

Antinociceptive Activity of Structural Analogues of Rotundifolone: Structure-Activity Relationship

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Rotundifolone, a monoterpene isolated from the essential oil of the leaves of *Mentha x villosa*, is a constituent of several essential oils and known to have antinociceptive activity. Our recent study demonstrated that the analogues of rotundifolone showed also a significant antinociceptive effect. In the present report, to investigate the correlation between the structure and antinociceptive activity, rotundifolone and its analogues were evaluated in the acetic acid-induced writhing test in mice. All compounds showed to be more antinociceptive than rotundifolone against the pain response induced by acetic acid. Comparing the antinociceptive effect of rotundifolone with limonene oxide and (+)-pulegone, the results demonstrated that the epoxide group contributes as much as the ketone group to the antinociceptive activity of rotundifolone. Similarly, pulgone oxide and carvone epoxide were more antinociceptive than rotundifolone, thereby suggesting that the position of the functional group on the ring also influences the antinociceptive activity. (–)-Carvone produced maximal inhibition of the writhing response and was slightly more active than (+)-carvone. The study showed that by appropriate structural modification it may be possible to develop novel antinociceptive agents.

Key words: Rotundifolone, Monoterpenes, Analgesic Activity, *p*-Menthanes

Introduction

The use of essential oils in the flavor and fragrance industry is very known. The fragrances of essential oils have been used in aromatherapy to induce mental tranquility or relaxation and to aid sleep in humans (Lavabre, 2001). The study of the pharmacological potential of essential oils of plants has been growing rapidly for the last several years. Many essential oils possess a great variety of biological activities, such as anxiolytic (de Almeida *et al.*, 2004), anticonvulsant (de Almeida *et al.*, 2003), antinociceptive (de Almeida *et al.*, 2001) and immunomodulatory (Mikhaeil *et al.*, 2003). Previous studies showed that some monoterpenes present in many essential oils also possess antinociceptive (Abdel-Fattah *et al.*, 2000), anticonvulsant (Elisabetsky *et al.* 1995), antimicrobial (Dhar *et al.*, 2004) and anaesthetic (Ghelardini *et al.*, 2001) activity in animal experiments. Recently, we demonstrated that derivative compounds of monoterpenes also exhibit several types of pharmacological properties, such as antinociceptive (de Sousa *et al.*, 2004), sedative (de Sousa *et al.*, 2006a) and antidepressant (de Sousa *et al.*, 2006b). The

essential oil of the plant *Mentha x villosa* Hudson (Lamiaceae), known popularly as “hortelã-da-folha-miúda”, has been shown to exhibit a central nervous system-depressant effect (Raya *et al.*, 1990). Rotundifolone is an important chemical constituent of the essential oil of many *Mentha* species such as *Mentha x villosa*. This monoterpene has cardiovascular (Guedes *et al.*, 2004), relaxant of intestinal smooth muscle (Sousa *et al.*, 1997) and antinociceptive (Almeida *et al.*, 1996) effects. The main aim of the present study was to determine the relationship between the chemical structure of rotundifolone and its antinociceptive activity to understand the influence of the functional groups of this monoterpene.

Materials and Methods

Chemicals

Compounds limonene oxide (Thomas and Besiere, 1989), pulgone oxide (Katsuhara, 1967), and carvone epoxide (Santos *et al.*, 1997) were prepared in our laboratory as previously described. (+)-Pulegone, (–)-carvone and (+)-carvone were purchased from Aldrich. Rotundifol-

one was isolated from essential oil of *Mentha × villosa* using a previously described procedure (Almeida *et al.*, 1996). All compounds were dissolved in 5% Tween 80 as an emulsion.

Animals

Male Swiss mice (28–34 g) were obtained from our research animal facility. The animals were maintained at constant room temperature [$(26 \pm 1)^\circ\text{C}$] and on a 12-h/12-h light-dark cycle (light from 06:00 to 18:00), with free access to food and water. All behavioral observations were conducted between 13:00 and 18:00.

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.

Acetic acid-induced writhing

The mice were divided into nine groups ($n = 8$). The compounds and morphine were dissolved in 5% Tween 80 and saline (0.9%), respectively. The first group was pretreated with 0.9% saline (control). The compounds (250 mg/kg i.p.) and morphine (3 mg/kg i.p.) were administered. After 30 min an acetic acid solution (0.8%; 0.1 mL/10 g i.p.) was injected. After further 10 min, the number of constrictions was recorded for 10 min (Almeida *et al.*, 1996).

Results and Discussion

Assessment of the antinociceptive activity of rotundifolone and the analogous compounds was performed using the acetic acid-induced writhing model in mice. It was used morphine (3 mg/kg) as a positive control. All six tested analogues (Fig. 1) were found to be more antinociceptive than rotundifolone itself (Fig. 2). In the comparison of rotundifolone (**1**) (having an α,β -unsaturated ketone and epoxide group) and limonene oxide (**2**) (having only an epoxide group), it was shown that the absence of the ketone group did not decrease the antinociceptive effect. Indeed, there is a significant increase of this pharmacologic activity. Comparing the antinociceptive effect of (**1**) and (+)-pulegone (**3**) (having only an α,β -unsaturated ketone group) showed that the absence of the epoxide group did not decrease the antinociceptive effect. Similarly,

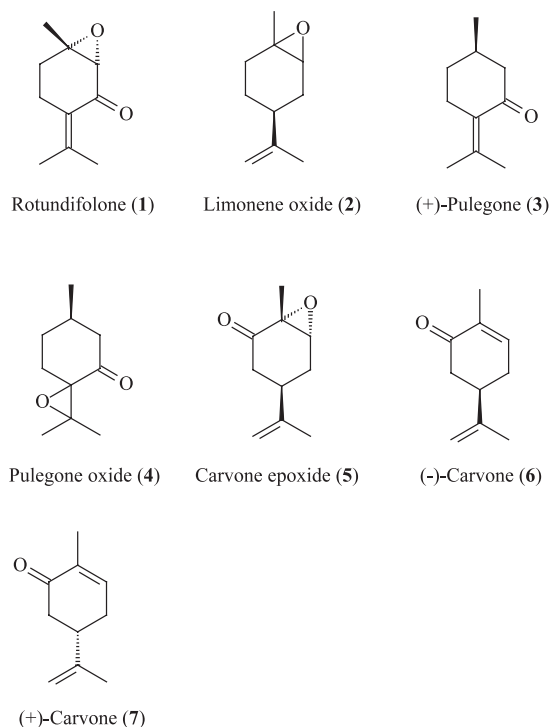


Fig. 1. Compounds used in this study.

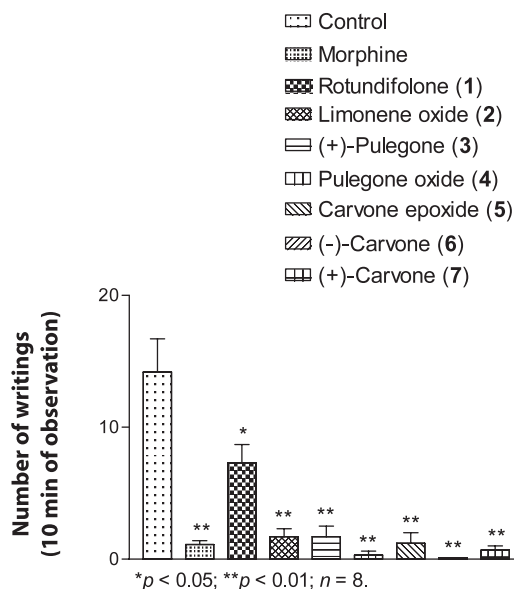


Fig. 2. Antinociceptive effect of morphine (3 mg/kg) and compounds (250 mg/kg) evaluated using acetic acid-induced writhing in mice.

there is a significant increase of this effect. In both cases, **1** versus **2** and **1** versus **3**, it was found that the epoxide group contributes as much as the ketone group to the antinociceptive activity of **1**. Interestingly, both limonene oxide (**2**) and (+)-pulegone (**3**) were more antinociceptive than rotundifolone (**1**), thereby implying that the presence of the two functional groups in the molecule does not result in an increase of the effects, but it was less active. These results also showed that the presence of the epoxide or ketone group in the structure of **1** was not a critical requirement. Differences were not observed between the effects of limonene oxide (**2**) and (+)-pulegone (**3**) in the acetic acid-induced writhing test.

To show if the position of the epoxide and ketone groups of the molecule affects the antinociceptive activity, rotundifolone (**1**) was compared with carvone epoxide (**5**) and pulegone oxide (**4**) (Fig. 2). Both **1** and **5** have a ring ketone group and differ in the position of this group. Carvone epoxide (**5**) was found to be significantly more antinociceptive than rotundifolone (**1**). Comparing the antinociceptive effect of pulegone oxide (**4**) (having an exocyclic epoxide group) with rotundifolone (**1**) (having an endocyclic epoxide group) showed that the position of the epoxide group in the molecule did affect the antinociceptive effect. Pulegone oxide (**4**) was more antinociceptive than rotundifolone (**1**) and all the other compounds tested, except for (–)- and (+)-carvone. These results showed that the position of the functional group on the ring also influence the antinociceptive activity.

The monoterpene (–)-carvone (**6**) produced maximal inhibition of the writhing response and

was slightly more antinociceptive than its enantiomer (+)-carvone (**7**). This difference in the effects showed the influence of the chirality of these enantiomers on the pharmacological activity. Among the monoterpenoids with only an α,β -unsaturated ketone group (**3**, **6** and **7**), (+)-pulegone (**3**) was found to be less active. It appeared that the compounds with an endocyclic double bond conjugated to a ketone group were more active.

The antinociceptive activity of other oxygenated monoterpenes has been shown. For example, 1,8-cineole (having an ether group), (–)-3-isothujone (having a ketone group) and menthol (having a hydroxy group) present in many plant essential oils were active on some types of nociception models in mice (Santos and Rao, 2000; Rice and Wilson, 1976; Galeotti *et al.*, 2002). We have reported in a previous paper that rotundifolone (**1**) showed analgesic activity in several methods of nociception. Its analgesic effect was blocked by naloxone pretreatment, indicating a possible action involving an opioid mechanism (Almeida *et al.*, 1996).

We have attempted in the present study to learn the structural relationship of rotundifolone (**1**) and its antinociceptive effect. It was found that the functional groups and their position on the ring of rotundifolone (**1**) contribute for its antinociceptive activity. Our experimental results also suggested that by appropriate structural modification of monoterpenes it may be possible to develop novel antinociceptive drugs.

Acknowledgements

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