B-induced sleeping time, by 2.7-fold, 1.9-fold and 1.7-fold, respectively, as compared with the control. The mean sleeping time of mice pretreated with DAT was longer than with DAU. Interestingly, U, T and 6-MU did not exhibit any prolonging effect on B-induced sleep.

Figure 1 shows the effects of DAU and DAT, selected from among the allyl compounds, on the DZ (5 mg/kg, i.p.)-induced motor incoordination. Both DAU and DAT enhanced the effect of DZ at a dose of 40 mg/kg, i.p., although DAU or DAT alone did not induce apparent motor incoordination at the same dose. DAU was more potent than DAT.

Discussion

The results of the present study indicate that the introduction of two allyl groups onto N^1 and N^3 of the 2,4-dioxopyrimidine ring resulted in an increase of the depressant effect. *N*-Allyl-substituted derivatives of U seem to exhibit more potent depressant effects on the central nervous system (CNS) than N^1- and/or N^3-allyl-substituted derivatives of barbituric acid according to our previous data.[3] Recently, we have reported that N^3-benzyluridine exerted hypnotic activity when given by i.c.v. injection,[19] and that N^3-allyluridine and N^3-allylthymidine enhanced the drug-induced central depressant effect.[20] A compound that has two substituent groups at N^1 and N^3 of the U ring may generally possess a potent central depressant effect. The other *N*-allyl derivatives of U, T and 6-MU alone did not show loss of righting reflex or anticonvulsant activity. The acute toxicity of DAU was lower than that of DAT or DA-6-MU.

The interaction study of the *N*-allyl compounds with PB showed that the compounds tested (160 mg/kg, i.p.), except for N^3-MAU, significantly prolonged the PB-induced sleeping time. Upon i.c.v. administration, only DAU showed a significant potentiation of PB-induced sleep. This might be due to direct action of DAU on the CNS. The other allyl compounds did not show any significant prolonging effects on PB-induced sleep by i.c.v. injection. However,

DAT, N^1-MAU and DAU significantly prolonged the B-induced sleeping time. Since B is excreted unmetabolized,[21] an increase in its action could be due either to an enhanced penetration into the CNS or to alteration of neuronal sensitivity to the drug. The potentiation of DZ-induced motor incoordination by DAU and DAT also supports the hypothesis that both compounds have a direct depressant effect on the CNS.

N^1-Monoallyl-substituted derivatives of T, including U were more potent CNS depressants than the corresponding N^3-monoallyl-substituted derivatives. A methyl group at the 5 or 6 position of the U ring seems to be unnecessary for action on the CNS. It is of interest that the *N*-allyl compounds derived from endogenous substances such as U and T showed such potent pharmacological activities.

These results indicate that DAU and related *N*-allyl compounds have a CNS-depressant effect, and suggest that these compounds could prolong PB-induced sleep as a result of their depressant effect on the CNS.

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