Synthesis and Pharmacological Effects in Mice of Halogenated Cannabinol Derivatives

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Eight halogenated drivatives of cannabinol (CBN) substituted on the aromatic ring at the 2 and/or 4 position were synthesized and their pharmacological effects were evaluated by intracerebroventricular injection (50 μg/mouse) in mice, using hypothermia, pentobarbital-induced sleep prolongation, catalepsy and anticonvulsant effect as indices. The hypothermic effects of monohalogenated drivatives of CBN were comparable to that of CBN, whereas the effects of dihalogenated derivatives of CBN except for the fluorinated derivative were attenuated. In the interaction with pentobarbital, two monochlorinated derivatives exhibited a significant prolongation of sleeping time, although other derivatives did not significantly affect the sleeping time. The cataleptogenic effects of monofluoro- and 4-bromo-CBN were stronger than that of CBN. 4-Bromo-CBN exhibited a significant prolongation of seizure latency induced by pentylenetetrazol. These data suggest that halogenation of CBN modifies the pharmacological profile of the cannabinoid.

Key words hypothermia; catalepsy; anticonvulsant; cannabinol: 4-bromo-cannabinol; halogenated cannabinol

Cannabinoids are C_{21} compounds composed of only C, H and O,[1] and possess many pharmacological activities, such as inducing hypothermia, catalepsy, and analgesia and having antiemetic activity in various animal species including humans.[2] It is rather difficult to separate potentially therapeutic effects from undesirable side effects.[3] Very recently the existence of cannabinoid receptors has been reported in the spleen[4] as well as in the brain.[5] Thus, knowledge is required of the structure–activity relationship of cannabinoids with respect to the receptor.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) has been considered to be responsible for most of the pyschotropic effects of marihuana. Another cannabinoid, cannabinol (CBN, **1**), has been reported to be inactive on the basis of animal and human experiments, although some workers observed marginal activity of CBN.[6,7] Yamamoto *et al.*[8] reported that 11-hydroxy-CBN, a major metabolite of CBN, was pharmacologically more potent than cannabinol.

Narimatsu *et al.*[9] reported that a nitrogen-containing CBN derivative had some psychotropic activity in mice. We anticipated that structural changes of cannabinoids might increase the pharmacological activities or alter the profile of them. Charalambous *et al.*[10] and Martin *et al.*[11] have reported pharmacological effects of halogenated derivatives of Δ^8- and Δ^9-THC. In the present study, we wish to report the synthesis and pharmacological activities in mice of CBN derivatives halogenated on the aromatic ring.

Experimental

The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-PMX 60 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s = singlet, br = broad. The mass spectra (MS) were recorded on a JEOL JMS-DX300 mass spectrometer.

THC was purified from cannabis leaves by the method of Aramaki *et al.*[12] THC was oxidized to CBN (**1**) by sulfur.[13] CBN derivatives were synthesized as described below. The purity of the cannabinoids was determined to be greater than 95% by gas chromatography.

Synthesis of Monofluoro-CBN (2) and 2,4-Difluoro-CBN (3) A solution of **1** (326 mg, 1.05 mmol) and *N*-fluoro-3,5-dichloropyridinium triflate (336 mg, 1.06 mmol) in CH_2Cl_2 (10 ml) was heated under reflux for 5 h. The reaction mixture was extracted with ether. The ether solution was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The oily residue was chromatographed on a silica gel column with benzene–*n*-hexane–diethylamine (25 : 10 : 1) to afford **2** (178 mg, 73.8%) and **3** (20 mg, 7.9%) as yellow oils. Compound **2** was a mixture of isomers (2- and 4-fluoro-CBN) on the basis of its NMR spectrum.

2; HREIMS *m/z*: 328.1837 (M^+). Calcd for $C_{21}H_{25}FO_2$: 328.1832. ^{1}H-NMR ($CDCl_3$) δ: 1.60 (6H, s, C_6-$(CH_3)_2$), 2.40 (3H, s, C_9-H), 4.48 (1H, br, C_1-OH), 6.32 (1/2H, s, C_2-H), 6.44 (1/2H, s, C_4-H), 7.14 (2H, s, C_7-H and C_8-H), 8.30 (1H, s, C_{10}-H).

3; HREIMS *m/z*: 346.1743 (M^+). Calcd for $C_{21}H_{24}F_2O_2$: 346.1738. ^{1}H-NMR ($CDCl_3$) δ: 1.72 (6H, s, C_6-$(CH_3)_2$), 2.40 (3H, s, C_9-CH_3), 7.08 (2H, s, C_7-H and C_8-H), 8.40 (1H, br, C_{10}-H).

Synthesis of 2-Chloro-CBN (4), 4-Chloro-CBN (5) and 2,4-Dichloro-CBN (6) A solution of **1** (95 mg, 0.306 mmol) and *N*-chlorosuccinimide (NCS) (41 mg, 0.306 mmol) in methanol was stirred at room temperature for 24 h. The reaction mixture was extracted with ether. The ether solution was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The oily residue was chromatographed on silica gel with *n*-hexane–ethyl acetate (40 : 1) to afford **4** (186 mg, 64.6%) as a yellow oil and **5** (24 mg, 22.8%) as a brown oil. Compound **6** (186 mg, 64.6%) was obtained by stirring **1** (236 mg, 0.761 mmol) and NCS (201.9 mg, 1.51 mmol) in methanol at room temperature for 24 h.

4; HREIMS *m/z*: 344.1544 (M^+). Calcd for $C_{21}H_{25}ClO_2$: 344.1537. ^{1}H-NMR ($CDCl_3$) δ: 1.60 (6H, s, C_6-$(CH_3)_2$), 2.40 (3H, s, C_9-CH_3), 6.26 (1H, s, C_1-OH), 6.52 (1H, s, C_4-H), 7.10 (2H, s, C_7-H and C_8-H), 8.32 (1H, br, C_{10}-H).

5; HREIMS *m/z*: 344.1544 (M^+). Calcd for $C_{21}H_{25}ClO_2$: 344.1522. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (6H, s, C_6-$(CH_3)_2$), 2.35 (3H, s, C_9-CH_3), 5.25 (1H, br, C_1-OH), 6.36 (1H, s, C_2-H), 7.12 (2H, s, C_7-H and C_8-H), 8.14 (1H, br, C_{10}-H).

6; HREIMS *m/z*: 378.1148 (M^+). Calcd for $C_{21}H_{24}Cl_2O_2$: 378.1161. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (6H, s, C_6-$(CH_3)_2$), 2.38 (3H, s, C_9-CH_3), 6.23 (1H, br, C_1-OH), 7.13 (2H, br, C_7-H and C_8-H), 8.33 (1H, br, C_{10}-H).

Synthesis of 4-Bromo-CBN (7) and 2,4-Dibromo-CBN (8) A solution of **1** (150 mg, 0.484 mmol) and *N*-bromosuccinimide (86.3 mg, 0.485 mmol) in dimethylformamide (2.4 ml) was stirred at room temperature for 24 h. The reaction mixture was extracted with CH_2Cl_2 and the solu-

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tion was dried over anhydrous Na_2SO_4, then evaporated *in vacuo*. The oily residue was chromatographed on silica gel with benzene–*n*-hexane–diethylamine (25:10:1) to afford **7** (39 mg, 20.7%) and **8** (41 mg, 18.1%) as brown oils.

7; HREIMS *m/z*: 388.1031 (M^+). Calcd for $C_{21}H_{25}BrO_2$: 388.1040. ^{1}H-NMR ($CDCl_3$) δ: 1.59 (6H, s, C_6-$(CH_3)_2$), 2.36 (3H, s, C_9-CH_3), 5.69 (1H, br, C_1-OH), 6.48 (1H, s, C_2-H), 7.04 (2H, s, C_7-H and C_8-H), 8.25 (1H, br, C_{10}-H).

8; HREIMS *m/z*: 466.0136 (M^+). Calcd for $C_{21}H_{24}Br_2O_2$: 466.0132. ^{1}H-NMR ($CDCl_3$) δ: 1.60 (6H, s, C_6-$(CH_3)_2$), 2.35 (3H, s, C_9-H), 5.57 (1H, br, C_1-OH), 7.07 (2H, s, C_7-H and C_8-H), 8.24 (1H, br, C_{10}-H).

Synthesis of 2,4-Diiodo-CBN (9) A solution of **1** (215 mg, 0.694 mmol) and *N*-iodosuccinimide (168.7 mg, 0.750 mmol) was stirred at room temperature for 24 h. The reaction mixture was extracted with ether. The ether solution was dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The oily residue was chromatographed on silica gel with petroleum ether to afford **9** (345 mg, 88.5%) as a brown oil.

9; HREIMS *m/z*: 561.9860 (M^+). Calcd for $C_{21}H_{24}I_2O_2$: 561.9866. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (6H, s, C_6-$(CH_3)_2$), 2.40 (3H, s, C_9-CH_3), 6.09 (1H, br, C_1-OH), 7.09 (2H, s, C_7-H and C_8-H), 8.20 (1H, br, C_{10}-H).

Animals and Drug Male ddN mice weighing 19—25 g were used in groups each consisting of 10 mice. All CBN derivatives were suspended in physiological saline containing 1% Tween 80 and administered intracerebroventricularly (i.c.v.) at a dose of 50.0 μg/head to examine potency in the central nervous system. Sodium pentobarbital and PTZ were purchased from Tokyo Kasei Kogyo Co., Ltd. and Mallinckrodt Chem. Works, respectively, and dissolved in saline. All animal experiments were carried out in an ambient temperature of 22—24 °C.

Hypothermia Rectal temperature was measured as described previously,[14] for 120 min after injection of the cannabinoids.

Pentobarbital-Induced Sleep Prolongation Sodium pentobarbital (40 mg/kg, i.p.) was injected 15 min after injection of the cannabinoids. Loss of the righing reflex was used as an index of sleep.

Catalepsy Cataleptogenic effects were assessed by the simple bar test as described previously 10 min after injection of the cannabinoids.[9,15]

Anticonvulsant Effect Against PTZ-Induced Seizures PTZ (120 mg/kg, s.c.) was injected into mice 20 min after the i.c.v. injection of the cannabinoids or vehicle. The latency for clonic and tonic seizures was recorded.[16]

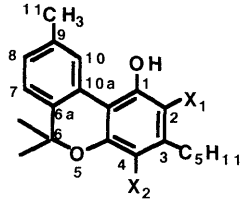
Statistical Analyses The statistical significance of differences was determined by means of the Bonferroni test.

Results and Discussion

Synthesis of Halogenated CBN Derivatives Structures of halogenated CBN derivatives synthesized in the present study are listed in Table I. Monofluoro-CBN (**2**) and 2,4-difluoro-CBN (**3**) were prepared by using *N*-fluoro-3,5-dichloropyridinium triflate.[17] However, **2** was a mixture of 2-fluoro-CBN and 4-fluoro-CBN from the ^{1}H-NMR spectrum and these products were not separable in the present study. 2-Chloro-CBN (**4**), 4-chloro-CBN (**5**) and 2,4-dichloro-CBN (**6**) were prepared by using *N*-chlorosuccinimide. 4-Bromo-CBN (**7**) and 2,4-dibromo-CBN (**8**) were prepared by using *N*-bromosuccinimide, whereas 2-bromo-CBN could not be obtained, presumably because of its instability. 2,4-Diiodo-CBN (**9**) was synthesized by the same method using *N*-iodosuccinimide, and although monoiodo-CBN was detected on thin layer chromatography, the product could not be isolated.

Pharmacological Effects The hypothermic effects of halogenated CBN derivatives are summarized in Table II. Fluorinated CBN derivatives (**2** and **3**) caused a maximal hypothermic effect of −2.3——2.5 °C in mice and their effects were comparable to that of **1**. Halogenated compounds, **4** and **5** retained the hypothermic effect, while **6** did not show any hypothermic effect at 50 μg/mouse, i.c.v. Compound **9** did not cause a significant hypothermic effect and compound **8** showed a slight hypothermic effect,

TABLE I. Structures of Halogenated CBN Derivatives



No.	X_1	X_2	MS (*m/z*)	^{1}H-NMR δ (in $CDCl_3$)
2[a]	F (H)	H (F)	328 (M^+)	6.52 (1H, s, C_2-H), 6.54 (1H, s, C_4-H)
3	F	F	346 (M^+)	8.40 (1H, br, C_{10}-H)
4	Cl	H	344 (M^+)	6.52 (1H, s, C_4-H)
5	H	Cl	344 (M^+)	6.36 (1H, s, C_2-H)
6	Cl	Cl	378 (M^+)	8.33 (1H, s, C_{10}-H)
7	H	Br	390 (M^+)	6.48 (1H, s, C_2-H)
8	Br	Br	460 (M^+)	8.24 (1H, br, C_{10}-H)
9	I	I	562 (M^+)	8.20 (1H, br, C_{10}-H)

a) Mixture of isomers.

TABLE II. Hypothermic Effects of Halogenated Derivatives of CBN

Cannabinoid	Maximal hypothermia (°C)
CBN (**1**)	−2.1±0.6 (30 min)[a]
Monofluoro-CBN (**2**)	−2.3±0.3 (45 min)
2,4-Difluoro-CBN (**3**)	−2.5±0.5 (30 min)
2-Chloro-CBN (**4**)	−1.5±0.8 (45 min)
4-Chloro-CBN (**5**)	−1.8±0.6 (45 min)
2,4-Dichloro-CBN (**6**)	−0.5±0.3 (30 min)
4-Bromo-CBN (**7**)	−1.7±0.4 (30 min)
2,4-Dibromo-CBN (**8**)	−0.8±0.7 (45 min)
2,4-Diiodo-CBN (**9**)	−0.1±0.5 (90 min)

Each group consisted of 10 mice. Hypothermia indicates the difference in body temperature of mice from that just before the injection (50 μg/mouse, i.c.v.). *a*) The time in parenthesis indicates the time of maximal hypothermia observed after injection of cannabinoids.

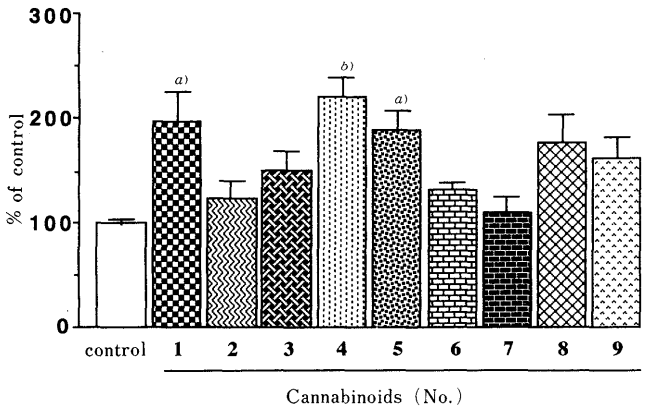


Fig. 1. Effects of CBN and Halogenated CBN Derivatives on Pentobarbital-Induced Sleeping Time

CBN and its derivatives (50 μg/mouse, i.c.v.) were administered 15 min prior to the injection of pentobarbital (40 mg/kg, i.p.). Each bar represents the mean±S.E. of 10 mice as % of the control. The mean sleeping time in control mice was 47.8±2.0 min. *a*) Significantly different from the control ($p<0.05$). *b*) Significantly different from the control ($p<0.01$).

whereas compound **7** was as potent as **1**.

The effects of halogenated CBN derivatives on pentobarbital-induced sleep are shown in Fig. 1. Compounds

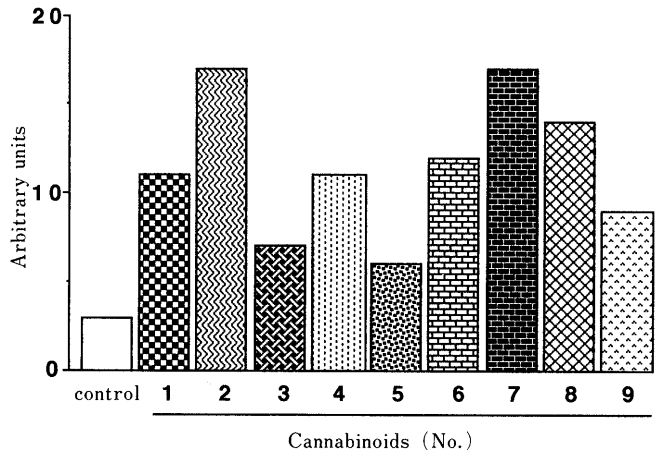


Fig. 2. Cataleptogenic Effects of CBN and Halogenated CBN Derivatives

CBN and its derivative were administered at a dose of 50 μg/mouse, i.c.v. Arbitrary units were calculated as follows; mice showing catalepsy lasting more than 15 s were regarded as positive (2 units), for 1 to 15 s as quasipositive (1 unit), and for less than 1 s as negative (0 units). Each group consisted of 10 mice.

TABLE III. Anticonvulsant Effects of CBN and 4-Bromo-CBN against PTZ-Induced Seizures

Treatment	Number of animals	Latent period (s, Mean±S.E.)	
		Clonic seizures	Tonic seizures
Control	10	87±15	247±43
1	10	103±14	322±17
7	10	168±34[a)]	484±73[b)]

a) Significantly different from control ($p<0.05$). *b*) Significantly different from control ($p<0.01$).

4 and **5** significantly prolonged pentobarbital-induced sleeping time. However, the other derivatives having hypothermic effects did not cause a significant prolongation of pentobarbital-induced sleeping time. These results suggest that the action sites of CBN derivatives for the hypothermic effect and the sleep prlongation may differ.

The cataleptogenic effects of halogenated CBN are shown in Fig. 2. Compounds **2** and **7** have a potency 1.6 times greater than that of **1**. Sixty percent of mice treated with **7** (50 μg/mouse) exhibited a cataleptogenic effect lasting more that 30 s.

As summarized in Table III, **7** showed apparent prolongation of seizure latency against both clonic and tonic convulsions induced by subcutaneous injection of pentylenetetrazol (PTZ) and afforded a higher protective effect than **1**.

Martin *et al.*[11] reported enhanced and decreased analgesic effects of 2-iodo-Δ^8-THC and 4-bromo-Δ^8-THC, respectively. In contrast, we found that 4-bromo-CBN exhibited a higher activity than CBN as regards the cataleptogenic and anticonvulsant effects. Recently, a cannabinoid receptor has been cloned[5] and characterized.[18] The importance of electron density distribution of THC for interaction with the cannabinoid receptor was suggested.

In conclusion, we synthesized eight CBNs halogenated on the aromatic ring and evaluated their pharmacological effects. The data suggest that some pharmacological effects of CBN could be separable by modification of the cannabinoid structure, and that the electronegativity of the substituted group on the aromatic ring may be an important factor in the interaction of the cannabinoid with the receptor for exhibiting the pharmacological effects.

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