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Effects of bioactive monoterpenic ketones on membrane organization. A langmuir film study

María Elisa Mariani, Mariela E. Sánchez-Borzone, Daniel A. García*

Instituto de Investigaciones Biológicas y Tecnológicas (IIByT-CONICET), Cátedra de Química Biológica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, Córdoba (5016), Argentina

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ABSTRACT

The cyclic ketones, thujone and dihydrocarvone, are lipophilic components of essential oils extracted from different plants, which have proven insecticidal activity. The $GABA_A$ receptor is activated by the neurotransmitter GABA and is the action site of widely used neurotoxic pesticides. Many compounds that regulate $GABA_A$ receptor function interact with membrane lipids, causing changes in their physical properties and consequently, in the membrane dynamic characteristics that modulate receptor macromolecules. In the present study, the biophysical effects of thujone (a gabaergic reference compound) and dihydrocarvone (structurally very similar) were explored by using monomolecular films of DPPC as a model membrane system, to gain insight into membrane-drug interaction. The compression isotherms showed that both ketones expand the DPPC isotherms and increase membrane elasticity. They penetrate the monolayer but their permanence depends on the possibility of establishing molecular interactions with the film component, favored by defects present in the membrane at the phase transition. Finally, by using Brewster angle microscopy (BAM) as a complementary technique for direct visualization of the study films, we found that incorporating ketone seems to reduce molecular repulsion among phospholipid headgroups. Our results reinforce the notion that changes in membrane mechanics may be occurring in the presence of the assayed ketones, suggesting that their interaction with the receptor's surrounding membrane may modulate or affect its functionality, possibly as part of the mechanism of the bioactivity described for thujone and DHC.

1. Introduction

Different essential oils and their components extracted from plants are known to possess insecticidal activities, among their other effects. The cyclic ketone, thujone, is an active ingredient of wormwood oil and some other herbal medicines and is reported to have antinociceptive, insecticidal, and anthelmintic activity (Höld et al., 2000). Dihydrocarvone (DHC) is present in oils extracted from the caraway plant and is used for its fragrance as flavoring and for medicinal purposes (Tripathi et al., 2003). Both naturally occurring ketones are highly lipophilic compounds, whose insecticidal activity was demonstrated in previous reports (Tripathi et al., 2003; Grainge, 1988).

$GABA_A$ is the major inhibitory receptor of the brain, and belongs to a superfamily of pentameric ligand-gated ion channels. It is operated by binding of GABA and is also recognized as a molecular target for many drugs (e.g. barbiturates, benzodiazepines, neuroactive steroids, anesthetics) and alcohol (Suzdak and Paul, 1987). In recent years, it has been shown that $GABA_A$ receptors are also targets for several insecticides and other toxicants (Eldefrawi and Eldefrawi, 1987), which act by recognition of the picrotoxinin or noncompetitive antagonist site to block $GABA_A$ (Chen et al., 2011). It is known that thujone is specifically a receptor antagonist and, by inhibiting GABA receptor activation, may make neurons fire more easily, causing muscle spasms and convulsions (Höld et al., 2000; Reiner et al., 2013a).

Although there is an increasing body of information, more study is required of the activities and molecular mechanisms responsible for the activity of some lipophilic compounds, which may be potential insecticides. Perturbation of physical membrane characteristics could be one of their modes of actions, as was suggested

* Corresponding author.
E-mail addresses: dagarcia@unc.edu.ar, dasmedgarcia@yahoo.com (D.A. García)

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for some antimicrobial agents (Lopes et al., 2009; Sirk et al., 2009) and anesthetics (Cantor, 1998).

In this work, thujone and DHC were selected due to their similarities at molecular structure level (Fig. 1). While thujone presents a bicyclic ring structure, DHC exhibits an aromatic hydrocarbon ring with a comparable substituent to thujone. The inclusion of thujone, as a reference compound acting on the $GABA_A$ receptor (Sanchez-Borzone et al., 2014), and DHC, which recently was found to act as a negative allosteric modulator of this receptor (unpublished results), allows us to gain insight into their effects on membranes, as part of the mechanism of action involved in receptor modulation. Such studies could be significant, taking into account that subtle structural differences may underlie the results observed in lipid interactions and changes of membrane properties.

The interaction between surface active compounds and phospholipids has been extensively studied in artificial model membrane systems, including liposomes and Langmuir monolayers (Dynarowicz-Latka et al., 2001; Peetla et al., 2009). Many crucial phenomena that take place in bilayers, as correspond to biological membranes, can be elucidated by using monolayers at the air-water interface (Feng, 1999), since plasma membranes are the first contact of lipophilic compounds with cells, and the drug-membrane interaction, being a potential regulatory point, is a fundamental condition for their function. Phospholipid monolayers constitute simple models to study intermolecular interactions (Demel, 1974; Bohm et al., 1993), given that the lipid interface can be easily modulated by changing the interfacial composition or lateral packing (Imbenotte and Verger, 1973; De Tullio et al., 2013; Daniele et al., 1996; Scott et al., 1990; Miyamoto and Kollman, 1992). It has been successfully employed to study the characteristics of membrane structure and the interaction between lipids and amphiphilic molecules, peptides, proteins (Brockman, 1999) and more recently with polysaccharides, such as chitosan (Krajewska, 2004). Furthermore, this technique makes it possible to study the effect of compounds on lipid and surrounding molecules, taking into consideration several physical changes (for example, changes in order parameters of monomolecular films, molecular areas, surface potential, surface tension, etc.) (Brockman, 1999; Nowotarska et al., 2014; Pathirana et al., 1998). Moreover, the partition of the receptor-containing membrane itself can cause changes in the physical environment of the receptor and it is known that the membrane fluidity in living organisms is highly regulated ((Søgaard et al., 2006; Hansen et al., 2013) and ref. therein).

The aim of the present work was to emphasize the differences in the membrane interactions of DHC and thujone using DPPC monolayers, which are widely used to perform membrane interaction studies of lipophilic compounds (Pathirana et al., 1998; Hansen et al., 2013; Amador Kane and Floyd, 2000; Reiner et al., 2013b). This approach allowed us to focus on the interaction between ketones and phospholipids, by recording π-area isotherms and calculating different physical parameters. Moreover, Brewster angle microscopy (BAM) of Langmuir monolayers was applied as a complementary technique for visualizing the effect of ketones on the bidimensional phase state of the films, since DPPC domains exhibit remarkable shapes.

2. Materials and methods

2.1. Materials

1,2-dipalmitoyl-sn-*glycero*-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL, USA). Thujone (1-isopropyl-4-methylbicyclo [3.1.0] hexan-3-one) and (+)-dihydrocarvone (2-methyl-5-prop-1-en-2-ylcyclohexan-1-one) (DHC) were purchased from Sigma Chem Co. (St. Louis, MO, USA). All other reagents were of the highest analytical grade. All ketones were prepared as 5 M stock solutions in pure DMSO, light-protected, and stored at 4 °C. Stock solutions were diluted before each experiment in DMSO and finally in phosphate buffer pH 7.4 (135 mM NaCl, 7.5 mM Na_2HPO_4 and 1.5 mM K_2HPO_4) maintaining a 0.25% (v/v) DMSO final concentration. Solutions were prepared with double-deionized water.

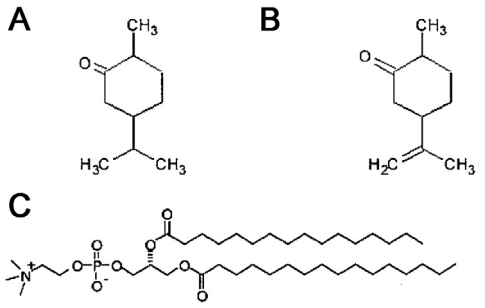

Fig. 1. Molecular structures of thujone (A), dihydrocarvone (B) and DPPC (C).

2.2. Lateral surface pressure (π) – mean molecular area (MMA) isotherms

π (mN/m) vs. MMA (Å^2/molecule) isotherms were performed by the compression of monolayers containing DPPC using a Minitrough II (KSV, Finland). Lipid monolayers on the air-water interface were prepared by spreading pure DPPC dissolved in chloroform/methanol (2:1) on the aqueous surface of a Teflon™ trough filled with phosphate buffer pH 7.4 as subphase. After 5 min solvent evaporation, the film was compressed isometrically at a constant rate of 4 ± 1 Å^2/min/molecule until reaching the target pressure. π was measured with a platinum plate by the Wilhelmy method (Verger and De Haas, 1973), at different MMA of the phospholipid, in the absence or presence of each ketone (20, 250 and 500 μM final concentration), mixed previously with the subphase. Control isotherms obtained in the presence of DMSO 0.25% (v/v) were not different from those at 0% DMSO (data not shown). The collapse point in monolayers (π_c) was characterized either by reaching a rapid decrease in the surface pressure or as a horizontal break in the isotherm. All assays were performed in duplicate at 25 ± 1 °C. Graphics were made by using Sigmaplot 12.5 (Systat Software Inc., USA).

2.3. Compressibility analysis of DPPC films

In order to analyze the elastic behavior of the film, the *compressibility modulus* (Cs^{-1}) was determined. The onset of phase transition points was identified from a minimum and π_c from a maximum in the variation of Cs^{-1} vs. MMA plot. Cs^{-1} values represent the reciprocal of the compressibility and were calculated directly from the slope of π-MMA isotherms applying Eq. (1):

$$Cs^{-1} = -(A_\pi)\,(d\pi/dA)_\pi$$

where A_π is the MMA at the indicated surface pressure (Kodama et al., 2004). All calculations were made by using the program PeakFit v4.12 (Systat Software Inc., USA). The maximal error of this parameter did not exceed 1%.

2.4. Penetration of ketones into the monolayer

To study the penetration of the ketones into DPPC lipid monolayers, the experiments were performed in a homemade

circular Teflon trough (15 mL of volume) containing phosphate buffer pH 7.4 as subphase. The determinations were made under continuous stirring (150–250 rpm) at different initial π (π_i), to measure the increment in π induced by the compound's penetration into the DPPC monolayer ($\Delta\pi$) as a function of time, at constant surface area. The phospholipid monolayer composed by pure DPPC was formed by spreading a chloroform/methanol solution of the lipid on the air-water interface, prior to ketone injection (100 μM final concentration) into the subphase. After achieving stabilization of the different target π (π_i) of study (between 5 and 10 min), the changes in π were recorded as a function of time until the equilibrium surface pressure was reached (changes in pressure less than 1 mN/m per hour). Finally, plots of $\Delta\pi$ vs. π_i were obtained to determine the "π_{cutoff}" value for both ketones by extrapolating the maximum π at which each drug would be able to penetrate. All experiments were performed in duplicate at 25 ± 1 °C.

To determine the extent of membrane pressure "alteration" produced immediately by the presence of ketones, the difference between the peak surface pressure observed, when the ketone is added to the subphase, and the surface pressure of stabilization was calculated (see Supplementary material: Table S1 and Fig.S1).

2.5. BAM imaging

Langmuir monolayers were prepared as described above. The Langmuir equipment was mounted on the stage of a Nanofilm EP3 imaging Ellipsometer (Accurion, Göttingen, Germany), which was used in the Brewster Angle Microscopy (BAM) mode. Minimum reflection was set with a polarized 532 λ laser incident on the bare aqueous surface at the experimentally calibrated Brewster angle (~53.1°). After DPPC monolayer formation, and during compression, the reflected light was collected with a 20x objective and an analyzer-polarizer lens connected to a CCD camera. The gray level at each pixel of the BAM images can be converted to reflectivity values after calibration factors are set for each individual experiment. For 2D isotropic films, the reflectivity obtained from BAM measurements is directly related to the square of the film thickness and to the refractive index of the film, and is polarized in the same direction as the incident beam (Lheveder et al., 2000; Fanani and Maggio, 2011). For a better visualization, the lower 0-100 gray level range (from the 0–255 original scale) was selected using the free software ImageJ 1.43 μ (Wayne Rasband-NIH, USA) to maintain the ratio of gray level to film thickness. The spatial resolution of the BAM was 50 μm. The subphase was the same as

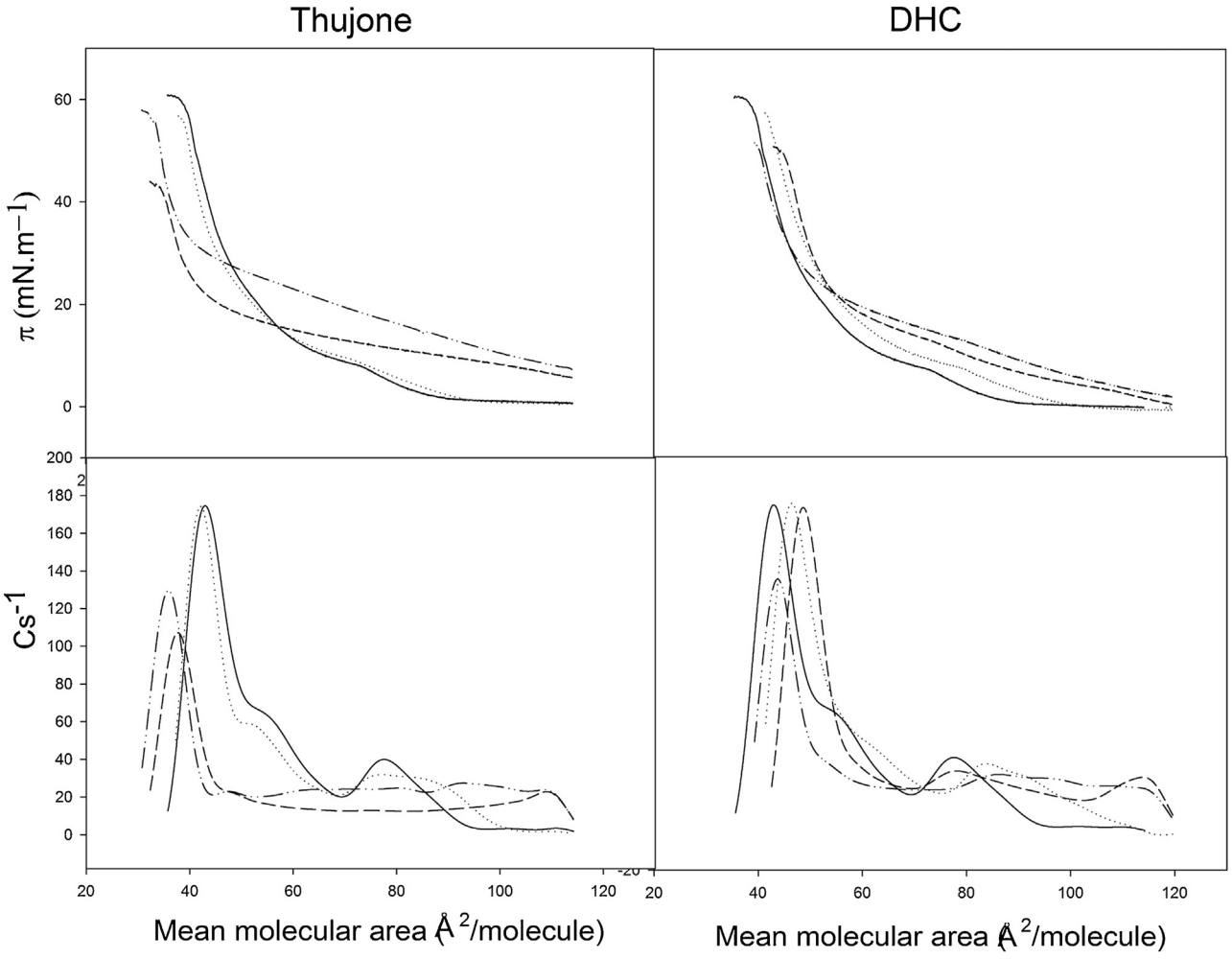

Fig. 2. Effects of ketones on DPPC isotherms. Surface pressure-area (upper panels) and Compressibility modulus-area (lower panels) isotherms of DPPC on buffered subphase in the absence (solid lines) or the presence of 20 μM (dotted lines), 250 μM (medium dash) and 500 μM (dash-dot-dot) of thujone (left panels) and DHC (right panels), respectively.

mentioned above and the ketones concentration assayed was 100 μM.

3. Results and discussion

3.1. Compression isotherms of thujone and DHC

π-MMA isotherms registered for DPPC monolayers obtained in the absence or presence of 20, 250 and 500 μM of DHC or thujone in the subphase are presented in Fig. 2 (upper panels). As expected, the DPPC monolayer showed the characteristic LC phase at low areas/lipid and the LE phase at larger areas/lipid, molecularly differentiated by a tight hexagonal packing or a more disordered packing of the hydrocarbon chains, respectively (Fig. 2 upper panels, straight line). The presence of ketones causes significant variations in the shape and position of the isotherms, demonstrating their ability to modify the interfacial characteristics of DPPC.

In order to emphasize the differences observed in the DPPC lipid monolayers in the absence or presence of ketone molecules in the subphase, three parameters: MMA occupied by a molecule in monolayers, compressibility modulus values at a selected π = 30 mN/m, and collapse pressure, are gathered in Table 1.

The area expansion of both phases (LE and LC) produced by the presence of DHC (Fig. 2 upper-right panel) is clearly due to the incorporation of DHC into the DPPC monolayer favored by less-packed structures in a concentration-dependent manner, showing slightly larger values of apparent MMA (Table 1) determined at π = 30 (within the LC phase), with respect to the MMA of pure DPPC monolayer, which correlates with the expanding effect observed. This "expanding effect" (Phillips et al., 1970) was recently described for DPPC monolayers spread over a subphase containing (−) carvone (Pathirana et al., 1998).

In contrast, the presence of thujone in the subphase produced expansion only of the LE phase and the compression isotherm was lifted off at lower MMA (Fig. 2 upper-left panel). This apparent "condensing effect" behavior (Gershfeld and Pagano, 1972) of the LC phase was previously described for (+) carvone in DPPC monolayers (Pathirana et al., 1998) and dipalmitoyl-lecithin/cholesterol mixed monolayers (Gershfeld and Pagano, 1972). The apparent MMA showed itself to be slightly smaller than the area of the pure DPPC monolayer (Table 1) which is in accordance with its condensing effect at the LC phase, suggesting an increased packing density of DPPC molecules in the presence of thujone.

Surface pressure isotherms of DPPC/thujone showed a liquid-expanded monolayer behavior with lower slopes than those observed for DPPC, indicating that the phase transition from LE into LC state occurred at a reduced rate. In contrast, surface pressure isotherms of DPPC/DHC showed no significant changes in slope and rate.

The PC groups exhibit considerable flexibility. They are able to accommodate both untilted and tilted crystalline hydrocarbon chains and to adopt different arrangements such as a space-saving saw-tooth-like (39–42 Å^2) or a spacious surface-aligned (48–54 Å^2) packing, which in presence of water can expand even further (60–70 Å^2) (Hauser et al., 1981). When a solution of an amphiphilic compound is dissolved in the subphase aqueous solution of a phospholipid monolayer, the adsorption of the compound at the air/water interface implies its insertion between the phospholipid molecules. With that in mind, the presence of DHC molecules may increase the distance between DPPC molecules, causing an expansion of the apparent MMA where the two-dimensional headgroup contact cannot exist. This behavior could be due only to the presence of guest molecules that act as spacers and form lateral bridges between host phospholipid molecules at the polar headgroup level, as was described for other compounds (Hauser et al., 1981; Castro et al., 2013). In contrast, thujone seems to be sterically accommodated, allowing the DPPC molecules to be closer to each other. The decrease in the apparent MMA suggests that phospholipids require a more space-saving arrangement in which the headgroups are inclined and/or alternately displaced ("inclined" model). Moreover, compression of the monolayer can lead to a loss of surface lipid molecules by vertical displacement into the multilamellar phase ("squeezed-out" model) (Hauser et al., 1981), which in turn is responsible for the decrease in the apparent MMA. Nevertheless, our study was unable to discriminate between an "inclined" or "squeezed-out" space-filling model.

The value of the MMA at the collapse pressure (MMA_c) (≈60 mN/m) found in the present work for DPPC monolayers (38 Å^2/molecule-Table 1) is in agreement with other reports and corresponds to the area required for the PC group in a space-saving saw-tooth-like arrangement (39–42 Å^2) (Hauser et al., 1981). However, the collapse pressure drops to lower values in the presence of ketones at any concentration assayed (Table 1 and Fig. 2). Despite the results mentioned above, these modifications were not simply correlated with ketone concentrations.

Although the π_c was almost unaffected up to 20 μM for both ketones, higher concentrations induced a decrease of π_c. In Fig. 2 (upper-right panel), the collapse pressure for DHC occurs at lower pressures for concentrations of 250 μM and 500 μM, determining the instability of the monolayer. The same observation was made for thujone at a concentration of 250 μM (Table 1). In conclusion, the maximum π of the compressibility curves was displaced to smaller molecular areas as the ketone concentration increased.

Another important change was observed in the phase transition of π-A isotherms where, in the presence of ketones, it becomes increasingly less marked compared with DPPC until it disappears (Fig. 2, upper panels). In turn, in compressibility modulus plots, it is evident that the phase transition region begins at a higher π for

Table 1
Molecular and rheological parameter of ketones from the π-A isotherms.

COMPOUNDS Concentration (μM)	collapse$_{(mN/m)}$ pressure	$MMA_{C(Å^2/molecule)}$	$Cs^{-1}{}_{(mN/m)}$	Phase state	$MMA_{(Å^2/molecule)}$
DIHYDROCARVONE					
0 (pure DPPC)	60.89	37.63	115.04	LC	47.01
20	57.89	41.44	119.43	LC	50.23
250	51.87	41.85	147.80	LC	50.71
500	51.87	39.75	84.69	LE	47.28
THUJONE					
0 (pure DPPC)	60.89	37.63	115.04	LC	47.01
20	58.30	34.15	113.48	LC	38.72
250	44.12	31.90	106.28	LC	32.52
500	58.66	29.29	22.52	LE	36.91

All data were taken from curves shown in Fig. 2. The values corresponding to Cs^{-1}, phase state and MMA were determined at a surface pressure of 30 mN/m. MMA_c: mean molecular area at the collapse surface pressure.

both ketones, shifting to larger areas for DHC and almost disappearing for thujone (Fig. 2 lower panels) in a concentration-dependent way. Since the sharpness of the phase transition depends on the number of molecules forced to cooperate in it (Aloia and Boggs, 1985), it may be that, in the presence of ketones, cooperativity is reduced.

Taking into account all the results, it is possible that the ketone molecules are inserted between DPPC molecules, thus diminishing the monolayer stability.

3.2. The effect of rheological properties on ketones penetrating monolayers

The DPPC bilayer system has been used as a model of bio-membranes in various studies, because its phase transition temperature (41.5 °C) is close to physiological temperature (Yin et al., 2014). It is important to consider that the phase behavior of DPPC monolayers gives considerable biophysical information since it displays continuous subtle changes between phases, with the richness of this behavior being indicative of frustration of the monolayer caused in part by differences in the cross-sectional area of the lipid headgroup and lipid tails, which induces monolayer deformation (Duncan and Larson, 2008).

The rheological properties of the Langmuir films were studied by determining the *compressibility modulus* (Cs^{-1}), the value of which is inversely related to the film elasticity. This parameter is an important element to assess the state of phase monolayers, which is expected to be low ($\leq$100 mN/m) for fluid monolayers (LE phases) and higher for condensed films (LC Phases) (Gaines, 1966). In LC phases, where the molecules are accommodated with the greatest possible packing, a high modulus means that the film responds to compression with a large increase in pressure. Conversely, in LE phases, where the molecules that form the film have lower molecular interaction and greater fluidity, low compressibility is determined as progressive changes occur in surface pressure as the MMA decreases (Gaines, 1966).

The plots of compressibility modulus (Cs^{-1}) in function of MMA shown in Fig. 2 (lower panels) enabled the discussion about the alterations of degree of monolayer condensation during the compression. The results show that the presence of ketones in the subphase, mainly at the higher concentrations assayed, leads to the modification of monolayer condensation that was especially observable for surface pressures above the phase transition, corresponding to the LC phase of the membrane. In the presence of thujone, the values of Cs^{-1} were almost similar or smaller than that observed for DPPC (Table 1), while DHC induced higher values of Cs^{-1} for 20 and 250 μM and a lower value for 500 μM. Experimental values of the Cs^{-1} obtained for DPPC monolayers are known to be of the same magnitude as the surface pressure for LE films, up to 250 mN/m for LC films and above 250 mN/m for solid films (Kodama et al., 2004; Vitovic et al., 2006). The compressibility plots clearly showed that the DPPC phase transition between LE and LC states, in the presence of any ketone, disappears or moves toward larger MMA. This decrease in Cs^{-1} indicated a softening effect of the ketones or an increase in the elasticity of the DPPC monolayer.

3.3. Adsorption and penetration of ketones into lipid monolayers

DHC and thujone are lipophilic molecules (Fig. 1) showing a theoretically estimated partition coefficient (Log *P*) of 2.63 and 2.47, respectively (calculated by using ChemSketch, version 14.01, –www.acdlabs.com-). The hydrophobicity of these compounds is an important characteristic, since it enables the interaction with lipid membranes.

In the first trials, the assays reveal that both compounds, DHC and thujone, were not able to form stable monolayers in the air-water interface (results not shown). When a solution of each ketone was injected into the subphase of a pre-formed lipid film, the further increase in the lateral pressure ($\Delta\pi$) of the film (Fig. 3) was interpreted in terms of penetration of the lipophilic molecule into the monomolecular film (Thompson, 2002; Winget et al., 2006). This change in surface pressure shows a rapid penetration/adsorption of both ketone molecules into the membrane, since around 90% of the extent of the interaction is achieved in 2–3 min (see Supplementary material: Fig. S1, π vs. time). The $\Delta\pi$ values were very similar (Fig. 3) for both ketones, matching their comparable hydrophobicity.

The values of $\Delta\pi$ observed after injection of the compounds at π_i corresponding to pure LE or LC phases, showed that neither compound produced a significant change in the membrane physical properties, but a noticeably large initial peak could be observed before the π stabilization ($\pi_{eq.}$), suggesting that a structural alteration is taking place. This alteration before the final stabilization could be due to a rapid penetration followed by a poor permanence of compounds in the membrane (Fig.S1 and Table S1).

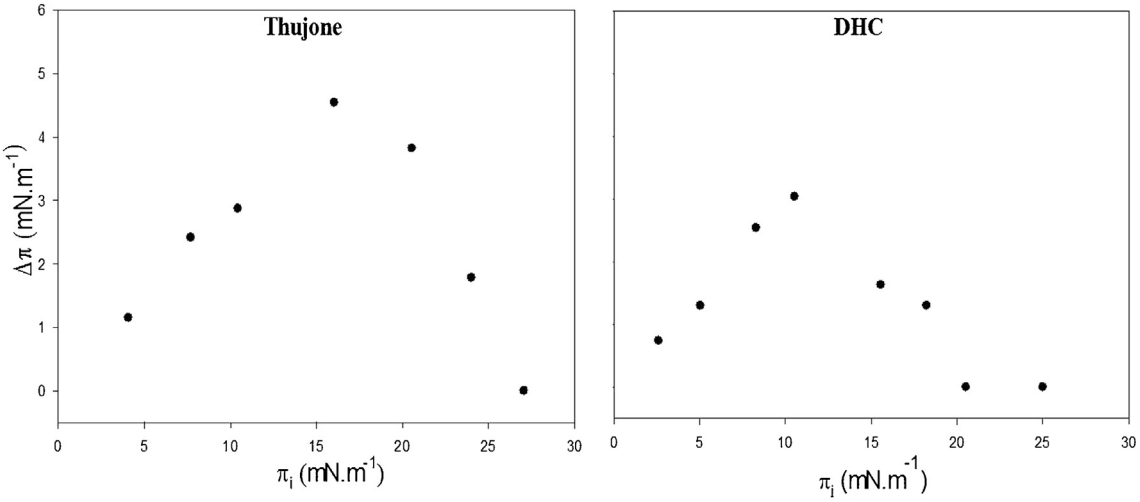

Fig. 3. Analysis of ketone penetration on DPPC monolayers. Points represent the $\Delta\pi$ versus π_i profiles of ketones calculated as indicated in Materials and Methods. The error value was estimated to be ±1 approximately.

Nevertheless, the $\Delta\pi$ values obtained at π_i around the DPPC phase transition, where the main defects of the membrane are present, showed a higher extent. This may suggest that these membrane defects facilitate the "contact" between DPPC and ketones. Thus, the interaction with membranes could be summarized as greater ketone molecule penetration at different monolayer stages with an elevated compound permanence in the proximity of the transition phase, where the monolayer structure would favor the stable insertion of ketones.

As observed in the $\Delta\pi$ vs. π_i plots in Fig. 3, the *cut off* values obtained (27 mN/m for thujone and 22 mN/m for DHC), indicate that both compounds have almost the same ability to penetrate the film. Considering these *cut off* values, penetration in natural membranes, whose average lateral pressure is about 30–35 mN/m (Demel et al., 1975; Marsh, 1996; Sanchez et al., 2004), could not be extensively expected. However, taking into account that the lateral pressure values accepted for natural membranes are an approximation of a complex and dynamic system, in which different domains present local pressures (Marsh, 1996; Feigenson, 2007; Samuli Ollila et al., 2007), it is very likely that both compounds may be integrated into biomembranes. This is confirmed by the bell-shaped plots observed in Fig. 3, in which the compound's incorporation is favored by the presence of membrane defects, as was explained above.

3.4. BAM analysis

The BAM images of pure DPPC monolayer (Fig. 4, upper panels) show the formation of LC domains at the onset of the phase transition during monolayer compression, with the characteristic structures curving in a counterclockwise direction as expected for pure L-DPPC (Reiner et al., 2013b; Kruger and Losche, 2000; Martin et al., 2011). In the presence of the compounds studied, the LC domains were usually smaller and without a clear curving direction compared to those observed in the control monolayer, with the strongest effect seen in DHC (Fig. 4, middle and lower panels).

Domain shape is valuable from physicochemical and biological perspectives. The different shapes are related to the structure of the molecules that constitute the domain and their packing and orientation within it. Microscopic studies of LC domains of DPPC determined that their handedness is directly related to the enantiomer configuration (Nandi and Vollhardt, 2007). For phospholipids, the impact of chirality at the microscopic level is determined by the headgroup size in relation to the cross-section of the aliphatic chains. Phosphatidic acids (PAs) and phosphatidylethanolamines (PEs), with small headgroup sizes, form circular domains (Kruger and Losche, 2000; Helm et al., 1987), while DPPC, with a larger headgroup, shows a chiral structure in the domain shape. The elimination of chiral shapes in the LC domains, induced by the presence of both ketones, indicates their location between phospholipid molecules, probably in the headgroup region, changing the molecular orientation within the domain, as was previously reported for propofol-derived compounds (Reiner et al., 2013b).

The size and shape of domains in phospholipid monolayers have been generally described as controlled by several forces, such as electrostatic repulsion between excess dipole moments within the condensed phase (Kruger and Losche, 2000). The contribution of in-plane (parallel to the interface) and out-of-plane (perpendicular to the interface) dipole moments of the phospholipid molecule is decisive in the domain shape (Nandi and Vollhardt, 2003; Thirumoorthy et al., 2007). The in-plane dipole moment in DPPC is relatively high, mainly due to its larger molecular tilt in the LC domains (Thirumoorthy et al., 2007). This larger dipolar repulsion is the main force that enables DPPC domains to develop

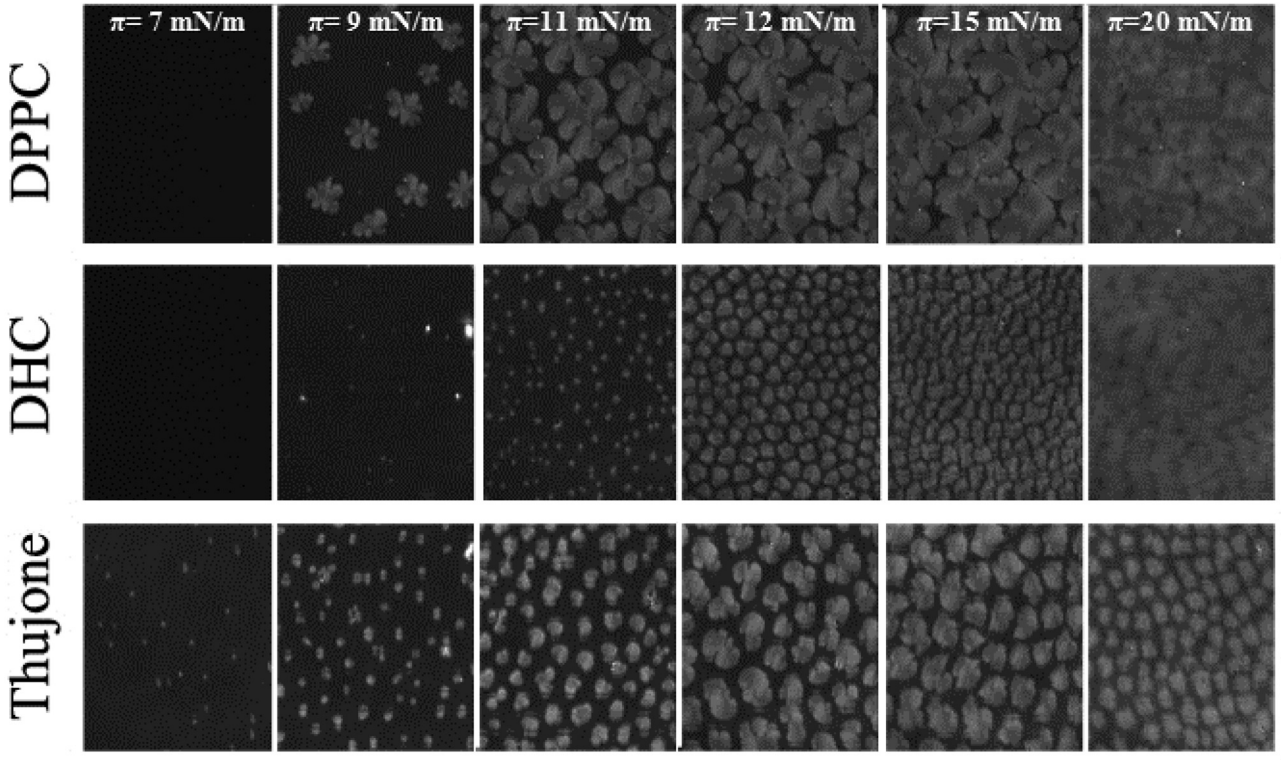

Fig. 4. Brewster angle microscopy visualization of DPPC monolayers in the absence or the presence of thujone or DHC in the subphase. Monolayers were compressed at a rate of 4 ± 1 Å^2/min/molecule at pH7.4. BAM images were recorded at different surface pressures as indicated in the figure. Representative images were taken from three independent experiments. The mixed monolayers proved to be stable enough (no large desorption of molecules, which would produce a loss of molecular area, was detected) to be manipulated under the BAM equipment for a lapse of approximately 30 min.

elongated arms. However, the presence of thujone or DHC between DPPC molecules would reduce this dipolar repulsion in the tilt direction, allowing the domain to grow in different directions. This behavior was previously also reported for phenolic compounds (with comparable dipole moment values ≈ 2 D) and for local anesthetics, whose interaction with DPPC monolayers could be explained as a molecular intercalation between DPPC molecules, reducing the molecular repulsion among phospholipid headgroups (Amador Kane and Floyd, 2000; Reiner et al., 2013b).

A direct interpretation of a probably more "ordered" state, imposed by the presence of ketones in the lipid interface, could be made, but remains to be elucidated. However, our results strongly support the hypothesis that the location of the ketone molecules reducing the repulsive forces among phospholipids headgroups allows a closer molecular packing, diminishing the mobility of the hydrocarbon chains.

4. Conclusions

We demonstrate that DHC and thujone are lipophilic compounds that can penetrate DPPC lipid monolayers, where they remain incorporated into the membrane in an organized interface, producing alterations of the lateral organization in the conditions assayed.

Compression isotherms show that increasing amounts of ketones are able to modulate the liquid expanded to liquid-condensed DPPC phase transition, which is also reflected in changes to the isothermal elasticity.

Changes in domain shape visualized in the presence of ketones reveal that their incorporation would reduce the molecular repulsion among phospholipid headgroups.

All together, these results reinforce the notion that changes in membrane mechanics could be occurring in the presence of the assayed ketones, suggesting that their interaction with the receptor's surrounding membrane may modulate or affect its functionality, possibly as part of the mechanism of action of the bioactivity described for thujone and DHC.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemphyslip.2016.05.002.

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