Sedative Effect of Monoterpene Alcohols in Mice: A Preliminary Screening

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Z. Naturforsch. **62 c**, 563–566 (2007); received January 17/March 12, 2007

Many essential oils and monoterpenes are used therapeutically as relaxing drugs and tranquilizers. In this study, ten structurally related monoterpene alcohols, present in many essential oils, were evaluated in mice to investigate their pharmacological potential in the central nervous system. Isopulegol (**1**), neoisopulegol (**2**), (±)-isopinocampheol (**3**), (–)-myrtenol (**4**), (–)-*cis*-myrtanol (**5**), (+)-*p*-menth-1-en-9-ol (**6**) and (±)-neomenthol (**8**) exhibited a depressant effect in the pentobarbital-induced sleep test, indicating a sedative property. (–)-Menthol (**7**), (+)-dihydrocarveol (**9**), and (±)-isoborneol (**10**) were ineffective in this test. The results show that these psychoactive monoterpenes have the profile of sedative drugs, and this pharmacological effect is influenced by the structural characteristics of the molecules.

Key words: Sedative Effect, *p*-Menthane, Monoterpenes

Introduction

Essential oils are natural products with many different applications, especially in the medical and cosmetic areas. The use of essential oils in the flavour and fragrance industry is well known, and their fragrances have been used in aromatherapy to induce mental tranquility or relaxation and to aid sleep in humans (Lavabre, 2001). In addition, many of them are found to exhibit varied biological properties, such as spasmolytic (Lis-Balchin and Hart, 1999) and anticonvulsant (Almeida *et al*., 2003) activities. These effects are probably due to the structural diversity of the essential oil constituents. This notion is supported by previous studies which showed that some monoterpenes present in many essential oils possess sedative activity in animal experiments, such as linalool (Elisabetsky *et al*., 1995), 1,8-cineole (Santos and Rao, 2000) and α-terpineol (Buchbauer *et al*., 1993). Some derivatives of monoterpenes also showed a sedative effect (De Sousa *et al*., 2004, 2006). These facts led us to evaluate ten structurally related monoterpene alcohols through a preliminary screening to verify their potential as sedative drugs. The investigated monoterpenes are present in the volatile oils of many plant species such as *Mentha piperita* (Galeotti *et al*., 2002), *Zanthoxylum schinifolium* (Paik *et al*., 2005) and *Mentha* x *villosa* (Arruda *et al*., 2006).

Materials and Methods

Chemicals

The following compounds were used (purity higher than 95%): Isopulegol (**1**) and neoisopulegol (**2**) [separated by column chromatography of technical grade isopulegol (Dierberger S. A., Barra Bonita, Brazil)]; (±)-isopinocampheol (**3**), (–)-myrtenol (**4**), (–)-*cis*-myrtanol (**5**), (+)-*p*-menth-1-en-9-ol (**6**), and (±)-isoborneol (**10**) (Aldrich, USA); (–)-menthol (**7**) (Usina Colombina S. A., Bebedouro, Brazil); (±)-neomenthol (**8**) and (+)-dihydrocarveol (**9**) (SCM Glidden Organics, Jacksonville, USA). All were dissolved in 5% Tween 80 as an emulsion. Pentobarbital and polyoxyethylene-sorbitane monolate (Tween 80) were purchased from Sigma (USA).

Animals

Male Swiss mice (26–36 g; 6–8 weeks old) were obtained from the Biology Department of Universidade Federal de São Carlos. The animals were maintained at constant room temperature [(23 ± 1) °C] and on a 12 h/12 h light-dark cycle (light from 07:00 to 19:00 h), with free access to food and water, for a minimum of 7 d before performing the experiments. All behavioural observations were conducted between 13:00 and 19:00 h.

0939–5075/2007/0700–0563 $ 06.00

Statistical analysis

The statistical analysis was performed using analysis of variance followed by Dunnet's test. A probability level of 0.05 was regarded as significant.

Experimental

Sodium pentobarbital at a hypnotic dose of 50 mg/kg was injected intraperitoneal (i.p.) in groups (n = 8) of mice 30 min after pretreatment with water/5% Tween 80 (control) and monoterpenes (150 mg/kg, i.p.), respectively. The duration of sleep time (loss and recovery of the righting reflex) was recorded (De Sousa *et al.*, 2004).

Results and Discussion

We have tested ten monoterpenes, typical components of essential oils of aromatic and medicinal plants, differing in their structure and in the position of their functional groups. The effects of these monoterpenes were evaluated for central pharmacological activity. The central nervous system (CNS)-depressant activity of the monoterpenes was investigated using the pentobarbital-induced hypnosis model in mice. The tested compounds **1**–**6** and **8** (Fig. 1) increased the sleep time of the mice indicating a sedative property, which may be attributed to an action on the central mechanisms involved in the regulation of sleep or an inhibition of the pentobarbital metabolism (Mattei and Franca, 2006; Chindo *et al.*, 2003). Neoisopulegol (**2**) and (+)-*p*-menth-1-en-9-ol (**6**) were found to be significantly more bioactive than the other compounds (Fig. 2). Isopulegol (**1**), (±)-isopinocampheol (**3**), and (−)-myrtenol (**4**) presented similar potency. In the comparison of (−)-*cis*-myrtanol (**5**) and (±)-neomenthol (**8**), it was shown that they are equipotent. Interestingly, (−)-menthol (**7**), (+)-dihydrocarveol (**9**), and (±)-isoborneol (**10**) do not present any effect. In this investigation, neoisopulegol (**2**) was slightly more potent than its isomer isopulegol (**1**). The present results show that the stereogenic centre at carbon atom 3 in these molecules is important in the interaction with the receptor. Similarly, comparing the sedative activity of (−)-myrtenol (**4**) and (−)-*cis*-myrtanol (**5**) shows that the presence of the double

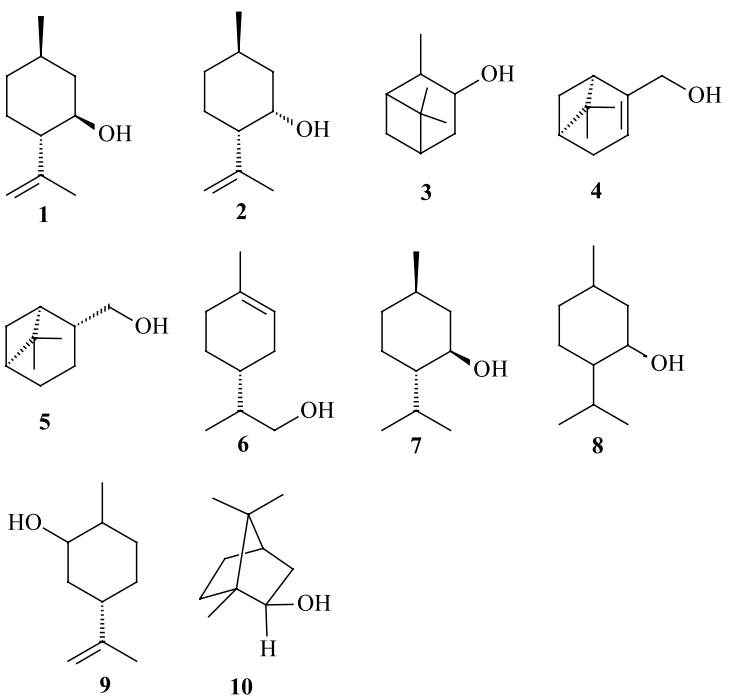

Fig. 1. Compounds used in this study: Isopulegol (**1**), neuoisopulegol (**2**), (±)-isopinocampheol (**3**), (−)-myrtenol (**4**), (−)-*cis*-myrtanol (**5**), (+)-*p*-menth-1-en-9-ol (**6**), (−)-menthol (**7**), (±)-neomenthol (**8**), (+)-dihydrocarveol (**9**), and (±)-isoborneol (**10**).

bond in the structure of **4** also influences the potency of the pharmacological effect. Surprisingly, (±)-neomenthol (**8**) increases the pentobarbital-induced sleep time in mice, but its isomer (−)-menthol (**7**) was ineffective in the test.

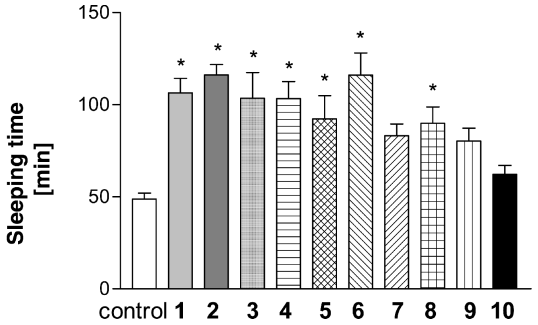

Fig. 2. Effect of monoterpenes **1**–**10** on pentobarbital-induced hypnosis in mice. Values are the mean ± SEM for 8–10 mice. $*p < 0.05$ as compared to vehicle (control); one-way ANOVA followed by Dunnet's test.

Monoterpenes such as isoborneol are able to increase the motility of the animals indicating an activating effect, but clearly decrease caffeine-induced overagitation in mice (both administrations of isoborneol were by the inhalation route) (Buchbauer *et al.*, 1993). In our study, isoborneol did not exhibit a sedative action or a stimulating profile in mice, after administration by the i. p. route. This result contrasts with the earlier observation possibly due to the different administration routes or the experimental model used to evaluate the effects.

The sedative effect of other oxygenated monoterpenes has been shown. For example, 1,8-cineole, having an ether group (Santos and Rao, 2000), and linalool, having a hydroxy group (Elisabetsky *et al.*, 1995), were able to increase the pentobarbital-induced sleeping time in mice. Linalool also induced a sedative effect on humans when administered by inhalation (Sugawara *et al.*, 1998). Significant sedative effects were achieved when mice were subjected to the inhalation of fragrance compounds and essential oils. The same results were also achieved when mice were artificially induced to overagitation after treatment with caffeine. Indeed, other terpene alcohols caused a distinct sedative effect under both conditions, like α-terpineol (Buchbauer *et al.*, 1993). Therefore, our data are in agreement with the results in mice submitted to inhalation of structurally similar monoterpenes, to the compounds evaluated in our work.

Several drugs with depressant effects in the CNS, such as anticonvulsants and anxiolytics, also increased the time of sleep in the pentobarbital-induced sleeping time test (Mattei and Franca, 2006). Therefore, the tested monoterpenes can present depressant specific effects in the CNS, like the anticonvulsant activity of the monoterpene linalool already investigated.

The present results show that a sedative effect of the tested monoterpenes is influenced by the structure of the molecules, but it is not possible to outline a structure-activity relationship on the basis of these findings. It can nevertheless be pointed out, when considering these data and those obtained previously with 1,8-cineole and linalool, that a sedative effect appears more frequently applying oxygenated monoterpenes. Our experimental results also suggest that the psychoactive monoterpenes reported in this paper present a pharmacologic profile of sedative drugs.

Acknowledgements

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP).

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