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Spasmolytic Activity of Chiral Monoterpene Esters

Damião P. de Sousa*, Genival A. S. Júnior, Luciana N. Andrade, and Josemar S. Batista

Laboratório de Química de Produtos Naturais e Sintéticos Bioativos (LAPROBIO), *Departamento de Fisiologia, Universidade Federal de Sergipe, CEP 49100-000, São Cristóvão, Sergipe, Brazil*

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Abstract: The present study aimed to investigate the correlation between structure and spasmolytic activity of racemate and enantiomers of linalyl and citronellyl acetates, chemical constituents of several bioactive essential oils, such as *Thymus leptophyllus* essential oil, which contains linalyl acetate as major constituent. The monoterpene esters showed significant spasmolytic activity in guinea-pig isolated ileum. Therefore, these result confirm that linalyl acetate should be involved at spasmolytic activity of the *Thymus leptophyllus* essential oil. The (+)-, (-)-, and (±)-linalyl acetates exhibited a relaxant effect equipotent. (+)- and (-)-Citronellyl acetates also showed a similar effect, however, synergistic action was presented on constituents of citronellyl acetate racemate. The study showed that the racemate and enantiomers of linalyl and citronellyl acetates are bioactives and that the position of the functional group on the molecule structures influences the effect of relaxation of the ileum.

Keywords: terpenes; essential oils; smooth muscle; spasmolytic activity; chirality; structure-activity relationships.

1. Introduction

The plant kingdom is rich in spasmolytic compounds. Some preparations are used as spasmolytic in the gastrointestinal tract and may be used for complaints such as indigestion and diarrhea. Essential oils such as those of peppermint, dill, and caraway are examples of plant-derived spasmolytics [1,2]. Another example is the *Thymus leptophyllus* essential oil that exhibit significant dose-dependent relaxations of acetylcholine-induced contractions in isolated rat duodenum and contains 68.5% of linalyl acetate [3]. Therefore, linalyl acetate possibly contributes to this pharmacological effect. Recently, we demonstrated that some monoterpenes present in many essential oils also possess spasmolytic activity, such as carvone and pulegone [4].

* Corresponding author: E-Mail:damiao_desousa@yahoo.com.br

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Pharmacological assessment of chiral compounds in an earlier 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. For various reasons, however, many racemates have been developed and for these compounds the pharmacological profile is considerably more complex than the single enantiomer activity profile. Therefore, in the present paper, we studied the spasmolytic activity of racemate and enantiomers of linalyl and citronellyl acetates and investigated the possible contribution of linalyl acetate in the relaxant effect of the *Thymus leptophyllus* essential oil on the intestinal smooth muscle.

2. Materials and Methods

2.1. Chemicals and solutions

(-)-Linalool, (-)-citronellol, and (+)-citronellol were purchased from Aldrich. Essential oil of *Coriandrum sativum* (coriander), (±)-citronellol, and (±)-linalool were purchased from company Dierberger Óleos Essenciais S.A., Barra Bonita, Brazil. All compounds were dissolved in 10% Tween 80 as an emulsion.

2.2. Acetylation of alcohols. General Method

The starting materials (1 g) were treated with a mixture of Ac_2O/py (6:10, vol), at reflux and stirred until complete disappearance of the starting material (alcohol). The reaction was monitored by TLC. Reaction times for linalools and citronellols were 48 and 1 h, respectively. After completion, the reaction mixture was quenched with cold water (30 mL), and product was extracted with $CHCl_3$ (90 mL), washed with saturated copper sulfate, water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexane–EtOAc (8:2) to afford the esters.

2.3. 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 23 ± 2^{o}C under 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 2009/04/06 with protocol number 19/08.

2.4. 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 under 1 g of resting tension in 10 mL organ bath containing Tyrode solution (composition in mmol L^{-1}: NaCl, 137; KCl, 2.7, $MgCl_2.6H_2O$, 0.5; $CaCl_2.2H_2O$, 1.8; NaH_2PO_4, 0.4; $NaHCO_3$, 12; glucose, 5.5) which was maintained at 37^{o}C and continuously bubbled with atmospheric air. The ileum strips were allowed to equilibrate for 60 minutes, meanwhile they were washed every 15 minutes 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 on a computer.

2.5. Experimental protocol

After the equilibration period (60 min), the tonus of ileum was elevated by addiction of 3μM bethanechol more 60 nM cloprostenol. 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 reduction of the bethanechol – more cloprostenol - induced tonus and converted to relaxation percentage. In order to compare the potencies of the esters in relation to spasmolytic actions, the concentration required to obtain half of the maximum response (EC_{50}) was obtained from the concentration-response curve of each ester by the method of nonlinear regression. Moreover, the maximum effects of the esters were obtained by the percentage of maximum reduction of the bethanechol- more cloprostenol-induced tonus.

2.6. Data presentation and statistical analysis

Data are presented as mean relaxation percentage (±SEM) of the bethanechol- more cloprostenol-induced tonus of guinea-pig ileum muscle strips prepared from six animals. The statistical analysis was performed using one way analysis of variance followed by Bonferroni's Test. A probability level of 0.05 was regarded as significant.

3. 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 comparative study the findings from the assessment of the spasmolytic activity of racemate and enantiomers of linalyl and citronellyl acetates using guinea-pig isolated ileum precontracted by 3μM bethanechol more 60 nM cloprostenol. Thus, acetylation of alcohols using a mixture of Ac_2O/py afforded the easily purified linalyl and citronellyl acetates, in about 46% yield. (+)-Linalyl acetate was obtained in 70% yield (w/w) after the acetylation of *Coriandrum sativum* essential oil, which contains (+)-linalool as a major component [5]. TLC analysis of the reaction products suggests that the esters were synthesized. From the spectral (IR, ^{13}C and ^{1}H NMR) characterization, chromatographic (TLC) behavior, specific rotation, and comparison with literature data [6-8], the isolated compounds were identified as (+)-linalyl acetate: $[\alpha]_D^{29}$ = +4.01 (*c* = 1.70 in $CHCl_3$), (-)-linalyl acetate: $[\alpha]_D^{29}$ = -4.12 (*c* = 1.70 in $CHCl_3$), (±)-linalyl acetate: $[\alpha]_D^{29}$ = 0 (*c* = 1.60 in $CHCl_3$), (+)-citronellyl acetate: $[\alpha]_D^{29}$ = +1.91 (*c* = 1.60 in $CHCl_3$), (-)-citronellyl acetate: $[\alpha]_D^{29}$ = -1.95 (*c* = 1.60 in $CHCl_3$), and (±)-citronellyl acetate: $[\alpha]_D^{29}$ = 0 (*c* = 1.60 in $CHCl_3$).

The spasmolytic activity of the chiral monoterpene esters (Figure 1) were then evaluated on smooth muscle (Table 1). In the comparison of racemate and enantiomers, the (+)-, (-)-, and (±)-linalyl acetates presented a relaxant effect equipotent. Therefore, linalyl acetate should be involved at spasmolytic activity of the *Thymus leptophyllus* essential oil. (+)- and (-)-Citronellyl acetates also were equipotent, however, the citronellyl racemate showed more potent spasmolytic effect. This indicates that the largest potency of the citronellyl racemate is determined possibly by synergistic action of its constituents. In general, linalyl acetates were slightly more effective than citronellyl acetates in relaxing guinea-pig isolated ileum. These experimental data suggest that monoterpene esters with acetate group attached to tertiary carbon have higher spasmolytic effect. Therefore, these results show that the position of the acetate group on the chemical structure of compounds tested influences the spasmolytic activity.

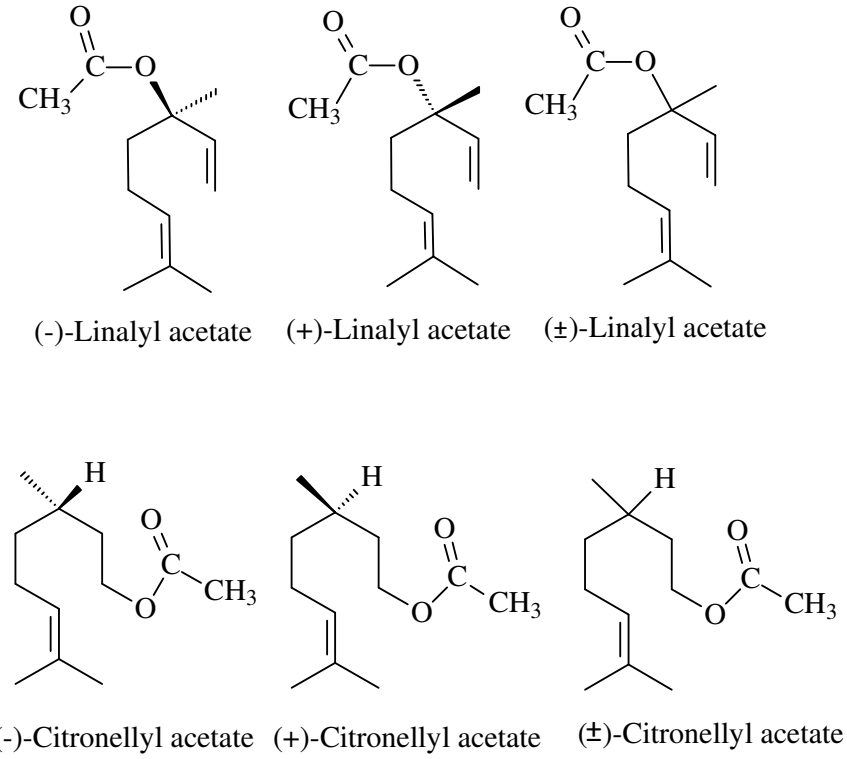

Figure 1. Chemical structures of the compounds used in this study.

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. This study shows that the chiral monoterpenes linalyl and citronellyl acetates present in bioactive essential oils should contribute in their spasmolytic activity. Several essential oils are reported to exhibit effect of relaxation on ileum smooth muscle [3,9-12]. Monoterpenes are the major components of these oils. The spasmolytic activity of some oxygenated monoterpenes present in these essential oils has been shown, such as α-pinene and β-pinene (hydrocarbons) [13], thymol (a phenol) and camphor (a ketone) [10]. Therefore, the effects observed are consistent with those reported for other compounds belonging to the same chemical class. Whereas the inhibition of contractile over-activity of the ileum is the basis of the treatment of some gasterointestinal disorders such as diarrhea, the monoterpene esters of this study and other structural analogues may have clinical benefits for the treatment of these conditions.

Table 1. EC_{50} and maximum effect values obtained of concentration-response curves in guinea pig isolated ileum.

Compound	EC_{50} (IC 95%)	Maximum effect (% of tonus reduction of BetOH)".
(-)-citronellyl acetate	3.3×10^{-4} ($2.6 \times 10^{-4} - 4.2 \times 10^{-4}$)*	100
(+)-citronellyl acetate	3.4×10^{-4} ($2.4 \times 10^{-4} - 4.7 \times 10^{-4}$)*	100
(±)-citronellyl acetate	6.6×10^{-5} ($5.3 \times 10^{-5} - 8.3 \times 10^{-5}$)	100
(-)-linalyl acetate	1.7×10^{-5} ($1.3 \times 10^{-5} - 2.4 \times 10^{-5}$)	100
(+)-linalyl acetate	2.3×10^{-5} ($1.9 \times 10^{-5} - 2.7 \times 10^{-5}$)	100
(±)-linalyl acetate	2.1×10^{-5} ($1.7 \times 10^{-5} - 2.6 \times 10^{-5}$)	100

* $p < 0{,}001$ in relation to (±)-citronellyl acetate

In the present study we demonstrated the spasmolytic activity of chiral monoterpene esters present in many essential oils and that the position of the functional group on the acyclic monoterpene structures and synergistic action influence the effect of relaxation on ileum smooth muscle.

Acknowledgements

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