# Synthesis and Pharmacological Activities in Mice of Halogenated $\Delta^9$-Tetrahydrocannabinol Derivatives

Noriyuki USAMI,[a] Kiyofumi KOBANA,[a] Hisatoshi YOSHIDA,[a] Toshiyuki KIMURA,[a] Kazuhito WATANABE,[a] Hidetoshi YOSHIMURA,[b] and Ikuo YAMAMOTO*,[a]

*Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University,*[a] *3-Ho Kanagawa-machi, Kanazawa 920–1181, Japan and Department of Food and Nutrition, Nakamura Gakuen University,*[b] *Fukuoka 814–0198, Japan.* Received March 2, 1998; accepted May 26, 1998

**Seven halogenated derivatives of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC, 1) substituted on the aromatic ring at the 2 and/or 4 position, 2 (4)-fluoro- (2), 2,4-difluoro- (3), 2-chloro- (4), 2-bromo- (5), 2,4-dibromo- (6), 2-iodo- (7) and 2,4-diiodo-$\Delta^9$-THC (8) were synthesized and pharmacological effects such as catalepsy, anticonvulsant effects, hypothermia, pentobarbital-induced sleep prolongation and locomotor activity evaluated by intracerebroventricular (i.c.v., 25 $\mu$g/head) and intravenous (i.v., 5 or 10 mg/kg) injections in mice. The cataleptogenic effects of 2 and 5 were about three-quarters and two-thirds, respectively, compared to those of 1 (i.v.), though other derivatives were much less active (i.c.v. and i.v.). 2 (for clonic seizures) exhibited a significant prolongation of seizure latency induced by pentylenetetrazol (i.v.). Hypothermic effects of monohalogenated derivatives were comparable to 1 when administered by i.v. injection, whereas the effects of dihalogenated derivatives of 1 were attenuated. In contrast, 3 and 8 exhibited a significant hyperthermic effect in mice. In synergy with pentobarbital, 4 and 5 exhibited a significant prolongation of sleeping time by 1.6- and 1.8-fold, respectively, compared with control (32.4±2.5 min), although other derivatives did not affect significantly the sleeping time (i.c.v.). However, by i.v. injection, 2, 4, 5 and 7 significantly prolonged pentobarbital-induced sleeping time and reduced locomotor activity. The sleep prolonging effects of 2, 4 and 7 (10 mg/kg, i.v.) were as potent as that of 1 (5 mg/kg, i.v.). 5 and 7 were the most potent derivatives among the synthetic cannabinoids examined in the present study. These results indicate that halogenation of 1 leads to modification of the pharmacological profile of THC.**

**Key words** $\Delta^9$-tetrahydrocannabinol; halogenated tetrahydrocannabinol; catalepsy; 2-bromo-$\Delta^9$-tetrahydrocannabinol; hypothermia; pentobarbital-induced sleep prolongation

Numerous cannabinoids[1,2] including $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC, **1**), a major psychoactive constituent of *Cannabis sativa* L. and its preparations (marihuana, hashish and ganja), have shown therapeutic activities[3] as well as psychotropic properties. Recently, these compounds have regained attention due to the discovery of a cannabinoid receptor in mammalian brain,[4] its subsequent cloning[5] and the isolation of anandamide as an endogenous ligand from brain[6] and spleen[7] that binds to the receptor. A new field of research may evolve from these findings and open the way for new therapeutic compounds.

Cannabinoids are known to be $C_{21}$ compounds composed of only C, H and O,[8] and possess unique pharmacological activities such as hypothermia, catalepsy, analgesic, antiemetic effects and so on in various animal species including humans.[9] Many investigators have demonstrated that some of these effects are useful in clinical medicine, although it is difficult to separate the valuable effects from undesirable side effects.[1] To attain this aim, it is necessary to further accumulate knowledge on the structure–activity relationships of cannabinoids as agonists or antagonists for the receptor.

Charalambous *et al.*[10] and Martin *et al.*[11] reported pharmacological effects of halogenated derivatives of $\Delta^8$- and $\Delta^9$-THC, although a systematic evaluation of the pharmacological effects of halogenated THC was not carried out. A previous study in our laboratory demonstrated that halogenated derivatives of cannabinol (CBN) exhibited cannabimimetic activity to some extent.[12] In the present study, we wish to report the synthesis and pharmacological activities in mice of aromatic ring halogenated derivatives of **1** (Table 1).

## Experimental

**1** and cannabidiol (CBD) were isolated and purified from cannabis leaves according to the method of Aramaki *et al.*[13] The purities of cannabinoid used were determined to be at least 98% by gas chromatography (GC). 18-Crown-6, *m*-chloroperbenzoic acid (MCPBA) and *N*-fluoro-3,5-dichloropyridinium triflate were obtained from Wako Pure Chemical Ind. (Osaka, Japan). All other chemical reagents and solvents used were of analytical reagent grade.

Proton and carbon nuclear magnetic resonance ($^1$H- and $^{13}$C-NMR) spectra were recorded on a JEOL JNM-GSX400 Fourier transform (FT)-NMR spectrometer using tetramethylsilane as an internal standard. $^1$H- and $^{13}$C-NMR chemical shift assignments were based on those of **1** reported previously.[14] The following abbreviations are used: s=singlet, d=doublet, t=triplet and m=multiplet. The mass spectra (MS) were recorded on a JEOL JMS-SX-102A mass spectrometer.

**General Halogenation Procedure** For the synthesis of halogenated derivatives, except for **2**, we followed a method described for halogenated resorcinols.[15] A suspension prepared from the potassium halide (50 mmol), 18-crown-6 (264 mg, 1 mmol), and **1** (10 mmol) in methylene dichloride ($CH_2Cl_2$, 35 ml) was stirred well at room temperature. It was then cooled to 0 °C and, with stirring, was treated with a solution of 80% MCPBA (2.59 g, 12 mmol) in $CH_2Cl_2$ (20 ml) over 5 min. The reaction mixture was then vigorously stirred for an additional 15 min and diethyl ether (70 ml) was added. The solution was successively washed with a 10% aqueous reducing solution (sodium hydrogen sulphite), 10% aqueous sodium hydrogen carbonate, and 10% aqueous sodium chloride. The diethyl ether solution was dried over anhydrous $Na_2SO_4$, and evaporated *in vacuo*. The oily residue was chromatographed on a silica gel column with petroleum ether–diethyl ether (95 : 5 or 90 : 10) and purities examined by GC.

The synthesis of **2** was carried out by using CBD instead of **1**, according to the method of Umemoto *et al.*[16] A solution of CBD (256 mg, 0.82 mmol) and *N*-fluoro-3,5-dichloropyridinium triflate (250 mg, 0.79 mmol) in $CH_2Cl_2$ (10 ml) was refluxed for 6 h. The reaction mixture was then extracted with diethyl ether. The diethyl ether solution was dried over anhydrous $Na_2SO_4$, and evaporated *in vacuo*. The oily residue was chromatographed on a silica gel column with petroleum ether–diethyl ether (95 : 5 or 90 : 10) to afford **2** as a mixture of isomers (2- and 4-fluoro-$\Delta^9$-THC) on the basis of NMR

* To whom correspondence should be addressed.

analysis.

**2 (4)-Fluoro-$\Delta^9$-THC (2) and 2,4-Difluoro-$\Delta^9$-THC (3)** **2**: 110 mg, 41% yield, as a brown oil. MS *m/z*: 332 ($M^+$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.88 (3H, t, $J$=7.0 Hz, $C_{5'}$-$CH_3$), 1.07 (3H, s, $C_{6\alpha}$-$CH_3$), 1.33 (4H, m, $C_{3'}$, $C_{4'}$-$CH_2$), 1.37 (1H, m, $C_{7\alpha}$-H), 1.40 (3H, s, $C_{6\beta}$-$CH_3$), 1.57 (2H, m, $C_{2'}$-$CH_2$), 1.69 (3H, s, $C_9$-$CH_3$), 1.92 (1H, m, $C_{7\beta}$-H), 2.15 (2H, m, $C_{8\alpha}$-H, $C_{8\beta}$-H), 2.57 (2H, t, $C_{1'}$-$CH_2$), 3.25 (1H, d, $J$=11.0 Hz, $C_{10a}$-H), 6.17/6.19 (1H, s, $C_2$-H/$C_4$-H), 6.39 (1H, d, $C_{10}$-H).

**3**: 83 mg, 23% yield, as a yellow oil. MS *m/z*: 350 ($M^+$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.89 (3H, t, $J$=7.0 Hz, $C_{5'}$-$CH_3$), 1.07 (3H, s, $C_{6\alpha}$-$CH_3$), 1.34 (4H, m, $C_{3'}$, $C_{4'}$-$CH_2$), 1.37 (1H, m, $C_{7\alpha}$-H), 1.40 (3H, s, $C_{6\beta}$-$CH_3$), 1.57 (2H, m, $C_{2'}$-$CH_2$), 1.69 (3H, s, $C_9$-$CH_3$), 1.92 (1H, m, $C_{7\beta}$-H), 2.15 (2H, m, $C_{8\alpha}$-H, $C_{8\beta}$-H), 2.52 (2H, t, $C_{1'}$-$CH_2$), 3.25 (1H, d, $J$=11.0 Hz, $C_{10a}$-H), 6.39 (1H, d, $C_{10}$-H).

**2-Chloro-$\Delta^9$-THC (4)** **4**: 23 mg, 14% yield, as a yellow oil. MS *m/z*: 348 ($M^+$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.89 (3H, t, $J$=7.0 Hz, $C_{5'}$-$CH_3$), 1.07 (3H, s, $C_{6\alpha}$-$CH_3$), 1.34 (4H, m, $C_{3'}$, $C_{4'}$-$CH_2$), 1.39 (1H, m, $C_{7\alpha}$-H), 1.40 (3H, s, $C_{6\beta}$-$CH_3$), 1.57 (2H, m, $C_{2'}$-$CH_2$), 1.68 (3H, s, $C_9$-$CH_3$), 1.91 (1H, m, $C_{7\beta}$-H), 2.16 (2H, m, $C_{8\alpha}$-H, $C_{8\beta}$-H), 2.58 (2H, t, $C_{1'}$-$CH_2$), 3.26 (1H, d, $J$=11.0 Hz, $C_{10a}$-H), 6.31 (1H, s, $C_4$-H), 6.36 (1H, d, $C_{10}$-H).

**2-Bromo-$\Delta^9$-THC (5) and 2,4-Dibromo-$\Delta^9$-THC (6)** **5**: 39 mg, 10% yield, as a brown oil. MS *m/z*: 392 ($M^+$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.90 (3H, t, $J$=7.0 Hz, $C_{5'}$-$CH_3$), 1.08 (3H, s, $C_{6\alpha}$-$CH_3$), 1.35 (4H, m, $C_{3'}$, $C_{4'}$-$CH_2$), 1.37 (1H, m, $C_{7\alpha}$-H), 1.41 (3H, s, $C_{6\beta}$-$CH_3$), 1.57 (2H, m, $C_{2'}$-$CH_2$), 1.68 (3H, s, $C_9$-$CH_3$), 1.90 (1H, m, $C_{7\beta}$-H), 2.15 (2H, m, $C_{8\alpha}$-H, $C_{8\beta}$-H), 2.62 (2H, t, $C_{1'}$-$CH_2$), 3.27 (1H, d, $J$=11.0 Hz, $C_{10a}$-H), 5.98 (1H, s, $C_4$-H), 6.34 (1H, d, $C_{10}$-H).

**6**: 41 mg, 19% yield, as a brown oil. MS *m/z*: 472 ($M^+$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.89 (3H, t, $J$=7.0 Hz, $C_{5'}$-$CH_3$), 1.08 (3H, s, $C_{6\alpha}$-$CH_3$), 1.33 (4H, m, $C_{3'}$, $C_{4'}$-$CH_2$), 1.37 (1H, m, $C_{7\alpha}$-H), 1.41 (3H, s, $C_{6\beta}$-$CH_3$), 1.57 (2H, m, $C_{2'}$-$CH_2$), 1.68 (3H, s, $C_9$-$CH_3$), 1.90 (1H, m, $C_{7\beta}$-H), 2.15 (2H, m, $C_{8\alpha}$-H, $C_{8\beta}$-H), 2.57 (2H, t, $C_{1'}$-$CH_2$), 3.27 (1H, d, $J$=11.0 Hz, $C_{10a}$-H), 6.34 (1H, d, $C_{10}$-H).

**2-Monoiodo-$\Delta^9$-THC (7) and 2,4-Diiodo-$\Delta^9$-THC (8)** **7**: 345 mg, 13% yield, as a brown oil. MS *m/z*: 440 ($M^+$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.90 (3H, t, $J$=7.0 Hz, $C_{5'}$-$CH_3$), 1.08 (3H, s, $C_{6\alpha}$-$CH_3$), 1.35 (4H, m, $C_{3'}$, $C_{4'}$-$CH_2$), 1.40 (1H, m, $C_{7\alpha}$-H), 1.41 (3H, s, $C_{6\beta}$-$CH_3$), 1.58 (2H, m, $C_{2'}$-$CH_2$), 1.68 (3H, s, $C_9$-$CH_3$), 1.93 (1H, m, $C_{7\beta}$-H), 2.15 (2H, m, $C_{8\alpha}$-H, $C_{8\beta}$-H), 2.59 (2H, t, $C_{1'}$-$CH_2$), 3.23 (1H, d, J=11.0 Hz, $C_{10a}$-H), 5.67 (1H, s, $C_4$-H), 6.31 (1H, d, $C_{10}$-H).

**8**: 41 mg, 4% yield, as a brown oil. MS *m/z*: 566 ($M^+$). $^1$H-NMR ($CDCl_3$) $\delta$: 0.89 (3H, t, $J$=7.0 Hz, $C_{5'}$-$CH_3$), 1.08 (3H, s, $C_{6\alpha}$-$CH_3$), 1.34 (4H, m, $C_{3'}$, $C_{4'}$-$CH_2$), 1.40 (1H, m, $C_{7\alpha}$-H), 1.41 (3H, s, $C_{6\beta}$-$CH_3$), 1.58 (2H, m, $C_{2'}$-$CH_2$), 1.68 (3H, s, $C_9$-$CH_3$), 1.93 (1H, m, $C_{7\beta}$-H), 2.15 (2H, m, $C_{8\alpha}$-H, $C_{8\beta}$-H), 2.54 (2H, t, $C_{1'}$-$CH_2$), 3.23 (1H, d, $J$=11.0 Hz, $C_{10a}$-H), 6.31 (1H, d, $C_{10}$-H).

**Animals and Drugs** Male ddY mice weighing 20.0—25.0 g were used in pharmacological experiments. All derivatives were suspended in physiological saline containing 1% Tween 80 and administered by intracerebroventricular injection (i.c.v.) at a dose of 25 $\mu$g/head to assess potency in the central nervous system or by intravenous injection (i.v.). Sodium pentobarbital and pentylenetetrazol (PTZ) were purchased from Tokyo Kasei Kogyo Co., Ltd. and Mallinckroft Chem. Works, respectively, and dissolved in physiological saline. All animal experiments were carried out at an ambient temperature of 22—24 °C.

**Catalepsy** Each group of 10 mice was injected with **1** or the halogenated derivatives (25 $\mu$g/head, i.c.v. or 5 mg/kg, i.v.). The cataleptogenic effect was assessed by the simple bar test, as previously described, 20 min after injection.[17]

**Anticonvulsant Effect against PTZ-Induced Seizures** PTZ (120 mg/kg, subcutaneous, s.c.) was injected in the mouse 20 min after the 25 $\mu$g/head, i.c.v. or 10 mg/kg, i.v. injection of the cannabinoids or the vehicle. The latency for clonic and tonic seizures was recorded.[18]

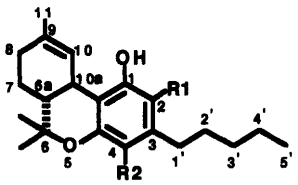
**Hypothermia** **1** and its derivatives (25 $\mu$g/head, i.c.v. or 20 mg/kg, i.v.) were injected into each group of 10 mice. The control mice received vehicle only. The rectal temperature of mice was measured by a thermistor thermometer (Natsume Seisakusho Co., Ltd.) just before administration and for 120 min after injection of the cannabinoids as previously described.[19] The mean initial body temperature of mice in each group was 38.02 to 38.22 °C.

**Pentobarbital-Induced Sleep Prolongation**[20] Each group of 10 mice was injected with **1** and its halogenated derivatives (25 $\mu$g/head, i.c.v. or 5 and 10 mg/kg, i.v.) or the vehicle. Sodium pentobarbital (40 mg/kg, intraperitoneal, i.p.) was injected at 20 min after injection of the cannabinoids. The loss of righting reflex was used as an index of sleep. Control mice were injected with the vehicle instead of cannabinoids.

**Locomotor Activity**[21] Animals were injected with **1** and its halogenated derivatives (25 $\mu$g/head, i.c.v. or 10 mg/kg, i.v.) in groups of 10 or 14 mice, and locomotor activities, consisting of horizontal (total distance moved) and vertical activity (numbers of rearing) were measured with a Muromachi animal behavior analyzer equipped with an NEC PC-9801 RX microcomputer (Muromachi Ind., Tokyo, Japan). The control mice received vehicle only. The total distance (cm) and numbers of rearing movements of mice were then measured every 15 min using a Muromachi BTA-1 equipment for 60 min.

**Statistical Analyses** The statistical significance of variation in data was calculated by Bonferroni multiple-comparison test using one way analysis of

Table 1. Structures of Cannabinoid Derivatives




| | | $R^1$ | $R^2$ | MS (*m/z*) | $^1$H-NMR $\delta$ (in $CDCl_3$) ppm | $^{13}$C-NMR $\delta$ (in $CDCl_3$) ppm |
|---|---|---|---|---|---|---|
| **1** | $\Delta^9$-THC | H | H | 314 ($M^+$) | 6.14 (C-2, s)<br>6.35 (C-4, s) | 108.0 (C-4, d)<br>110.0 (C-2, d) |
| **2** | 2(4)-F-$\Delta^9$-THC[a] | F (H) | H (F) | 332 ($M^+$) | 6.17, 6.18 (C-2, d)<br>6.39 (C-4, s) | 108.9 (C-4, d)<br>110.5 (C-2, d) |
| **3** | 2,4-Di-F-$\Delta^9$-THC | F | F | 350 ($M^+$) | — | — |
| **4** | 2-Cl-$\Delta^9$-THC | Cl | H | 348 ($M^+$) | 6.31 (C-4, s) | 110.6 (C-4, d)<br>111.3 (C-2, s) |
| **5** | 2-Br-$\Delta^9$-THC | Br | H | 392 ($M^+$) | 6.34 (C-4, s) | 110.2 (C-4, d)<br>111.8 (C-2, s) |
| **6** | 2,4-Di-Br-$\Delta^9$-THC | Br | Br | 472 ($M^+$) | — | — |
| **7** | 2-I-$\Delta^9$-THC | I | H | 440 ($M^+$) | 6.38 (C-4, s) | 111.0 (C-4, d)<br>111.6 (C-2, s) |
| **8** | 2,4-Di-I-$\Delta^9$-THC | I | I | 566 ($M^+$) | — | — |

*a*) Mixture of isomers.

variance (ANOVA).

## Results

**Synthesis of Halogenated Derivatives** Structures, MS and NMR of the halogenated derivatives of **1** synthesized in the present study are listed in Table 1. **3**, **4**, **5**, **6**, **7** and **8** were prepared according to the method of Srebnik *et al.*[15b] The proton at the 2-position of **1** ($\delta$ 6.41 ppm) disappeared in the $^1$H-NMR spectra of monohalogenated derivatives. The two protons at the 2- and 4-positions of **1** ($\delta$ 6.41 and 6.35 ppm) disappeared in the $^1$H-NMR spectra of dihalogenated derivatives. The MS and NMR spectra of the halogenated derivatives support the assigned structures. **2** was synthesized from CBD using *N*-fluoro-3,5-dichloropyridinium triflate. The conversion of CBD to $\Delta^9$-THC has been reported by Razdan *et al.* using $BF_3$ as a catalyst.[22] The same mechanism may occur in this case with *N*-fluoro-3,5-dichloropyridinium triflate. However, **2** was obtained as a mixture of 2- and 4-fluoro-$\Delta^9$-THC, as shown by the NMR spectrum, and these isomers were not separable in the present study.

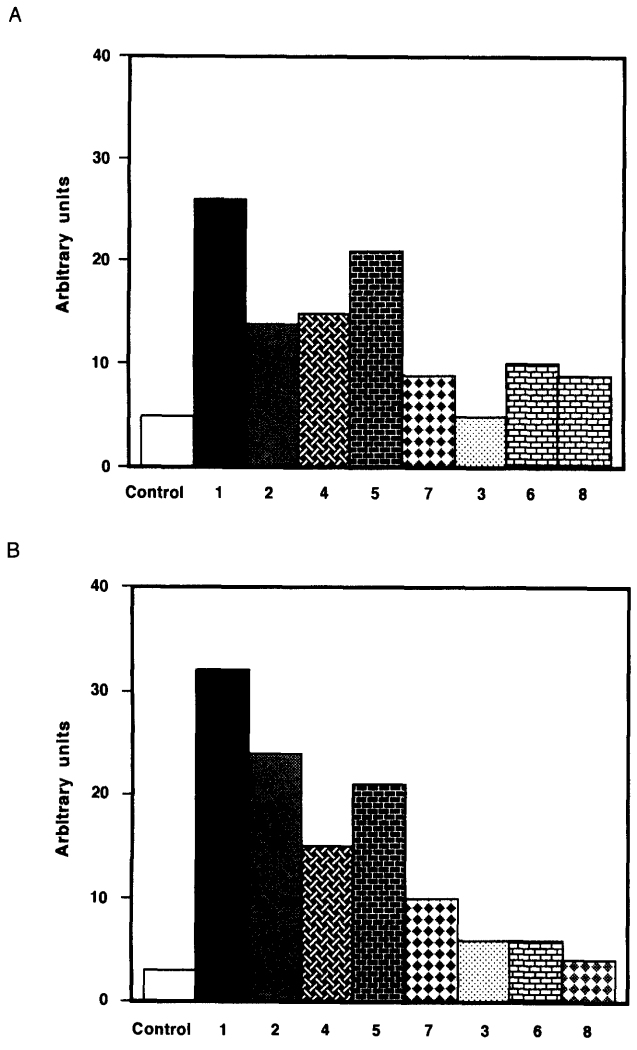



Fig. 1. Cataleptogenic Effects of $\Delta^9$-THC and Its Halogenated Derivatives in Mice by I.c.v. and I.v. Injection

Arbitrary units were calculated as follows; mice showing catalepsy lasting more than 60 s were regarded as positive (5 units), for 46 to 60 s as quasipositive (4 units), for 31 to 45 s as quasipositive (3 units) for 16 to 30 s as quasipositive (2 units) for 1 to 15 s as quasipositive (1 unit) and for less than 1 s as negative (0 unit). A, monohalogenated $\Delta^9$-THCs (25 $\mu$g/head, i.c.v.); B, monohalogenated $\Delta^9$-THCs (5 mg/kg, i.v.). Each group consisted of 10 mice. **1**, $\Delta^9$-THC; **2**, 2(4)-F-$\Delta^9$-THC; **3**, 2,4-Di-F-$\Delta^9$-THC; **4**, 2-Cl-$\Delta^9$-THC; **5**, 2-Br-$\Delta^9$-THC; **6**, 2,4-Di-Br-$\Delta^9$-THC; **7**, 2-I-$\Delta^9$-THC; **8**, 2,4-Di-I-$\Delta^9$-THC. **2** was a mixture of isomers.

**Pharmacological Effects** Catalepsy: The cataleptogenic effects of halogenated derivatives of **1** are shown in Fig. 1. **5** had a potency about 20—35% lower than that of **1**. In the i.c.v. injection, 50% of mice treated with **5** (25 $\mu$g/head) showed a cataleptogenic effect of more than 30 s. Moreover, in the i.v. injection, 20% of mice treated with **5** (5 mg/kg) showed a cataleptogenic effect of more than 30 s. **2** and **4** exhibited cataleptogenic effects to some extent, whereas the other halogenated derivatives were much less active.

Anticonvulsant: As summarized in Table 2, **5** tended to prolong seizure latency against both the clonic and tonic seizures induced by PTZ, and exhibited higher protection effect than **1**. In the prolongation of seizure latency against both clonic and tonic convulsions, **5** (25 $\mu$g/head, i.c.v.) has about 1.6- and 1.5-fold greater potency than that of **1**, respectively. Moreover, **5** (10 mg/kg, i.v.) had about 1.4- and 1.2-fold greater potency than **1** in the prolongation of seizure latency induced by PTZ. Furthermore, **2** exhibited a significant prolongation of seizure latency induced by PTZ by i.v. injection. Other derivatives did not exhibit any significant protection against PTZ induced seizures.

Hypothermia: The hypothermic effects of halogenated derivatives of **1** are shown in Fig. 2. By i.v. injection, **2**, **4**, **5** and **7** caused a significant hypothermic effect in mice, and their effects were comparable to that of **1**, while **3** and **8** displayed a significant hyperthermic effect (Fig. 2-C and D). **2**, **4**, **5** and **7** caused maximal hypothermic effects of $-1.37\pm0.12$ (30 min), $-1.37\pm0.13$ (45 min), $-1.56\pm0.20$ (60 min) and $-1.95\pm0.11$ °C (90 min) in mice, respectively. The effects of **5** and **7** were comparable to that of **1**, and **2** and **4** retained the hypothermic effect. By i.c.v. injection, **5** showed a significant hypothermic effect and **3** exhibited a significant hyperthermic effect (Fig. 2-A and B). **5** induced a maximal hypothermic effect of $-2.49\pm0.18$ °C (30 min) in mice and

Table 2 Anticonvulsant Effects of $\Delta^9$-THC and Its Halogenated Derivatives in Mice by I.c.v. and I.v. Injection

| | Treatment | Number of animals | Latent period (s, mean±S.E.) | |
|---|---|---|---|---|
| | | | Clonic seizures | Tonic seizure |
| | I.c.v. | | | |
| | Control | 20 | 273± 66 | 499± 88 |
| **1** | $\Delta^9$-THC | 20 | 299± 47 | 494± 63 |
| **2** | 2(4)-F-$\Delta^9$-THC[a] | 10 | 290± 50 | 290± 50 |
| **4** | 2-Cl-$\Delta^9$-THC | 10 | 394± 65 | 418± 59 |
| **5** | 2-Br-$\Delta^9$-THC | 10 | 483±152 | 734±155 |
| **7** | 2-I-$\Delta^9$-THC | 10 | 255± 56 | 469± 71 |
| **3** | 2,4-Di-F-$\Delta^9$-THC | 10 | 329± 75 | 471± 98 |
| **6** | 2,4-Di-Br-$\Delta^9$-THC | 10 | 244± 48 | 347± 76 |
| **8** | 2,4-Di-I-$\Delta^9$-THC | 10 | 393±112 | 440±108 |
| | I.v. | | | |
| | Control | 20 | 185± 24 | 439± 51 |
| **1** | $\Delta^9$-THC | 20 | 211± 31 | 471± 36 |
| **2** | 2(4)-F-$\Delta^9$-THC[a] | 10 | 336± 66[b] | 606± 46 |
| **4** | 2-Cl-$\Delta^9$-THC | 10 | 287± 34 | 686±157 |
| **5** | 2-Br-$\Delta^9$-THC | 10 | 303± 52 | 551± 61 |
| **7** | 2-I-$\Delta^9$-THC | 10 | 165± 34 | 459± 44 |
| **3** | 2,4-Di-F-$\Delta^9$-THC | 10 | 141± 17 | 317± 61 |
| **6** | 2,4-Di-Br-$\Delta^9$-THC | 10 | 121± 23 | 274± 32 |
| **8** | 2,4-Di-I-$\Delta^9$-THC | 10 | 117± 12 | 289± 63 |

*a*) Mixture of isomers. Each group consisted of 10 mice. *b*) significantly different from control ($p<0.05$).

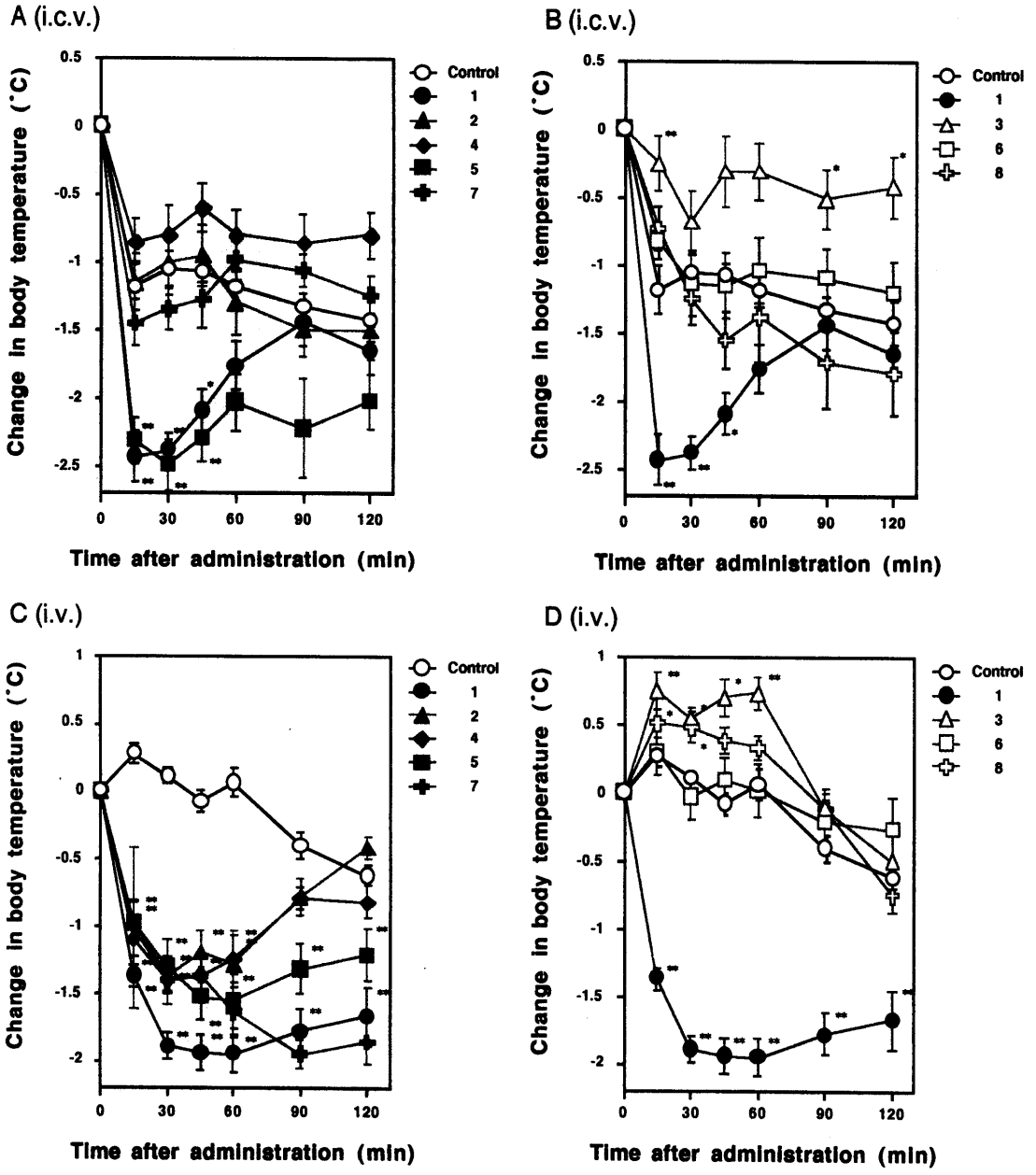



Fig. 2. Hypothermic Effects of $\Delta^9$-THC and Its Halogenated Derivatives in Mice by I.c.v. and I.v. Injection

Hypothermia indicates the difference in body temperature of mice from that just before the injection. A, monohalogenated $\Delta^9$-THCs (25 $\mu$g/head, i.c.v.); B, dihalogenated $\Delta^9$-THC (25 $\mu$g/head, i.c.v.); C, monohalogenated $\Delta^9$-THCs (20 mg/kg, i.v.); D, dihalogenated $\Delta^9$-THC (20 mg/kg, i.v.). Each group consisted of 10 mice. **1**, $\Delta^9$-THC; **2**, 2(4)-F-$\Delta^9$-THC; **3**, 2,4-Di-F-$\Delta^9$-THC; **4**, 2-Cl-$\Delta^9$-THC; **5**, 2-Br-$\Delta^9$-THC; **6**, 2,4-Di-Br-$\Delta^9$-THC; **7**, 2-I-$\Delta^9$-THC; **8**, 2,4-Di-I-$\Delta^9$-THC. **2** was a mixture of isomers. * significantly different from control ($p<0.05$). ** significantly different from control ($p<0.01$).

was comparable to that of **1**.

Pentobarbital-Induced Sleep Prolongation: The effects of the halogenated derivatives on pentobarbital-induced sleep are shown in Fig. 3. I.c.v. injection (25 $\mu$g/head) of **4** (50.6±6.1 min) and **5** (57.3±3.8 min) significantly prolonged pentobarbital-induced sleeping time. However, the other derivatives did not cause a significant prolongation of sleeping time (Fig. 3-A). On the other hand, all monohalogenated derivatives of **1** dose-dependently prolonged pentobarbital-induced sleeping time by i.v. injection (Fig. 3-B). With a dose of 10 mg/kg, the sleeping times of mice treated with **2**, **4**, **5** and **7** were 47.7±3.0, 57.9±4.8, 94.1±7.7 and 56.7±2.7 min, respectively, compared to the control group with 18.4±2.0 min.

Locomotor Activity: Locomotor activities were analyzed separately into vertical and horizontal components (Fig. 4). Fig. 4-A and C show the horizontal activity, measuring the total distance of the animal's movement. In this index, as well as for the vertical activity, the time period for recording the mouse behavior was divided into short terms of every 15 min for 60 min and the last terms for each component were for the animals returning to normal activity. For both activities (Fig. 4), a significant suppression was observed upon injection of **1**, **5** and **7**. During the first 15 min, horizontal and vertical activities for the groups treated with **5** and **7** by i.v. injection were 776±271 and 866±349 cm (total distances) and 0.3±0.2 and 6.6±4.3 (numbers of rearing), respectively (Fig. 4-C and D). These results were significantly lower than those of the control group (total distance: 2199±331 cm; numbers of rearing: 46.6±4.8).

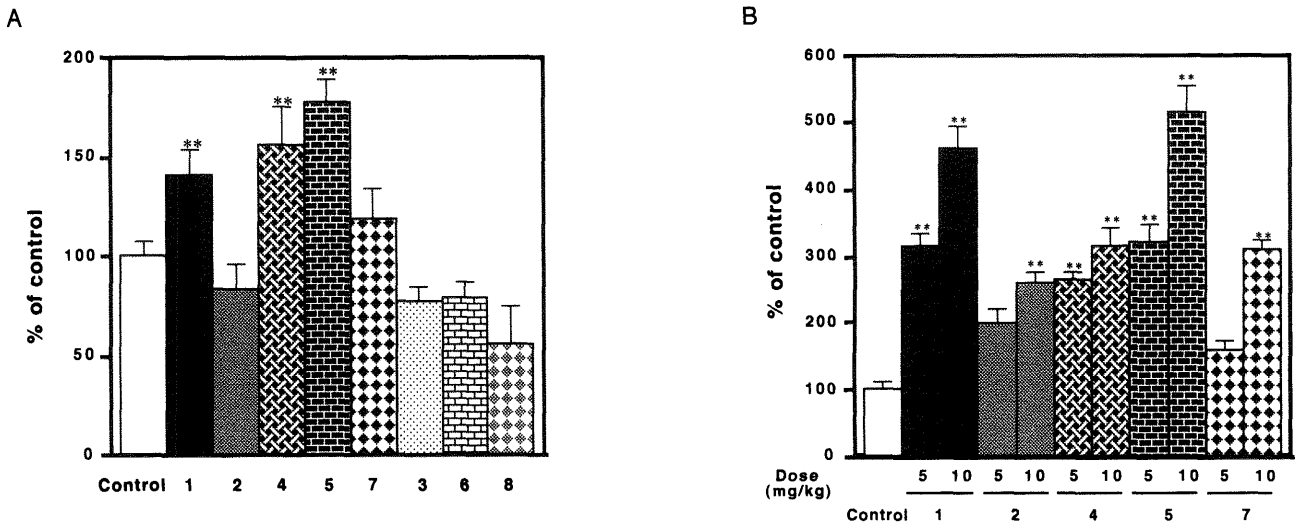



Fig. 3. Effects of $\Delta^9$-THC and Its Halogenated Derivatives on Pentobarbital-Induced Sleeping Time in Mice by I.c.v. and I.v. Injection

Pentobarbital was injected to mice i.p. at 40 mg/kg 20 min after the injection of the cannabinoids. A, monohalogenated $\Delta^9$-THCs (25 $\mu$g/head, i.c.v.); B, monohalogenated $\Delta^9$-THCs (5 or 10 mg/kg, i.v.). The mean sleeping time in control mice was 32.4±2.5 (i.c.v.) or 18.4±2.0 (i.v.) min, respectively. Each group consisted of 10 mice. **1**, $\Delta^9$-THC; **2**, 2(4)-F-$\Delta^9$-THC; **3**, 2,4-Di-F-$\Delta^9$-THC; **4**, 2-Cl-$\Delta^9$-THC; **5**, 2-Br-$\Delta^9$-THC; **6**, 2,4-Di-Br-$\Delta^9$-THC; **7**, 2-I-$\Delta^9$-THC; **8**, 2,4-Di-I-$\Delta^9$-THC. **2** was a mixture of isomers. ** significantly different from control ($p<0.01$).

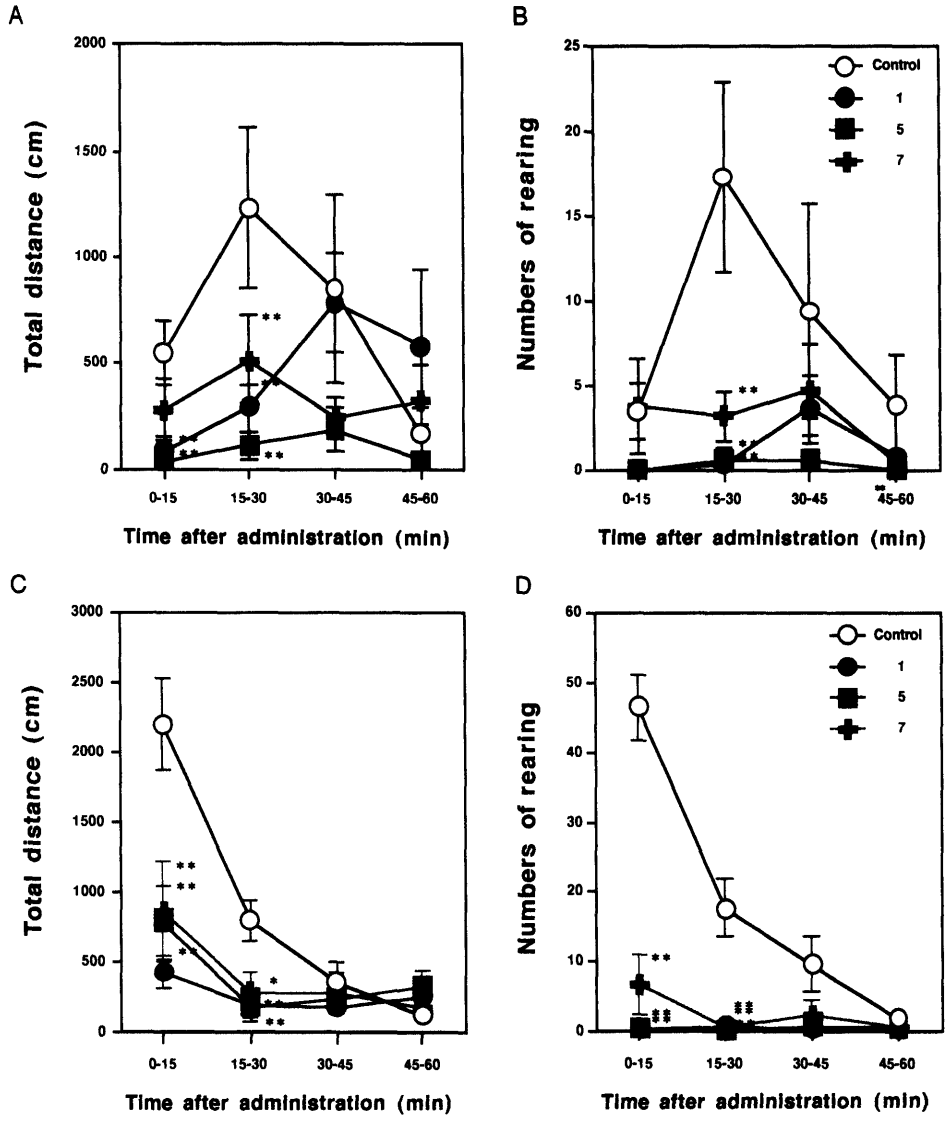



Fig. 4. Effects of $\Delta^9$-THC and Its Monohalogenated Derivatives on Locomotor Activity of Mice by I.c.v. and I.v. Injection

A, total distance (cm) (25 $\mu$g/head, i.c.v.); B, number of rearing (25 $\mu$g/head, i.c.v.); C, total distance (cm) (10 mg/kg, i.v.); D, number of rearing (10 mg/kg, i.v.). Each group consisted of 10 (i.c.v.) or 14 (i.v.) mice. **1**, $\Delta^9$-THC; **5**, 2-Br-$\Delta^9$-THC; **7**, 2-I-$\Delta^9$-THC. * significantly different from control ($p<0.05$). ** significantly different from control ($p<0.01$).

## Discussion

**1** has been considered to be responsible for most of the psychotropic effects of marihuana. CBN is another related cannabinoid that has been reported to be inactive, on the basis of animal and human experiments, although some workers have described marginal activity.[23,24] We reported that a nitrogen-containing CBN derivative[21] and halogenated CBN[12] had some psychotropic activities in mice. We expect that structural modification of cannabinoids may increase pharmacological activities. In fact, 2-chloro-CBN exhibited a prolongation of sleeping time induced by pentobarbital and cataleptogenic effects greater than those of 4-chloro-CBN.[12]

Martin *et al.*[11] reported enhanced and decreased potencies for 2-iodo-$\Delta^8$-THC and 4-bromo-$\Delta^8$-THC, respectively, when examining pharmacological effects such as rectal temperature, analgesia, spontaneous activity and immobility index. Some of the cannabinoids studied here had already been analyzed by computer modeling as part of their structure–activity relationships during the search for non-psychotropic therapeutic derivatives.[25] A major advancement in this field was achieved when a therapeutic cannabinoid, devoid of THC-like psychotropic effects was discovered.[26] These authors suggested the importance of the electron density map of THC for interaction with the cannabinoid receptor.

In the present study, we synthesized seven halogenated derivatives of **1** and their pharmacological activities in mice were compared with those of **1** using catalepsy, hypothermia, pentobarbital-induced sleep prolongation, anticonvulsant effect and locomotor activity as indices for systematic evaluation. All of the halogenated derivatives, except for **2**, were successfully synthesized by halogenation of **1** with potassium halides and 18-crown-6 in methylene chloride. **2** was synthesized from CBD with *N*-fluoro-3,5-dichloropyridinium triflate.

Monohalogenated derivatives (particularly **5** and **7**) exhibited a hypothermic effect as potent as that of **1**, whereas dihalogenated derivatives had lower potency than **1**. Differences observed in the hypothermic effects of halogenated derivatives between i.c.v. and i.v. administration may be due to metabolic activation of these derivatives by the i.v. route. Cataleptogenic effect of **2** was as high as that of **1** (i.v.). **2** was also significantly more potent than **1** in anticonvulsant effect against PTZ-induced clonic and tonic seizures (i.c.v.). Monohalogenated derivatives significantly prolonged pentobarbital-induced sleeping time by i.v. injection, while **4** and **5** also prolonged significantly the sleeping time by i.c.v. injection. The order of potency for halogenated derivatives was slightly different for the hypothermic effect and sleep prolongation. Moreover, **5** and **7** reduced the locomotor activity of mice.

These results indicate that the pharmacological effects of monohalogenated derivatives of **1**, such as **5** and **7** were comparable to those of **1** by i.v. injection, whereas dihalogenated derivatives were much less active. The present study suggests that the pharmacological effects of **1** may be separable by suitable modification of the cannabinoid structure, and that electronegativity of a substituent group on the aromatic ring may be an important factor for exhibiting pharmacological effects.

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