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Potential of Barbiturate-Induced Narcosis by *N,N'*- (or *N,S*-) Diallyl-Substituted Derivatives of Phenobarbital, Amobarbital and Thiopental in Mice

YUJI TATEOKA,^a TOSHIYUKI KIMURA,^a KAZUHIITO 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.*

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N- (or *S*-) Allyl derivatives of phenobarbital (PheB), amobarbital (AB) and thiopental (TP) were prepared, and their pharmacological activities (hypnotic activity and anticonvulsant activity against pentylenetetrazol-induced seizures) were investigated in mice. *N*-Monoallyl-substituted derivatives (MAPheB, MAAB and MATP) of PheB, AB and TP* exhibited hypnotic and anticonvulsant activities. *N,S*-Diallylthiopental (*N,S*-DATP) possessed hypnotic activity, but failed to show anticonvulsant activity even at a dose of 250 mg/kg (i.p.). *N,N'*-Diallyl-substituted derivatives (DAPheB, DAAB and DATP) were devoid of not only the hypnotic activity, but also the anticonvulsant activity. The interactions of these allyl derivatives with various barbiturates were then studied in order to characterize whether the allyl compounds have antagonistic or potentiating properties. MAPheB, DAPheB, MAAB and DAAB showed dose-dependent potentiation of the sleep induced by the corresponding mother compounds. All the allyl compounds tested prolonged the pentobarbital-induced sleeping time more than 7- to 19-fold as compared with the control. MAAB markedly prolonged the PheB-induced sleeping time by 25-fold, but it did not potentiate the barbital-induced sleep. These results indicate that *N*- (or *S*-) allyl derivatives of PheB, AB and TP interact differently with the barbiturates.

Keywords—*N,N'*-diallylphenobarbital; *N,N'*-diallylamobarbital; *N,N'*-diallylthiopental; *N,S*-diallylthiopental; *N*-monoallylbarbiturate; central depressant effect; hypnotic activity; anticonvulsant activity; acute toxicity; barbiturate-induced sleep

In previous studies,¹⁻³⁾ we demonstrated that *N*-allyl substituted derivatives of pentobarbital (PB), barbital (B) and barbituric acid exhibited different pharmacological activities in mice. *N*-Monoallylbarbital displayed more potent hypnotic activity than B.²⁾ Further, these allyl derivatives possessed a potent synergistic effect on sedative hypnotic-induced responses. In contrast, *N,N'*-diallylpentobarbital apparently showed a shortening effect on the B-induced sleeping time.¹⁾ These results indicate the complexity of action of allyl-substituted barbiturates. In connection with the above findings, the present study was designed to investigate systematically the pharmacological activities of allyl-substituted phenobarbital (PheB), amobarbital (AB) and thiopental (TP), although syntheses and/or studies of the hypnotic activity of *N*-allyl derivatives of PheB and AB have already been reported.⁴⁻⁷⁾

In order to evaluate the antagonistic or potentiating properties of these allyl compounds and to compare the results with those obtained previously,¹⁻³⁾ we further examined the pharmacological activities of allyl derivatives of PheB, AB and TP. For this purpose, three kinds of barbiturates having an ethyl group and a phenyl, 3-methylbutyl or 1-methylbutyl group at the 5-position, and having =O or =S at the 2-position of the barbiturate ring were chosen.

TABLE I. *N*- (or *S*)-Allyl-Substituted Derivatives of Barbiturates

Compd.	R ₁	R ₂	X	Yield (%)	mp (°C) (Lit.)	Recryst. solvent	Formula	Analysis (%)			IR $\nu_{\text{max}}^{\text{KBr}}$ (cm ⁻¹)	¹ H-NMR (in CDCl ₃) δ
								Calcd (Found)				
								C	H	N		
MAPheB		H	O	73	68—70 (68—69) ^{a)}	MeOH:H ₂ O (1:1)	C ₁₅ H ₁₆ N ₂ O ₃	66.16 (66.15)	5.92 6.00	10.29 10.25	3120 (NH) 1690 (C=O)	4.58 (2H, d, <i>J</i> = 6 Hz, N-CH ₂ -), 5.04—5.44 (2H, m, =CH ₂), 5.72—6.06 (1H, m, -CH=)
DAPheB		CH ₂ =CHCH ₂ -	O	80	36—40 (40) ^{a)}	MeOH:H ₂ O (1:1)	C ₁₈ H ₂₀ N ₂ O ₃	69.21 (69.18)	6.45 6.55	8.97 8.95	1680 (C=O)	4.61 (4H, d, <i>J</i> = 6 Hz, (N-CH ₂) ₂), 5.18—5.45 (4H, m, (=CH ₂) ₂), 5.76—6.06 (2H, m, (-CH=) ₂)
MAAB	(CH ₃) ₂ C ₃ H ₅ -	H	O	68	37—40	MeOH:H ₂ O (1:1)	C ₁₄ H ₂₂ N ₂ O ₃	63.14 (63.08)	8.33 8.46	10.52 10.53	3250 (NH) 1685 (C=O)	4.57 (2H, d, <i>J</i> = 6 Hz, N-CH ₂ -), 5.23—5.54 (2H, m, =CH ₂), 5.74—6.07 (1H, m, -CH=)
DAAB	(CH ₃) ₂ C ₃ H ₅ -	CH ₂ =CHCH ₂ -	O	19	Oil ^{a)}		C ₁₇ H ₂₆ N ₂ O ₃	66.64 (66.59)	8.55 8.64	9.14 9.10	1690 (C=O)	4.61 (4H, d, <i>J</i> = 6 Hz, (N-CH ₂) ₂), 5.25—5.54 (4H, m, (=CH ₂) ₂), 5.87—6.20 (2H, m, (-CH=) ₂)
MATP	C ₃ H ₇ (CH ₃)CH-	H	S	7	Oil ^{a)}		C ₁₄ H ₂₂ N ₂ O ₂ S	59.55 (59.33)	7.85 7.93	9.92 9.79	3250 (NH) 1680 (C=O)	4.96 (2H, d, <i>J</i> = 6 Hz, N-CH ₂ -), 5.18—5.46 (2H, m, =CH ₂), 5.68—6.11 (1H, m, -CH=)
DATP	C ₃ H ₇ (CH ₃)CH-	CH ₂ =CHCH ₂ -	S	2	Oil ^{a)}		C ₁₇ H ₂₆ N ₂ O ₂ S	63.32 (63.41)	8.12 8.15	8.68 8.41	1690 (C=O)	5.04 (4H, d, <i>J</i> = 6 Hz, (N-CH ₂) ₂), 5.19—5.32 (4H, m, (=CH ₂) ₂), 5.68—6.10 (2H, m, (-CH=) ₂)
<i>N,S</i> -DATP ^{b)}				70	Oil ^{a)}		C ₁₇ H ₂₆ N ₂ O ₂ S	63.32 (63.00)	8.12 8.17	8.68 8.69	1680 (C=O)	3.92 (2H, d, <i>J</i> = 7 Hz, S-CH ₂ -), 4.54 (2H, d, <i>J</i> = 6 Hz, N-CH ₂ -), 5.14—5.48 (4H, m, (=CH ₂) ₂), 5.62—6.15 (2H, m, (-CH=) ₂)

a) Oily compounds were purified by column chromatography on silica gel.

b) This novel compound has the following structure:

Results

1) Pharmacological Activity

Analytical data for *N*- (or *S*-) allyl barbiturates synthesized for the studies are listed on Table I. Table II summarizes the pharmacological activities of *N*- (or *S*-) allyl-substituted derivatives. *N*-Monoallyl-substituted derivatives of PheB, AB and TP (MAPheB, MAAB and MATP) possessed some hypnotic and anticonvulsant activities. However, the activities of these *N*-monoallyl compounds were less potent than those of the parent compounds. MATP had the most potent anticonvulsant activity among these allyl compounds. *N,S*-Diallyl-thiopental (*N,S*-DATP) possessed some hypnotic activity, although the compound did not show any anticonvulsant activity even at 250 mg/kg, i.p. On the other hand, *N,N'*-diallyl-substituted derivatives of PheB, AB and TP (DAPheB, DAAB and DATP) exhibited neither hypnotic nor anticonvulsant activity. The acute toxicities of these allyl compounds were lower than those of the corresponding parent compounds.

2) Interaction

2.1) MAPheB and DAPheB—The time course (1, 15, 30 and 60 min) of the effect of pretreatment with MAPheB and DAPheB (80 mg/kg, i.p.) on the PheB (150 mg/kg, i.p.)-induced sleeping time was examined. The peak effects of MAPheB and DAPheB on the PheB-

TABLE II. Pharmacological Activities of *N*- (or *S*-) Allyl-Substituted Derivatives of Barbiturates

Compd.	HD ₅₀ (mg/kg, i.p.)	PTZ-ED ₅₀ (mg/kg, i.p.)	LD ₅₀ (mg/kg, i.p.)
PheB	96.3 (77.0—120) ^{a)}	19.2 (13.8—26.6)	226 (213—240)
MAPheB	186 (173—200)	62.0 (51.0—75.3)	488 (456—522)
DAPheB	None (1400) ^{b)}	> 250	1270 (1207—1336)
AB	67.0 (53.4—84.1)	17.5 (15.6—19.6)	185 (171—200)
MAAB	176 (147—210)	75.5 (62.1—91.8)	650 (620—682)
DAAB	None (1200)	> 250	855 (770—949)
TP	53.0 (42.7—65.7)	7.10 (4.73—10.7)	193 (183—203)
MATP	107 (84.7—135)	29.1 (24.2—35.0)	> 320
DATP	None (240)	> 160	> 240
<i>N,S</i> -DATP	275 (215—351)	> 250	575 (501—660)

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

TABLE III. Effects of MAPheB and DAPheB on Barbiturate-Induced Sleep

Barbiturate	Sleeping time (min)		
	Control	MAPheB 80 mg/kg, i.p.	DAPheB 80 mg/kg, i.p.
B (300 mg/kg, i.p.)	32 ± 6 (9)	63 ± 6 ^{b)} (12)	51 ± 6 ^{a)} (11)
PheB (150 mg/kg, i.p.)	76 ± 18 (8)	233 ± 24 ^{b)} (10)	146 ± 34 (8)
AB (100 mg/kg, i.p.)	82 ± 7 (8)	555 ± 56 ^{b)} (8)	324 ± 26 ^{b)} (7)
PB (40 mg/kg, i.p.)	21 ± 3 (8)	259 ± 29 ^{b)} (8)	253 ± 36 ^{b)} (8)
TP (50 mg/kg, i.p.)	9 ± 2 (7)	87 ± 29 ^{a)} (8)	15 ± 3 (7)

MAPheB and DAPheB were administered 15 min prior to the injection of barbiturates. 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 ($p < 0.01$).

Fig. 1. Dose-Response Curves for Effect of MAPheB and DAPheB on the PheB-Induced Sleep

MAPheB and DAPheB were administered i.p. 15 min prior to the i.p. injection of PheB. The control group was pretreated with 1% Tween 80-saline (vehicle). Each point corresponds to the mean sleeping time of 8 mice. Vertical bars indicate S.E. of the mean.

●—●, MAPheB + PheB 180 mg/kg; ●—●, MAPheB + PheB 150 mg/kg; ●—●, MAPheB + PheB 120 mg/kg; ○—○, DAPheB + PheB 180 mg/kg; ○—○, DAPheB + PheB 150 mg/kg; ○—○, DAPheB + PheB 120 mg/kg.

Fig. 2. Dose-Response Curves for Effect of MAAB and DAAB on the AB-Induced Sleep

MAAB and DAAB were administered i.p. 15 min prior to the i.p. injection of AB. The control group was pretreated with 1% Tween 80-saline (vehicle). Each point corresponds to the mean sleeping time of 8 mice. Vertical bars indicate S.E. of the mean.

●—●, MAAB + AB 100 mg/kg; ●—●, MAAB + AB 80 mg/kg; ●—●, MAAB + AB 60 mg/kg; ○—○, DAAB + AB 100 mg/kg; ○—○, DAAB + AB 80 mg/kg; ○—○, DAAB + AB 60 mg/kg.

TABLE IV. Effects of MAAB and DAAB on Barbiturate-Induced Sleep

Barbiturate	Sleeping time (min)		
	Control	MAAB 80 mg/kg, i.p.	DAAB 80 mg/kg, i.p.
B (300 mg/kg, i.p.)	32 ± 6 (9)	39 ± 8 (8)	103 ± 29 ^a (11)
PheB (150 mg/kg, i.p.)	61 ± 9 (6)	1573 ± 61 ^b (5)	405 ± 95 ^a (6)
AB (100 mg/kg, i.p.)	79 ± 7 (8)	317 ± 33 ^b (8)	784 ± 72 ^b (8)
PB (40 mg/kg, i.p.)	13 ± 2 (7)	110 ± 21 ^b (8)	97 ± 21 ^b (8)
TP (50 mg/kg, i.p.)	4 ± 1 (5)	11 ± 1 ^b (8)	10 ± 2 (8)

MAAB and DAAB were administered 15 min prior to the injection of barbiturates. 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 ($p < 0.01$).

induced sleep were obtained at 15–30 min, as reported previously.^{1,2}) As shown in Table III, MAPheB (80 mg/kg, i.p.) markedly prolonged the barbiturate-induced sleeping time in all cases. DAPheB (80 mg/kg, i.p.) also significantly prolonged the B- ($p < 0.05$), AB- ($p < 0.01$) and PB- ($p < 0.01$) induced sleeping times, but not that of PheB or TP. Potentiation of the PB-induced sleep by DAPheB was about the same as that of MAPheB, although the effect of DAPheB on the AB-induced sleeping time was only about half of that by MAPheB. Figure 1 shows the dose-response relationship for the effects of MAPheB and DAPheB on the parent compound (PheB)-induced sleep. MAPheB and DAPheB (20, 40 and 80 mg/kg, i.p.) prolonged the PheB (120, 150 and 180 mg/kg, i.p.)-induced sleeping time. Potentiation by

TABLE V. Effects of MATP, DATP and *N,S*-DATP on the PB-Induced Sleep

Compd.	Dose (mg/kg, i.p.)	Sleeping time (min)
Control		34 ± 9 (5)
MATP	80	423 ± 43 ^{b)} (6)
DATP	80	438 ± 102 ^{a)} (6)
<i>N,S</i> -DATP	80	663 ± 45 ^{b)} (6)

MATP, DATP and *N,S*-DATP were administered 15 min prior to the 40 mg/kg, i.p. injection of PB. 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 ($p < 0.01$).

both compounds of the PheB-induced sleep was dose-dependent. When 150 or 180 mg/kg, i.p. of PheB was given, MAPheB exhibited a more potent prolonging effect than DAPheB.

2.2) MAAB and DAAB—The peak effects of MAAB and DAAB on the AB-induced sleep were at 30 and 15 min, respectively. As shown in Table IV, MAAB significantly ($p < 0.01$) prolonged the barbiturate-induced sleeping times, except in the case of B. In particular, MAAB potentiated the PheB-induced sleep 25-fold over the control level. DAAB also exhibited a prolonging effect on the barbiturate-induced sleep, except in the case of TP. DAAB was more potent than MAAB in potentiating B- and AB-induced sleeping times. Figure 2 shows the dose-response relationship (5, 20 and 40 mg/kg, i.p.) for the effects of MAAB and DAAB on the AB (60, 80 and 100 mg/kg, i.p.)-induced sleep. Potentiation of the AB-induced sleep by MAAB was evident at doses lower than HD_{50} of MAAB. The slope of the plot for the DAAB-treated group is steeper than that in the case of the MAAB-treated group. Potentiation of the AB-induced sleeping time by DAAB was stronger than that by MAAB.

2.3) MATP, DATP and *N,S*-DATP—Table V shows the interactions of MATP, DATP and *N,S*-DATP with PB. MATP and DATP significantly ($p < 0.01$ and $p < 0.05$, respectively) prolonged the PB-induced sleeping time 12-fold and 13-fold, respectively, as compared with the control. The novel compound, *N,S*-DATP, also markedly prolonged the PB-induced sleeping time 20-fold. Potentiation of the PB-induced sleep by DATP was as effective as that by MATP.

Discussion

MAPheB, MAAB, MATP, and *N,S*-DATP exhibited some hypnotic activity, although these derivatives were less active than the parent barbiturates. MAPheB, MAAB and MATP also showed some anticonvulsant activity. It has been reported that monomethoxymethyl derivatives of PheB possess potent anticonvulsant activity⁸⁾ and *N*-monoallyl-B has potent hypnotic activity.²⁾ However, the present study indicated that introduction of an allyl group into the barbiturate ring resulted in a decrease of the depressant effect of the parent compound. The results were the same as in the case of PB reported previously.¹⁾ DAPheB, DAAB and DATP did not show hypnotic and anticonvulsant activities. The acute toxicities (LD_{50} 's) of allyl compounds were lower than those of the corresponding parent compound.

MAPheB, which has some hypnotic activity, prolonged the sleeping times induced by all barbiturates. Among all allyl derivatives of barbiturates tested, MAAB had the most potent prolonging effect on the PheB-induced sleep. However, the compound did not show any prolonging effect on the B-induced sleep. The reason for this difference is not clear. B and PheB have been classified as long-acting barbiturates. However, each barbiturate may have a

different mechanism of action. On the other hand, DAPheB potentiated the B-induced sleeping time, as well as the AB- and PB-induced sleep, and DAAB also showed potentiation of the B-, PheB- and PB-induced sleep. Further, MAPheB, DAPheB, MAAB and DAAB dose-dependently potentiated the parent compound (PheB or AB)-induced sleeping time. The prolonging effect of DAAB on the AB-induced sleep was stronger than that of MAAB. Therefore, it is suggested that DAPheB and DAAB possess some depressant effect on the central nervous system. DAAB did not attenuate the B-induced sleep, while *N,N'*-diallylpentobarbital antagonized the B-induced sleep.¹⁾ The difference in the effect of the *N,N'*-diallyl derivatives on the B-induced sleep indicates that the position of the methyl group on the methylbutyl side chain of AB and PB may affect the antagonistic action against B. Allyl derivatives of TP markedly prolonged the PB-induced sleeping time. DATP and MATP showed a similar effect on the PB-induced sleep. The central depressant effect of MATP and *N,S*-DATP is responsible for their prolonging effect on the PB-induced sleep. It has been reported that *N*-methyl-TP has a very high lipid/water partition coefficient⁹⁾ and that the concentration of *N*-methyl-TP in fat was 40 times the plasma concentration at 6 h after administration in dogs.¹⁰⁾ TP can bind to serum albumin more strongly than comparable barbiturates and will partially displace the latter from serum albumin.¹¹⁾ Allyl derivatives of TP, which have higher lipid solubility, may affect the binding of PB to serum albumin, so that the concentration of free PB in plasma increases, leading to a higher concentration of PB in the brain.

In conclusion, the results of the present study indicate that allyl derivatives of PheB, AB and TP have different pharmacological activities and that diallyl derivatives of these barbiturates, as well as the *N*-monoallylbarbiturates, potentiate the barbiturate-induced sleep *via* their central depressant effect.

Experimental

Materials—Animals: Male ddN strain mice weighing 22–28 g were used. Animals were housed under a normal light-dark cycle (7:00–19:00) at ambient temperature ($23 \pm 1^\circ\text{C}$). Food and water were given *ad lib*.

Chemicals: Preparations of allyl compounds were carried out according to the method described in our previous paper.³⁾ Sodium PheB (Linasen, Daiichi Seiyaku Co., Ltd.), AB (Isomytal, Nippon Shinyaku Co., Ltd.), sodium TP (Ravonal, Tanabe Seiyaku Co., Ltd.), sodium B (E. Merck A G.), sodium PB (Nembutal, Abbott Laboratories) and pentylenetetrazol (PTZ) (K & K Laboratories) were used. All allyl compounds and AB were suspended in 1% Tween 80–saline solution and the other drugs used were dissolved in saline. Allyl compounds and barbiturates were administered intraperitoneally (i.p.) to mice.

Animal Experiments—Measurement of Sleeping Time: Sleeping time was considered to be the interval between loss and recovery of an effective righting reflex. An effective righting reflex was considered to be recovery from a side position within 1 min.

Hypnotic Activity [50% Hypnotic Dose (HD_{50}): Hypnotic activity was determined as the loss of righting reflex regardless of the duration of the effect. The number of mice that slept was recorded for each dose, and the dose required to induce sleep in 50% of the animals (HD_{50} with 95% confidence limits) was determined.

Anticonvulsant 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, by using a modification of the method described by Vida *et al.*⁸⁾ Compounds were administered 20 min prior to the subcutaneous injection of PTZ, 120 mg/kg. The blocking of tonic-extensor convulsion was considered to be evidence of anticonvulsant activity.

Acute Toxicity [50% Lethal Dose (LD_{50}): The LD_{50} values of the compounds tested were also determined. Mortality was observed for a period of 3 d.

Interaction with Barbiturates: Allyl derivatives were injected 15 min prior to the administration of barbiturates. The effects of MAPheB, DAPheB, MAAB and DAAB (80 mg/kg, i.p.) on the sleep induced by several barbiturates (B, PheB, AB, PB and TP) were studied. Control groups received the vehicle, 1% Tween 80–saline solution. Dose-response experiments on the effects of MAPheB and DAPheB (20, 40 and 80 mg/kg, i.p.) on the PheB (120, 150 and 180 mg/kg, i.p.)-induced sleeping time were carried out. In the experiments on the interaction of MAAB and DAAB with AB, the allyl compound (5, 20 and 40 mg/kg, i.p.) was administered prior to the injection of AB (60, 80 and 100 mg/kg, i.p.). The effects of MATP, DATP and *N,S*-DATP (80 mg/kg, i.p.) on the PB (40 mg/kg, i.p.)-induced

sleep were also evaluated.

Statistical Analysis— HD_{50} , $PTZ-ED_{50}$, LD_{50} and their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.¹²⁾ Statistical significance was analyzed by using Student's *t*-test [a), $p < 0.05$; b) $p < 0.01$].

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