Stereo-Selective Activity of Menthol on $GABA_A$ Receptor

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ABSTRACT Menthol is a naturally occurring compound, which has three chiral centers that define eight possible optically actives stereoisomers. Neuroactivity of menthol and related agents by affecting neuronal intracellular signaling or by modulation of neurotransmitter-gated currents has been reported. Furthermore, stereo-selectivity of menthol in its analgesic activity as well as in its sensory properties and other biological activities was also described. The present study is the first contribution to the description of stereo-selectivity of $GABA_A$ receptor against the most possible isomers of menthol, discussed in terms of their chirality. The results showed that only (+)-menthol, among the five stereoisomers analyzed, was active, stimulating in a dose-response manner the binding of an allosteric $GABA_A$ receptor ligand. Taking into account these results, and comparing them with those of some active phenolic compounds, it is strongly suggested that the existence of a relative spatial location of its substituents with respect to the ring (equatorial position of all substituents and (1*S*,2*R*,5*S*)-configuration) as well as the presence in the cyclic molecule of an aliphatic non polar group (isopropyl) with free rotation near to a polar group (hydroxyl) are crucial points to demonstrate activity on the receptor. *Chirality 21:525–530, 2009.* © 2008 Wiley-Liss, Inc.

KEY WORDS: menthol; stereo-isomers; stereo-selectivity; [^{3}H]-flunitrazepam binding; $GABA_A$ receptor

INTRODUCTION

Menthol (2-isopropyl-5-methyl-cyclohexanol) is a naturally occurring compound of plant origin present in the volatile oil of several species of mint plants such as peppermint (*Mentha piperita* L), which is among the most popular single ingredient herbal teas.[1,2]

In vitro and in vivo studies demonstrated that peppermint has significant antimicrobial and antiviral activities, strong antioxidant and antitumor actions, and some antiallergenic potential as well as a relaxation effect on gastrointestinal tissue, analgesic and anesthetic effects in the central and peripheral nervous system, immuno-modulating actions and chemopreventive (anti-carcinogenic) potential.[2]

Menthol constitutes the main volatile component identified in the essential oil of peppermint (33–60%).[2] (−)-Menthol is the most widely occurring isomer in nature and therefore sometimes is referred to as "natural" menthol, albeit all other isomers are found in peppermint oils as well.[3] The analysis of enantiomeric composition of menthol in *Mentha piperita,* from different Spanish regions, showed that menthol and isomenthol occurred, in general, as pure enantiomers, prevailing the (−)-enantiomer for the former and the (+)-enantiomer for the latter. In contrast, in the case of neomenthol, the presence of both enantiomers with similar proportions was found in all cases.[4] Differences in sensory properties and biological activities between pairs of enantiomers have already been reported before.[5] Specifically for menthol, some researchers have reported that only (−)-menthol, of the eight stereoisomers of menthol [i.e., (+/−)-menthol, (+/−)-neoisomenthol, (+/−)-neomenthol and (+/−)-isomenthol, see Scheme 1], exhibits the greatest cooling activity as well as the typical peppermint odor. [Refs. 4, 6, and references cited therein.]

Analgesic effects have long been described for menthol, and it was suggested that antinociceptive and local anaesthetic effect of menthol might be mediated via blockade of voltage-operated sodium channels.[7] However, while the local anaesthetic effect would be independent of the assayed enantiomer,[1] the analgesic activity exhibited stereo-selectivity being only (−)-menthol able to induce analgesia.[1]

It was reported the neuroactive properties of menthol and related agents by affecting neuronal intracellular signaling or by a modulation of neurotransmitter-gated currents. For instance, it was demonstrated that menthol induced Ca^{2+} release from intracellular stores, resulting in enhanced neurotransmission at sensory synapses.[8] Other studies showed that this compound enhanced GABA

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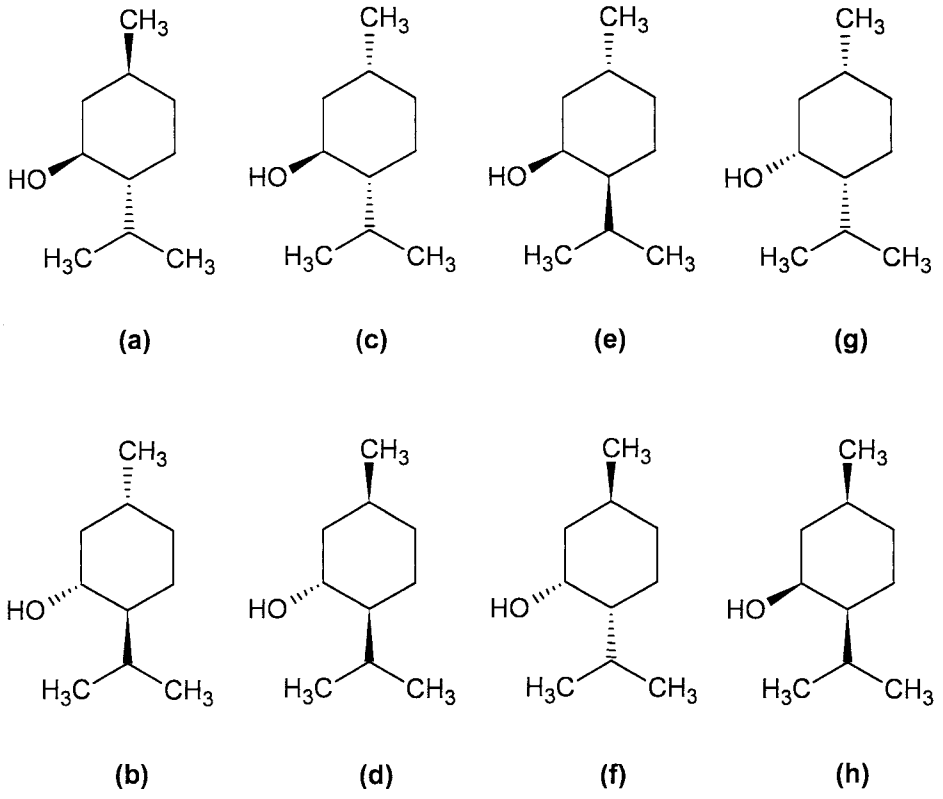

Scheme 1. Structural formulas of the possible stereoisomers of menthol (2-isopropyl-5-methyl-cyclohexanol). (**a**) (+)-menthol; (**b**) (−)-menthol; (**c**) (+)-isomenthol; (**d**) (−)-isomenthol; (**e**) (+)-neomenthol; (**f**) (−)-neomenthol; (**g**) (+)-neoisomenthol; (**h**) (−)-neoisomenthol.

currents, (+)-menthol proving more potent than (−)-menthol, revealing a neuroactive role for menthol as a stereoselective modulator of $GABA_A$ receptor.[9] However, this gabaergic activity could not be confirmed by García et al.,[10] perhaps because the menthol used in this previous work was a commercial mixture with unknown proportions of each enantiomer.

Since the $GABA_A$ receptor is recognized as an important target for modulation by sedative, anxiolytic, general anesthetic, and convulsant agents,[11–13] and due to the ambiguous results reported, in the present work we study the ability of five different menthol stereoisomers to modify the binding of [^{3}H]-flunitrazepam ([^{3}H]-FNZ), an allosteric modulator of the $GABA_A$ receptor.

MATERIAL AND METHODS

Materials

[^{3}H]-FNZ (85 Ci/mmol) was purchased from New England Nuclear Chemistry (E.I.DuPont de Nemours, Boston, MA). (1*R*,2*S*,5*R*)(−)-5-methyl-2-(1-methylethyl)-cyclohexanol [(−)-menthol], (1*S*,2*R*,5*S*)(+)-5-methyl-2-(1-methylethyl)-cyclohexanol [(+)-menthol], (1*S*,2*R*,5*R*)(+)-5-methyl-2-(1-methylethyl)-cyclohexanol [(+)-isomenthol], (1*S*,2*S*,5*R*)(+)-5-methyl-2-(1-methylethyl)-cyclohexanol [(+)-neomenthol], (1*R*,2*R*,5*S*)(−)-5-methyl-2-(1-methylethyl)-cyclohexanol [(−)-neomenthol], 5-methyl-2-(1-methylethyl)-phenol (thymol), diazepam, and GABA were from Sigma-Aldrich (St. Louis, MO). Other drugs and solvents were of analytical grade.

Animals

Chicks (Cobb, of both sexes) were obtained from a commercial hatchery, INDACOR (Argentina). Birds in groups of 20 were housed in brooders (50 × 90 cm^2) on the evening of the day of hatch. The brooders were placed in a room (3 × 3 m^2) isolated from external noises, with constant temperature (32°C) and humidity at a 12 h light: 12 h dark cycle (lights on at 0700) with food and water freely available and maintained in these conditions until they became 15 days old.

Binding of [^{3}H]-FNZ to Membranes from Chick Forebrain

All the procedures were carried out at 4°C. The membrane receptor preparation was obtained as follows: whole brains from recently sacrificed birds were homogenized in 20 volumes of ice-cold 0.32 M sucrose/g of original tissue, using a Potter glass Teflon homogenizer and centrifuged at 1000*g* for 10 min. The supernatants were centrifuged at 10,000*g* for 20 min and the pellets were resuspended in water to induce a hypo-osmotic shock to liberate intracellular GABA. These suspensions were homogenized and centrifuged at 30,000*g* during 20 min; the pellets were resuspended in 100 mM NaCl-50 mM Tris-HCl buffer pH 7.4 and maintained at −20°C. Immediately before their use,

these samples were defrosted and diluted by adding 10 volumes of bidistilled water, centrifuged again at 30,000*g* during 20 min and the pellets resuspended in the buffer indicated earlier, at the protein concentration required for the experiment.

The incubation system contained, in a final volume of 250 μl, the membrane suspension at a final protein concentration of 0.25 mg/ml approximately, 2-3 nM [^{3}H]-FNZ, 100 mM NaCl-50 mM Tris-HCl buffer pH 7.4 containing (nonspecific) or not (total) diazepam 10 μM (final concentration). Menthols were prepared as 400 mM stock solutions in DMSO, light protected, and stored at 4°C. Stock solutions were diluted before each experiment in the incubation system at variable concentrations depending on the solubility of each enantiomer, maintaining a 0.25% (v/v) DMSO final concentration. In some experiments, the incubation system contained GABA at variable concentrations (0–200 μM) or thymol (500 μM) instead the menthols. Samples were incubated at 4°C in the dark for 1 h and then they were immediately filtered through GF/B filters with an automatic harvester apparatus. The filters were rinsed and dried in the air, and placed in vials containing 2.5 ml of scintillation liquid (25% v/v Triton X-100, 0.3% p/v diphenyloxazole in toluene) and the retained radioactivity was determined with a scintillation counter Rackbeta 1214 (Pharmacia-LKB, Finland) with an efficiency of 60% for tritium. Protein concentration in the incubation system was adjusted at 0.2–0.3 mg/ml and was determined by the Lowry's method.[14]

Data Analysis

Data shown represent the mean ± standard error of mean (SEM). Sigmoid curves were fitted to concentration-response data and statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA). A two tailed Student's *t*-test, as well as one-way analysis of variance (ANOVA), was used.

RESULTS AND DISCUSSION

To determine whether menthol stereoisomers could be active on $GABA_A$ receptor, we measured the binding of a known positive allosteric modulator, FNZ, in the presence of different concentrations of each stereoisomer in membranes from chick forebrain.

To check the response of $GABA_A$ receptor in our membrane preparation, we performed a concentration-response curve for GABA-induced [^{3}H]-FNZ specific binding (Fig. 1). The sigmoid curve obtained was comparable with that reported by Perillo et al.[15] in a similar system, with EC_{50} value of 1.6×10^{-7} M (log EC_{50}= −6.796 ± 1.168). Figure 1 also shows an increment of (139.27 ± 0.85)% induced by a single concentration of thymol (500 μM), similar to the reported previously in the same membrane system.[16]

(+)-Menthol induced a concentration-dependent increase in [^{3}H]-FNZ specific binding in membranes from chick forebrain with an EC_{50} value of 1.55×10^{-6} M (log EC50 = −5.809 ± 7.122) and a maximum response of (123.2 ± 1.77)% according to a sigmoid curve fitted to the

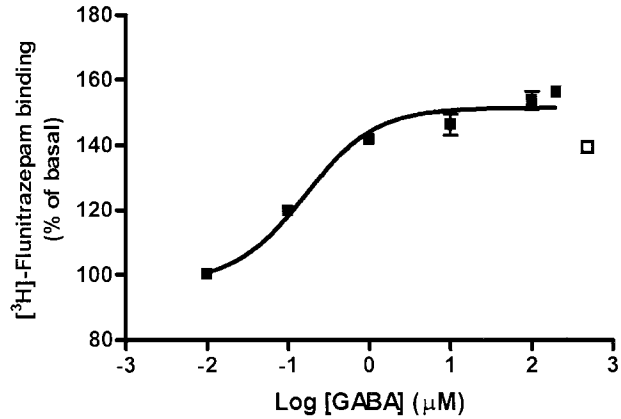

Fig. 1. Concentration-response curve for GABA-induced [^{3}H]-FNZ specific binding in chick brain membranes. Membranes were exposed to different concentrations of GABA (0–200 μM) and at a constant concentration of [^{3}H]-FNZ (2–3 nM) (filled symbols) (see details of the incubation system in Material and Methods section). The empty symbol corresponds to the response at 500 μM thymol used as a reference. The values represent the percentages of increment with respect to basal binding in the absence of the test agent. Each point represents the mean ± S.E.M. of 2–3 independent experiments, each performed in triplicate.

concentration-response data showed in Figure 2a. (−)-Menthol and (+)–isomenthol did not induce significant increments in the [^{3}H]-FNZ specific binding with respect to the control in all the concentration range (up to 1000 and 100 μM, respectively) (Figs. 2b and 2c). In spite of the fact that (+)-neomenthol and (−)-neomenthol induced a significant increase in the specific binding at the highest assayed concentration (1000 μM), it was not possible to fit a sigmoid curve to the data (Figs. 2d and 2e).

To discuss these results taking into consideration the menthol stereo-chemistry, it is necessary to describe more in detail its possible configurations. The compound menthol (2-isopropyl-5-methylcyclohexanol) has three chiral centers corresponding to the hydroxyl, isopropyl, and methyl groups, respectively, as shown in Scheme 1. Hence, eight (2^3) optically actives stereoisomers are possible, defining four pairs of diastereoisomers (two enantiomorphic structures for each diastereoisomer) (Scheme 1). It is known that enantiomers [i.e., (+) and (−)-menthol] do not differ in their physical or chemical properties except in the sign of their optical rotation, whereas diastereoisomers differ in these former properties. Thermodynamically, (+) and (−)-menthol is the most stable pair of enantiomers because all the three substituents are equatorially oriented, diminishing at a minimum the strain due to steric interactions between these groups. On the other hand, the rest of the stereoisomers contain axial substituents, which introduce certain degree of steric strain in the molecule.[17–18]

Considering that only (+)-menthol, among the stereoisomers studied, shows an evident positive effect on the [^{3}H]-FNZ binding, it is possible to suggest that the molecular requirements to be active are not only the presence of the substituents in equatorial positions but also the relative positions of them in the molecule: (1*S*,2*R*,5*S*) configu-

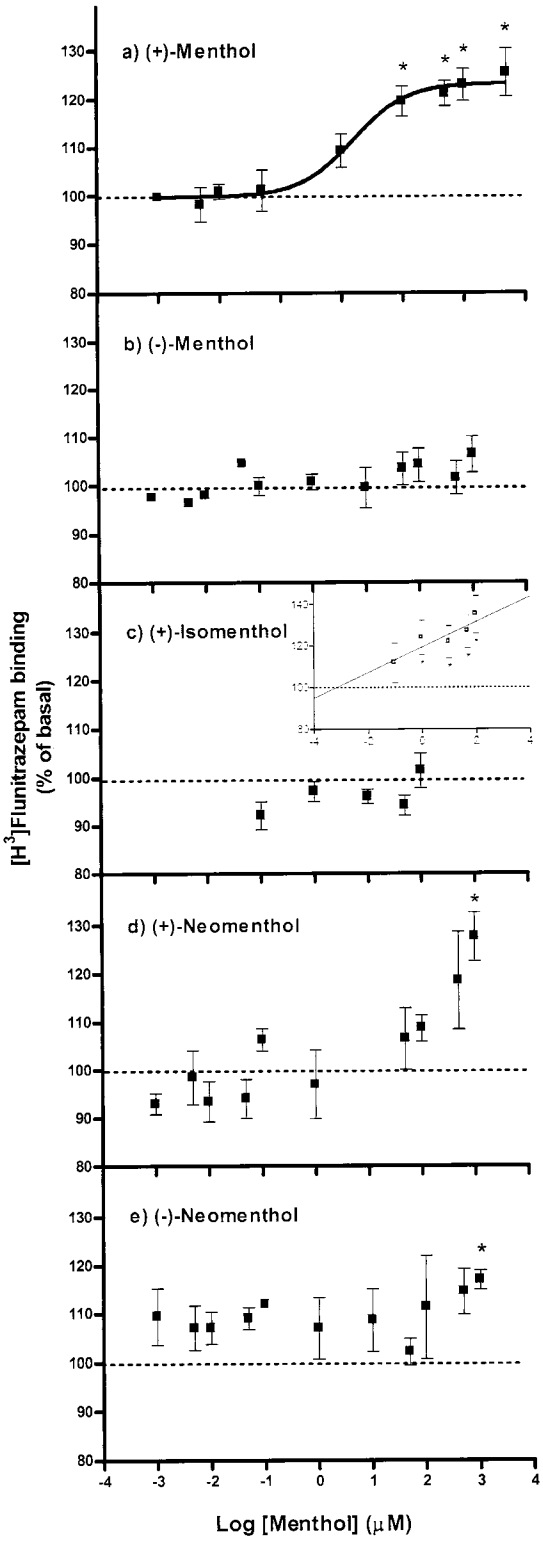

Fig. 2. Concentration-response curves for [^{3}H]-FNZ (2–3 nM) specific binding in the presence of **(a)** (+)-menthol, **(b)** (−)-menthol, **(c)** (+)-isomenthol, **(d)** (+)-neomenthol and **(e)** (−)-neomenthol. Results are expressed as the percentages with respect to basal binding in the absence of the test agent. Each point represents the mean ± S.E.M. (n = 4–6) independent experiments, each performed in triplicate. Inset: effect of (+)-isomenthol on the [^{3}H]-FNZ non-specific binding; a straight line was adjusted to the data (r^2=0.82; ordinate= 119.2 ± 2.3; slope= 6.08 ± 1.7). *, Significantly different from Basal (taken as 100%) ($P < 0.05$, Student's t-test).

ration. This result is partially in agreement with those described by Hall et al.[9] in electrophysiological studies where (+)-mentol showed an activity higher than that of its enantiomer (−)-menthol. The slight activity demonstrated for (−)-menthol was not found in the present work perhaps due to the different subunit composition of $GABA_A$ receptor. While Hall et al.[9] used $GABA_A$ receptor-expressing oocytes (from cDNAs encoding only for the α1, β2, and γ2s subunits of human $GABA_A$ receptors), in this work we employed membranes from chick forebrains, which possess several receptor subtypes formed by different combinations of subunits, whose expression varies in different brain cells and brain regions and whose pharmacological properties also differ.[12,19]

Thymol, a naturally occurring phenolic monoterpene, and the anaesthetic propofol, a synthetic phenol, have been widely described as positive allosteric modulators of $GABA_A$ receptor.[10,20,21] Menthol, a neutral cyclohexanol, shares with thymol and propofol the presence of an isopropyl residue adjacent to their respective hydroxyl groups. The presence of an aliphatic chain near the alcohol group was previously included in pharmacophoric models as an essential requirement for the activity of these compounds on the $GABA_A$ receptor.[10,21] An important structural difference between menthol and these phenolic compounds is the fact that menthol possesses a molecular chair-like structure with three quiral centers, as was described earlier, while thymol and propofol are nonquiral molecules with a planar ring structure. However, an interesting similarity occurs in the free rotation of the isopropyl group in menthol, as well as in both phenolic compounds. Comparing the different configurations of the menthol isomers assayed with respect to the thymol structure, it is important to remark that (+) and (−) menthol exhibit a relative spatial situation of their substituents, with respect to the central ring, comparable with that present in thymol, due to the equatorial positions of the substituents. Thus, it can be proposed that, for the active association with $GABA_A$ receptor binding site, it is necessary that the presence of the substituents with certain relative positions to establish precise interactions with different groups of the protein. The loss of activity of (−)-menthol would be due to the relative enantiomeric configuration of its substituents (1*R*,2*S*,5*R*) as was described before. This compound, in spite of to present adequate relative positions between its substituents exhibits an opposite configuration with respect to (+)-menthol as both constitute mirror images of each other.

According to the increases induced by (+) and (−)-neomenthol only at the highest assayed concentration (1000 μM), both compounds were considered pharmacologically inactive for the system used in the present work because the typical concentration-response sigmoid curve could not fit to the data. In addition, it would be taken into account that slightly higher concentrations (>1000 μM) presented clear symptoms of insolubility (formation of visible crystals or marked increment of the turbidity—results not shown) for all the isomers, except (+)-isomenthol that demonstrated insolubility at lower concentrations (>100 μM).

Menthol, with an octanol/water partition coefficient about 1000 ($\log P \approx 3$), is a lipophilic compound capable to interact with membranes as was recently described by Turina et al.[22] These authors demonstrated that this compound can penetrate in artificial model membranes located within the polar head group region of membrane components. Considering that the lipophilic drug partitioning in the membrane may modulate the drug–receptor interaction through changes in the molecular organization of membrane-bound receptors surroundings.[15,23,24] and, that the FNZ nonspecific interaction with natural membranes can be affected by the physical state of the membrane,[25] in the present work we analyzed separately this nonspecific binding. (+)-Menthol, (−)-menthol, (+)-neomenthol, and (−)-neomenthol did not affect significantly the non-specific [^{3}H]-FNZ binding within the whole concentration range assayed (results not shown). However, (+)-isomenthol induced a concentration dependent increment of the nonspecific binding, which could be fitted to a straight line ($r^2 = 0.82$) (Fig. 2c, inset). These results, discard any artefactual effect of the nonspecific interactions on the positive increment induced by (+)-menthol on the specific binding. Furthermore, the lower solubility and the noticeable effect on the [^{3}H]-FNZ nonspecific binding demonstrated by (+)-isomenthol could be expected in view of the fact that diastereoisomers can differ in their physical or chemical properties as was explained before.

CONCLUSIONS

It has been widely described in many cases, for different chiral compounds and in different systems, the importance of the chiral recognition to exhibit biological activity. In this sense, it is extensively known the stereo-selectivity of many proteins (for review see Ref. 26) and particularly the stereo-specificity present in $GABA_A$ receptor described in several articles [Refs. 27–30, and references therein].

The present study constitutes the first contribution to the description of stereo-selectivity presents in the $GABA_A$ receptor against the most of the possible isomers of menthol, discussed in terms of its chirality. The results showed that only (+)-menthol, among the five stereoisomers analyzed, was active, stimulating in a dose-response manner the binding of an allosteric $GABA_A$ receptor ligand. (+) and (−)-neomenthol were considered inactive because they were able to increment the binding only at a very high concentration and because dose-response curves could not be fitted to the data. Taking into account these results, and comparing them with those of some active phenolic compounds, it is strongly suggested that the existence of a relative spatial location of its substituents with respect to the ring (equatorial position of all substituents and an (1S,2R,5S)-configuration) as well as the presence in the cyclic molecule of an aliphatic non polar group (isopropyl) with free rotation near to a polar group (hydroxyl) are crucial points to demonstrate activity on the receptor.

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LITERATURE CITED

1. Galeotti N, Di Cesare Mannelli L, Mazzanti G, Bartolini A, Ghelardini C. Menthol: a natural analgesic compound. Neurosci Lett 2002;322: 145–148.
2. McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytother Res 2006;20:619–633.
3. Oertling H, Reckziegel A, Surburg H, Bertram HJ. Applications of menthol in synthetic chemistry. Chem Rev 2007;107:2136–2164.
4. del Castillo ML, Blanch GP, Herraiz M. Natural variability of the enantiomeric composition of bioactive chiral terpenes in *Mentha piperita*. J Chromatogr A 2004;1054:87–93
5. Perillo MA, Zygadlo JA. Terpenes. Stereochemistry and bioactivities. Curr Topics Phytochem Rev 2005;7:89–104.
6. Eccles R, Griffiths DH, Newton CG, Tolley NS. The effects of menthol isomers on nasal sensation of airflow. Clin Otolaryngol Allied Sci 1988;13:25–29.
7. Haeseler G, Maue D, Grosskreutz J, Butler J, Nentwig B, Piepenbrock R, Dengler R, Leuwer M. Voltage-dependent block of neuronal and skeletal muscle sodium channels by thymol and menthol. Eur J Anaesthesiol 2002;19:571–579.
8. Tsuzuki K, Xing H, Ling J, Gu JG. Menthol-induced Ca^{2+} release from presynaptic Ca^{2+} stores potentiates sensory synaptic transmission. J Neurosci 2004;24:762–771.
9. Hall AC, Turcotte CM, Betts BA, Yeung WY, Agyeman AS, Burk LA. Modulation of human $GABA_A$ and glycine receptor currents by menthol and related monoterpenoids. Eur J Pharmacol 2004;506:9–16.
10. García DA, Bujons J, Vale C, Suñol C. Allosteric positive interaction of thymol with the $GABA_A$ receptor in primary cultures of mouse cortical neurons. Neuropharmacology 2006;50:25–35.
11. MacDonald RL, Olsen RW. $GABA_A$ receptor channels. Annu Rev Neurosci 1994;17:569–602.
12. Korpi ER, Gründer G, Lüddens H. Drug interactions at $GABA_A$ receptors. Prog Neurobiol 2002;67:113–159.
13. Rudolph U, Möhler H. Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu Rev Pharmacol Toxicol 2004; 44:475–498.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275.
15. Perillo MA, Garcia DA, Marin RH, Zygadlo JH. Tagetona modulates the coupling of flunitrazepam and GABA binding sites at $GABA_A$ receptor from chick brain membranes. Mol Membr Biol 1999;16:189–194.
16. Sánchez M., Turina AV, García DA, Nolan MV, Perillo MA. Surface activity of thymol: implications for an eventual pharmacological activity. Coll Surf (B) 2004;34:77–85.
17. Krishnaswamy NR. Learning organic chemistry through natural products, Part 2: Determination of absolute stereochemistry. Resonance 1996;1:40–46.
18. Hembury GA, Borovkov VV, Inoue Y. Chirality-sensing supramolecular systems. Chem Rev 2008;108:1–73.
19. Darlison MG, Pahal I, Thode C. Consequences of the evolution of the $GABA_A$ receptor gene family. Cell Mol Neurobiol 2005;25:607–624.
20. Mohammadi B, Haeseler G, Leuwer M, Dengler R, Krampfl K, Bufler J. Structural requirements of phenol derivatives for direct activation of chloride currents via $GABA_A$ receptors. Eur J Pharmacol 2001;421:85–91.
21. Krasowski MD, Hong X, Hopfinger AJ, Harrison NL. 4D-QSAR analysis of a set of propofol analogues: mapping binding sites for an anesthetic phenol on the $GABA_A$ receptor. J Med Chem 2002;45:3210–3221.
22. Turina AV, Nolan MV, Zygadlo JA, Perillo MA. Natural terpenes: self-assembly and membrane partitioning. Biophys Chem 2006;122:101–113.

23. García DA, Marín RH, Perillo MA. Stress-induced decrement in the plasticity of the physical properties of chick brain membranes. Mol Membr Biol 2002;19:221–230.
24. Søgaard R, Werge TM, Bertelsen C, Lundbye C, Madsen KL, Nielsen CH, Lundbæk JA. $GABA_A$ receptor function is regulated by lipid bilayer elasticity. Biochemistry 2006;45:13118–13129.
25. García DA, Perillo MA. Supramolecular events modulate flunitrazepam partitioning into natural and model membranes. Coll Surf (B) 1997;9:49–57.
26. Lamzin VS, Dauter Z, Wilson KS. How nature deals with stereoisomers. Curr Opin Struct Biol 1995;5:830–836.
27. Covey DF, Nathan D, Kalkbrenner M, Nilsson KR, Hu Y, Zorumski CF, Evers AS. Enantioselectivity of pregnanolone-induced γ-aminobutyric acid (A) receptor modulation and anesthesia. J Pharmacol Exp Ther 2000;293:1009–1016.
28. Verdon B, Zheng J, Nicholson RA, Ganelli CR, Lees G. Stereoselective modulatory actions of oleamide on $GABA_A$ receptors and voltage-gated Na^+ channels in vitro: a putative endogenous ligand for depressant drug sites in CNS. Br J Pharmacol 2000;129:283–290.
29. Cordato DJ, Chebib M, Mather LE, Herkes GK, Johnston GA. Stereoselective interaction of thiopentone enantiomers with the $GABA_A$ receptor. Br J Pharmacol 1999;128:77–82.
30. Tomlin SL, Jenkins A, Lieb WR, Franks NP. Preparation of barbiturate optical isomers and their effects on $GABA_A$ receptors. Anesthesiology 1999;90:1714–1722.