

Structure and Spasmolytic Activity Relationships of Monoterpene Analogues Found in Many Aromatic Plants

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Rotundifolone, a monoterpene isolated from the essential oil of the leaves of *Mentha × villosa*, is a constituent of several essential oils and known to have spasmolytic activity. The present study aimed to investigate the correlation between structure and spasmolytic activity of rotundifolone and its analogues in ileum isolated from guinea-pig. Five of the seven tested analogues were found to have a spasmolytic effect more potent than rotundifolone itself, except for pulegone and (+)-limonene. The comparison between rotundifolone and limonene oxide showed that the absence of the keto group did not decrease the relaxant effect. Comparison of the spasmolytic activity between rotundifolone and (+)-pulegone showed that the absence of the epoxy group did not decrease the relaxation of the ileum. Carvone epoxide was found to be significantly more potent than rotundifolone. The monoterpene (–)-carvone produced ileum relaxation and was more potent than its enantiomer (+)-carvone. (+)-Limonene and pulegone oxide showed a similar effect. The study showed that the functional groups and their position at the ring of rotundifolone contributed to the relaxation activity of the ileum. The absence of the oxygenated molecular structure is not a critical requirement for the molecule to be bioactive.

Key words: Terpenes, Essential Oils, Smooth Muscle

Introduction

The plant kingdom is rich in spasmolytic compounds. Some preparations are used as spasmolytics for the gastro-intestinal tract and may also be used for other disorders such as indigestion and diarrhea. Essential oils such as those of peppermint, dill, and caraway are examples of plant-derived spasmolytics (Williamson *et al.*, 1996).

About 3000 essential oils have been identified and several hundred are commercially available. The subtlety of nature shows up here, because a natural essential oil may include hundreds of different types of compounds, and its impact may be changed by removing those even present in very minute concentration. A major use for essential oils is in the blending of perfumes, but they are also used to flavour foods, cosmetics, toothpaste, chewing gum, and pharmaceutical preparations (Atkins, 2003; Erickson, 1976).

In folk medicine as well as in phytotherapy, essential oils have been used to treat several dis-

eases. They are used for example as sedatives, relaxants or anticonvulsants (Lawless, 2002; Almeida *et al.*, 2003). Studies of the pharmacological potential of essential oils of plants have been grown rapidly in the last several years. Previous studies showed that some monoterpenes present in many essential oils possess pharmacological properties, such as anticonvulsant (De Sousa *et al.*, 2007a, 2006a), analgesic (Gonçalves *et al.*, 2008; Amaral *et al.*, 2007; De Sousa *et al.*, 2007b), sedative (De Sousa *et al.*, 2007c) and anxiolytic-like (Silva *et al.*, 2007) activity in animal experiments. Recently, we demonstrated that derivatives of monoterpenes also exhibit several types of pharmacological properties, such as antinociceptive (Oliveira *et al.*, 2008; De Sousa *et al.*, 2004), sedative (De Sousa *et al.*, 2006b) and antidepressant (De Sousa *et al.*, 2006c). The essential oil of the plant *Mentha × villosa* Hudson (Lamiaceae), popularly known as “hortelã-da-folha-miúda”, has shown 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 × villosa*. This monoterpene has cardiovascular (Guedes *et al.*, 2004), antinociceptive (De Sousa *et al.*, 2007b) and relaxant of intestinal smooth muscle (Sousa *et al.*, 1997) effects. The main aim of the present study was to determine the relationship between the chemical structure of rotundifolone and its spasmolytic activity to understand the influence of the functional groups of this monoterpene.

Materials and Methods

Chemicals and solutions

The compounds limonene oxide (Thomas and Bessiere, 1989), pulegone 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. (+)-Limonene was purchased from Dierberger Óleos Essenciais S. A., Barra Bonita, Brazil. Rotundifolone was isolated from the essential oil of *Mentha × villosa* using a previously described procedure (Almeida *et al.*, 1996). All compounds were mixed with 10% Tween 80 to give an emulsion.

Animals

Male guinea-pigs (weighing 300–400 g), obtained from Central Animal House of the Federal University of Sergipe, São Cristóvão, Brazil, were used. Two days before the experiments, the animals were housed at 25–30 °C under a light and dark cycle (6–18 h light and 18–6 h dark) in the Animal House of the Department of Physiology. The animals were fasted for 16 h prior to the beginning of the experiments, but were allowed free access to water. The use of animals in this experimental protocol was approved by the Ethics Committee on Research Animals of the Federal University of Sergipe, São Cristóvão, Brazil on 2007/06/25 with protocol number 43/07.

Tissue preparation

The animals were killed by cervical dislocation and bleeding through cut of the carotid arteries. A 2.0 cm long whole segment of the distal portion of the ileum (1 cm proximal to the ileocaecal sphincter) was removed and suspended in 1 g of resting tension in 10 mL organ bath containing Tyrode solution (composition in mmol L⁻¹: NaCl, 137;

KCl, 2.7, MgCl₂ · 6H₂O, 0.5; CaCl₂ · 2H₂O, 1.8; NaH₂PO₄, 0.4; NaHCO₃, 12; glucose, 5.5) which was maintained at 37 °C and continuously bubbled with air. The ileum strips were allowed to equilibrate for 60 min, meanwhile they were washed every 15 min with Tyrode solution. The muscle strips were connected to a force transducer coupled to an amplifier-recorder (GOLD, Ohio, USA), and the isometric contraction was recorded using a computer. In Tyrode solution with elevated potassium level (60 mM), NaCl was decreased to 76.3 mM to maintain the solution's osmolality.

Experimental protocol

After an equilibration period, the tonus of the ileum was elevated by washing the isolated organ system two times successively with Tyrode solution containing 60 mM of KCl. When the muscle tension was stabilized, the compounds were cumulatively added in separate preparations to obtain concentration-relaxant response curves. The relaxation was then measured by the reduction of the 60 mM K-induced tonus and converted to relaxation percentage. In order to compare the potencies of the compounds in relation to their spasmolytic actions, the concentration required to obtain half of the maximum response (EC₅₀) was obtained from the concentration-response curve of each compound by the method of nonlinear regression. Moreover, the maximum effects of the compounds were obtained by the percentage of maximum reduction of the 60 mM K-induced tonus.

Data presentation and statistical analysis

Data are presented as mean relaxation percentage (± SEM) of the 60 mM potassium-induced contraction of guinea-pig ileum muscle strips prepared from at least five animals. The statistical analysis was performed using analysis of variance followed by Tukey's test. A probability level of 0.05 was regarded as significant.

Results and Discussion

Since monoterpenes are common in many plant species and are used in cosmetic, non-cosmetic and pharmaceutical preparations, as well as in the food industry, it is interesting to know the spasmolytic effects of these compounds. Chiral recognition by receptors and enzymes is well demonstrated in biochemical, pharmaceutical, and chemosensory research. We report in this compar-

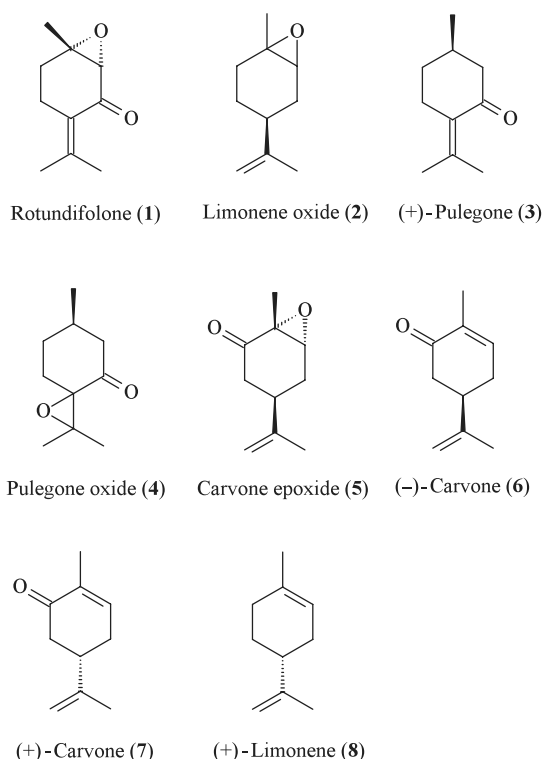


Fig. 1. Chemical structures of the compounds used in this study.

active study the findings from the assessment of the spasmolytic activity of rotundifolone (**1**) and analogous compounds using precontracted by 60 mM potassium ileum isolated from guinea-pig. Five tested analogues (Fig. 1) were found to have a spasmolytic effect more potent than rotundifolone itself (Table I), except for pulegone and (+)-limonene. In

the comparison of rotundifolone (**1**) (having an α,β -unsaturated keto and epoxy group) and limonene oxide (**2**) (having only an epoxy group), it was shown that the absence of the keto group did not decrease the relaxant effect. Indeed, there is a significant increase of this pharmacological activity ($p < 0.05$). The comparison between the spasmolytic effect of rotundifolone (**1**) and (+)-pulegone (**3**) (having only an α,β -unsaturated keto group) showed that the absence of the epoxy group did not decrease the effect of relaxation of the ileum ($p < 0.001$). Similarly, there is a significant increase in this effect. In both cases, **1** versus **2** and **1** versus **3**, it was found that both the epoxy and keto groups contribute to the spasmolytic activity of rotundifolone (**1**). Interestingly, both limonene oxide (**2**) and (+)-pulegone (**3**) presented a spasmolytic effect more potent than that of rotundifolone (**1**), thereby implying that the presence of the two functional groups in the molecule does not result in an increase of this effect, indeed it was less active. These results also showed that the presence of the epoxy or keto group in the molecule of **1** is not a critical requirement. In our evaluation, differences between the effects of **2** and **3** were not observed.

In order to investigate whether the position of the epoxy and keto groups in the molecule affects the spasmolytic activity, rotundifolone (**1**) was compared to carvone epoxide (**5**) and pulegone oxide (**4**) (Table I). Both **1** and **5** have a ring keto group and differ from each others in the position of this group. Carvone epoxide was found to have a significantly spasmolytic effect more potent than that of rotundifolone ($p < 0.001$). The comparison between the relaxant effect of pulegone oxide (having an exocyclic epoxy group) and rotundifolone (having an endocyclic epoxy group) showed that the po-

Table I. EC₅₀ values and maximum effect of the compounds relative to the relaxant activity in ileum isolated from guinea pig.

Compound	EC ₅₀ (CI 95%)	Maximum effect (% of reduction of 60 mM K-induced tonus)
Rotundifolone (1)	$1.1 \cdot 10^{-3}$ ($9.0 \cdot 10^{-4}$ – $1.4 \cdot 10^{-3}$)	121.8 ± 6.61
Limonene oxide (2)	$5.7 \cdot 10^{-4}$ ($4.3 \cdot 10^{-4}$ – $7.6 \cdot 10^{-4}$)	$108.5 \pm 2.56^*$
(+)-Pulegone (3)	$4.1 \cdot 10^{-4}$ ($2.7 \cdot 10^{-4}$ – $6.3 \cdot 10^{-4}$)	121.4 ± 8.18
Pulegone oxide (4)	$3.0 \cdot 10^{-3}$ ($2.5 \cdot 10^{-3}$ – $3.7 \cdot 10^{-3}$)	120.8 ± 3.32
Carvone epoxide (5)	$3.7 \cdot 10^{-4}$ ($3.3 \cdot 10^{-4}$ – $4.3 \cdot 10^{-4}$)	120.2 ± 3.9
(-)-Carvone (6)	$5.9 \cdot 10^{-5}$ ($4.7 \cdot 10^{-5}$ – $7.3 \cdot 10^{-5}$)	128.7 ± 1.45
(+)-Carvone (7)	$6.5 \cdot 10^{-4}$ ($5.0 \cdot 10^{-4}$ – $8.6 \cdot 10^{-4}$)	136.8 ± 8.57
(+)-Limonene (8)	$3.3 \cdot 10^{-3}$ ($2.5 \cdot 10^{-3}$ – $4.3 \cdot 10^{-3}$)	$110.4 \pm 2.51^{**}$

* $p < 0.05$ in relation to (+)-carvone; ** $p < 0.01$ in relation to (+)-carvone.

sition of the epoxy group in the molecule did affect the spasmolytic effect. Pulegone oxide was less potent than rotundifolone ($p < 0.001$). These results showed that the position of the functional group at the ring also influences the spasmolytic activity.

The pharmacological assessment of chiral compounds in an early research phase can lead to the selection of a single isomer for development. This selection process can maximize the potential for specific activity and minimize the potential for side-effects. The monoterpene (–)-carvone (**6**) produced an effect of relaxation of the ileum and was more potent than its enantiomer (+)-carvone (**7**) ($p < 0.001$) and all other compounds tested. This difference in the effects shows the influence of chirality of these enantiomers on the pharmacological activity. Among the monoterpenoids with only one α,β -unsaturated keto group (**3**, **6** and **7**), (+)-pulegone (**3**) and (+)-carvone (**7**) were found to be equipotent. (+)-Limonene (**8**) is a hydrocarbon (consisting entirely of hydrogen and carbon atoms). This compound and pulegone oxide (**4**) showed a similar effect. Apparently the absence of an oxygenated molecular structure is not a critical requirement for the molecule to be bioactive. Among the tested compounds (+)-carvone had a greater effect on ileum relaxation than limonene oxide and (+)-limonene.

Several essential oils are reported to exhibit spasmolytic activity (Prakash *et al.*, 2006; Astudillo *et al.*, 2004; El Tantawy *et al.*, 1999; Gamez *et al.*, 1990; Zafra-Polo *et al.*, 1989). Monoterpenes are the major components of these oils. The spasmolytic activity of some oxygenated monoterpenes present in these essential oils has been shown, for example, thymol (having a phenol group) and

camphor (a keto group) (Astudillo *et al.*, 2004). The tested monoterpenes (+)-pulegone (**3**), (–)-carvone (**6**), and (+)-carvone (**7**) have the same functional group like camphor. Therefore the effects observed are consistent with those reported for other compounds belonging to the same chemical class. In our study (+)-limonene (**8**) presented this effect. Other hydrocarbons also showed spasmolytic activity, such as α -pinene, β -pinene, and γ -terpinene (Sadraei *et al.*, 2001; Astudillo *et al.*, 2004). Interestingly, the effect of a mixture of α -pinene and β -pinene was, however, less than the sum of their separate effects. A synergistic action was not observed. Whereas the inhibition of contractile over-activity of the ileum is the basis of the treatment of some gastro-intestinal disorders such as diarrhea, the monoterpenes of this study and other analogues may have clinical benefits for the treatment under these conditions.

In the present study we have attempted to learn the relationship between the structure of rotundifolone and its spasmolytic activity. All monoterpenes tested, which are chemical constituents of essential oils of many aromatic plants, are relaxants of intestinal smooth muscles. It was found that the functional groups and their position at the ring of rotundifolone contribute to their effect of relaxation of the ileum. Our experimental results also suggested that by appropriate structural modification of monoterpenes it may be possible to develop novel spasmolytic drugs.

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Almeida R. N., Hiruma C. A., and Barbosa-Filho J. M. (1996), Analgesic effect of rotundifolone in rodents. *Fitoterapia* **67**, 334–338.
Almeida R. N., Motta S. C., and Leite J. R. (2003), Óleos essenciais com propriedades anticonvulsivantes. *Bol. Latinoam. Caribe Plantas Med. Aromat.* **2**, 3–6.
Amaral J. F., Pequeno M. R., Neto M. R. A., Neto P. F. T., Moura B. A., Melo C. T. V., Araújo F. L. O., De Sousa D. P., Vasconcelos P. F., Vasconcelos S. M. M., and Sousa F. C. F. (2007), Antinociceptive effect of the monoterpene *R*-(+)-limonene in mice. *Biol. Pharm. Bull.* **30**, 1217–1220.

Astudillo A., Hong E., Bye R., and Navarrete A. (2004), Antispasmodic activity of extracts and compounds of *Acalypha phleoides* Cav. *Phytother. Res.* **18**, 102–106.
Atkins P. W. (2003), *Atkins' Molecules*. 2nd ed. Cambridge University Press, Cambridge, p. 148.
De Sousa D. P., Raphael E., Brocksom U., and Brocksom T. J. (2004), Antinociceptive profile of 2-phenylselenenyl-1,8-cineole in mice. *Biol. Pharm. Bull.* **27**, 910–911.
De Sousa D. P., Gonçalves J. C. R., Quintans-Júnior L., Cruz J. S., Araújo D. A. M., and De Almeida R. N. (2006a), Study of anticonvulsant effect of citronellol, a

- monoterpene alcohol, in rodents. *Neurosci. Lett.* **401**, 231–235.
- De Sousa D. P., Oliveira F. S., and De Almeida R. N. (2006b), Evaluation of the central activity of hydroxydihydrocarvone. *Biol. Pharm. Bull.* **29**, 811–812.
- De Sousa D. P., Schefer R. R., Brocksom U., and Brocksom T. J. (2006c), Synthesis and antidepressant evaluation of three *para*-benzoquinone mono-oximes and their oxy derivatives. *Molecules* **11**, 148–155.
- De Sousa D. P., Quintans J. L., and Almeida R. N. (2007a), Evolution of the anticonvulsant activity of α -terpineol. *Pharm. Biol.* **45**, 69–70.
- De Sousa D. P., Júnior E. V. M., Oliveira F. S., Almeida R. N., Nunes X. P., and Barbosa-Filho J. M. (2007b), Antinociceptive activity of structural analogues of rotundifolone: structure-activity relationship. *Z. Naturforsch.* **62c**, 39–42.
- De Sousa D. P., Raphael E., Brocksom U., and Brocksom T. J. (2007c), Sedative effect of monoterpene alcohols in mice: a preliminary screening. *Z. Naturforsch.* **62c**, 563–566.
- El Tantawy M. E., El Sakhawy F. S., El Sohly M. A., and Ross S. A. (1999), Chemical composition and biological activity of the essential oil of the fruit of *Taxodium distichum* L. rich growing in Egypt. *J. Essent. Oil Res.* **11**, 386–392.
- Erickson R. E. (1976), The industrial importance of monoterpenes and essential oils. *Lloydia* **39**, 8–19.
- Gamez M. J., Jimenez J., Navarro C., and Zarzuelo A. (1990), Study of the essential oil of *Lavandula dentata* L. *Pharmazie* **45**, 69–70.
- Golçalves J. C. R., Oliveira F. S., Benedito R. B., De Sousa D. P., De Almeida R. N., and Araújo D. A. M. (2008), Antinociceptive activity of (–)-carvone: evidence of association with decreased peripheral nerve excitability. *Biol. Pharm. Bull.* **31**, 1017–1020.
- Guedes D. N., Silva D. F., Barbosa-Filho J. M., and Medeiros I. A. (2004), Endothelium-dependent hypotensive and vasorelaxant effects of the essential oil from aerial parts of *Mentha × villosa* in rats. *Phytomedicine* **11**, 490–497.
- Katsuhara J. (1967), Absolute configuration of pulegone oxide and piperitenone dioxide. *J. Org. Chem.* **32**, 797–799.
- Lawless J. (2002), In: *Encyclopedia of Essential Oils*. Thorsons, London, pp. 35–145.
- Oliveira F. S., De Sousa D. P., and De Almeida R. N. (2008), Antinociceptive effect of hydroxydihydrocarvone. *Biol. Pharm. Bull.* **31**, 588–591.
- Prakash O., Kasana V. K., Pant A. K., Zafar A., Hore S. K., and Mathela C. S. (2006), Phytochemical composition of essential oil from seeds of *Zingiber roseum* Rosc. and its antispasmodic activity in rat duodenum. *J. Ethnopharmacol.* **106**, 344–347.
- Raya M. D. P., Utrilla M. P., Navarro M. C., and Jimenez J. (1990), CNS activity of *Mentha-rotundifolia* and *Mentha-longifolia* essential oil in mice and rats. *Phytother. Res.* **4**, 232–234.
- Sadraei H., Asghari G. R., Hajhashemi V., Kolagar A., and Ebrahimi M. (2001), Spasmolytic activity of essential oil and various extracts of *Ferula gummosa* Boiss. on ileum contractions. *Phytomedicine* **8**, 370–376.
- Santos R. B., Brocksom T. J., and Brocksom U. (1997), A convenient deoxygenation of α,β -epoxy ketones to enones. *Tetrahedron Lett.* **38**, 745–748.
- Silva M. I. G., Neto M. R. A., Neto P. F. T., Moura B. A., Amaral J. F., De Sousa D. P., Vasconcelos S. M. M., and Sousa F. C. F. (2007), Central nervous system activity of acute administration of isopulegol. *Pharmacol. Biochem. Behav.* **88**, 141–147.
- Sousa P. J. C., Magalhães P. J. C., Lima C. C., Oliveira V. S., and Leal-Cardoso J. H. (1997), Effects of piperitenone oxide on the intestinal smooth muscle of the guinea pig. *Braz. J. Med. Biol. Res.* **30**, 787–791.
- Thomas A. F. and Bessiere Y. (1989), Limonene. *Nat. Prod. Rep.* **6**, 291–309.
- Williamson E. M., Okpako D. T., and Evans F. J. (1996), Selection, preparation and pharmacological evaluation of plant material. In: *Pharmacological Methods in Phytotherapy Research*, Vol. I. John Wiley & Sons Ltd, West Sussex, pp. 25–26.
- Zafra-Polo M. C., Blazquez M. A., and Villar A. (1989), Spasmolytic and antimicrobial activity of the essential oils from *Thymus leptophyllus* and *T. webbianus*. *Fitoterapia* **60**, 469–473.