Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: www.informahealthcare.com/journals/iphb20

Dihydromagnolol – a Potent Anxiolytic-like Agent in Mice

Hisashi Kuribara, Eiko Kishi, Masayuki Kimura, Susan T. Weintraub, Yoshihisa Ito & Yuji Maruyama

To cite this article: Hisashi Kuribara, Eiko Kishi, Masayuki Kimura, Susan T. Weintraub, Yoshihisa Ito & Yuji Maruyama (2003) Dihydromagnolol – a Potent Anxiolytic-like Agent in Mice, Pharmaceutical Biology, 41:1, 31-36, DOI: 10.1076/phbi.41.1.31.14702

To link to this article: https://doi.org/10.1076/phbi.41.1.31.14702

Published online: 29 Sep 2008.

Pharmaceutical Biology
2003, Vol. 41, No. 1, pp. 31–36

1388-0209/03/4101-031$16.00

Dihydromagnolol – a Potent Anxiolytic-like Agent in Mice

Hisashi Kuribara[1], Eiko Kishi[1], Masayuki Kimura[2], Susan T. Weintraub[3], Yoshihisa Ito[4] and Yuji Maruyama[1]

[1]Department of Neuropsychopharmacology (Tsumura), Gunma University School of Medicine, Gunma, Japan; [2]Central Research Laboratories, Tsumura & Co., Yoshiwara, Ibaraki, Japan; [3]Department of Biochemistry, The University of Texas Health Science Center at San Antonio, Texas, USA; and [4]Department of Pharmacology, College of Pharmacy, Nihon University, Funabashi, Chiba, Japan

Abstract

Oral administration of a single 1 mg/kg dose of dihydromagnolol [DHM; 5′-(2-propenyl)-5-propyl-(1,1′-biphenyl)-2,2′-diol] produced a significant anxiolytic-like effect in mice, as assessed in an elevated plus-maze test. This dose did not cause any marked change in ambulatory activity. The anxiolytic-like effect peaked at 1 h and persisted for more than 3 h after administration. Significant anxiolytic-like activity at a dose of 1 mg/kg of dihydromagnolol was also indicated by use of Vogel's conflict test. Although dihydromagnolol enhanced $^{36}Cl^-$ uptake into mouse cortical synaptoneurosomes in the absence of muscimol in *in vitro* experiments, it did not alter the activities of diazepam, flumazenil or bicuculline *in vivo*. The results of these studies suggest that the anxiolytic-like activity of DHM occurs through action at a site(s) in the nervous system other than the $GABA_A$/benzodiazepine receptor complex which is the site of action of the traditional benzodiazepine anxiolytics.

Keywords: Anxiolytic-like effect, $^{36}Cl^-$ uptake, dihydromagnolol, elevated plus-maze test, motor activity, Vogel's conflict test.

Introduction

It has previously been shown in this laboratory (Maruyama et al., 1998) that honokiol (Fig. 1), a substituted biphenyl isolated from magnolia bark (Fujita et al., 1973), exhibits anxiolytic-like activity in mice when examined in an elevated plus-maze test. A minimum oral dose of 20 mg/kg of honokiol was required for acute administration while 0.2 mg/kg was found to be effective after p.o. administration for 7 days. We subsequently discovered that a partially reduced analog of honokiol [DHH-B; 3′-(2-propenyl)-5-propyl-(1,1′-biphenyl)-2,4′-diol; Fig. 1] was approximately 100 times more effective as an acute anxiolytic agent than honokiol (Kuribara & Maruyama, 1996), and, furthermore, demonstrated that DHH-B did not cause any benzodiazepine-like side effects (Kuribara et al., 2000b).

Magnolol (Fig. 1), an isomer of honokiol, is also derived from magnolia bark [*Magnolia obovata* Thunberg (Magnoliaceae)] (Fujita et al., 1973). Magnolol exhibits only mild anxiolytic-like activity in mice following single or repeated treatment (Maruyama et al., 1998), although there are central depressant effects at high doses (Watanabe et al., 1975, 1983a,b). *In vivo* studies in rats suggested that a portion of the circulating magnolol becomes hydrogenated to tetrahydromagnolol [5,5′-dipropyl-(1,1′-biphenyl)-2,2′-diol] (Hattori et al., 1984, 1986). Since neither magnolol nor tetrahydromagnolol exhibits significant anxiolytic-like activity, we postulated that an intermediary metabolite such as dihydromagnolol [DHM; 5′-(2-propenyl)-5-propyl-(1,1′-biphenyl)-2,2′-diol; Fig. 1], generated from magnolol, might be responsible for the observed anti-anxiolythic-like effects. Analogous to our findings with DHH-B, we envisioned that DHM might likewise have enhanced anxiolytic-like efficacy as compared to the parent di-propenyl-biphenyl, magnolol. To test this possibility, we used both the elevated plus-maze (Kuribara & Maruyama, 1996; Kuribara et al., 1996) and Vogel's conflict test (Vogel et al., 1971) in mice to evaluate the acute efficacy of DHM. Furthermore, we studied the effects of DHM on $^{36}Cl^-$ uptake into mouse cortical synaptoneurosomes (Schwartz et al., 1986) to gain insight into the mechanism of action.

Accepted: April 30, 2002

Address correspondence to: Professor Yuji Maruyama, Center for Cooperative Research, Medical Division, Gunma University, 3-39-22 Showa-Machi, Maesbashi-shi Gunma, 371-8511 Japan. Tel.: +81 27 220 8115; Fax: +81 27 220 8116; E-mail: yuji@med.gunma-u.ac.jp

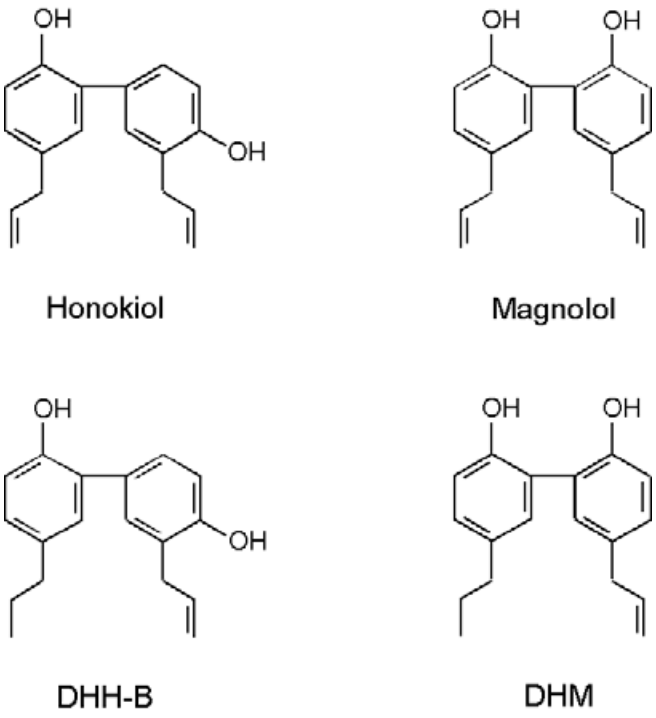

Figure 1. Chemical structures of honokiol, magnolol and partially reduced analogs DHH-B and DHM.

Materials and methods

Animals

Male ddY mice (7 weeks old, 33–36 g; Japan SLC, Hamamatsu, Japan) were used for all experiments. The mice were housed in randomly chosen groups of ten in polycarbonate cages (20 cm × 30 cm × 15 cm with woodchip bedding), with free access to a standard solid diet and tap water. The conditions of the animal room were controlled as follows: temperature, 23 ± 1 °C; relative humidity, 55 ± 3%; and a 12-h light-dark cycle, with lights turned on at 7:00 am). All experimental protocols were approved by The Committee of Animal Experiments in Gunma University School of Medicine and met "The Guideline for Animal Experimentation of the Japan Association of Laboratory Animal Science."

Drugs

Diazepam (Cercine Inj.) was purchased from Takeda Chemical Industries, Ltd. (Osaka, Japan) and bicuculline from Sigma Chemical Co. (St. Louis, MO, USA). Flumazenil was a generous gift from Hoffman-La Roche (Nutley, NJ, USA). DHM and DHH-B were synthesized as previously described (Kuribara et al., 2000a).

DHM and DHH-B were first dissolved in 50 µl of ethanol and then diluted with physiological saline (0.9% aqueous NaCl) containing 0.1% Tween-80, so that the final ethanol concentration in the drug solution was 0.5%. The injectable preparation of diazepam was diluted with physiological saline. Flumazenil and bicuculline were suspended in Tween-80/physiological saline. The concentration of each drug solution or suspension was adjusted so that the volume administered was 0.1 ml/10 g mouse body weight. Animals in the control groups received vehicle alone. In separate experiments, it was confirmed that none of the components of the vehicles influenced either the behavioral or the biochemical tests.

Apparatus and experimental procedures

Elevated plus-maze test

The elevated plus-maze used in this study was a modified version of the original apparatus described for mice (Lister, 1987). The design of the elevated plus-maze has been presented in detail in previous publications from this laboratory (Kuribara & Maruyama, 1996; Kuribara et al., 1996).

For testing on the elevated plus-maze, a mouse was placed on the central platform facing one of the closed-sided arms, and the cumulative time spent in the open-sided arms during the ensuing 5-min observation period was recorded by a trained observer. A mouse was considered to have entered an open-sided arm if all four paws crossed the border between the central platform and the open-sided arm.

Activity test

Immediately after the end of the plus-maze test, the motor activity of each mouse was measured for 5 min using a tilting ambulometer (SMA-1; O'Hara & Co., Tokyo, Japan).

Vogel's conflict test

Vogel's conflict test in mice (Kuribara et al., 1989; Umezu, 1999), which was a slight modification of the original method used for rats (Vogel et al., 1971), was carried out using a Plexiglas chamber (13 cm × 9 cm × 9 cm), fitted with control and recording units (models VC-3002-L and VC-2050-L, respectively) produced by O'Hara & Co. (Tokyo, Japan). The following test protocol was employed. A mouse that had been deprived of water for 24 h was placed in a Plexiglas chamber and allowed free intake of water from a spout for 30 min. The mouse was then returned to its regular cage without access to water. On the following day, the mouse was placed in the same chamber with access to the water spout. However, during this stage, the mouse was allowed to drink water for 30 min but received an electric foot shock (45 V, 0.2 mA, 50 Hz AC, for 0.3 sec) delivered through the stainless steel floor grid of the chamber for each 0.05 ml intake of water. The number of shocks delivered during the 30 min experimental period was recorded.

Measurement of $^{36}Cl^-$ uptake into cortical synaptoneurosomes

The effect of DHM on $^{36}Cl^-$ uptake into mouse cortical synaptoneurosomes was evaluated according to the method

described elsewhere (Ito et al., 1992, 1995, 1996). Briefly, mouse cerebral cortices were homogenized in 5 vol of ice-cold Krebs-Henseleit buffer (pH 7.4). The homogenates were diluted with 20 vol of the same buffer and then filtered through three layers of nylon cloth and a 10 µm Millipore filter (LCP 047: Millipore Corp., Bedford, MA, USA). The filtrates were centrifuged at 1000 ×g for 15 min and the resulting pellets suspended in an ice-cold solution containing 118 mM NaCl, 4.7 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 20 mM HEPES-Tris (pH 7.4, Buffer-A). Aliquots of the synaptoneurosomal fraction (1.5–1.8 mg protein) were preincubated at 30 °C for 30 min and then the test solution was added. In agreement with earlier studies (Ito et al., 1996), maximal $^{36}Cl^-$ uptake was observed following an incubation period of 3 sec at 30 °C. The reaction was terminated by addition of 0.1 mM picrotoxin in 5 ml ice-cold Buffer-A followed by rapid filtration under vacuum through a Whatman GF/C glass-fiber filter pretreated with 0.05% polyethyleneimine. The filters were washed with two 5 ml portions of the same buffer. Radioactivity trapped on the filters was then assessed using a scintillation counter. Protein concentrations were determined according to the method of Lowry et al. (1951).

Statistical analysis

Statistical significance was assessed though use of a one- or two-way analysis of variance (ANOVA), as indicated, followed by a Student-Neuman Keuls test. Values of P less than 0.05 were considered significant.

Results

Acute anxiolytic-like activity

The results for evaluation of anxiolytic-like activity by means of the elevated plus-maze test are shown in Table 1 where it can be seen that analogous to diazepam and DHH-B, DHM was effective after a single oral dose without causing marked changes in motor activity. Table 2 shows the time course of anxiolytic-like activity following p.o. and i.p. administration of DHM. The activity of DHM was maximal at 1 h and persisted for at least 3 h following p.o. administration. In contrast, after i.p. administration the anxiolytic-like effect of DHM peaked at 30 min and returned to basal levels by 2 h. A similar pattern was observed for p.o. administration of DHH-B, although peak activity occurred later for DHH-B (3 h) than for DHM (Kuribara et al., 2000a). As shown in Table 3, when Vogel's conflict test was employed, significant anxiolytic-like activity was indicated after p.o. administration of 1 and 5 mg/kg DHM as for 1 mg/kg diazepam.

Combined drug effects

The effects of combining DHM and diazepam with flumazenil and bicuculline can be seen in Table 4. When diazepam

Table 1. Anxiolytic-like activity of honokiol and magnolol analogs after acute oral administration, evaluated by the elevated plus-maze test.

Treatment[a]		Time in Open-sided Arms (sec)[b]	Activity (counts/5 min)[b]
Drug	N		
DHM			
Control	30	3.9 ± 0.9	34.1 ± 1.7
0.5 mg/kg	10	9.9 ± 2.7	28.3 ± 2.7
1.0 mg/kg	20	19.9 ± 3.6*	28.0 ± 1.8
DHH-B			
Control	22	7.8 ± 1.7	32.0 ± 3.8
0.5 mg/kg	20	16.9 ± 4.6	40.2 ± 5.4
1.0 mg/kg	20	23.6 ± 5.8**	35.4 ± 2.9
Diazepam			
Control	10	9.7 ± 2.8	48.4 ± 6.3
1.0 mg/kg	10	37.5 ± 7.5**	36.6 ± 3.5

[a] All drugs were administered orally at the following times before evaluation on the elevated plus-maze: DHM, 1 h; DHH-B, 3 h; diazepam, 10 min; animals in control groups received only the corresponding vehicle; N, number of mice per test group.
[b] Values represent the mean ± standard error of the mean; statistical significance compared to the corresponding vehicle-treated group was assessed by a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test: * $P < 0.05$; ** $P < 0.01$.

Table 2. Time course for anxiolytic-like activity of dihydromagnolol (DHM).

Time after Treatment (hr)	Time in Open-sided Arms (sec)[a]	
	Control[b]	DHM[b]
p.o. administration		
0.5	5.8 ± 2.5	11.0 ± 2.0
1.0	7.8 ± 2.1	23.9 ± 5.6*
2.0	6.0 ± 2.2	14.5 ± 4.0
3.0	4.6 ± 2.1	13.9 ± 4.2
6.0	7.8 ± 2.1	10.5 ± 4.3
8.0	7.8 ± 3.6	7.5 ± 3.5
12.0	7.0 ± 2.1	6.6 ± 3.7
i.p. administration		
0.25	4.0 ± 1.5	7.4 ± 2.5
0.5	4.0 ± 1.2	18.2 ± 3.6*
1.0	4.5 ± 1.2	13.8 ± 5.3
2.0	3.8 ± 1.9	2.4 ± 1.1
3.0	4.0 ± 1.5	4.0 ± 1.5

[a] Values represent the mean ± standard error of the mean for 10 animals in each test group; statistical significance compared to the corresponding vehicle-treated group was assessed by a two-way ANOVA followed by a Student-Newman-Keuls test: * $P < 0.05$.
[b] Treatment: Control, vehicle; DHM (p.o.), 1 mg/kg; DHM (i.p.), 1 mg/kg.

Table 3. Anxiolytic-like activity of dihydromagnolol (DHM) after acute oral administration, evaluated by Vogel's Conflict Test.

Treatment		
Drug[a]	N	Shocked drinks[b]
DHM		
Control	20	5.8 ± 0.3
1.0 mg/kg	20	10.6 ± 1.5*
5.0 mg/kg	20	11.9 ± 1.7**
Diazepam		
Control	30	4.3 ± 1.5
1 mg/kg	30	12.8 ± 2.7*

[a] All drugs were administered orally before evaluation by Vogel's conflict test as follows: DHM, 1 h; diazepam, 10 min; animals in control groups received only the corresponding vehicle; N, number of mice per test group.
[b] Values represent the mean ± standard error of the mean; statistical significance compared to the corresponding vehicle-treated group was assessed by a one-way ANOVA followed by a Student-Newman-Keuls test: * $P < 0.05$; ** $P < 0.01$.

was administered in combination with DHM, the resulting anxiolytic-like effect was significantly greater than after administration of DHM alone; however, the times spent in the open-sided arms were not statistically different from the value recorded after a single dose of diazepam. Acute administration of flumazenil caused a minimal, but significant increase in the time spent in the open-sided arms of the elevated plus-maze. While both flumazenil and bicuculline significantly inhibited the anxiolytic-like effect of diazepam, neither agent modified the activity of DHM. No significant changes in motor activity were observed with any of the drug combinations.

$^{36}Cl^-$ uptake

The effects of DHM on $^{36}Cl^-$ uptake into mouse cortical synaptoneurosomes were examined in the absence and presence of muscimol. As can be seen in Table 5, in the absence of muscimol, DHM induced a significant increase in ion uptake. However, DHM did not significantly enhance muscimol-stimulated $^{36}Cl^-$ uptake.

Table 4. Anxiolytic-like activity of dihydromagnolol (DHM) in combination with other agents.

Treatment[a]			
Drug	N	Time in Open-sided Arms (sec)[b]	Activity (counts/5 min)[b]
Vehicle	30	4.5 ± 1.5	34.4 ± 1.4
Diazepam	40	25.7 ± 3.2**	32.7 ± 5.4
DHM	30	16.4 ± 2.1**	30.8 ± 4.4
DHM + Diazepam	10	32.8 ± 8.5**	31.6 ± 4.5
Vehicle	20	4.9 ± 2.1	31.3 ± 3.9
Flumazenil	50	9.2 ± 1.2*	27.5 ± 3.4
DHM	30	16.0 ± 1.8*	29.6 ± 4.6
DHM + Flumazenil	30	18.2 ± 5.0*	31.6 ± 4.2
Diazepam	20	24.3 ± 3.5*	31.5 ± 4.0
Diazepam + Flumazenil	20	6.7 ± 1.6[$]	39.4 ± 4.2
Vehicle	30	4.8 ± 1.0	28.6 ± 3.0
Bicuculline	50	7.2 ± 3.4	28.6 ± 3.7
DHM	20	15.8 ± 4.7*	29.7 ± 2.7
DHM + Bicuculline	20	11.7 ± 3.5	27.3 ± 3.0
Diazepam	20	25.3 ± 7.2*	32.0 ± 4.5
Diazepam + Bicuculline	20	8.7 ± 1.0[$]	40.9 ± 4.1

[a] Drug administration was as follows: Diazepam, 1 mg/kg (p.o.), 10 min before plus-maze test; flumazenil, 0.3 mg/kg (i.p.), 10 min; bicuculline, 0.1 mg/kg (s.c.), 10 min; DHM, 1 mg/kg (p.o.), 1 h; N, number of mice per test group.
[b] Values represent the mean ± standard error of the mean; statistical significance was assessed by a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test. Significance compared to the corresponding vehicle-treated group: * $P < 0.05$; ** $P < 0.01$; significance compared to the corresponding diazepam-treated group: [$] $P < 0.05$.

Table 5. Effect of dihydromagnolol (DHM) on $^{36}Cl^-$ uptake into mouse cortical synaptoneurosomes.

Treatment	$^{36}Cl^-$ Uptake (% Control)[a]
Vehicle (Muscimol 0 µM)	100
+ DHM 5 µM	119.5 ± 5.9
+ DHM 17 µM	121.5 ± 4.2*
+ DHM 50 µM	107.4 ± 12.8
Muscimol 25 µM	104.3 ± 4.1
+ DHM 5 µM	111.1 ± 6.4
+ DHM 17 µM	121.6 ± 15.9
+ DHM 50 µM	139.9 ± 18.3
Muscimol 250 µM	147.6 ± 7.9*
+ DHM 5 µM	135.5 ± 9.9
+ DHM 17 µM	140.9 ± 7.9
+ DHM 50 µM	151.8 ± 7.5

[a] Values are shown as the percent of control (0 µM muscimol; 21.6 ± 1.2 mmol/mg protein/3 sec) and represent the mean ± standard error of the mean for 6 samples analyzed in triplicate; statistical significance compared to the corresponding vehicle-treated (0 µM muscimol) group was assessed by a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test: * $P < 0.05$.

Discussion

In our previous studies using the elevated plus-maze test, we found that magnolol exhibited only mild anxiolytic-like efficacy following p.o. administration for seven days (Maruyama et al., 1998). In contrast, the experiments described in the present report demonstrated that acute oral administration of 1 mg/kg of DHM, a partially reduced derivative of magnolol, yielded a potent anxiolytic-like effect, as evidenced by a significant increase in the time spent in the open-sided arms of the plus-maze, without causing any marked change in the motor activity. We have previously reported a similar observation for honokiol in that a partially reduced analog, DHH-B, exhibited significantly greater anxiolytic-like activity than the parent compound (Kuribara et al., 2000a). It is, therefore, probable that reduction of one of the two propenyl substituents is important for efficacy as an anxiolytic-like agent.

The anxiolytic-like properties of DHM were also confirmed by Vogel's conflict test which is considered a simple and reliable assessment of anxiety-like behavior in rodents (Commissaris, 1993). As shown in Table 3, acute administration of DHM produced effects similar to diazepam by significantly increasing the number of shocked drinks of water. In the Vogel's test, both DHM and diazepam were effective at 1 mg/kg, the same dose that elicited a significant response in the elevated plus-maze.

It has been previously established that binding of benzodiazepine anxiolytics to the $GABA_A$/benzodiazepine receptor complex is required for development of anxiolytic activity (Olsen & Tobin, 1990; Sieghart, 1992). In support of those observations, in this report we demonstrated that the anxiolytic-like effect of diazepam could be diminished by either a benzodiazepine receptor antagonist (flumazenil) or a $GABA_A$ receptor antagonist (bicuculline). In contrast, only flumazenil significantly reduced the anxiolytic-like activity of DHH-B (Kuribara et al., 2000a), and, as shown in Table 4, neither flumazenil nor bicuculline altered the anxiolytic-like action of DHM. In addition, the combined administration of DHM with diazepam did not result in any potentiation of the anxiolytic-like effect, that is, the time spent in the open-sided arms was not significantly higher than following administration of diazepam alone. Further information in this area was gained from the results of *in vitro* $^{36}Cl^-$ uptake experiments which showed that DHM increased $^{36}Cl^-$ uptake into mouse synaptoneurosomes. This is in agreement with results for DHH-B (Maruyama et al., 2001) and a variety of benzodiazepine receptor antagonists (Miller et al., 1988a,b). However, in our previous experiments we found that DHH-B also enhanced muscimol-induced ion uptake (Maruyama et al., 2001), suggesting some degree of interaction of DHH-B with the $GABA_A$/benzodiazepine receptor complex. While DHM caused an apparent increase in 25 µM muscimol-induced $^{36}Cl^-$ uptake, the results were not statistically significant. Thus, there may be differences in the mechanism of action of DHM and DHH-B, despite their close similarity in chemical structure. And, although receptor binding assays have not as yet been carried out with these biphenyl analogs, the present experiments also suggest differences in the modes of action of DHM and DHH-B as compared to diazepam and may indicate that DHM and DHH-B act as anxiolytic agents through binding to sites other than $GABA_A$/benzodiazepine receptor complex. Studies are underway to further elucidate mechanism(s) for the anxiolytic-like efficacy of DHM and DHH-B.

Acknowledgment

We thank Hoffman-La Roche (Nutley, NJ, USA) for the generous gift of flumazenil.

References

Commissaris RL (1993): Conflict behaviors as animal models for study of anxiety. In: Haaren FV, ed., *Methods in Behavioral Pharmacology*. Amsterdam, Elsevier, pp. 443–474.

Fujita M, Itokawa H, Sashida Y (1973): Studies on the components of *Magnolia obovata* Thunb. III. Occurrence of magnolol and honokiol in *M. obovata* and other allied plants. *Yakugaku Zasshi 93*: 429–434.

Hattori M, Endo Y, Takabe S, Kobashi K, Fukasaku N, Namba T (1986): Metabolism of magnolol from Magnoliae cortex. II. Absorption, metabolism and excretion of [ring-^{14}C] magnolol in rats. *Chem Pharm Bull 34*: 157–167.

Hattori M, Sakamoto T, Endo Y, Kakiuchi N, Kobashi K, Mizuno T, Namba T (1984): Metabolism of magnolol from Magnoliae cortex. I. Application of liquid chromatography-

mass spectrometry to the analysis of metabolites of magnolol in rats. *Chem Pharm Bull 32*: 5010–5017.

Ito Y, Koshiba T, Doi M, Asami S, Fukuda H, Murakoshi Y (1992): Characterization of antagonistic activity and binding properties of SR 95531, a pyridazinyl-GABA derivative, in rat brain and cultured cerebellar neuronal cells. *Synapse 10*: 326–333.

Ito Y, Miyasaka T, Fukuda H, Akahane K, Kimura Y (1996): Inhibition of $GABA_A$ receptor chloride channel by quinolones and norfloxacin-biphenylacetic acid hybrid compounds. *Neuropharmacology 35*: 1263–1269.

Ito Y, Segawa K, Fukuda H (1995): Functional diversity of $GABA_A$ receptor ligand-gated chloride channels in rat synaptoneurosomes. *Synapse 19*: 188–196.

Kuribara H, Haraguchi H, Tadokoro S (1989): An anticonflict effect of caffeine: investigation by punished and hypertonic NaCl solution procedure in mice. *Jpn J Alcohol Drug Depend 24*: 144–153.

Kuribara H, Kishi E, Kimura M, Weintraub ST, Maruyama Y (2000a): Comparative assessment of the anxiolytic-like activities of honokiol and derivatives. *Pharmacol Biochem Behav 67*: 597–601.

Kuribara H, Kishi E, Maruyama Y (2000b): Does dihydrohonokiol, a potent anxiolytic compound, develop benzodiazepine-like side effects? *J Pharm Pharmacol 52*: 1017–1022.

Kuribara H, Maruyama Y (1996): The anxiolytic effect of oriental herbal medicines by an improved plus-maze test in mice: Involvement of benzodiazepine receptors. *Jpn J Neuropsychopharmacol 18*: 179–190.

Kuribara H, Morita M, Ishige A, Hayashi K, Maruyama Y (1996): Investigation of the anxiolytic effect of the extracts derived from Saiboku-to, an oriental herbal medicine, by an improved plus-maze test in mice. *Jpn J Neuropsychopharmacol 18*: 643–653.

Lister RG (1987): The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacol 92*: 180–185.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the Folin phenol reagent. *J Biol Chem 193*: 265–275.

Maruyama Y, Kuribara H, Kishi E, Weintraub ST, Ito Y (2001): Confirmation of the anxiolytic-like effect of dihydrohonokiol following behavioural and biochemical assessments. *J Pharm Pharmacol 53*: 721–725.

Maruyama Y, Kuribara H, Morita M, Yuzurihara M, Weintraub ST (1998): Identification of magnolol and honokiol as anxiolytic agents in extracts of Saiboku-to, an oriental herbal medicine. *J Nat Prod 61*: 135–138.

Miller LG, Greenblatt DJ, Roy RB, Summer WR, Shader RI (1988a): Chronic benzodiazepine administration. II. Discontinuation syndrome is associated with up regulation of γ-aminobutyric acid A receptor binding and function. *J Pharmacol Exp Ther 246*: 177–182.

Miller LG, Weill CL, Roy RB, Gaver A (1988b): Lorazepam administration during embryonic development alters $GABA_A$ receptor binding and function. *Dev Brain Res 44*: 241–246.

Olsen RW, Tobin AJ (1990): Molecular biology of $GABA_A$ receptors. *FASEB J 4*: 2240–2253.

Schwartz RD, Suzudak PD, Paul SM (1986): γ-Aminobutyric acid (GABA)- and barbiturate-mediated $^{36}Cl^-$ uptake in rat brain synaptoneurosomes: Evidence for rapid desensitization of the GABA receptor-coupled chloride ion channel. *Molec Pharmacol 30*: 419–426.

Sieghart W (1992): $GABA_A$ receptors: Ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. *Trends Pharmacol Sci 12*: 446–450.

Umezu T (1999): Effects of psychoactive drugs in the Vogel conflict test in mice. *Jpn J Pharmacol 80*: 111–118.

Vogel JR, Beer B, Clody DE (1971): A simple and reliable conflict procedure for testing antianxiety agents. *Psychopharmacologia 21*: 1–7.

Watanabe H, Watanabe K, Hagino K (1983a): Chemostructural requirement for centrally acting muscle relaxant effect of magnolol and honokiol, neolignane derivatives. *J Pharmacobiodyn 6*: 184–190.

Watanabe K, Watanabe H, Goto Y, Yamaguchi M, Yamamoto N, Hagino K (1983b): Pharmaclological properties of magnolol and honokiol extracted from *Magnolia officinalis*: Central depressant effects. *Planta Med 49*: 103–108.

Watanabe K, Watanabe H, Goto Y, Yamamoto N, Yoshizaki M (1975): Studies on the active principles of magnolia bark. Centrally acting muscle relaxant activity of magnolol and honokiol. *Japan J Phamacol 25*: 605–607.