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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07C 39/00 — A2

(11) International Publication Number: **WO 00/40532**

(43) International Publication Date: 13 July 2000 (13.07.00)

(21) International Application Number: PCT/US99/30168

(22) International Filing Date: 17 December 1999 (17.12.99)

(30) Priority Data:
09/224,188 31 December 1998 (31.12.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/224,188 (CON)
Filed on 31 December 1998 (31.12.98)

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: SYNTHESIS OF DIHYDROHONOKIOL COMPOSITIONS

(57) Abstract

The synthesis and use of the anxiolytic compound dihydrohonokiol, its derivatives, analogs and homologs are disclosed. A method for reducing anxiety in a mammal is also disclosed.

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DESCRIPTION

SYNTHESIS OF DIHYDROHONOKIOL COMPOSITIONS

BACKGROUND OF THE INVENTION

1.1 Field of the Invention

The present invention relates to the synthesis of dihydrohonokiol, its derivatives, analogs and homologs, and to methods of use for the dihydrohonokiol compounds. Also included are compositions particularly useful for treatment of anxiety disorders.

1.2 Description of Related Art

Anxiety and anxiety-related disorders are extremely common. Anxiety-related conditions can be relatively mild or can be sufficiently severe as to be disabling. Also noteworthy is that anxiety, while infrequently a "disease" in itself, is an almost inevitable and often exacerbating consequence of many other medical and surgical conditions. Estimates of the number of patients suffering from various anxiety disorders range between 12 and 35 million persons in seven major industrialized nations.

Saiboku-to, a Chinese herbal medicine, has long been used to treat anxiety and neurotic disorders (Hosaya and Yamamura, 1988; Nartia, 1990). A disadvantage of Saiboku-to is that it requires approximately seven days or more of daily administration before an anxiolytic effect is observed (Maruyama *et al.*, 1998). Fractionation of Saiboku-to has identified the two putative principal active anxiolytic components as magnolol (5,5′-di-(2-propenyl)-1,1′-biphenyl-2,2′-diol) and its positional isomer, honokiol (3,5′-di-(2-propenyl)-1,1′-biphenyl-2′,4diol) (Maruyama *et al.*, 1998). Behavioral tests indicate that honokiol is at least 5000 times more potent than Saiboku-to. But honokiol still requires several days of administration to elicit an

anxiolytic effect (Maruyama *et al.*, 1998). The delay in the onset of anxiolytic activity of honokiol is thought to be due to either changes in receptors or a slow build-up of honokiol metabolites within the body.

The metabolic pathway of honokiol has not been fully established. The positional isomer magnolol is known to be metabolized to a number of compounds including the hydrogenated products dihydromagnolol (5-(2-propenyl)-5′-n-propyl-1,1′biphenyl-2,2′diol) and tetrahydromagnolol (5,5′–δι-n-propyl-1,1′biphenyl-2,2′-diol) (Hattori *et al.*, 1984a). The excretion of the reduced metabolites in feces and urine increased significantly in amount after repeated daily administration of magnolol. This pattern was possibly the result of induction of the enzymes responsible for the metabolism.

Benzodiazapines, such as diazepam and alprazolam, represent the most commonly used class of anxiolytic agents administered for the treatment of anxiety. Benzodiazapines can act to counteract anxiety by depressing the electrical afterdischarge in the limbic system, and appear to potentiate neural inhibition that is mediated by gamma-aminobutyrate (GABA) (Baldessarini, 1990). These compounds have proven to be effective at reducing anxiety, but they also have significant side effects including sedation, ataxia, amnesia, dependence, tolerance, and behavioral disturbances and act as skeletal muscle relaxers. These side effects can render these compounds unsuitable for many patients, particularly those whose anxiety is coupled to another form of illness.

In addition to benzodiazepines, other drugs used to treat anxiety include barbiturates, certain anticholinergic agents, antihistamines, and azaspirodecanediones (Baldessarini, 1990). These drugs are sedatives, or at least have many properties in common with traditional sedatives. The barbiturates are general neuronal depressants. The use of certain anticholinergic agents and antihistamines for treating anxiety appears to be based on their sedative properties. The azaspirodecanediones, buspirone in particular, are used in treating anxiety but require weeks of administration before anxiolytic activity is noted. The mechanism of action of the azaspirodecanediones is unknown.

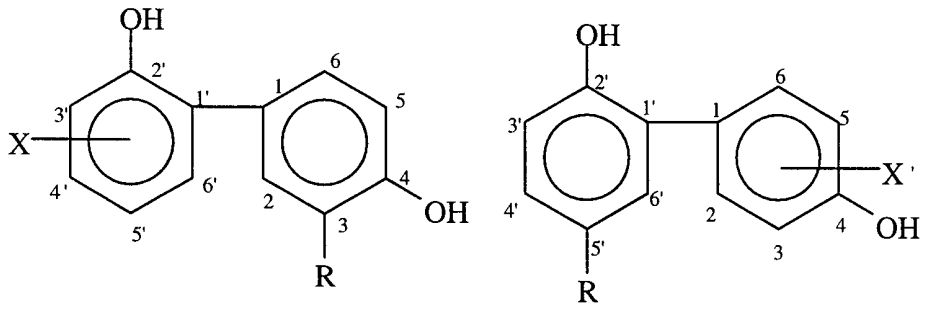
2.0 SUMMARY OF THE INVENTION

The present invention addresses problems associated with commonly-used anxiolytic drugs, with Saiboku-to therapy in general, and with honokiol in particular, for treatment of anxiety. The present invention provides compounds which exhibit anxiolytic activity within hours without many of the negative side effects associated with well-known anxiolytic compounds, such as the benzodiazepines. The invention also provides a method of treating anxiety disorders. Further, the invention provides methods of synthesis of novel anxiolytic compounds.

2.1 Novel Anxiolytic Compounds

The present invention provides new anxiolytic compounds that do not have the adverse side effects of the benzodiazepines. In particular embodiments, the invention relates to the use of dihydrohonokiol, its derivatives, analogs and homologs, as anxiolytic agents. The inventors have demonstrated that dihydrohonokiol, for example, exerts an anxiolytic effect more rapidly than honokiol and exhibits greater potency and fewer side effects than diazepam, a benzodiazepine anxiolytic.

An aspect of the present invention encompasses novel compositions of matter comprising compounds of the formula:



In a preferred embodiment, R is CH_2-CH_2-CH_3 and X is 5′-CH_2-CH=CH_2 and X′ is 3-CH_2-CH=CH_2.

In other embodiments, the group R represents -CH_2-CH=CH_2, CH=CH-CH_3, or -CH_2-CH_2CH_3. The group X represents from one to two substituents in any of the 3-, 4-, 5-, or 6-positions and is separately and independently fluorine, hydroxy, methoxy, (1-adamantyl), C_1-C_5 alkyl, C_2-C_3 alkenyl, C_2-C_3 alkylcarbonyl or C_1 -C_4

carboxyalkyl. The C_1-C_5 alkyl group may be substituted with one or more fluorine or hydroxyl. Excepted from this list are the compounds where R is -CH_2-CH=CH_2 and X is 5′-CH_2-CH_3, 5′-CH_2-CH=CH_2, 5′-OH, 3′–OH, 5′-CH_2-CH-CH_3, and 3′–OCH_3, 5′-CH_2-CH=CH_2, and where R is -CH_2-CH_2-CH_3, and X is 3-CH_2-CH_2-CH_3.

In particular, X may represent: 5′-CH=CH_2; 5′-CH_2-CH_3; 5′-CH=CH_2, 3′-OH; 5′–CH_2-CH=CH_2; 5′-CH_2-CH=CH_2, 3′-OH; 5′-CH_2-CH_2-CH_3, 3′-OH; 5′-CH_2-CH=CH_2, 3′-OCH_3; 5′-CH_2-CH_2-CH_3, 3′-OCH_3; 5′-CH=CH-CH_3, 3′-OH; 5′-CH=CH-CH_3, 3′–OCH_3; 5′-CH_3; 5′-$CH(CH_3)_2$; 5′-$CH_2CH(CH_3)_2$; 5′-$C(CH_3)_3$; 5′-$CH(CH_3)_2C_2H_5$; 5′–(1-adamantyl); 5′-$CH(CH_3)_2$, 6 -CH_3; 5′-$CH(CH_3)_2$, 4 -CH_3; 5′-C(=O)-CH_3; 5′–CH(-OH)-CH_3; 5′-CH_2-C(=O)-CH_3; 5′-CH_2.CH(-OH)-CH_3; 5′-CH_2-COOH; 5′–CH_2CH_2-COOH; 5′-$CH_2CH_2CH_2$-COOH; 5′-CH_2-COOH, 3-OH; 5′-OH; 5′-OCH_3; 3′-F; 4′-F; 5′-F; 3′-F, 5′-CH_3; 3′-F, 5′-CH_2-CH_3; 3′-F, 5′-CH_2-OH; 3′-F, 5′-CH_2-CH_2-OH; 3′-F, 5′-COOH; 3′-F, 5′-CH_2-COOH; 3′-F, 5′-CH=CH-CH_3; 3′-F, 5′-CH_2-CH_2-CH_3; 3′-F, 5′-CH_2-CH=CH_2; 3′-F, 5′-CH_2-CHF-CH_3; 3′-F, 5′-CH_2-CHF-CH_2F; 4′-F, 5′-CH_3; 4′-F, 5′-CH_2-OH; 3′-F, 5′-CH_2-CH_3; 6′-F, 5′-CH_2-CH_3; 6′-F, 5′-CH_2-OH; and 6′-F, 5′-COOH.

In other embodiments, the group X′ represents from one to two substituents in any of the 3-, 4-, 5-, or 6-positions and is separately and independently fluorine, hydroxy, methoxy, C_1-C_4 alkyl, C_3 alkenyl, C_1-C_3 alkylcarbonyl, C_1 -C_3 carboxyalkyl or C3 carboxyalkenyl. The alkyl group may be substituted with one or more fluorine or hydroxyl. Excepted from this list are where R = -CH_2-CH=CH_2 and X′ = 3-CH_2-CH_3, 3′-CH_2-CH=CH_2, and where R = -CH_2-CH_2-CH_3 and X′ = 3-CH_2-CH_2-CH_3.

In particular, X′ may represent: 3-CH_3; 3-CH_2-CH_3; 3-CH_2-CH=CH_2; 3-CH_2-CH=CH_2, 5-OH; 3-CH_2-CH_2-CH_3, 5-OH; 3-CH_2-CH=CH_2, 5-OCH_3; 3-$CH_2CH_2CH_3$, 5-OCH_3; 3-CH=CH-CH_3; 3-CH_3, 6-$CH(CH_3)_2$; 3,5 di-CH_3; 2,6 di-CH_3; 3-$CH(CH_3)_2$; 3-$CH(CH_3)_2$, 6-CH_3; 3-$CH_2CH(CH_3)_2$; 3-C(=O)-CH_3; 3-CH(-OH)-CH_3; 3-CH_2-C(=O)-CH_3; 3-CH_2-CH(-OH)-CH_3; 3-COOH, 6-OH; 2-COOH; 3-OCH_3; 3-CH_2COOH; 3-CH_2CH_2COOH; 3-CH=CHCOOH; 3-CHO, 2-OH; 3-CH_2OH, 2-OH; 3-CHO, 5-OCH_3; 3-CH_2OH, 5-OCH_3; 3-CHO, 5-CH_3; 3-CH_2OH, 5-CH_3, 3-F; 2F; 2-F, 3-CH_3; 6-F, 3-CH_3; 5-F, 3-CH=CH-CH_3; 5-F,3-CH_2-CH_2-CH_3; 5-F, 3-CH_2-CH=CH_2; 5-F, 3-CH_2-CHF-CH_3; or 5-F, 3-CH_2-CHF-CH_2F.

2.1.2 Pharmaceutical Compositions

Another aspect of the present invention includes novel compositions comprising dihydrohonokiol, its derivatives, analogs and homologs. It will, of course, be understood that one or more dihydrohonokiol, dihydrohonokiol derivative, analogs and homologs may be used in the methods and compositions of the invention. The maximum number of novel anxiolytic compounds of the present invention that may be administered is limited only by practical considerations, such as the possibility of eliciting an adverse effect.

Compositions employing the novel anxiolytic compounds will contain a biologically effective amount of the anxiolytic compound. As used herein, a "biologically effective" amount of an anxiolytic compound refers to an amount effective to alter, modulate, or reduce anxiety or related conditions. As disclosed herein, different amounts of the novel anxiolytic compounds may be effective.

Clinical doses will of course be determined by the nutritional status, age, weight and health of the patient. The quantity and volume of the composition administered will depend on the subject and the route of administration. The precise amounts of active anxiolytic compounds required will depend on the judgment of the practitioner and may be peculiar to each individual. However, in light of the data presented here, the determination of a suitable dosage range for use in different mammals will be straightforward.

Pharmaceutical compositions prepared in accordance with the present invention find use in several applications, including inhibition or reduction of anxiety. Such methods generally involve administering to a mammal a pharmaceutical composition comprising an anxiolytically effective amount of dihydrohonokiol, its derivatives, analogs or homologs.

Therapeutic kits comprising dihydrohonokiol, its derivatives, analogs and homologs comprise another aspect of the present invention. Such kits will generally contain, in suitable container means, a pharmaceutically acceptable formulation of dihydrohonokiol, its derivatives, analogs and homologs. The kit may have a single container means that contains the novel composition(s) or it may have distinct

container means for the novel composition(s) and other reagents which may be included within such kits.

2.2 Method of Treating Anxiety Disorders

The invention also includes a method of treating anxiety disorders with dihydrohonokiol, its derivatives, analogs and homologs. This method comprises administration of an anxiolytic amount of a suitable composition containing dihydrohonokiol, its derivatives, analogs and homologs, to a subject in need thereof. Administration is preferably by oral dosage, but any route of administration capable of delivering an effective dose my be used. The choice of the composition to be used for treatment, the amount of the composition to be administered, and the duration of treatment will depend on the judgment of the practitioner and may be peculiar to each individual, as disclosed above. The treatment may be maintained as long as necessary and may be used in conjunction with other forms of treatment.

2.3 Methods of Synthesis of Anxiolytic Compounds

In yet another aspect, the invention relates to synthetic methods for producing dihydrohonokiol and its various derivatives, analogs and homologs. The compounds may be synthesized from honokiol derived from plant sources. This reaction is described herein as a hemi-synthesis. Alternatively, the total synthesis may be accomplished from the asymmetric coupling of 4-allylphenylalkyl ether with 4-alkoxy haloaryls, followed by dealkylation of the dialkoxyl biaryl formed.

In the hemi-synthesis of dihydrohonokiol, honokiol is partially hydrogenated by reacting hydrogen atoms in the presence of a tris-(triphenylphosphine) rhodium(I) chloride catalyst with honokiol dissolved in a non-polar solvent. In preferred embodiments, the non-polar solvent is selected from toluene, benzene or hexane. Under conditions of a limited supply of hydrogen, the predominant reaction product has only one of the two similar allylic groups of the biphenyl molecule reduced. Tetrahydrohonkiol is formed as a minor component, thus enabling unreacted honokiol to be recovered and reused. The partially hydrogenated honokiol products are collected and the two dihydrohonokiol isomers are isolated and purified.

The total synthesis of 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol, one of the two isomers of dihydrohonokiol, designated herein as I, utilizes 4-allylanisole as the starting chemical compound. This starting chemical compound is reacted with *tert*-butyllithium and anhydrous zinc chloride. The intermediate compound so formed is coupled with 2-propyl-4-iodo-anisole in the presence of a catalyst, resulting in 2′,4-dimethoxy-3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl, which after demethylation generates 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) as one of the reaction products. The synthesis of the other isomer of dihydrohonokiol, 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol, hereinafter designated as II, can be accomplished in a similar manner, using 4-propylanisole as the starting chemical compound and reacting it with *tert*-butyllithium and anhydrous zinc chloride. This intermediate compound is coupled with 2-allyl-4-iodo-anisole in the presence of a catalyst, resulting in 2′,4-dimethoxy-5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl, which after demethylation generates 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II) as one of the reaction products. The two structural isomers of dihydrohonokiol can be isolated from the reaction mixtures by procedures known in the art, such as preparative reverse phase high pressure liquid chromatography or preparative thin layer chromatography.

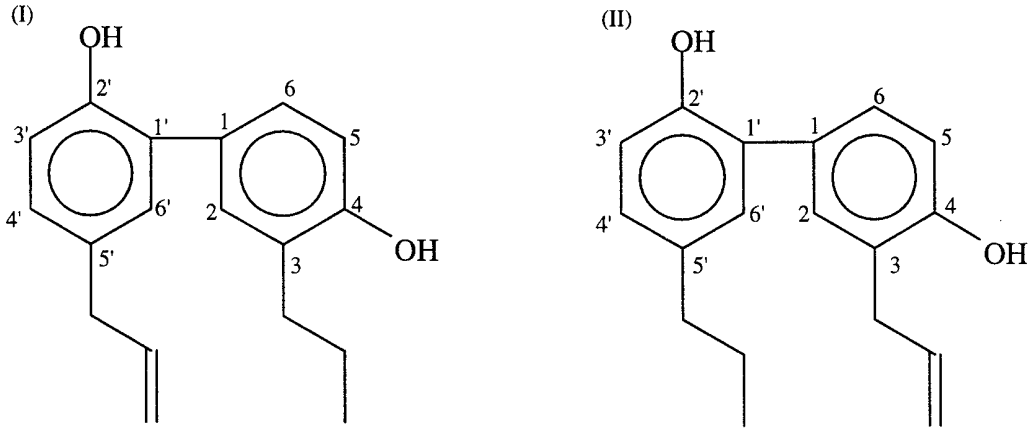
Analogs and homologs of 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) and 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II) are synthesized from substituted 4-allyl or alkylphenyl alkyl ethers which are converted to the corresponding 2-alkoxy-5-allylphenyl metal halide or 2-alkoxy-5-alkylphenyl metal halide by a directed *ortho* metalation reaction. The products of such reactions are, in turn, reacted with various 4-alkoxyhaloaryls *in-situ* to generate the required homologs or analogs of compounds I and II. Carboxylic groups may be protected as esters (*e.g.*, ethyl ester) during the synthetic process. The dihydrohonokiol analog formed after the coupling reaction and demethylation can be deprotected by base catalyzed hydrolysis of the ester group. Alcoholic groups may be protected as acyl esters, *e.g.*, by base catalyzed acetylation with acetic anhydride. The dihydrohonokiol analog formed after the coupling and demethylation can be deprotected by base catalyzed removal of acyl groups.

3.0 DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The anxiolytic compounds of the present invention, including dihydrohonokiol, its derivatives, analogs and homologs, in contrast to the benzodiazepines, can be administered without significant side effects. The benzodiazepines have side effects including sedation, ataxia, amnesia, dependence, tolerance, and behavioral disturbances. These side effects have not been observed with therapeutic doses of the dihydrohonokiol isomers I and II.

In tests in mice maximum anxiolytic activity of dihydrohonokiol was observed three hours after oral administration. This contrasts with up to at least seven days for anxiolytic activity to manifest in mice treated with honokiol.

The novel dihydrohonokiols shown below have been synthesized:



The nomenclature adopted for the dihydrohonokiols, 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) and 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II), is based on the original nomenclature for honokiol and tetrahydrohonokiol as used by Takeya *et al*, (1986).

The dihydrohonokiol compounds I and II can be synthesized by partial hydrogenation of honokiol as described in Example 1, § 5.1.2. This methods allows for the hydrogenation of only one of the two very similar allylic groups on the biphenyl moiety. In a typical reaction of the exemplified synthesis, 55 % of the reaction products was dihydrohonokiol, 35.5% unreacted honokiol, and 9.5%

tetrahydrohonokiol. Thus dihydrohonkiol represents almost 86% of the consumed honokiol. This method allows for the recovery of unreacted honokiol, the reuse of which in the synthesis of the dihydrohonokiols isomers markedly increases the potential efficiency of this synthesis.

Limiting the supply of hydrogen was important in preventing conversion to tetrahydrohonkiol. Thus increasing the total amount of hydrogen in the reaction mixture, *e.g.*, by increasing the volume of solvent and associated dissolved hydrogen, or increasing the time hydrogen is passed through the reaction mixture after the addition of honokiol, increases the amount of tetrahydrohonokiol produced. Preferred reaction conditions were those which resulted in the reaction of 0.6 to 1.2 mol of hydrogen per mol of honokiol. Despite use of a variety of conditions, dihydrohonkiol could not be prepared by hydrogenation using either 5% or 10% palladium on activated carbon as catalyst. With palladium/carbon, either unreacted honokiol or fully reduced tetrahydrohonokiol was recovered from the reaction mixture.

Alternatively, compounds I and II can be synthesized by a total synthesis, details of which are given in Example 1, § 5.1.3. Briefly, 4-allyl or alkyl phenyl ethers are converted to the respective 2-alkoxy 5-allyl or 5-alkyl phenyl metal halides by a directed *ortho* metalation reaction. Such intermediates are reacted with 4-alkoxy haloaryls in the presence of a palladium catalyst to yield unsymetrical biphenyl coupling products. The removal of alkyl protecting groups by boron tribromide under mild conditions, followed by preparative high pressure liquid chromatographic purification, produces purified compounds I and II.

In like fashion, homologs and analogs of 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) may be generated by converting 4-allylphenyl alkyl ethers to 2-alkoxy-5-allylphenyl metal halides by a directed ortho metalation reaction, which in turn will be reacted with various 4-alkoxyhaloaryls *in-situ*. Similarly, various substituted-phenyl alkyl ethers will be converted to 2-alkoxy-substituted phenyl metal halides and be reacted *in-situ* with various 3-allyl-4-alkoxyhalophenyls to generate the homologs and analogs of 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II).

3.1 Anxiety

As used herein, the term "anxiety" is intended to refer to a condition of apprehension, uncertainty, dread, or fear unattached to a clearly defined stimulus accompanied by numerous physiological and psychological symptoms such as tachycardia, dyspnia, tension, restlessness, inattentiveness, and loss of appetite, skeletal motor function, initiative, cognitive logic, short- and long-term memory, and the like. Practice of the method of the present invention can combat, *i.e.*, reduce or alleviate, some, most, or all of these physiological symptoms.

A suitable subject to be treated by the present method is an animal, such as a human or other mammal (*e.g.*, house pets such as dogs and cats, or other commercially valuable or domestic animals), which experience anxiety-related symptoms due to some external or internal stimulus that are desirably combated. Preferably, the subject is human.

As used herein the term "treating" includes prophylaxis of a physical and/or mental condition or amelioration or elimination of the developed physical and/or mental condition once it has been established or alleviation of the characteristic symptoms of such condition.

The term "antianxiety dose", as used herein, represents an amount of compound necessary to prevent or treat a human susceptible to or suffering from anxiety following administration to such human. The active compounds are effective over a wide dosage range. For example, dosages per day will normally fall within the range of about 0.005 to about 500 mg/kg of body weight. In the treatment of adult humans, the range of about 0.05 to about 100 mg/kg, in single or divided doses, is preferred. However, it will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances including the condition to be treated, the choice of compound to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. While the present compounds are preferably administered orally to humans susceptible to or suffering from anxiety, the compounds may also be administered by a variety of other routes such as the transdermal, parenteraly, subcutaneous, intranasal,

intramuscular and intravenous routes. Such formulations may be designed to provide delayed or controlled release using formulation techniques which are known in the art.

Examples of anxiety disorders which may preferably be treated using an effective amount of a named compound or pharmaceutically acceptable salt thereof include, but are not limited to: Panic Attack; Agoraphobia; Acute Stress Disorder; Specific Phobia; Panic Disorder; Psychoactive Substance Anxiety Disorder; Organic Anxiety Disorder; Obsessive-Compulsive Anxiety Disorder; Posttraumatic Stress Disorder; Generalized Anxiety Disorder; and Anxiety Disorder NOS.

The named anxiety disorders have been characterized in the DSM-IV-R. Diagnostic and Statistical Manual of Mental Disorders, Revised, 4th Ed. (1994). The DSM-IV-R was prepared by the Task Force on Nomenclature and Statistics of the American Psychiatric Association, and provides clear descriptions of diagnostic categories. The skilled artisan will recognize that there are alternative nomenclatures, nosologies, and classification systems for pathologic psychological conditions and that these systems evolve with medical scientific progress. Social anxiety disorder may also be preferably treated using an effective amount of this compound or pharmacologically acceptable salt thereof.

3.2 Antidepressant Activity

The compounds of the present invention are also contemplated to be useful for the treatment of depression. There is considerable support the involvement of GABA in mood disorders. Somatic treatment for depression and mania upregulate the GABA B receptor similar to the effect of GABA agonists. Decreased GABA function accompanies depressed or manic mood states. Low GABA levels are found in brain, cerebrospinal fluid and plasma in patients with depression and in plasma of patients with mania. Low GABA function is proposed to be an inherited biological marker of vulnerability for development of mood disorders.

Benzodiazepines are GABA A receptor agonists and augment the activity of GABA while generally not affecting biogenic amine uptake or metabolism. A metaanalysis of the use of benzodiazepines in the treatment of depression found that alprazolam had activity comparable to standard antidepressants.

The anxiolytic activity of the mixture of compound I and II and the benzodiazepine diazepam in the elevated plus maize model was blocked by the GABA antagonist bicuculline. But the anxiolytic activity of diazepam but not the I/II mixture was blocked by flumazenil, a benzodiazepine GABA site antagonist. While these results differentiate the mechanism of action of compounds I and II from that of benzodiazepines, they also indicate a possible augmenting action of dihydrohonokiol compounds on the GABA system, an action indicative of antidepressant activity.

4.2 Pharmaceutical Compositions

Another aspect of the present invention includes novel compositions comprising the novel dihydrohonokiol, its derivatives, analogs and homologs. In a preferred embodiment the composition will comprise dihydrohonokiol, either 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) or 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II). Alternatively, compositions may comprise any ratio of the two isomers such as 10-90% of I and 90-10% of II. The composition may further comprise derivatives, analogs or homologs of dihydrohonokiol, or one or more other pharmacologically-active compounds, and particularly one or more anxiety-altering compounds. The methods of the invention may thus entail the administration of one, two, three, or more, of the new dihydrohonokiol, its derivatives, analogs and homologs. The maximum number of species that may be administered is limited only by practical considerations, such as the particular effects of each compound. Of course, a composition of derivatives of I or II may be preferable, depending on their particular physical properties and physiological effects.

The compositions of the invention may include a dihydrohonokiol, its derivatives, analogs and homologs, modified to render it biologically protected. Biologically protected compounds have certain advantages over unprotected compounds when administered to human subjects and, may exhibit increased pharmacological activity.

Compositions employing the novel compounds will contain a biologically effective amount of the compounds. As used herein a "biologically effective amount" of a compound or composition refers to an amount effective to alter, modulate or reduce anxiety or related conditions. For oral administration, a satisfactory result may

be obtained employing the compounds in an amount within the range of from about 0.001 mg/kg to about 50 mg/kg, preferably from about 0.005 mg/kg to about 35 mg/kg and more preferably from about 0.01 mg/kg to about 20 mg/kg alone or in combination with one or more additional anti-anxiety compounds in an amount within the range from about 0.001 mg/kg to about 50 mg/kg, preferably from about 0.005 mg/kg to about 35 mg/kg and more preferably from about 0.01 mg/kg to about 20 mg/kg both being employed together in the same oral dosage form or in separate oral dosage forms taken at the same time. For parenteral administration, *e.g.*, subcutaneously or intramuscularly, the dihydrohonokiol compound(s) will be employed in an amount within the range of from about 0.005 mg/kg to about 10 mg/kg and preferably from about 0.01 mg/kg to about 1 mg/kg, alone or with the additional anti-anxiety compounds in an amount within the range of from about 0.005 mg/kg to about 20 mg/kg and preferably from about 0.01 mg/kg to about 2 mg/kg.

It will also be understood that, if desired, the disclosed dihydrohonokiol, its derivatives, analogs and homologs, may be administered in combination with additional agents, such as, *e.g.*, proteins or polypeptides or various pharmaceutically active agents. So long as the composition comprises a dihydrohonokiol, its derivatives, analogs and homologs, there is virtually no limit to other components which may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The dihydrohonokiol, its derivatives, analogs and homologs, may thus be delivered along with various other agents as required in the particular instance.

In carrying out the method of the present invention, the pharmaceutical compound(s) alone or in combination with one or more anxiety-modulating compounds may be administered to mammalian species, such as monkeys, dogs, cats, rats and humans, and as such may be incorporated in a conventional systemic dosage form, such as a tablet, capsule, elixir or injectable. The above dosage forms will also include the necessary carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid of sodium bisulfite) or the like. Oral dosage forms are preferred, although parenteral forms such as intramuscular, intraperitoneal, or intravenous are quite satisfactory as well. The dose administered must be carefully adjusted according to age, weight and condition of the

patient, as well as the route of administration, dosage form and regimen and the desired result.

The pharmaceutical compositions disclosed herein may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of solutions, suspensions, elixirs, troches, tablets, pills, capsules, sustained release formulations, powders, syrups, wafers and the like, and contain about 0.1% to about 95% of active ingredient, preferably about 1% to about 70%. Normally employed excipients may include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of the unit. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compounds sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Tablets of various sizes can be prepared, *e.g.*, of about 30 to 900 mg in total weight, containing one or more of the active substances in the ranges described above, with the remainder being a physiologically acceptable carrier of other materials according to accepted pharmaceutical practice. These tablets can, of course, be scored to provide for fractional doses. Gelatin capsules can be similarly formulated. Liquid formulations can also be prepared by dissolving or suspending one or the combination of active substances in a conventional liquid vehicle acceptable for pharmaceutical administration so as to provide the desired dosage in one to four teaspoonfuls. Such dosage forms can be administered to the patient on a regimen of one to four doses per day.

According to another modification, in order to more finely regulate the dosage schedule, the active substances may be administered separately in individual dosage units at the same time or carefully coordinated times. Since blood levels are built up and maintained by a regulated schedule of administration, the same result is achieved by the simultaneous presence of the two substances. The respective substances can be individually formulated in separate unit dosage forms in a manner similar to that described above.

In formulating the compositions, the active substances, in the amounts described above, are compounded according to accepted pharmaceutical practice with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, *etc.*, in the particular type of unit dosage form.

The active compounds may also be administered parenterally or intraperitoneally. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the

conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The dihydrohonokiol compound(s) described above may be administered in the dosage forms as described above in single or divided doses of one to four times daily. It may be advisable to start a patient on a low dose combination and work up gradually to a high dose combination.

The formulations as described above may be administered for a prolonged period, that is, for as long as the potential for onset of anxiety remains or the symptoms of anxiety continue. Sustained release forms of such formulations which may provide such amounts biweekly, weekly, monthly and the like may also be

employed. A dosing period of at least one to two weeks are required to achieve minimal benefit.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the active compound with one or more conventional solid carriers, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10%, preferably about 1 to about 2%.

Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 to 0.2M concentration with respect to the said active compound.

Formulations suitable for transdermal administration may also be delivered by iontophoresis (see, for example, *Pharmaceutical Research,* 3(6):318, 1986) and typically take the form of an optionally buffered aqueous solution of the active compound. Suitable formulations comprise citrate or bis tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M active ingredient.

The compositions are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated, including, *e.g.*, age, physical condition and degree of symptoms presented. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are of the order of several hundred micrograms active ingredient per dose. Suitable regimes for initial administration and booster doses are also variable, but are typified by an initial administration followed by subsequent administrations.

Clinical doses will of course be determined by the nutritional status, age, weight and health of the patient. The quantity and volume of the composition administered will depend on the subject and the route of administration. The precise amounts of active compound required will depend on the judgment of the practitioner and may be peculiar to each individual. However, in light of the data presented herein, the determination of a suitable dosage range for use in humans will be straightforward.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

4.2.1 Liposomes and Nanocapsules

In certain embodiments, the inventors contemplate the use of liposomes and/or nanocapsules for the introduction of one or more of the disclosed pharmaceutical composition into a host cell. Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the honokiol derivatives and/or analogs disclosed herein.

The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur *et al.*, 1977 which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy of intracellular bacterial infections and diseases). More recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon and Papahadjopoulos, 1988; Allen and Choun, 1987).

In one instance, the disclosed composition may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. The term "liposome" is intended to mean a composition arising spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991).

Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyano-

acrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be are easily made, as described (Couvreur *et al.*, 1977; 1988). Methods of preparing polyalkyl-cyano-acrylate nanoparticles containing biologically active substances and their use are described in U.S. Patent 4,329,332, U.S. Patent 4,489,055, and U.S. Patent 4,913,908.

Pharmaceutical compositions containing nanocapsules for the oral delivery of active agents are described in U.S. Patent 5,500,224 and U.S. Patent 5,620,708. U.S. Patent 5,500,224 describes a pharmaceutical composition in the form of a colloidal suspension of nanocapsules comprising an oily phase consisting essentially of an oil containing dissolved therein a surfactant and suspended therein a plurality of nanocapsules having a diameter of less than 500 nanometers. U.S. Patent 5,620,708 describes compositions and methods for the oral administration of drugs and other active agents. The compositions comprise an active agent carrier particle attached to a binding moiety which binds specifically to a target molecule present on the surface of a mammalian enterocyte. The binding moiety binds to the target molecule with a binding affinity or avidity sufficient to initiate endocytosis or phagocytosis of the particulate active agent carrier so that the carrier will be absorbed by the enterocyte. The active agent will then be released from the carrier to the host's systemic circulation. In this way, degradation of the disclosed pharmaceutical compounds in the intestines can be avoided while absorption of compound form the intestinal tract is increased.

An excellent review of nanoparticles and nanocapsular carriers is provided by Arshady 1996. Arshady notes that one of the major obstacles to the targeted delivery of colloidal carriers, or nanocapsules, is the body's own defense mechanism in capturing foreign particles by the reticuloendothelial system (RES). This means that following intravenous administration, practically all nanometer size particles are captured by the RES (mainly the liver). The review describes recent initiatives on the design of macromolecular homing devices which seem to disguise nanoparticles from the RES and, hence, are of potential interest to the targeted delivery of nanocapsular carriers. The idea is based on a graft copolymer model embodying a link site for attachment to the carrier, a floating pad for maintaining the particles afloat in the

blood stream, an affinity ligand for site-specific delivery and a structural tune for balancing the overall structure of the homing device.

Yu and Chang, 1996 describe the use of nanocapsules containing hemoglobin as potential blood substitutes. They use different polymers including polylactic acid and polyisobutyl-cyanoacrylate and modify the surface of the nanocapsules with polyethylene glycol (PEG) or with PEG 2000 PE. The surface modified nanocapsules containing hemoglobin survive longer in the circulation.

U.S. Patent 5,451,410 describes the use of modified amino acid for the encapsulation of active agents. Modified amino acids and methods for the preparation and used as oral delivery systems for pharmaceutical agents are described. The modified amino acids are preparable by reacting single amino acids or mixtures of two or more kinds of amino acids with an amino modifying agent such as benzene sulfonyl chloride, benzoyl chloride, and hippuryl chloride. The modified amino acids form encapsulating microspheres in the presence of the active agent under sphere-forming conditions. Alternatively, the modified amino acids may be used as a carrier by simply mixing the amino acids with the active agent. The modified amino acids are particularly useful in delivering peptides or other agents which are sensitive to the denaturing conditions of the gastrointestinal tract.

4.3 Diagnostic and Therapeutic Kits

Therapeutic kits comprising dihydrohonokiol, its derivatives, analogs and homologs, comprise another aspect of the present invention. Such kits will generally contain, in suitable container means, a therapeutically-effective amount of a pharmaceutically acceptable composition of dihydrohonokiol, its derivatives, analogs or homologs, and a pharmaceutically acceptable excipient. The diagnostic/therapeutic kits comprising the pharmaceutical compositions disclosed herein will generally contain, in suitable container means, a therapeutically-effective amount of dihydrohonokiol, its derivatives, analogs and homologs, in a pharmaceutically acceptable excipient. The kit may have a single container means that contains the dihydrohonokiol, its derivatives, analogs and homologs, and a suitable excipient or it may have distinct container means for each compound.

The components of the kit may be provided as liquid solution(s), or as dried powder(s). When the components are provided in a liquid solution, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

When the components of the kit are provided in one or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. The dihydrohonokiol, its derivatives, analogs and homologs, may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, or other such like apparatus, from which the formulation may be administered into the body, preferably by injection or even mixed with the other components of the kit prior to injection. The dihydrohonokiol, its derivatives, analogs and homologs, to be administered may be a single compound, or a composition comprising two or more of dihydrohonokiol, its derivatives, analogs and homologs, in a single or multiple dose for administration. Alternatively, one or more dihydrohonokiol, its derivatives, analogs and homologs, may be administered consecutively or concurrently with other agents as deemed appropriate by the clinician. Dosage of each of the compositions will vary from subject to subject depending upon severity of conditions, size, body weight, *etc.* The calculation and adjustment of dosages of pharmaceutical compositions is well-known to those of skill in the art.

In an alternate embodiment, components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the dihydrohonokiol, its derivatives, analogs and homologs, may be placed, preferably, suitably allocated. Where two or more of dihydrohonokiol, its derivatives, analogs and homologs, are provided, the kit will also generally contain a second vial or other container into which this additional compound may be formulated. The kits may also comprise a

second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent.

The kits of the present invention will also typically include a means for containing the vials in close confinement for commercial sale, such as, *e.g.*, injection or blow-molded plastic containers into which the desired vials are retained. Alternatively, the vials may be prepared in such a way as to permit direct introduction of the composition into an intravenous drug delivery system.

Irrespective of the number or type of containers, the kits of the invention may also comprise, or be packaged with, an instrument for assisting with the injection/administration or placement of the ultimate dihydrohonokiol, its derivatives, analogs and homologs, composition within the body of an animal. Such an instrument may be a syringe, pipette, forceps, measured spoon, eye dropper or any such medically approved delivery vehicle.

5.0 EXAMPLES

5.1 Example 1• Synthesis of Dihydrohonokiols

5.1.1 Materials and Methods:

Materials

4-Allyl anisole, *t*-butyllithium (1.7 M solution in anhydrous pentane), anhydrous tetrahydrofuran, anhydrous dichloromethane, anhydrous zinc chloride (1.0 M solution in anhydrous diethyl ether), bis(triphenylphosphine) palladium(II)chloride, diisobutylaluminum hydride (1.0 M solution in hexane), hexane, tris-(tiphenylphosphine) rhodium(I) chloride, toluene, deuterated chloroform, tetramethylsilane and florisil were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin).

Honokiol was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Alternatively, honokiol may be extracted by supercritical carbon dioxide extraction from suitable portions of Magnolia trees (Chandra and Nair, 1995).

Tetrahydrohonokiol was prepared by the hydrogenation in the presence of 5% Palladium on active carbon catalyst as described by Fujita *et al.* (1972).

2-Propyl-4-iodo anisole was obtained from Rann Research Laboratory (San Antonio, Texas).

Ammonium chloride, sodium chloride, anhydrous diethyl ether and anhydrous sodium sulfate were purchased from Fisher Scientific (Fair Lawn, New Jersey). HPLC grade Ethyl acetate, acetone, water and acetonitrile were obtained from Burdick and Jackson Inc. (Muskegon, Michigan).

Analytical and preparative TLC plates (20 × 20 cm glass plates coated with 250 micron and 1 mm thickness of silica gel (G) were purchased from Analtech Inc. (Newark, Delaware).

HPLC

The analytical and preparative high pressure liquid chromatography were performed on a Milton Roy HPLC equipment consisting of a CM 4000 solvent delivery system, the autoinjector A1000 and the spectro-monitor 3100 variable wavelength detector from LCD Analytical (Riviera Beach, Florida). A reverse phase HPLC column (Econosil C18, 10μ, 250 × 10 mm, Catalog #C231) was purchased from Alltech Associates, Inc. (Deerfield, Illinois). The solvent for elution consisted of a mixture of acetonitrile and water (6:4). The elution of the honokiol and its analogs was detected at a wavelength of 280 nm. The chart speed was 0.5 cm/min, solvent flow rate was 5 ml/min and the detection was performed at the sensitivity of 0.05 and 2.0 absorbance units full scale (for analytical and preparative use, respectively). The proton magnetic resonance spectra were recorded on a 300 MHz GE QE 300 NMR spectrometer. The samples were dissolved in deuterated chloroform and tetramethylsilane was used as the internal standard.

5.1.2 Synthesis of 3-*n*-Propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) and 5′-*n*-Propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II) by the Partial Hydrogenation of Honokiol:

Hydrogen gas was passed for two hours with stirring in a solution of tris-(triphenylphosphine) rhodium(I) chloride (10 mg) in toluene (4 ml) at room temperature. Then, honokiol (20 mg) dissolved in toluene (1 ml) was added slowly into the reaction flask with stirring and the hydrogen gas was passed for another 10

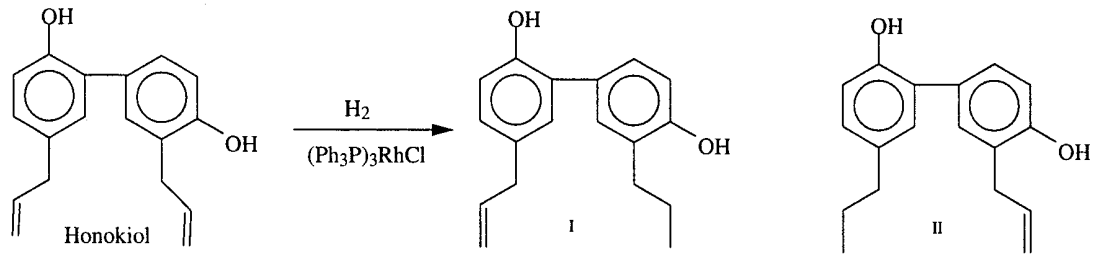
min after the complete addition of honokiol solution. The reaction mixture was stirred overnight at room temperature. The reaction mixture was passed through a column of florisil (5 gm), which was finally washed with 50 ml of dry diethyl ether. The solvents were evaporated to obtain crude residue which was resuspended in aforesaid HPLC eluant to be injected in the HPLC system for analysis. The HPLC analysis indicated the presence of three peaks:

(a) peak 1: Retention time- 9 min, honokiol.

(b) peak 2 (86.4% based on honokiol consumed): Retention time- 11 min.

(c) peak 3 (13.6% based on honokiol consumed): Retention time- 14.2 min, m/z 270, confirmed to be tetrahydrohonokiol when compared with standard tetrahydrohonokiol.

The compounds eluting as peak 2 were identified as the two isomers of dihydrohonokiol. The first component of the peak (up to the begining of the shoulder) was 92% of the total peak, [m/z 268; ^{1}H NMR($CDCl_3$): δ: 0.994 (t,3H), 1.663 (dt,3H), 2.629 (t,2H), 3.347 (d,2H), 5.03-5.11 (m,2H), 5.90-6.04 (m,1H), 6.88-7.20 (m, 6H) with a small shoulder (approximately 8% of total peak 2) [^{1}H NMR($CDCl_3$): δ: 0.994 (t,3H), 1.663 (dt,3H), 2.55(t,2H), 3.45(d,2H), 5.13(m,2H), 5.90-6.04 (m,1H), 6.88-7.20 (m,6H). The dihydrohonokiol isomers in peak 2 were isolated by preparative HPLC purification. The major component (approximately 92%) was assigned the structure as 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I), while the minor component (approximately 8%). was assigned the structure as 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol, (II). The HPLC retention times for the purified isomers was 11 min for isomer I and 12 min for isomer II. A typical reaction resulted in a product mixture consisting of 55% dihydrohonokiols-, 35.5% unreacted honokiol and 9.5% tetrahydrohonokiol. The dihydrohonokiol isomers represented approximately 86% of the dihydrohonokiol consumed in the reaction.



Synthesis of 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) and 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II) from Honokiol by Partial Hydrogenation

5.1.3 Total Synthesis of 3-*n*-Propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) and 5′-*n*-Propyl-3-(2-propenyl)-1,1′biphenyl-2′,4-diol (II)

5.1.3.1 3-*n*-Propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I):

To a solution of 4-allylanisole (148 mg, 1.0 mmol) in 1 ml anhydrous tetrahydrofuran was added a solution of tert-butyllithium in pentane (1.5 mmol; as 0.88 ml of 1.7 M solution in anhydrous pentane) with stirring under the nitrogen atmosphere at -78°C. After two hours, the mixture was warmed to -10°C and the solution was reacted with anhydrous zinc chloride (1 ml, 1.0 mmol; as 1.0 M solution in anhydrous diethyl ether). Then, the reaction mixture was stirred at room temperature for 1 h. The palladium catalyst was prepared separately. Thus, an anhydrous hexane solution of diisolbutyl aluminum hydride (0.066 ml, 0.066 mmol; 1.0 M solution in hexane) was added to a solution of bis-(triphenylphosphine)palladium(II) chloride (22 mg; 0.033 mmol) in 1 ml anhydrous tetrahydrofuran with stirring under nitrogen atmosphere. This organic catalyst solution was then reacted with 2-propyl-4-iodoanisole (193 mg, 0.70 mmol) in 2 ml of anhydrous tetrahydrofuran and a solution of the aryl zinc chloride, prepared as above. The mixture was stirred for 2 h at room temperature and then quenched with 5 ml saturated ammonium chloride solution. The aqueous layer was extracted with three 10 ml portions of ethyl acetate. The combined extract was washed once with brine and dried over anhydrous sodium sulphate. After the removal of solvent in vacuo, the crude product was chromatographed on a 1 mm thickness 20 × 20 cm silica

gel plate (silica gel G; eluent: hexane, ethyl acetate; 9:1) to get 2′,4-dimethoxy-3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl (95 mg, 45.9%; based on the amount of 2-propyl-4-iodoanisole): m/z: 296; ^{1}H NMR ($CDCl_3$): δ: 0.90 (t, 3H, 3-CH_2.CH_2.$C\underline{H}_3$), 1.45 (qt,3H, 3-CH_2.$C\underline{H}_2$.CH_3), 2.53 (t,2H, 3-$C\underline{H}_2$.CH_2.CH_3), 3.38 (d,2H, 5′-$C\underline{H}_2$.HC=CH_2), 3.71 (s, 3H, 4-$OC\underline{H}_3$), 3.77 (s, 3H, 2′-$OC\underline{H}_3$), 4.99 (dd,2H, 5′-CH_2.HC=$C\underline{H}_2$), 5.85-5.98 (m, 1H, 5′-CH_2.$\underline{H}$C=CH_2), 6.79-7.28 (m, 6H, aromatic $\underline{H}$).

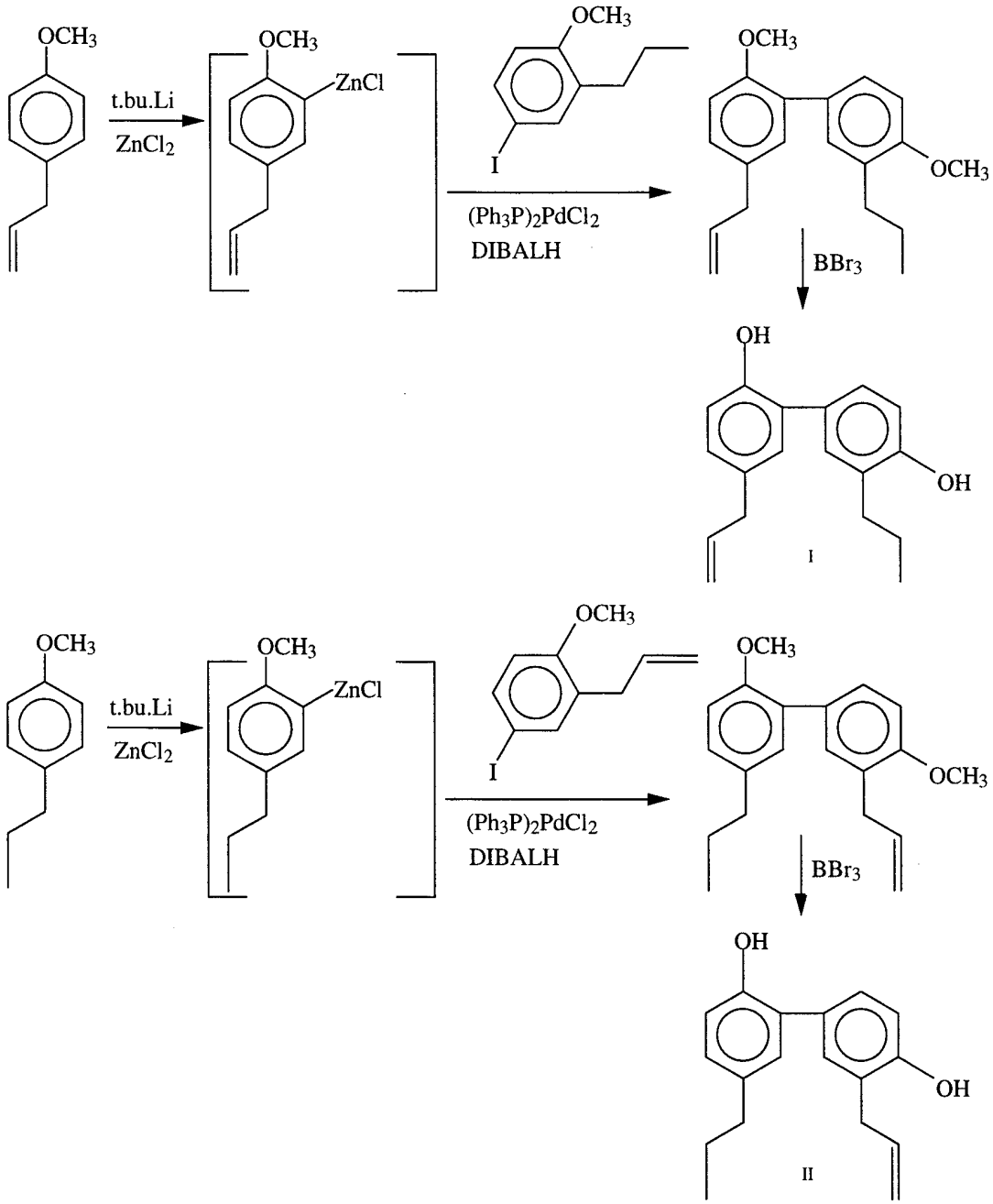
To a solution of 2′,4-dimethoxy-3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl (10 mg: 0.03 mmol) in 1 ml of anhydrous dichloromethane was slowly added a dichloromethane solution of boron tribromide (75 microliters, 1M solution in dichlormethane) at -78°C with magnetic stirring for one h. Then, the reaction mixture was stirred at room temperature overnight. Next day, the reaction was cooled and subjected to an aqueous quenching. The aqueous phase was extracted three times with 2 ml portions of dichloromethane. The combined organic layer was washed once with brine (1 ml) and dried over anhydrous sodium sulfate. The solid was filtered off and the solvent was evaporated under nitrogen. The crude product was chromatographed on a 250 micro thickness 20 × 20 cm silica gel plate (silica gel G; eluent: hexane, ethylacetate, 80:20) to get 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (6.5 mg; 72%). In a HPLC analysis, the retention time of total synthetic 3-*n*-propyl-5′-(2-propenyl)-1,1′- biphenyl-2′,4-diol was comparable to that of the hemi-synthetic sample made earlier; and in a mixed co-injection both co-eluted together. The structure was further confirmed by the proton magnetic resonance analysis on a HPLC pure sample. ^{1}H NMR ($CDCl_3$): δ: 0.994 (t, 3H, 3-CH_2.CH_2.$C\underline{H}_3$), 1.663 (qt,3H, 3-CH_2.$C\underline{H}_2$.CH_3), 2.629 (t,2H, 3-$C\underline{H}_2$.CH_2.CH3), 3.347 (d,2H, 5′-$C\underline{H}_2$.HC=CH_2), 5.03-5.11 (dd,2H, 5′CH_2.HC=$C\underline{H}_2$), 5.90-6.04 (m,1H, 5′-CH_2.$\underline{H}$C=CH_2), 6.88-7.20 (m, 6H, aromatic $\underline{H}$).

5.1.3.2 5′-*n*-Propyl-3-(2-propenyl)-1,1′biphenyl-2′,4-diol (II):

The same method as described above in § 5.1.3.1 for the synthesis 2′,4-dimethoxy-3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl can be used to synthesize 2′4-dimethoxy-5′-n-propyl-3-(2-propenyl)-1,1′-biphenyl. Thus, 4-propylanisole is

reacted with tert-butyllithium and anhydrous zinc chloride to form an aryl zinc chloride which is then coupled with 2-allyl-4-iodo-anisole, in the presence of the palladium catalyst made in a separate reaction of bis-(triphenylphosphine)palladium(II) chloride and diisolbutyl aluminum hydride. The product, 2′4-dimethoxy-5′-n-propyl-3-(2-propenyl)-1,1′-biphenyl, is purified by extraction and thin layer chromatography as described above.

The methyl protecting groups are removed from 2′4-dimethoxy-5′-n-propyl-3-(2-propenyl)-1,1′-biphenyl as described above in § 5.1.3.1 for 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol. The product, 5′-*n*-propyl-3-(2-propenyl)-1,1′biphenyl-2′,4-diol (II), is purified by extraction and thin layer chromatography, as described above.



Total synthesis of 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) and 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol (II)

5.2 Example 2• Synthesis of Analogs and Homologs of Dihydrohonokiol

For the synthesis of analogs and homologs of dihydrohonokiol, 4-allylphenyl alkyl ethers can be converted to 2-alkoxy, 5-allylphenyl metal halides by a directed

ortho metalation reaction, which in turn is reacted with various 4-alkoxyhaloaryls *in situ* to generate the homologs and analogs of 3-*n*-propyl, 5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol. Similarly, various substituted-phenyl metal halides are reacted *in situ* with various 5′-*n*-propyl, 3-(2-propenyl)-1,1′-biphenyl-2′,4-diol.

Carboxylic groups may be protected as esters (*e.g.*, ethyl ester) during the synthetic process. The dihydrohonokiol analog formed after the coupling reaction and demethylation can be deprotected by based catalyzed hydrolysis of the ester group. Thus, for example, for the synthesis of 3-*n*-propyl-5′-(3-carboxy propyl)-1,1′-biphenyl-2′,4-diol, the starting compound 3-(4-methoxyphenyl)-propionic acid, available from Aldrich (Milwaukee, WI) can be converted to its acid chloride and then to the ethyl ester, *i.e.* 3-(4-methoxyphenyl)-propionate. This compound may be treated with *tert*-butyllithium followed by anhydrous zinc chloride to form the intermediate 2-methoxy, 5-(3-ethoxy carbonyl propyl)-phenyl zinc chloride. This intermediate can be coupled with 2-n-propyl-4-iodo anisole in the presence of the palladium catalyst (prepared *in situ* by the reaction of bis-(triphenyl phosphine) palladium (II) chloride and diisobutyl aluminum hydride) to synthesize 2′,4-dimethoxy-3-*n*-propyl-5′-(3-ethoxycarbonyl propyl)-1,1′-biphenyl. The demethylation of this compound with borontribromide will give 3-*n*-propyl-5′-(3-ethoxycarbonyl propyl)-1,1′-biphenyl-2′,4-diol. Finally, the base catalyzed hydrolysis of the ester group, *e.g.*, with potassium hydroxide, will generate 3-*n*-propyl-5′-(3-carboxy propyl)-1,1′-biphenyl-2′,4-diol.

Alcoholic groups may be protected as acyl esters, *e.g.*, by base catalyzed acetylation with acetic anhydride. The dihydrohonokiol analog formed after the coupling and demethylation can be deprotected by base catalyzed removal of acyl groups. Thus, for example, for the synthesis of 3-*n*-propyl-5′-(2-hydroxy propyl)-1,1′-biphenyl-2′,4-diol, the intermediate 1-(4-methoxyphenyl)-2-propanol is made by sodium borohydride reduction of 4-methoxyphenyl acetone, available from Aldrich (Milwaukee, WI). The 1-(4-methoxyphenyl)-2-propanol can be reacted with acetic anhydride in basic medium to get 1-(4-methoxyphenyl)-2-propyl acetate. This compound may be treated with *tert*-butyllithium followed by anhydrous zinc chloride to get 2-methoxy, 5-(2-acetoxypropyl)-phenyl zinc chloride as an intermediate. This

intermediate can be coupled with 2-*n*-propyl-4-iodo anisole in the presence of the palladium catalyst (prepared *in situ* by the reaction of bis-(triphenyl phosphine) palladium (II) chloride and diisobutyl aluminum hydride) to synthesize 2′,4-dimethoxy-3-*n*-propyl-5′-(2-acetoxypropyl)-1,1′-biphenyl. The demethylation of this compound with borontribromide will yield 2′,4-dimethoxy-3-*n*-propyl-5′-(2-acetoxypropyl)-1,1′-biphenyl-2′,4-diol. Finally the base catalyzed hydrolysis of the acetyl group will generate 3-n-propyl-5-(2-hydroxy propyl)-1,1-biphenyl-2,4-diol.

5.3 Example 3• Anxiolytic Effect of a Mixture of Dihydrohonokiol Isomers in the Elevated Plus-Maze Test in Mice

5.3.1 Materials and Methods

5.3.1.1 Animals

Male mice of the Balb/C strain (Harlan, Indianapolis, IN) were used at about 6 wk of age and weighing of 22-27 g. Groups of 5 mice each were housed in standard polycarbonate cages (15W × 25L×12H cm) with woodchip bedding with free access to a standard solid diet and tap water. The environment of the animal room was well controlled with a temperature of 23±1°C, humidity 40-55% and a 12:12-h light-dark cycle; lights on between 0600-1800h).

All the experimental procedures were carried out according to "The Guide for the Care and Use of Laboratory Animals and Animal Welfare Act, and the experimental protocol (97031-34-01-A) was approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

5.3.1.2 Drugs

For oral administration, the mixture of dihydrohonokiol isomers I and II prepared by the partial hydrogenation of honokiol (designated I/II mixture) as described in § 5.1.2, was first dissolved in a very small amount of ethanol, and the solution was diluted with physiological saline containing Tween-80 (0.1%) (vehicle). Diazepam was suspended in the Tween-80/physiological saline. The concentration of

each drug solution or suspension was adjusted so that each volume administered was constant at 0.1 ml/10 g body weight of the mouse.

5.3.1.3 Apparatus and Experimental Procedures

The elevated plus-maze test: The elevated plus-maze used was the same as that used in previous studies (Kuribara *et al.*, 1996; Maruyama *et al.*, 1997), and was an improvement of the original apparatus for rats (Pellow *et al.*, 1985) and for mice (Lister, 1987). Briefly, the plus-maze consisted of two closed-arms and two open-arms (6W × 30L cm). The arms extended from a center platform (8 × 8 cm). The closed arms had side-walls (10H cm), and they were made of non-transparent polyvinyl chloride of gray color. The open arms were made of transparent acrylic fiber, and had no side-walls. The platform was made of non-transparent polyvinyl chloride of gray color. This plus-maze was set 40 cm above the base. Each mouse was placed at the center platform facing one of the closed arms, and the cumulative time of entering into the open arms during the 5 min observation period was recorded. The criterion of the mouse's entering into the open arms was crossing with all four paws the borderline separating the open arm and the center platform.

Activity test: Mouse activity was measured with a tilting-type ambulometer which had a bucket-like Plexiglas activity cage 20 cm in diameter (SMA-1: O'Hara and Co., Tokyo). Only a horizontal movement (ambulation) of the mouse caused a slight tilt of the activity cage, and was detected by microswitches attached to the cage. The duration of measurement was 5 min for each mouse. This activity test was carried out immediately after the end of the plus-maze test.

Traction test: The traction test was carried out to assess muscle strength. This experimental procedure was described elsewhere (Kuribara *et al.*, 1977). Briefly, a wire (1.6 mm in diameter, and 30 cm in length) was set horizontally 30 cm above the base. The mouse was first allowed to grip the wire with the fore paws, and the duration of clinging to the wire was measured up to 60 sec. The trial was held twice for each mouse, and the longer duration of clinging was used in the calculation of mean value. When the duration of clinging was over 60 sec, the mouse was released from the wire, and the clinging time was recorded as 60 sec. The traction test was carried out immediately after the end of the activity test.

5.3.1.4 Statistical Analysis

The time in open arms in the elevated plus-maze test, the activity counts in the activity test, and the durations of clinging in the traction test were compared by the Fisher's protected least significant difference statistical test. Values of *P* less than 0.05 were considered significant.

5.3.2 Dose Response of Dihydrohonokiol Isomers I/II Mixture

TABLE 1

Dose Effect in the Mouse of I/II Mixture Assessed Using the Elevated Plus-Maze and Motor Activity

Treatment, P.O.		Time (sec) spent in open arm		Motor Activity	
	mg/kg	1 h post adm.	3 h post adm.	1 h post adm.	3 h post adm.
Vehicle		13.2 ± 2.7	13.1 ± 4.7	30.6 ± 1.7	31.2 ± 1.5
I/II	0.1		26.0 ± 8.4		30.5 ± 1.5
I/II	0.2	27.6 ± 6.2	37.9 ± 6.2*	36.1 ± 2.3	21.9 ± 3.3
I/II	0.5	33.8 ± 4.9	87.3 ± 12.8*	32.7 ± 2.7	26.0 ± 1.8
I/II	1.0	53.8 ± 7.7	90.0 ± 9.2*	32.0 ± 1.7	30.8 ± 1.5
I/II	2.0	50.2 ± 5.7	95.3 ± 8.7*	34.6 ± 2.0	27.0 ± 1.7

The data for the plus-maze are mean times ± SEM spent in the open arm during the observation period of 5 min. The data for the activity test are mean counts ± SEM during the observation period of 5 min. The group size was 10. *$p<0.002$. Increasing doses of the I/II isomer mixture cause an increasing anxiolytic effect- a dose/effect relationship. A 0.2 mg/kg oral dose of the I/II mixture shows significant anxiolytic effects at 3 h post administration, while a higher dose of 1 mg/kg is significantly anxiolytic at only one hour. Since the inventors used the 0.2 mg/kg dose orally in this series the tests were performed 3 h after dosing of the I/II mixture.

Motor activity was unchanged by dose or time except for the 0.2 mg/kg dose at 3 h post administration.

5.3.3 Duration of I/II Mixture Induced Anxiolytic Activity

TABLE 2

Duration of Pharmacological Action Following 0.5 mg/kg I/II Mixture Given Orally

Treatment mg/kg		Hours post treatment	Time spent in open arm, sec	Motor activity
Vehicle		0	12.9 ± 3.8	26.9 ± 2.1
I/II	0.5	1/2	15.5 ± 3.7	28.6 ± 1.7
I/II	0.5	1	33.8 ± 4.9	32.7 ± 2.7
I/II	0.5	2	43.0 ± 7.5*	25.8 ± 1.1
I/II	0.5	3	87.3 ± 12.8*	26.0 ± 1.8
I/II	0.5	4	77.7 ± 13.2*	26.9 ± 2.0
I/II	0.5	6	37.4 ± 11.0	22.3 ±1.8
I/II	0.5	8	46.2 ± 9.1*	27.5 ± 2.1
I/II	0.5	12	47.0 ± 13.1*	24.0 ± 2.0
I/II	0.5	24	14.0 ± 5.0	17.3 ± 3.1
I/II	0.5	48	11.5 ± 4.5	27.3 ± 1.5

The data for the plus-maze test are mean times ± SEM spent in the open arm during the observation period of 5 min. The data for the activity test are the mean counts ± SEM during the test period of 5 min. The group size was 10 mice. *$p<0.02$ vs control vehicle.

The duration of anxiolytic activity of the I/II mixture following an oral dose of 0.5 mg/kg extends for at least 12 h post administration, ending between 12 h and 24 h after oral administration. The anxiolytic activity was not measured between 12 and 24 h. This long duration of action indicates the possibility of a one single dose per

day schedule. Single dosage greatly improves patient compliance. The motor activity was unchanged.

5.3.4 Duration of Diazepam Anxiolytic Activity

TABLE 3

Duration of Pharmacological Action Following 1.0 mg/kg Diazepam Given Orally

Treatment, dose in mg/kg	Hours post treatment	Time spent in open arm, sec.	Motor activity	Traction
Vehicle	0	12.3 ± 2.6	24.9 ± 1.7	60.0 ± 0.0
Diazepam 1.0	0.17	43.5 ± 6.1*	38.9 ± 3.2*	42.6 ± 6.3*
Diazepam 1.0	0.5	41.2 ± 5.2*	23.2 ± 3.8	53.3 ± 3.7
Diazepam 1.0	1	29.7 ± 6.9*	32.1 ± 2.3	60.0 ± 0.0
Diazepam 1.0	2	27.0 ± 8.0	32.2 ± 4.1	60.0 ± 0.0
Diazepam 1.0	4	21.7 ± 6.3	31.6 ± 3.1	60.0 ± 0.0
Diazepam 1.0	8	13.2 ± 5.9	27.0 ± 2.4	60.0 ± 0.0
Diazepam 1.0	24	12.2 ± 2.7	29.5 ± 3.4	60.0 ± 0.0

The data for the plus-maze test are mean times ± SEM spent in the open arm during the observation period of 5 min. The data for the activity test are the mean counts ± SEM during the test period of 5 min. The group size was 10 mice. *$p<0.04$ vs control.

The benzodiazepine compound diazepam was used as a positive control for anxiolytic activity in this series of tests because of wide use and its acceptance as representative of the benzodiazepine series of compounds. The time of peak anxiolytic action of diazepam is 0.17 h (10 min.). The anxiolytic tests in the subsequent series were done on diazepam 10 min after oral dosing to obtain the peak response. The duration of diazepam following the effective dose of 1 mg/kg orally is shown to be 1 h for its anxiolytic activity and 0.17 h for its effect on motor activity and its detrimental affect on traction (ataxia production).

5.3.5 Effect of Multiple Doses of I/II Mixture and Diazepam

TABLE 4

Effect of seven days treatment with 0.2 mg/kg po I/II Mixture and 1.0 mg/kg po diazepam

Treatment	Time in open arm (sec)	Motor activity	Traction
vehicle	15.6±5.0	25.9±3.8	60.0±0.0
I/II	44.9±7.7*	31.9±4.1	60.0±0.0
vehicle	12.3±2.6	24.9±1.7	60.0±0.0
Diazepam	43.5±6.1*	38.9±3.2*	42.6±6.2*

*$p<0.016$ vs vehicle control

The data for the plus-maze are mean times ± SEM spent in the open arm during the observation period of 5 min. The data for the activity test are mean counts ± SEM during the test period of 5 min. Traction was evaluated by the ability of the mouse to hold on to a bar for 60 sec. The group size was 10 mice.

Tolerance following chronic use, that is diminished anxiolytic activity requiring increasing doses in long term use, is evaluated in this data. The I/II mixture and diazepam were given orally each day for 7 days and then the anxiolytic activity was tested. There was no decrease in the effectiveness of either agent. The dose of each compound produced the same anxiolytic activity after 7 days of dosing as occurred after one dose. The I/II mixture still did not change motor activity. Diazepam still retained its change of motor activity and diminution of traction.

5.3.6 Synergistic Effect of Combination of I/II Mixture and Diazepam

TABLE 5

Synergy in the anxiolytic activity of I/II Mixture and Diazepam

Treatment mg/kg po	Time in open arm, in sec	Motor Activity	Traction
Vehicle	13.1 ± 4.7	31.2±1.5	60.0±0.0
I/II 0.2	37.9 ± 6.2*	21.9±3.1	60.0±0.0
Diazepam 1.0	43.5 ± 6.1*	38.9±3.2#	42.6±6.3*
I/II + Diazepam	137.0 ± 11.0**	27.1±4.5	44.0±4.8*

*p<0.02 vs vehicle control.

**p<0.0001 vs I/II mixture or diazepam alone.

#p<0.02 vs I/II mixture + diazepam.

The I/II mixture was given 3 h before testing and diazepam was given 10 min before testing. The data for the plus-maze are mean times ± SEM spent in the open arm during the observation period of 5 min. The data for the motor activity are mean counts ± SEM during the test period of 5 min. Traction was evaluated by the ability of the mouse to hold on to a bar for 60 sec. The group size was 10 mice.

To establish whether the I/II mixture and diazepam interact, an effective dose of the I/II mixture and diazepam were given to separate mice to show the anxiolytic activity of each compound when given alone. The effective dose of each compound given using appropriate timing was then combined mice. The combination of I/II mixture and diazepam resulted in a 3 fold increase in anxiolytic activity over either agent given separately. The compounds act synergistically as anxiolytics. The effect of diazepam on motor activity is abolished by combining with the I/II mixture UT_2 but the I/II mixture has no effect on the ataxia produced by diazepam.

5.3.7 Effect of Coadministration of Flumazenil

TABLE 6

The Effect of the Benzodiazepine Receptor Antagonist Flumazenil on the Anxiolytic Effect of the I/II Mixture and Diazepam

Treatment	Challenge	Time in Open Arms
Vehicle	saline	13.0 ± 3.4
Vehicle	flumazenil	6.7 ± 2.9
I/II	saline	37.9 ± 6.2
I/II	flumazenil	31.2 ± 3.8
Diazepam	saline	43.5 ± 6.1
Diazepam	flumazenil	13.5 ± 3.8*

*$p<0.0001$ vs the mice pretreated only with diazepam.

The time in the open arm of the elevated plus maze was observed during a 5 min period. The I/II mixture (0.2 mg/kg po) was given 3 h before testing. Diazepam (1 mg/kg po) was given 10 min before testing. Flumazenil (0.3 mg/kg sc) was given 10 min before testing.

Flumazenil is a benzodiazepine receptor blocking agent which when given blocks the anxiolytic effects of benzodiazepines. Flumazenil blocks the anxiolytic activity of diazepam but does not block the anxiolytic activity of the I/II mixture. This indicates that the I/II mixture does not physiologically act at the same site as the benzodiazepine anxiolytics.

TABLE 7

The Effects of the Benzodiazepine Receptor Antagonist Flumazenil on Motor Activity and Traction Measured Following Administration of Diazepam or I/II mixture

Treatment	Challenge	Motor Activity	Traction
Vehicle	saline	29.0 ± 2.4	60.0 ± 0.0
Vehicle	flumazenil	25.6 ± 3.0	60.0 ± 0.0
I/II	saline	21.9 ± 3.1	60.0 ± 0.0
I/II	flumazenil	28.6 ± 2.7	60.0 ± 0.0
Diazepam	saline	38.9 ± 3.2*	42.6 ± 6.3#
Diazepam	flumazenil	27.9 ± 1.8**	54.5 ± 2.9##

*$p < 0.01$ vs control

**$p < 0.007$ vs diazepam alone

#$p < 0.0001$ vs control

##$p < 0.004$ vs diazepam alone

The data for the activity test are for an observation period of 5 min. The data for the traction test are the mean duration of clinging. The I/II mixture (0.2 mg/kg po) was given three hours before testing. Diazepam (1 mg/kg po) was given ten min before testing as was flumazenil (0.3 mg/kg sc).

Motor activity and traction are not changed by the I/II mixture or flumazenil. Flumazenil blocks the motor activity effect of diazepam and significantly decreases the diazepam alteration of traction. These serious side effects of the benzodiazepine anxiolytics appear to reside in that receptor interaction.

5.3.8 Effect of Coadministration of Bicuculline

TABLE 8

The Effect of the GABA Antagonist Bicuculline Administered with the I/II mixture and Diazepam

Treatment mg/kg po	Bicuculline, mg/kg	Time spent in open arm, sec.	Motor Activity	Traction
Vehicle	saline	13.0±3.5	29.0±2.4	60.0±0.0
Vehicle	0.1	7.1±2.8	21.5±2.7	60.0±0.0
I/II 0.2	saline	37.9±6.2*	21.9±3.1	60.0±0.0
I/II 0.2	0.1	16.7±5.3	36.1±2.7	60.0±0.0
Diazepam 1.0	saline	43.5±6.1*	38.9±3.2*	42.6±6.3*
Diazepam 1.0	0.1	17.4±4.9	37.9±2.3*	46.6±6.9*

*$p<0.5$ vs control vehicle.

The I/II mixture was given 3 h before testing and diazepam and bicuculline were given 10 min before testing. The data for the plus-maze are mean times ± SEM spent in the open arms during the observation period of 5 min. The data for the motor activity are mean counts ± SEM during the test period of 5 min. Traction was evaluated by the ability of the mouse to hold on to a bar for 60 sec. The group size was 10 mice.

The GABAergic antagonist bicuculline abolished the anxiolytic activity of both the I/II mixture and diazepam identifying a common effect on the GABAergic system. Bicuculline did not block the effect of diazepam on motor activity or traction.

5.3.9 Effect of Coadministration of Caffeine

TABLE 9

Effect of Caffeine on the Pharmacological Activity of Diazepam and I/II Mixture

Treatment	Challenge	Time in Open	Motor Activity	Traction
Vehicle	saline	14.0±3.8	28.9±2.4	60.0±0.0
Vehicle	Caffeine	13.9±9.0	60.5±7.4***	60.0±0.0
I/II	saline	37.9±6.2	21.9±3.1	60.0±0.0
I/II	Caffeine	6.1±2.2*	61.1±5.0#	60.0±0.0
Diazepam	saline	43.5±6.1	38.9±3.2	42.6±6.3+
Diazepam	Caffeine	93.8±11.3**	55.1±7.1##	50.4±5.1+

*p<0.003 vs I/II mixture

**p<0.0001 vs diazepam

***p<0.0001 vs control

#p<0.0001 vs I/II mixture

##p<0.03 vs diazepam

+ p<0.05 vs vehicle control

I/II mixture, 0.2 mg/kg, was given po 3 hr before testing. Diazepam 1.0 mg/kg was given po 10 min before testing. Caffeine 30 mg/kg was given ip 15 min before testing. The time in sec in the open arms is reported as means ± SEM during a 5 min observation period. The motor activity is reported as mean ± SEM for the five min test period. Traction reports the ability of the mouse to hold to a bar for 60 sec mean ± SEM. Group size was 10 mice.

Caffeine is an axiogenic agent in high doses and increases motor activity. Given at a high dose with the I/II mixture, caffeine abolished the anxiolytic activity of the I/II mixture. A series of doses would be needed to establish a dose effect curve. Diazepam combined with caffeine resulted in increased anxiolytic activity and motor activity but did not change the ataxia production.

5.3.10 Effect of Coadministration of CCK

TABLE 10

The Effect of CCK on the Pharmacological Activity of the I/II Mixture and Diazepam

Treatment	Challenge	Time in Open	Motor Activity	Traction
Vehicle	saline	13.7 ± 4.9	27.1 ± 1.9	60.0 ± 0.0
Vehicle	CCK	2.2 ± 1.1	29.2 ± 1.9	60.0 ± 0.0
I/II	saline	37.9 ± 6.2	21.9 ± 3.1	60.0 ± 0.0
I/II	CCK	11.1 ± 3.6*	20.2 ± 3.7	60.0 ± 0.0
Diazepam	saline	43.5 ± 6.1	38.9 ± 3.2	42.6 ± 6.3
Diazepam	CCK	57.3 ± 13.0	34.1 ± 5.1	41.3 ± 7.7

*$p<0.008$ vs I/II mixture alone.

The I/II mixture was given 0.2 mg/kg po 3 h before the test. Diazepam 1.0 mg/kg po and CCK 50 ug/kg ip were given 10 min before the test. The data for the plus-maze test are mean ± SEM time spent in the open arms during the 5 min observation period. The data for the motor activity are mean counts ± SEM during the 5 min test period. Traction was evaluated by the ability of the mouse to hold on to a bar for 60 sec. The group size was 10 mice. CCK is cholecystokinin Ac-fragment 26-29 amide non-sulfated.

Cholecystokinin (CCK) is anxiogenic and its administration is used as a screen for discovery of new anxiolytic agents. CCK in this initial test shows it anxiogenic effect and effectively abolished the anxiolytic effect of I/II mixture. It had no effect on any of the three effects of diazepam.

TABLE 11

The Effect of a Constant Dose of CCK on Three Doses of the I/II Mixture

Treatment	Dose mg/kg	Challenge	Time in Open	Motor Activity
Vehicle	0	saline	13.7 ± 4.9	27.1 ± 1.9
Saline	0	CCK	2.2 ± 1.1	29.2 ± 2.2
I/II	0.2	saline	37.9 ± 6.3	21.9 ± 3.1
I/II	0.2	CCK	11.1 ± 3.5	20.2 ± 3.7
I/II	0.5	saline	87.3 ± 12.8	26.0 ± 1.8
I/II	0.5	CCK	28.5 ± 5.4*	35.5 ± 4.7#
I/II	2.0	saline	95.3 ± 8.7	27.0 ± 1.7
I/II	2.0	CCK	48.3 ± 15.0*#	32.3 ± 2.1

*$p<0.05$ vs CCK

# $p<0.05$ vs control vehicle.

The I/II mixture was given orally 3 h before the test. CCK, 50 ug/kg, was given ip 10 min before the test. The data for the plus-maze are mean times ± SEM spent in the open arms during the 5 min observation period. The data for the motor activity are mean counts ± SEM during the 5 min test interval. The group size was 10 mice. CCK is cholecystokinin Ac-fragment 26-29 amide non-sulfated.

CCK at a constant dose of 50 micrograms/kg abolishes the anxiolytic activity of the I/II mixture at a dose of 0.2 mg/kg po. CCK is antagonized in a dose dependent manner by 0.5 mg/kg and 2 mg/kg doses of the I/II mixture.

5.4 Example 4• Evaluation of Dependence Liability of the Dihydrohonokiol Isomer Mixture

TABLE 12

Number of Mice in Groups of Ten Showing Symptoms When Treated with Flumazenil Following 12 Days Administration of Diazepam or I/II Mixture

Drug	Flumaz.	Hr	Tr	Cc	Tc	Tf	Rf
Saline	0	1	0	0	0	0	0
Saline	10 mg/kg	3	0	0	0	1	0
Diazepam 0.5 mg/kg	10	7	0	0	0	0	0
1.0	10	10	0	0	0	0	3
2.0	10	10	5	0	0	2	5
5.0	10	10	7	0	0	1	8
10.0	10	10	5	3	1	1	10
I/II 0.2 mg/kg	10	4	0	0	0	0	0
0.5	10	0	0	0	0	0	0
2.0	10	3	0	0	0	0	0

Saline and Tween 80, I/II mixture and Diazepam were given orally once a day for twelve days. The challenge with 10 mg/kg I p Flumazenil followed 24 h after the last treatment. The number represents the number of mice of the ten treated mice showing symptoms during the 20 min observation period following Flumazenil treatment.

Hr: hyper-reactivity indicated by vocalization caused by light pressure on the back

Tr: tremor

Cc: clonic convulsions

Tc: tonic convulsions

Tf: tail flick

Rf: running fit evoked by auditory stimulus

To evaluate dependence liability, I/II mixture and diazepam were given for 12 days. This was followed by administration of flumazenil the benzodiazepine antagonist. No dihydrohonokiol (I/II mixture) antagonist is known. Six signs of withdrawal following flumazenil were observed and recorded. Diazepam showed extensive withdrawal signs following all doses 0.5 mg/kg to 10 mg/kg. The I/II mixture showed no withdrawal response at the tested doses of 0.2 mg/Kg, 0.5 mg/Kg or 2.0 mg/Kg. The 2.0 mg/Kg dose is 10 times the effective dose. The hyper-reactivity response of the I/II mixture given flumazenil was no different from flumazenil given alone. The 0.5 mg/kg dose of the I/II mixture followed by flumazenil produced no hyper-reactivity response at all.

5.5 Example 5• Evaluation of Sedative Potential of the Dihydrohonokiol Isomer Mixture

TABLE 13

Effect of I/II Mixture, Diazepam and I/II mixture plus Diazepam on the Duration of Hexobarbital Induced Sleep

Treatment, dose mg/kg po following hexobarbital		Additional drug, dose mg/kg po		Duration of sleep in sec
Vehicle				2552 ± 209
I/II	0.1			2822 ± 138
I/II	0.2			2580 ± 255
I/II	0.5			2753 ± 296
I/II	1.0			2440 ± 37
I/II	2.0			2870 ± 191
Vehicle				2902 ± 84
Diazepam	1.0			4476 ± 336*
Diazepam	1.0	I/II	0.1	4342 ± 195*
Diazepam	1.0	I/II	0.2	4248 ± 66*
Diazepam	1.0	I/II	0.5	4526 ± 467*
Diazepam	1.0	I/II	1.0	4052 ± 217*
Diazepam	1.0	I/II	2.0	4758 ± 299*

*$p<0.0003$ vs vehicle containing hexobarbital control.

All mice received 100 mg/kg hexobarbital ip. The I/II mixture was given orally 3 h prior to hexobarbital to obtain the maximum anxiolytic effect of the I/II mixture. Diazepam was given 10 min before hexobarbital to obtain the maximum effect. The time from loss of righting reflex in each mouse to the return of the righting reflex was recorded as the sleep duration.

To evaluate the sedative effects of the I/II mixture and diazepam all mice received a sedative dose of hexobarbital. The duration of the loss of righting reflex

(ability of the mouse to change from lying on the side to upright posture) was recorded. Doses of the I/II mixture up to 2 mg/kg which is 10 times the effective anxiolytic doses caused no increase in sleep time. Diazepam at the effective anxiolytic dose of 1 mg/kg increased sleep time significantly. The I/II mixture when combined with diazepam, and hexobarbital, caused no increase in sleep time over that of diazepam plus hexobarbital.

5.6 Example 6• Evaluation of Effect of Dihydrohonokiol Isomer Mixture on Cognitive Function

TABLE 14

Effect of Diazepam and I/II Mixture on Learning and Memory

Treatment	Dose, po mg/kg	Training Latency	Retention Latency
Vehicle	saline	59.1 ± 10.1	32.4 ± 9.4
Diazepam	1.0	41.8 ± 9.3	109.2 ± 16.9*
Vehicle	saline	71.7 ± 10.4	31.9 ± 9.9
Diazepam	1.0	85.3 ± 13.2	37.1 ± 5.8
Vehicle	saline	59.1 ± 9.8	25.6 ± 3.3
I/II	0.2	59.3 ± 8.8	26.2 ± 3.1
I/II	2.0	121.0 ± 12.7*	46.0 ± 6.6*
Vehicle	saline	62.8 ± 12.1	25.2 ± 2.8
I/II	0.2	60.1 ± 9.1	24.3 ± 2.8
I/II	2.0	71.9 ± 13.1	29.9 ± 5.3

*$p<0004$ vs control vehicle and saline

The I/II mixture was given 3 h before testing and Diazepam was given 10 min before testing. Group size was 10 mice.

To test the cognitive effects of the I/II mixture and diazepam, training latency and retention latency were evaluated using the elevated plus-maze. On one of two tests diazepam show significant retention latency at the effective anxiolytic doses of 1 mg/kg po. In one of two tests the I/II mixture showed only at 10 times the effective anxiolytic dose a training and retention latency.

5.7 Example 7• Evaluation of Effect of Dihydrohonokiol Isomer Mixture on Conditioned Place Preference

TABLE 15

The Effect of the I/II mixture and Diazepam on Conditioned Place Preference

Treatment mg/kg orally for six days		Time in sec in light compartment after conditioning
Saline		112.7±10.1†
Diazepam	1.0	182.9±16.8*†
Saline		105.4±6.9†
I/II	0.2	105.2±10.8†

*$p<0.002$ vs saline

†$n=10$ mice.

The elevated plus-maze test was used to evaluate the ability of the drugs to engender a desire to continue the drug, time spent at the site of six days prior administration of the drug was observed. The time spent at the site of administration following diazepam was significantly increased over saline administration. The time spent at the site following the I/II mixture administration was no different from that of saline administration.

5.8 Example 8• Anxiolytic Activity of 3-*n*-Propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol (I) and 5′-*n*-Propyl-3-(2-propenyl)-1,1′biphenyl-2′,4-diol (II)

The I/II mixture consists of the two dihydrohonokiol isomers in the ratio of 92:08. These isomers were separated by preparative HPLC as described in § 5.1.1 and their anxiolytic activity compared using a modified elevated plus-maze. The major modification change from the maze used in the previous studies was the use of a camcorder placed above and to the side of the maze so that no human observer was visible to the mouse running the maze. This change resulted in an increase in the time that the mouse remained on the transparent arm. The control time increased from 13 sec to 41 sec and the treated times also increased. The small amount of the II isomer (5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol) separated limited the inventors' group size to 5 instead of 10 mice. The oral doses were diazepam 1 mg/kg and I isomer (3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol) and II isomer each at 0.2 mg/kg. As shown in Table 16 and Table 17, both isomers were active but the I isomer was more active than the II isomer.

TABLE 16

Anxiolytic Activity of Isomer I and Isomer II in the Modified Elevated Plus Maze Test

	Mean	Std. Dev.	Std. Error	Count
Maze, Total	93.195	69.503	15.541	20
Maze, CONTROL	41.220	18.693	8.360	5
Maze, Group A	134.860	43.782	19.580	5
Maze, Group B	113.280	62.607	27.999	5
Maze, Group C	83.420	102.905	46.020	5

Group A: Diazepam

Group B: Isomer I

Group C: Isomer II

TABLE 17

Statistical Analysis of Anxiolytic Activity of Isomer I and Isomer II in the Modified Elevated Plus Maze Test

	Mean Dif.	Crit. Diff.	P-Value
CONTROL, Group A	-93.6400	86.8267	0.0362
CONTROL, Group B	-72.0600	86.8267	0.0976
CONTROL, Group C	-42.2000	86.8267	0.3182
Group A, Group B	21.5800	86.8267	0.6055
Group A, Group C	51.4400	86.8267	0.2272
Group B, Group C	29.8600	86.8267	0.4765

Fisher's PLSD for Maze. Descriptive statistics split by Group.

5. 8.1 Further Studies on the Anxiolytic Activity of Isomer I

The I isomer was further tested as shown in Tables 17 and 18. The anxiolytic activity of isomer I at an oral dose of 0.2 mg/kg was compared to diazepam at an oral dose of 1 mg/kg using the new elevated plus maze system with camcorder. Both compounds showed significant anxiolytic effect.

TABLE 18

Anxiolytic Activity of Isomer I and Isomer II in the Modified Elevated Plus Maze Test

	Mean	Std. Dev.	Std. Error	Count
Maze, Total	83.585	57.096	10.988	27
Maze, CONTROL	41.167	26.178	8.726	9
Maze, Group A	118.089	57.168	19.056	9
Maze, Group B	91.500	56.633	18.878	9

Group A: Diazepam

Group B: Isomer I

TABLE 19

Statistical Analysis of Anxiolytic Activity of Isomer I in the Modified Elevated Plus Maize Test

	Mean Difference	Crit. Difference	P-Value
CONTROL, Group A	-76.9222	47.5334	0.0027 S
CONTROL, Group B	-50.3333	47.5334	0.0388 S
Group A, Group B	26.5889	47.5334	0.2597

Fisher's PLSD for Maze. Descriptive statistics split by Group.

5.8.2 Evaluation of Effect of Isomer I on Ultrasonic Vocalization

TABLE 20

The Effect of Isomer I, Gepirone and the Anxiogenic Compound WAY on Conditioned Ultrasonic Distress Vocalizations in Adult Male Rats

Treatment	Number of Ultrasonic Vocalizations, 10 min.
Control	114.4 ± 10.7
Vehicle	154.8 ± 11.7
Vehicle	94.6 ± 10.8
Isomer I 1.0 mg/kg po	48.1 ± 4.7*
Vehicle	62.5 ± 8.4
Gepirone 0.5 mg/kg sc	20.0 ± 14.6*
Vehicle	30.0 ± 7.4
WAY 0.25 mg/kg sc	107.1 ± 19.2*

*$p<0.0001$ vs vehicle.

The rats were divided into four groups depending on their level of ultrasonic vocalization. Each of these groups was divided into an ultrasonic vocalization matched control and drug test group consisting of three rats each.

The conditioned ultrasonic distress vocalization test for anxiolytic activity in rats was used to compare isomer I to Gepirone, an anxiolytic of the azapirone class of compounds. The azapirone class of anxiolytics differ structurally and pharmacologically from the benzodiazepines. Their exact mechanism is unknown. The primary action appears to be binding to serotonin receptors in the brain. Only Buspirone is marketed and it is not prescribed, often the benzodiazepines being preferred. In this test, single pure isomer I reduced vocalization effectively indicating it is effective in aversive conditioning test for anxiolytic activity.

5.9 Example 9– Methods for Assessing Antianxiety Activity of the Disclosed Compounds

This example describes protocols to facilitate preclinical and clinical studies of the compounds of the present invention. The various elements of conducting preclinical and clinical trials, including animal and patient treatment and monitoring, will be known to those of skill in the art in light of the present disclosure. The following information is being presented as a general guideline for use in preclinical and clinical antianxiety studies.

5.9.1 Punished Responding

This procedure has been used to establish antianxiety activity in clinically established compounds. Response of rats or pigeons is maintained by a multiple schedule of food presentation. In one component of the schedule, responding produces food pellet presentation only. In a second component, responding produces both food pellet presentation and is also punished by presentation of a brief electric shock. Each component of the multiple schedule is approximately 4 min in duration, and the shock duration is approximately 0.3 sec. The shock intensity is adjusted for each individual animal so that the rate of punished responding is approximately 15 to 30% of the rate in the unpunished component of the multiple schedule. Sessions are conducted each weekday and are approximately 60 min in duration. Vehicle or a dose of compound are administered 30 min to 6 hr before the start of the test session by the subcutaneous or oral route. Compound effects for each dose for each animal are calculated as a percent of the vehicle control data for that animal. The data are expressed as the mean +- the standard error of the mean.

5.9.2 Monkey Taming Model

The antianxiety activity of a compound may be established by demonstrating that the compounds are effective in the monkey taming model. Plotnikoff, (1973) described the response of rhesus monkeys to pole prodding as a method of evaluating the antiaggressive activity of a test compound. In this method, the antiaggressive activity of a compound was considered to be indicative of its antianxiety activity. Hypoactivity and ataxia were considered to be indicative of a sedative component of

the compound. In one study, the pole prod response-inhibition induced by a compound of this invention may be analyzed and compared with that of a standard antianxiety compound such as diazepam as a measure of antiaggressive potential, and to obtain an indication of the duration of action of the compound.

Male and female rhesus, cynomolgus or squirrel monkeys, selected for their aggressiveness toward a pole, are housed individually in a primate colony room. Compounds or appropriate vehicle are administered orally or subcutaneously and the animals are observed by a trained observer at varying times after drug administration. A minimum of three days (usually a week or more) elapses between treatments. Treatments are assigned in random fashion except that no monkey receives the same compound two times consecutively. Aggressiveness and motor impairment are graded by response to a pole being introduced into the cage. The individuals responsible for grading the responses are unaware of the dose levels received by the monkeys.

5.9.3 Human Clinical Trials

Antianxiety activity may be demonstrated by human clinical trials. A study may be designed as a double-blind, parallel, placebo-controlled multicenter trial. Patients are randomized into four groups, placebo and three different appropriate doses of the test compound either once, twice or three times per day depending on the pharmacokinetics of the drug. The dosages may be administered orally with food. Patients are then observed during four visits to provide baseline measurements, and then visits 5 and beyond may be used as the treatment phase for the study.

During the visits, patients and their caregivers may be questioned and observed for signs of agitation, mood swings, vocal outbursts, suspiciousness, and fearfulness. Each of these behaviors are indicative of the effect of the test compound on an anxiety disorder.

6.0 REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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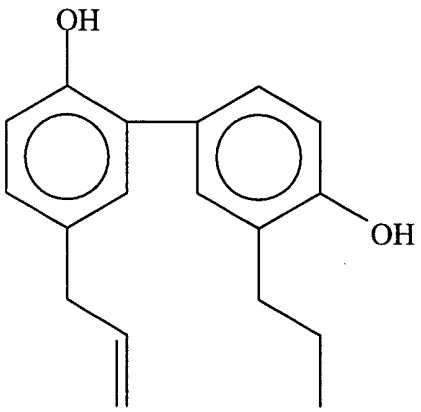
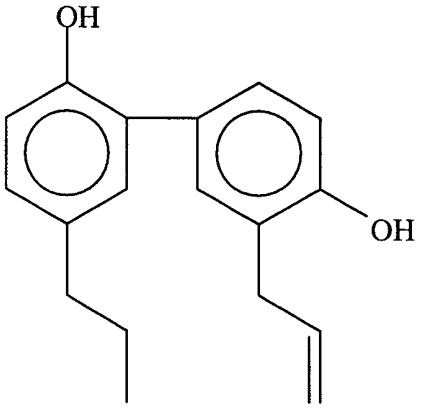
CLAIMS:

1. A compound of the formula:



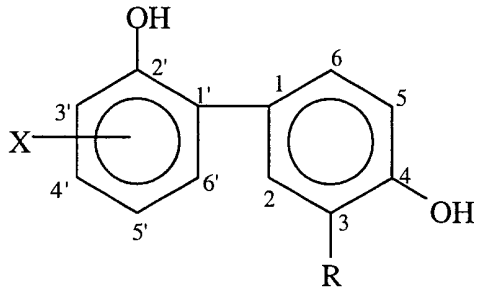
or a salt thereof.

2. A compound of the formula:



or a salt thereof.

3. A compound of the formula:



wherein R is $-CH_2-CH=CH_2$, $-CH=CH-CH_3$, and $-CH_2-CH_2-CH_3$; and

X is from one to two substituents in any of the 3'-, 4-', 5'-, or 6'-positions and is separately and independently fluorine, hydroxy, methoxy, (1-adamantyl), C_1-C_5 alkyl, C_2-C_3 alkenyl, C_2-C_3 alkylcarbonyl, and C_1 -C_4 carboxyalkyl, wherein the C_1-C_5 alkyl group may be substituted with one or more fluorine or hydroxyl; or a salt thereof;

providing, wherein R is $-CH_2-CH=CH_2$, X is not 5'-CH_2-CH_3; 5'-$CH_2-CH=CH_2$; 5'-OH; 3'–OH, 5'-$CH_2-CH-CH_3$; and 3'–OCH_3, 5'-$CH_2-CH=CH_2$; or

wherein R is $-CH_2-CH_2-CH_3$, X is not 5'-$CH_2-CH_2-CH_3$.

4. The compound according to claim 3, wherein R is $-CH_2-CH=CH_2$.

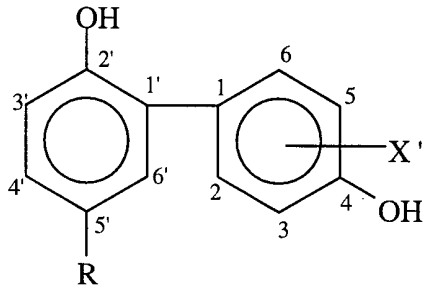
5. The compound according to claim 3, wherein R is $-CH_2-CH_2-CH_3$.

6. The compound according to claim 3, wherein R is $-CH_2=CH-CH_3$.

7. The compound according to claim 3, wherein X is from one to two substituents in any of the 3'-, 4-', 5'- or 6'-positions and is independently 5'-$CH=CH_2$; 5'-CH_2-CH_3; 5'-$CH=CH_2$, 3'-OH; 5'-$CH_2-CH=CH_2$; 5'-$CH_2-CH=CH_2$, 3'-OH; 5'-$CH_2-CH_2-CH_3$, 3'–OH; 5'-$CH_2-CH=CH_2$, 3'-OCH_3; 5'-$CH_2-CH_2-CH_3$, 3'-OCH_3; 5'-$CH=CH-CH_3$, 3'-OH; 5'-$CH=CH-CH_3$, 3'-OCH_3; 5'-CH_3; 5'-$CH(CH_3)_2$; 5'-$CH_2CH(CH_3)_2$; 5'-$C(CH_3)_3$; 5'–$CH(CH_3)_2C_2H_5$; 5'-(1-adamantyl); 5'-$CH(CH_3)_2$, 6 -CH_3; 5'-$CH(CH_3)_2$, 4 -CH_3; 5'–CH(-OH)-CH_3; 5'-$CH_2-CH(-OH)-CH_3$; 5'-CH_2-COOH; 5'-CH_2CH_2-COOH; 5'–$CH_2CH_2CH_2-COOH$; 5'-CH_2-COOH, 3-OH; 5'-OH; 5'-OCH_3; 3'-F; 4'-F; 5'-F; 3'-F, 5'-CH_3; 3'-F, 5'-CH_2-CH_3; 3'-F, 5'-CH_2-OH; 3'-F, 5'-CH_2-CH_2-OH; 3'-F, 5'-COOH; 3'-F, 5'-CH_2-COOH; 3'-F, 5'-$CH=CH-CH_3$; 3'-F, 5'-$CH_2-CH_2-CH_3$; 3'-F, 5'-$CH_2-CH=CH_2$; 3'-F, 5'-$CH_2-CHF-CH_3$; 3'-F, 5'-$CH_2-CHF-CH_2F$; 4'-F, 5'-CH_3; 4'-F, 5'-CH_2-OH; 3'-F, 5'-CH_2-CH_3; 6'-F, 5'-CH_2-CH_3; 6'-F, 5'-CH_2-OH; and 6'-F, 5'-COOH.

8. The compound according to claim 3, wherein X is independently 5′-C(=O)-CH_3 and 5′-CH_2-C(=O)-CH_3.

9. A compound of the formula:



wherein R is -CH_2-CH=CH_2, -CH=CH-CH_3, and -CH_2-CH_2-CH_3; and

X′ is from one to two substituents in any of the 2-, 3-, 5-, or 6-positions and is separately and independently fluorine, hydroxy, methoxy, C_1-C_4 alkyl, C_3 alkenyl, C_1-C_3 alkylcarbonyl, C_1-C_3 carboxyalkyl, and C_3 carboxyalkenyl, wherein the C_1-C_4 alkyl group may be substituted with one or more fluorine or hydroxyl; or a salt thereof;

providing, wherein R = -CH_2-CH=CH_2, X′ is not 3-CH_2-CH_3 and 3′-CH_2-CH=CH_2; or wherein R = -CH_2-CH_2-CH_3, X′ is not 3-CH_2-CH_2-CH_3.

10. The compound according to claim 9, wherein R is -CH_2-CH=CH_2.

11. The compound according to claim 9, wherein R is -CH_2-CH_2-CH_3.

12. The compound according to claim 9, wherein R is -CH_2=CH-CH_3.

13. The compound according to claim 9 wherein X′ is from one to two substituents in any of the 2-, 3-, 5- or 6-positions and is independently 3-CH_3; 3-CH_2-CH_3; 3-CH_2-CH=CH_2;3-CH_2-CH=CH_2, 5-OH; 3-CH_2-CH_2-CH_3, 5-OH; 3-CH_2-CH=CH_2, 5-OCH_3; 3-$CH_2CH_2CH_3$, 5-OCH_3; 3-CH=CH-CH_3; 3-CH_3, 6-$CH(CH_3)_2$; 3,5 di-CH_3; 2,6 di-CH_3; 3-$CH(CH_3)_2$; 3-$CH(CH_3)_2$, 6-CH_3; 3-$CH_2CH(CH_3)_2$; 3-CH(-OH)-CH_3; 3-CH_2-CH(-OH)-CH_3; 3-COOH, 6-OH; 2-COOH; 3-OCH_3; 3-CH_2COOH; 3-CH_2CH_2COOH; 3-CH=CHCOOH; 3-CH_2OH, 2-OH; 3-CH_2OH, 5-OCH_3; 3-

CH_2OH, 5-CH_3, 3-F; 2F; 2-F, 3-CH_3; 6-F, 3-CH_3; 5-F, 3-CH=CH-CH_3; 5-F,3-CH_2-CH_2-CH_3; 5-F, 3-CH_2-CH=CH_2; 5-F, 3-CH_2-CHF-CH_3; and 5-F, 3-CH_2-CHF-CH_2F.

14. The compound according to claim 9 wherein X′ is from one to two substituents in any of the 2-, 3- or 5-positions and is independently 3-C(=O)-CH_3; 3-CH_2-C(=O)-CH_3; 3-CHO, 2-OH; 3-CHO, 5-OCH_3; and 3-CHO, 5-CH_3.

15. A pharmaceutical composition comprising a suitable carrier and the compound of claim 1 or the compound of claim 2 or the compound of claim 1 and the compound of claim 2.

16. The composition of claim 15, wherein said compound of claim 1 or said compound of claim 2 or said compound of claim 1 and said compound of claim 2 are present in an anxiolytically effective amount.

17. A pharmaceutical composition comprising a suitable carrier and one or more compounds of claim 3 or claim 9.

18. The composition of claim 17, wherein said one or more compounds of claim 3 or claim 9 are present in an anxiolytically effective amount.

19. A method of reducing or preventing anxiety in mammals, which comprises administering to a mammal in need of such treatment an effective amount of the compound of claim 1 or the compound of claim 2 or the compound of claim 1 and the compound of claim 2.

20. A method of reducing or preventing anxiety in mammals, which comprises administering to a mammal in need of such treatment an effective amount of one or more compounds of claim 3 or claim 9.

21. A method of alleviating an anxiety-related disorder in a patient comprising administering to a patient in need of such treatment an amount of a compound of

claim 3 or claim 9 or a plurality of compounds of claim 3 or claim 9 effective in reducing or preventing anxiety and the symptoms associated with such disorder.

22. A method according to claim 21, wherein said anxiety-related disorder is selected from the group consisting of panic disorder, generalized anxiety disorder, agoraphobia, simple phobias, social phobia, posttraumatic stress disorder, obsessive-compulsive disorder, and avoidant personality disorder.

23. A process for preparing dihydrohonokiol comprising the steps of:

a) admixing tris-(triphenylphosphine) rhodium(I) chloride catalyst with non-polar organic solvent;

b) admixing honokiol with said catalyst and said non-polar solvent to form a mixture; and

c) saturating said mixture with hydrogen to form dihydrohonokiol such that 0.6 to 1.2 mol of hydrogen reacts per mol of honokiol.

24. The process of claim 22, wherein said non-polar organic solvent is selected from the group consisting of toluene, benzene, pentane, hexane, heptane and octane.

25. The process of claim 22, further comprising chromatographic separation and purification of said dihydrohonokiol into the two positional isomers 3-*n*-propyl-5′-(2-propenyl)-1,1′-biphenyl-2′,4-diol and 5′-*n*-propyl-3-(2-propenyl)-1,1′-biphenyl-2′,4-diol.