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

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Rotundifolone, a monoterpene isolated from the essential oil of the leaves of Mentha \textit{villosoa}, 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 spasmodytics (Williamson \textit{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 diseases. They are used for example as sedatives, relaxants or anticonvulsants (Lawless, 2002; Almeida \textit{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 \textit{et al.}, 2007a, 2006a), analgesic (Gonçalves \textit{et al.}, 2008; Amaral \textit{et al.}, 2007; De Sousa \textit{et al.}, 2007b), sedative (De Sousa \textit{et al.}, 2007c) and anxiolytic-like (Silva \textit{et al.}, 2007) activity in animal experiments. Recently, we demonstrated that derivatives of monoterpens present in many essential oils possess pharmacological properties, such as antinociceptive (Oliveira \textit{et al.}, 2008; De Sousa \textit{et al.}, 2004), sedative (De Sousa \textit{et al.}, 2006b) and antidepressant (De Sousa \textit{et al.}, 2006c). The essential oil of the plant Mentha \textit{villosoa} Hudson (Lamiaceae), popularly known as “hortelã-da-folha-miuña”, has shown a central nervous system-depressant effect (Raya \textit{et al.}, 1990). Rotundifolone is an important chemical
constituent of the essential oil of many Mentha species such as Mentha × villosa. This monoter-
pene has cardiovascular (Guedes et al., 2004), anti-
ociceptive (De Sousa et al., 2007b) and relaxant of intestinal smooth muscle (Sousa et al., 1997) ef-
tects. The main aim of the present study was to
determine the relationship between the chemical
structure of rotundifolone and its spasmylytic ac-
tivity 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 de-
scribed. (+)-Pulegone, (−)-carvone and (+)-car-
vone were purchased from Aldrich. (+)-Limonene
was purchased from Dierberger Oleos Essenciais
S. A., Barra Bonita, Brazil. Rotundifolone was iso-
lated from the essential oil of Mentha × villosa us-
ing 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), ob-
tained from Central Animal House of the Federal
University of Sergipe, São Cristóvão, Brazil, were
used. Two days before the experiments, the ani-
mals 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 be-
ginning of the experiments, but were allowed free
access to water. The use of animals in this exper-
imental protocol was approved by the Ethics Com-
mittee on Research Animals of the Federal Uni-
vversity 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 sphinc-
ter) 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 equili-
brate for 60 min, meanwhile they were washed ev-
ery 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 po-
tassium level (60 mM), NaCl was decreased to
76.3 mM to maintain the solution’s osmolarity.

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 ten-
sion was stabilized, the compounds were cumula-
tively added in separate preparations to obtain
concentration-relaxant response curves. The relaxa-
tion was then measured by the reduction of the
60 mM K-induced tonus and converted to relaxa-
tion percentage. In order to compare the potencies
of the compounds in relation to their spasmylytic
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 re-
duction of the 60 mM K-induced tonus.

Data presentation and statistical analysis

Data are presented as mean relaxation percent-
age (± 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 spas-
ymolytic effects of these compounds. Chiral rec-
ognition by receptors and enzymes is well dem-
onstrated in biochemical, pharmaceutical, and
chemosensory research. We report in this compar-
Fig. 1. Chemical structures of the compounds used in this study.

Table I. EC<sub>50</sub> values and maximum effect of the compounds relative to the relaxant activity in ileum isolated from guinea pig.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (CI 95%)</th>
<th>Maximum effect (% of reduction of 60 mm K-induced tonus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotundifolone (1)</td>
<td>1.1 · 10⁻³ (9.0 · 10⁻⁴ – 1.4 · 10⁻³)</td>
<td>121.8 ± 6.61</td>
</tr>
<tr>
<td>Limonene oxide (2)</td>
<td>5.7 · 10⁻⁴ (4.3 · 10⁻⁴ – 7.6 · 10⁻⁴)</td>
<td>108.5 ± 2.56*</td>
</tr>
<tr>
<td>(+)-Pulegone (3)</td>
<td>4.1 · 10⁻⁴ (2.7 · 10⁻⁴ – 6.3 · 10⁻⁴)</td>
<td>121.4 ± 8.18</td>
</tr>
<tr>
<td>Pulegone oxide (4)</td>
<td>3.0 · 10⁻³ (2.5 · 10⁻³ – 3.7 · 10⁻³)</td>
<td>120.8 ± 3.32</td>
</tr>
<tr>
<td>Carvone epoxide (5)</td>
<td>3.7 · 10⁻⁴ (3.3 · 10⁻⁴ – 4.3 · 10⁻⁴)</td>
<td>120.2 ± 3.9</td>
</tr>
<tr>
<td>(-)-Carvone (6)</td>
<td>5.9 · 10⁻⁵ (4.7 · 10⁻⁵ – 7.3 · 10⁻⁵)</td>
<td>128.7 ± 1.45</td>
</tr>
<tr>
<td>(+)-Carvone (7)</td>
<td>6.5 · 10⁻⁴ (5.0 · 10⁻⁴ – 8.6 · 10⁻⁴)</td>
<td>136.8 ± 8.57</td>
</tr>
<tr>
<td>(+)-Limonene (8)</td>
<td>3.3 · 10⁻³ (2.5 · 10⁻³ – 4.3 · 10⁻³)</td>
<td>110.4 ± 2.51**</td>
</tr>
</tbody>
</table>

* 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 spasmodic 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 spasmodic 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 monoterpenes ($\alpha$)-carvone (6) produced an effect of relaxation of the ileum and was more potent than its enantiomer ($\beta$)-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 $\alpha,\beta$-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 spasmodic 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 spasmodic 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), ($\alpha$)-carvone (6), and ($\beta$)-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 spasmodic activity, such as $\alpha$-pinene, $\beta$-pinene, and $\gamma$-terpinene (Sadraei et al., 2001; Astudillo et al., 2004). Interestingly, the effect of a mixture of $\alpha$-pinene and $\beta$-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 spasmodic 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 spasmodic drugs.

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