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The interaction of abietic acid with phospholipid membranes

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Abstract

Abietic acid is a major component of the oleoresin synthesized by many conifers and constitutes a major class of environmental toxic compounds with potential health hazard to animal, including human, and plant life. Being an amphipathic molecule, the study of the influence of abietic acid on the structure of membranes would be important to get insight into the mechanism of toxic action of the molecule. The interaction of abietic acid with model membranes of dipalmitoylphosphatidylcholine (DPPC) and dielaidoylphosphatidylethanolamine (DEPE) has been studied by differential scanning calorimetry and ^{31}P-nuclear magnetic resonance spectroscopy. It has been found that abietic acid greatly affects the phase transition of DPPC, shifting the transition temperature to lower values, giving rise to the appearance of two peaks in the thermogram and to the presence of fluid immiscible phases. In a similar way, the phase transition of DEPE, in the presence of abietic acid, was shifted to lower temperatures, and two peaks appeared in the thermograms. The temperature of the lamellar to hexagonal H_{II} phase transition was also decreased by the presence of abietic acid, but phase immiscibilities were not detected. The possible implications of these effects on the action of abietic acid on biological membranes are discussed.

Keywords: Abietic acid; Model membrane; DSC; NMR, ^{31}P-; Lipid polymorphism

1. Introduction

The amount and variety of aromatic hydrocarbons and their derivatives used in industrial and commercial activities has been increasing over the years. The release of these compounds into the environment is of a great concern because of their potential health hazard to animal and plant life. Abietic acid (Scheme 1) is a major component of the rosin fraction of oleoresin synthesized by grand fir (*Abies grandis*), lodgepole pine (*Pinus contorta*), and many other conifer species [1]. The production of oleoresins by conifer species is an important component of the defense response against insect attack and fungal pathogen infection [2]. Resin acids are a major class of toxicants discharged in the effluent of pulp and paper mills to aquatic environments [3] and they have been shown to be lethal to fish affecting respiration and energy metabolism [4] and causing liver dysfunction [5]. It is known that the dehydro derivative of abietic acid is toxic to the rat, impairing co-ordination

Abbreviations: DEPE, dielaidoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; ^{31}P-NMR, ^{31}P-nuclear magnetic resonance; T_c, main gel to liquid-crystalline phase transition temperature

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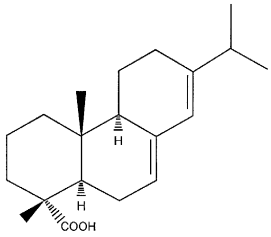

Scheme 1. Structure of abietic acid (13-isopropylpodocarpa-7,13-dien-15-oic acid).

and producing paralysis [6]. It has been suggested that abietic acid might be the specific etiologic agent in the development of acute and chronic lung disease in workers exposed to resin derived from pine wood [7].

The mechanism whereby abietic acid causes the toxic effects is still unknown. Since many amphipathic compounds affect the function and integrity of cell membranes, it seems possible that resin acids, also amphipathic compounds, might act in a similar manner. In this respect, it has been shown that the dehydro derivative of abietic acid affects the physical state of cytoskeletal proteins and the lipid bilayer of erythrocyte membranes [8]. Abietic acid and derivatives have been reported to act as non-specific inhibitors of platelet aggregation [9], they affect the permeability of synaptosomal membranes [10] and have a direct lytic activity to alveolar epithelial cells producing profound lung epithelial injury [11]. The above facts, together with the marked amphipathicity of resin acids, points to biological membranes as one of their target sites of toxic action. From this point of view, the knowledge of the interaction between abietic acid and membrane lipids may help to get insight into the mechanism of action of the amphipathic toxin.

The purpose of this study was to investigate in detail the interaction of abietic acid with membranes using lipid vesicles as model systems. Membrane model systems are widely used to study the mechanism of interaction of membrane active molecules with lipids [12–15]. It is known that dispersion of individual or mixtures of phospholipids of biological origin or synthetic ones can adopt several non-bilayer structures, in addition to the familiar bilayer organization. The ability of certain lipids to adopt these different structures is known as 'lipid polymorphism'. These non-bilayer structures include the micellar phase, the hexagonal H_{II} phase and lipidic particles [16], which can greatly affect the functional behavior of the membrane [17], being intermediates in vesicle fusion and involved in lipid flip-flop. Non-bilayer structures may also modulate the energetics and kinetics of transport across membranes [18]. There is also strong evidence that the function of membrane proteins can be affected by the modification of the properties of the lipid matrix in which they are embedded [19].

Since lipid polymorphism has such potential biological importance, in this investigation we have checked whether abietic acid might modulate it. We have studied the interaction between abietic acid and phosphatidylethanolamine because this phospholipid, being a major component of eukaryotic membranes, spontaneously adopts hexagonal H_{II} phases in the presence of excess aqueous buffer at physiological temperatures. At the same time, we have investigated the interaction of abietic acid with phosphatidylcholine, the most important class of membrane phospholipids. We have used differential scanning calorimetry (DSC) and ^{31}P-nuclear magnetic resonance (^{31}P-NMR) to assess the influence of abietic acid on the phase behavior of the phospholipids. The modulation of DPPC thermotropic properties and DEPE polymorphic behavior by abietic acid is discussed in the light of its possible mechanism of toxic action.

2. Materials and methods

2.1. Materials

Dipalmitoylphosphatidylcholine and dielaidoylphosphatidylethanolamine were obtained from Avanti Polar Lipids (Birmingham, AL, USA). Abietic acid was obtained from Aldrich Chemie (Steinheim, Germany). Organic solvents were obtained from Merck (Darmstadt, Germany). Deionized and twice distilled water was used.

2.2. Differential scanning calorimetry

The lipid mixtures for calorimetry measurements were prepared by combination of chloroform/

methanol (1:1) solutions containing 4 μmol of the phospholipid and the appropriate amount of abietic acid as indicated. The organic solvents were evaporated under a stream of dry N_2, free of O_2, and the last traces of solvents were removed by a further 3 h evaporation under high vacuum. After the addition of 1 ml of 0.1 mM EDTA, 100 mM NaCl, 10 mM Hepes (pH 7.4) buffer, multilamellar liposomes were formed by mixing, using a bench-vibrator, always keeping the samples at a temperature above the lamellar gel to lamellar liquid-crystalline phase transition temperature of the lipid. The suspensions were centrifuged at 13 000 rpm in a bench microfuge and the pellets were collected and placed into small aluminum pans. Pans were sealed and scanned in a Perkin–Elmer DSC-4 calorimeter, using a reference pan containing buffer. The heating rate was 4 C°/min in all the experiments. The DSC instrument was set at a sensitivity of 1 mcal/s full scale. For the determination of the total phospholipid contained in a pan, this was carefully opened, the lipid was dissolved with chloroform/methanol (1:1) and the phosphorus content were determined using the method of Bötcher et al. [20]. The instrument was calibrated using indium as standard.

The points used to construct the boundary lines in the phase diagrams where obtained from the beginning and the end of the transition peaks in the heating thermograms.

2.3. ^{31}P-Nuclear magnetic resonance

The samples for ^{31}P-NMR were prepared by combination of chloroform/methanol (1:1) solutions containing 50 mg of phospholipid and the appropriate amount of abietic acid. Evaporation of the solvents and multilamellar vesicles were formed as described above. The suspensions were centrifuged at 13 000 rpm in a bench microfuge and pellets were placed into conventional 5 mm NMR tubes and ^{31}P-NMR spectra were obtained in the Fourier Transform mode in a Varian Unity 300 spectrometer. All chemical shift values are quoted in parts per million (ppm) with reference to pure lysophosphatidylcholine micelles (0 ppm), positive values referring to low-field shifts. All spectra were obtained in the presence of a gated-broad band decoupling (10 W input power during acquisition time) and accumulated free induction decays were obtained from up to 1600 scans. A spectral width of 25 000 Hz, a memory of 32 K data points, a 2 s interpulse time and a 80° radio frequency pulse were used. Prior to Fourier transformation, an exponential multiplication was applied resulting in a 100 Hz line broadening.

3. Results

The DSC profiles for pure DPPC and mixtures of DPPC with abietic acid are shown in Fig. 1. The pure phospholipid showed its main lamellar gel to lamellar liquid-crystalline phase transition temperature (T_c) at 40.3 $\pm$ 0.2°C, in agreement with previous reports [21]. The thermotropic pretransition of DPPC was greatly affected by the presence of a very low concentration of abietic acid, being already abolished at an abietic acid mol fraction of 0.03. Increasing the concentration of abietic acid in the membrane produced the broadening and shifting of the transition peak to lower temperatures. At an abietic acid mol fraction of

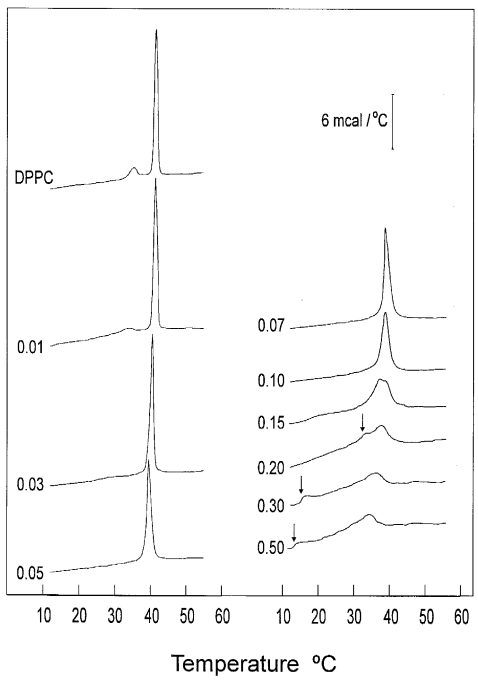

Fig. 1. DSC thermograms for mixtures of DPPC/abietic acid. The concentration of abietic acid in the membrane (mol fraction) is expressed on the curves. The profiles correspond to heating scans. Arrows indicate the peak chosen to determine the beginning of the transition.

Table 1
The enthalpy changes (ΔH, kcal/mol) for the lamellar gel to lamellar liquid-crystalline phase transition of mixtures of DPPC/abietic acid and DEPE/abietic acid at different abietic acid mol fractions (values are means ± standard deviation of four different experiments)

Abietic acid mol fraction	ΔH (kcal/mol)	
	DPPC	DEPE
0	8.5 ± 0.6	8.3 ± 0.3
0.01	7.8 ± 0.9	8.0 ± 0.4
0.03	8.5 ± 0.5	6.4 ± 0.6
0.05	7.9 ± 0.2	6.7 ± 0.6
0.07	7.6 ± 0.6	6.5 ± 0.1
0.10	7.4 ± 0.1	5.8 ± 0.5
0.15	6.3 ± 0.8	6.2 ± 0.6
0.20	7.0 ± 0.2	5.8 ± 0.2
0.30	8.9 ± 0.9	6.5 ± 1.0
0.50	7.6 ± 0.6	6.7 ± 0.3

0.15 two different peaks were present in the thermogram. The incorporation of higher concentrations of abietic acid produced the shifting of both peaks to lower temperatures, this effect being greater on the low temperature transition peak than on the high temperature one. As shown in Table 1, the presence of abietic acid did not produce a drastic decrease of ΔH of the lamellar gel to lamellar liquid-crystalline phase transition of DPPC. A direct relationship between ΔH and the abietic acid concentration was not observed.

In order to check whether abietic acid affects the phase behavior of DPPC, ^{31}P-NMR measurements at different temperatures were carried out. As shown in Fig. 2, the presence of abietic acid did not change the phospholipid phase organization, which remained in the lamellar phase over the whole range of temperatures under study. These data indicated that the different endotherms presented in the various thermograms corresponded solely to lamellar gel to lamellar liquid-crystalline phase transitions. However, the spectra obtained at high concentrations of abietic acid showed a reduced chemical shift anisotropy (Fig. 2D), suggesting that the presence of the toxicant increased the mobility of the DPPC molecules.

From the beginning and the end temperatures of the heating thermograms we constructed the partial phase diagram for DPPC/abietic acid system (shown in Fig. 3). This system is a mixture of a phospholipid (DPPC) and a molecule with a very different structure (abietic acid) which does not undergo any phase transition in the range of temperature under study, and therefore, all the observed thermotropic transitions arose from the phospholipid component in the

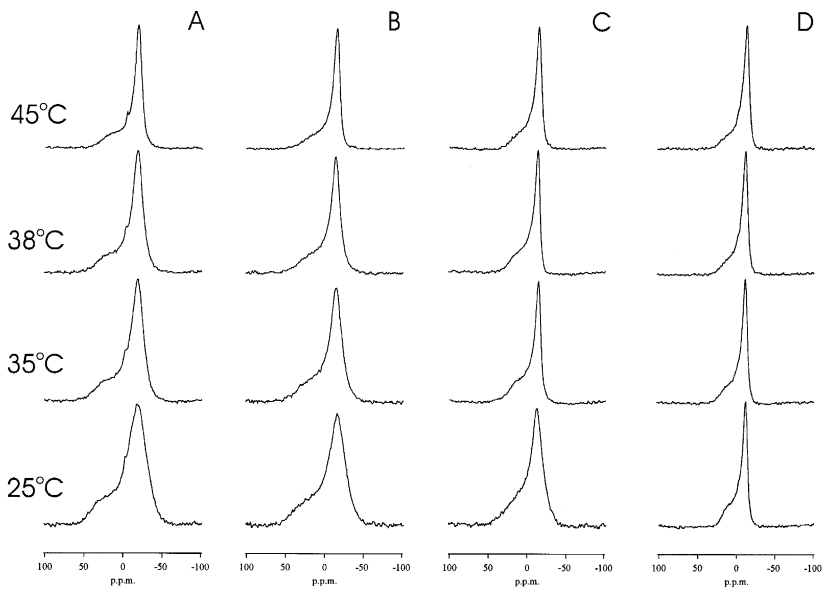

Fig. 2. ^{31}P-NMR spectra at different temperatures corresponding to pure DPPC (A); DPPC/abietic acid, 0.05 mol fraction (B); DPPC/abietic acid, 0.10 mol fraction (C) and DPPC/abietic acid, 0.30 mol fraction (D).

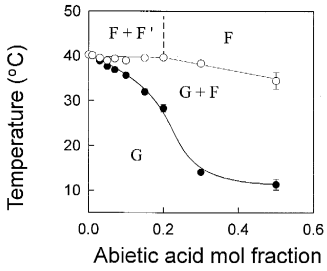

Fig. 3. Partial phase diagram for DPPC in mixtures of DPPC/abietic acid. Black (●) and open circles (○) were obtained from the begining and the end temperatures of the transitions, respectively (see text for details). G indicates a lamellar gel phase, F and F′ indicate different lamellar liquid-crystalline phases. Points represent the average of four experiments (error bars are shown when larger than the symbols).

mixture. Because of that, this phase diagram corresponds to a partial phase diagram for the DPPC component in mixtures of the phospholipid and abietic acid. To simplify the partial phase diagram, we did not consider the pretransition of DPPC. The thermotropic analysis of the pretransition is difficult as it is abolished at very low abietic acid content (see Fig. 1). The beginning and the end temperatures of the transitions gave us the points to obtain the solid and fluid lines, respectively. The temperature of the solid line decreased upon increasing the concentration of abietic acid. The fluid line kept horizontal, i.e., constant temperature, up to 0.2 mol fraction of abietic acid, whereas higher concentrations of abietic acid produced a decline of the fluid line, i.e., a decrease of temperature. The phospholipid component evolved from a lamellar gel phase (G phase) to a lamellar liquid-crystalline phase (F phase) through a broad region of phase coexistence (G + F), but in a concentration range of abietic acid from 0 to 0.2 mol fraction, a fluid phase immiscibility was observed (F + F′).

The effect of abietic acid on the thermotropic phase transitions of DEPE is shown in Fig. 4. Aqueous dispersion of DEPE can undergo a gel to liquid-crystalline phase transition in the lamellar phase and in addition a lamellar to hexagonal H_{II} structural phase transition [22]. This is shown in the thermogram corresponding to DEPE dispersed in buffer (Fig. 4, upper part). The lamellar gel to lamellar liquid-crystalline phase transition occurs around 36°C and the lamellar to hexagonal H_{II} structural phase transition occurs around 64°C in agreement with previous data [22]. The latter has a much smaller transition enthalpy due to the fluid character of both the lamellar and the hexagonal H_{II} phase [23]. As shown in Fig. 4, the presence of increasing concentrations of abietic acid produced a broadening and shifting of the lamellar gel to lamellar liquid-crystalline phase transition peak to lower temperatures. At 0.07 and 0.10 abietic acid mol fraction a shoulder at the lower temperature side of the endotherm is clearly observed. The effect of abietic acid on the lamellar to hexagonal H_{II} phase transition was even more drastic, it shifted the transition to lower temperatures such that at an abietic acid mol fraction of 0.15 the transition could no longer be observed (Fig. 4). The presence of abietic acid only produced a slight decrease in the enthalpy change of the lamellar gel to lamellar liquid crystalline phase transition of DEPE (Table 1).

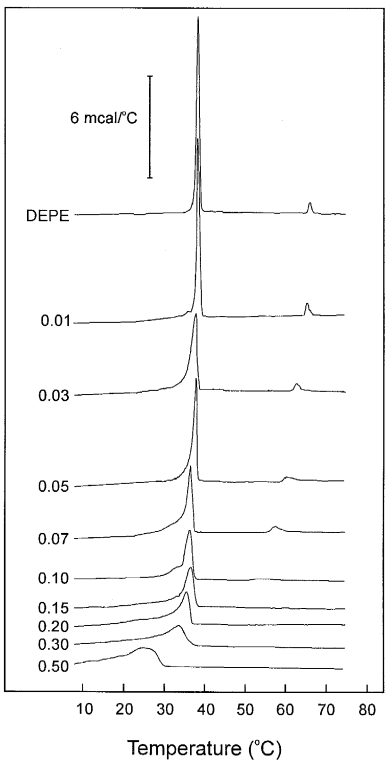

Fig. 4. DSC thermograms for the mixture DEPE/abietic acid. The concentration of abietic acid in the membrane (mol fraction) is expressed on the curves. The profiles correspond to heating scans.

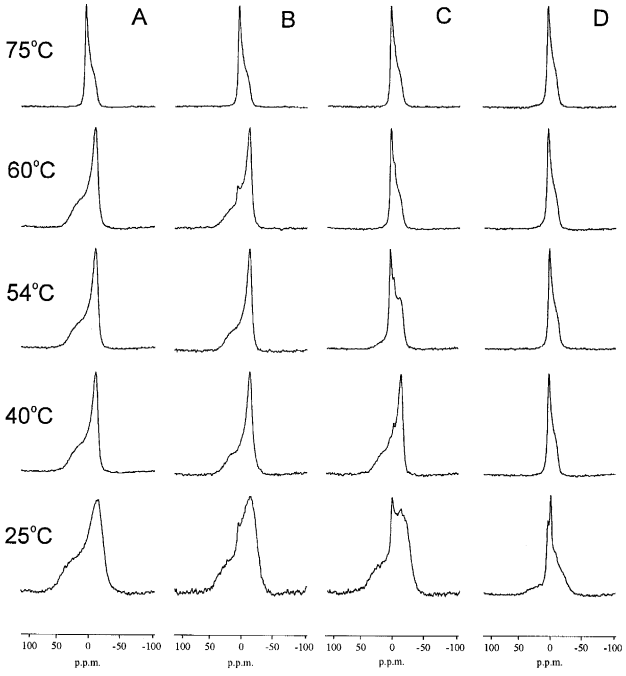

Fig. 5. ^{31}P-NMR spectra at different temperatures corresponding to pure DEPE (A); DEPE/abietic acid, 0.05 mol fraction (B); DEPE/abietic acid, 0.10 mol fraction (C) and DEPE/abietic acid, 0.30 mol fraction (D).

The effect of abietic acid on the thermotropic phase transitions of DEPE was further investigated by ^{31}P-NMR (Fig. 5). DEPE when organized in bilayer structures gives rise to a characteristic asymmetrical ^{31}P-NMR line-shape, with a high-field peak and a low-field shoulder [24]. The chemical shift anisotropy (measured as 3 times the chemical shift difference between the 90 degrees edge and the position of isotropically-moving lipid molecules) is approximately 40 ppm in the liquid-crystalline state, in agreement with previous data [24], characteristically of an axially symmetrical tensor (Fig. 5A). In the lamellar gel state, the lineshape is broadened compared with the lamellar liquid-crystalline state where the lineshape is considerably narrower. For DEPE organized in hexagonal H_{II} phase (Fig. 5A, 75°C) additional motional averaging is experienced due to diffusion of the phospholipids around the cylinders of which this phase is composed. This results in a 2-fold reduction in effective chemical shift anisotropy and a reversed asymmetry (i.e., a high-field shoulder and a low-field peak) [16,25]. The incorporation of abietic acid at a 0.05 mol fraction produces the appearance of a small signal at 60°C (Fig. 5B), superimposed to the bilayer lineshape. The resonance position of this signal coincides with the chemical shift of the low-field peak of the hexagonal H_{II} phase lineshape observed at higher temperatures. This is in agreement with the shift to lower temperatures of the lamellar to hexagonal H_{II} phase observed by DSC (Fig. 4). At an abietic acid 0.10 mol fraction the characteristic spectrum corresponding to the hexagonal H_{II} phase appeared at 54°C (Fig. 5C) and at an abietic acid 0.30 mol fraction the hexagonal H_{II} is already present at 40°C (Fig. 5D).

Using the DSC data and the information of phospholipid structural organization obtained from ^{31}P-NMR, we constructed a partial phase diagram for DEPE in mixtures of DEPE/abietic acid (Fig. 6). In a concentration range of abietic acid from 0 to 0.1 mol fraction, both the main lamellar gel to lamellar liquid-crystalline phase transition and the structural lamellar to hexagonal H_{II} phase transition of DEPE were clearly distinguishable (Fig. 4). We used the temperature of the beginning and the end of the lamellar gel to lamellar liquid-crystalline phase transition to obtain the solid and fluid lines, respectively, whereas the temperature of the beginning and the end of the lamellar to hexagonal H_{II} phase transition were used to obtain the lamellar and hexagonal lines respectively. At concentrations of abietic acid higher than 0.1 mol fraction, the lamellar to hexagonal H_{II} phase transition peak is not observed by DSC (Fig. 4). In these cases we used the temperature of the

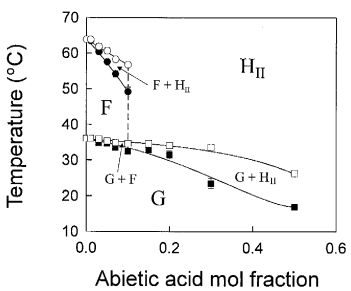

Fig. 6. Partial phase diagram for DEPE in mixtures of DEPE/abietic acid. Black and open symbols were obtained from the beginning and the end temperatures of the transitions (see text for details). G indicates a lamellar gel phase, F indicates a lamellar liquid-crystalline phases and H_{II} indicates an hexagonal H_{II} phase. Points represent the average of four experiments (error bars are shown when larger than the symbols).

beginning and the end of the observed transition to obtain the solid and fluid lines. According to ^{31}P-NMR data, the boundaries defined by these solid and fluid lines are different from those defined at lower abietic acid content. The temperature of all the boundary lines decreased as the concentration of abietic acid increased. Up to a concentration of abietic acid of 0.1 mol fraction the system evolved from a lamellar gel phase (G phase) to a lamellar liquid-crystalline phase (F phase) through a very narrow coexistence region (G + F), and then to a hexagonal H_{II} phase (H_{II} phase) through a coexistence region (F + H_{II}). At concentrations of abietic acid higher than 0.1 mol fraction, the system evolved from a lamellar gel phase (G phase) to a hexagonal H_{II} phase (H_{II} phase) through a coexistence region (G + H_{II}), apparently without an intervening lamellar liquid-crystalline phase.

4. Discussion

Abietic acid is a tricyclic diterpenoid carboxylic acid with an isopropyl substituent. The amphipathic nature of this toxic molecule makes biological membranes one of its most likely sites of action. Using DSC and ^{31}P-NMR, we have carried out the study of the interaction between abietic acid and lipid vesicles formed by two different classes of phospholipid of major relevance, phosphatidylcholine and phosphatidylethanolamine. We used DSC in order to characterize the influence of abietic acid on the thermotropic properties of the phospholipids. The profile of a DSC thermogram of a phospholipid phase transition is determined by the transition temperature and the enthalpy change. Determining the temperatures of the transitions allows the construction of phase diagrams, which provide information regarding the equilibrium between different phases.

For DPPC/abietic acid systems we observed a progressive broadening of the transition peak and a shift of the T_c to lower temperatures (Fig. 1). Abietic acid perturbs the cooperative behavior of the phospholipid. This could be explained by the establishment of a molecular interaction between the phospholipid acyl chain and the abietic acid molecule. This interaction would be the consequence of the intercalation of the abietic acid molecules between the DPPC ones. These observations are compatible with the abietic acid molecule aligning itself with the prevailing directions of the phospholipid acyl chains. The polar carboxyl group of abietic acid will prevent the molecule to be located in the terminal methylene region of the fatty acyl chains of the phospholipids, which otherwise would be the preferred location for a completely hydrophobic molecule. The carboxyl group tends to locate itself near the polar group of the phospholipids, i.e., near the water interface, where it could form hydrogen bonding with water and might also establish other types of interactions with the polar part of the phospholipids. This would restrict the location of the hydrophobic rings of the abietic acid molecule to the upper part of the phospholipid palisade. Due to the length of the abietic acid molecule, this region would probably be that comprised between methylene C1 and methylene C7 of the fatty acyl chain of the phospholipid. Such a location would perturb the phospholipid acyl chains, and consequently we found that abietic acid drastically decreased the onset temperature for the lamellar gel to lamellar liquid-crystalline phase transition. At concentrations higher than 0.10 mol fraction of abietic acid, the thermograms are more complex and several components are observed. This might be due to a lateral phase separation of abietic acid rich domains. Nevertheless, enough abietic acid seemed to remain in the bulk phase so that there was a further decrease of T_c of the components of the thermograms. It seems that the presence of abietic acid perturbs the packing of some phospholipids but these perturbed molecules undergo a transition with a ΔH similar to that of the unperturbed phospholipids (Table 1). Similar effects on the lamellar gel to lamellar liquid-crystalline phase transition of DPPC, i.e., a decrease of T_c with no change in ΔH, have been previously reported for C_5–C_{10} alcohols and C_7–C_{10} fatty acids [26].

Adding a foreign molecule (abietic acid) to a phospholipid system (DPPC) would normally be expected to change the transition temperature of the phospholipid, if both molecules are miscible. We observed that the temperature of the solid line decreased as more abietic acid is present in the system (Fig. 3). This indicates that DPPC and abietic acid are miscible in the lamellar gel phase, and the intercalation of the abietic acid molecule into the phospho-

lipid palisade will perturb the thermotropic properties of the phospholipid. We found that the fluid line kept horizontal in a concentration range of abietic acid from 0 to 0.2 mol fraction, at the temperature of the end of the transition of pure DPPC. The fact that no perturbation in the temperature is observed indicates that a fluid immiscibility occurs, suggesting the formation of an abietic acid/DPPC domain (F phase) immiscible with free DPPC (F′ phase) in the lamellar liquid-crystalline phase. This is an interesting observation which has been reported for unsaturated fatty acids [26], diglycerides [27] and retinol [28]. This type of immiscibility was predicted on the basis of theoretical calculations for mixtures of DPPC and anaesthetics [29], where a relatively strong interaction between the anaesthetic molecules was supposed, so that clusters were formed. Above 0.2 mol fraction of abietic acid, the temperature of the fluid line decreased indicating a miscibility in the lamellar liquid-crystalline phase at these concentrations of the toxicant.

Abietic acid produces a broadening and a shifting to lower temperature of the main lamellar gel to lamellar liquid-crystalline phase transition of DEPE. However this effect is less severe than that observed in DPPC (compare Fig. 1 with Fig. 4). The effect of abietic acid on the lamellar to hexagonal H_{II} phase transition of DEPE is more pronounced. Incorporation of increasing concentrations of the toxicant results in a progressive decrease of enthalpy and temperature of this transition. At a 0.15 molar fraction of abietic acid this transition cannot be observed any more. This can be interpreted that upon abietic acid incorporation, the DEPE molecules interacting with the toxicant give rise to a broad lamellar liquid-crystalline to hexagonal H_{II} phase transition which is shifted to lower temperatures. When the content of abietic acid is higher than 0.10 molar fraction a very broad lamellar to hexagonal H_{II} phase transition is present. In the presence of these high amounts of abietic acid, the structural lamellar to hexagonal H_{II} phase transition occurs at such a low temperature that it takes place concomitantly with the chain melting (lamellar gel to lamellar liquid-crystalline) transition of the phospholipid. The endothermic transitions observed in the thermograms corresponding to high mol fraction of abietic acid originate from the melting of the acyl chains. These endotherms mask the lamellar to hexagonal H_{II} phase transition which has a low heat content due to the liquid-crystalline state of the acyl chains in both phases. Our ^{31}P-NMR experiments confirmed the above interpretations, showing that abietic acid is able to promote hexagonal H_{II} structures at temperatures lower than for pure DEPE. At an abietic acid concentration of 0.05 mol fraction the transition to the hexagonal H_{II} phase is shifted down by about 4 C° (Fig. 5B). The effect on this transition is greater at higher abietic acid concentrations (Fig. 5, C and D) where shifts of 10 and 25 C° were observed.

The location of abietic acid in the upper part of the phospholipid palisade would enable the polar part of the molecule to establish hydrogen bonding with the polar head of DEPE molecules, and this could lead to an effective reduction of the headgroup area of the phospholipid, explaining the facilitating effect of abietic acid on the formation of hexagonal H_{II} structures. We found the presence of a minor isotropic signal in the spectra corresponding to 0.1 and 0.3 mol fraction of abietic acid in the gel phase (25°C) (Fig. 5, C and D). The presence of an isotropic signal in phosphatidylethanolamine systems have been previously reported [30–32]. From the phase diagram (Fig. 6), it can be concluded that the system does not present immiscibilities in any of the described phases as the temperature of all boundary lines decreased as more abietic acid is present. Interestingly, at concentrations of abietic acid higher than 0.10 mol fraction the system evolved directly from the lamellar gel phase to the hexagonal H_{II} phase.

The main result of this study is that abietic acid is able to be incorporated in phospholipid membranes, probably aligning itself with the phospholipid acyl chains, perturbing the packing of the lipids and affecting their thermotropic properties. When considering the possible action of abietic acid in biological membranes, it is interesting to note that, as shown above, the system may present immiscibilities in the fluid phase, a remarkable effect, given the usual fluid condition of biological membranes. This fluid-phase immiscibilities of abietic acid could give rise to the formation of domains, where the concentration of the toxic molecule could be specially high. On the other hand abietic acid is able to affect lipid polymorphism on phosphatidylethanolamine systems, promoting the hexagonal H_{II} phase.

Comparing the abietic acid action on model membranes described in this study with the abietic acid effects on biological membranes, it seems likely that the observed effects on biological membranes might partly be the consequences of the events described in the present investigation. For example, the promotion of non-lamellar structures by abietic acid found in this study might contribute to the observed lytic activity in alveolar epithelial cells [11]. The perturbing effect of abietic acid on membrane structure together with its ability to form enriched domains in the lamellar liquid-crystalline phase suggest that this molecule would alter membrane function, affecting not only the lipids but also the proteins of the membrane. This might be involved in the alteration of membrane permeability of synaptosomal membranes [10], the effects on erythrocyte membranes [8] and the inhibition of platelet aggregation [9] recently described.

In summary, the results of this work clearly show that abietic acid drastically affects the structural and polymorphic properties of the most abundant natural phospholipids in biological membranes, i.e., phosphatidylcholines and phosphatidylethanolamines. We believe that these results are useful to get insight into the interaction of abietic acid with membranes and to understand the effects of this toxic molecule, opening the possibility that some of the specific effects of abietic acid might be exerted through the alteration of membrane function produced by its interaction with the lipidic component of the membrane.

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References

[1] D.B. Mutton, in: W.E. Hillis (Ed.), Wood Extractives, Academic Press, New York, 1962, pp. 331–363.
[2] K.F. Raffa, A.A. Berryman, J. Simasko, W. Teal, B.L. Wong, Environ. Entomol. 14 (1985) 552–556.
[3] A. Oikari, B. Hombom, in: T.M. Poston, R. Purdy (Eds.), Aquatic Toxicology and Environmental Fate, American Society for Testing and Materials, Philadelphia, 1986, vol. 9, pp. 252–267.
[4] A. Oikari, M. Nikinmaa, S. Lindgien, B.-E. Lonn, Ecotoxicol. Environ. Safety 9 (1985) 378–384.
[5] L. Mattsoff, A. Oikari, Comp. Biochem. Physiol. 88C (1987) 263–268.
[6] D.C. Villeneuve, A.P. Yagminas, I.A. Marino, G.C. Becking, Bull. Env. Contam. Toxicol. 18 (1977) 42–47.
[7] P.S. Burge, M.G. Harries, I.M. O'Brien, J. Pepys, Clin. Allergy 10 (1980) 137–140.
[8] D.A. Butterfield, C.H. Trad, N.C. Hall, Biochim. Biophys. Acta 1192 (1994) 185–189.
[9] H.T.A. Cheung, S.-L. Fu, M.A. Smal, Arzneim.-Forsch./Drug Res. 44 (1994) 17–25.
[10] R.A. Nicholson, Biochem. Soc. Trans. 22 (1994) 226S.
[11] G.H. Ayars, L. Altman, C.E. Frazier, E.Y. Chi, J. Allergy Clin. Immunol. 83 (1989) 610–618.
[12] F.J. Aranda, J. Villalaín, J.C. Gómez-Fernández, Biochim. Biophys. Acta 1234 (1995) 225–234.
[13] E.A.J. Keukens, T. de Vrije, C.H.J.P. Fabrie, R.A. Demel, W.M.F. Jongen, B. de Kruijff, Biochim. Biophys. Acta 1110 (1992) 127–136.
[14] N. Kitson, M. Monck, K. Wong, J. Thewalt, P. Cullis, Biochim. Biophys. Acta 1111 (1992) 127–133.
[15] M.C. Sabra, K. Jørgensen, O.G. Mouritsen, Biochim. Biophys. Acta 1233 (1995) 89–104.
[16] P.R. Cullis, M.J. Hope, B. de Kruijff, A.J. Verkleij, C.P.S. Tilcock, in: J.F. Kuo (Ed.), Phospholipid and Cellular Regulation, CRC Press, Boca Ratón, 1985, vol. 1, pp. 1–60.
[17] B. de Kruijff, P.R. Cullis, A.J. Verkleij. M.J. Hope, C.J.A. van Echteld, T.F. Taraschi, in: A.N. Martonosi (Ed.), The Enzymes of Biological Membranes (2nd ed.), Plenum Press, New York, 1985, vol. I, pp. 131–204.
[18] J.M. Seddon, Biochim. Biophys. Acta 1031 (1990) 1–69.
[19] J. Navarro, M. Toivio-Kinnucan, E. Racker, Biochemistry 26 (1984) 5439–5447.
[20] C.F.J. Bötcher, C.M. van Gent, C. Priest, Anal. Chim. Acta 24 (1961) 203–204.
[21] D. Marsh, in: Handbook of Lipid Bilayers, CRC Press, Boca Ratón, 1990.
[22] J. Gallay, B. de Kruijff, Eur. J. Biochem. 142 (1984) 105–112.
[23] P.J. Cullis, B. de Kruijff, Biochim. Biophys. Acta 559 (1979) 399–420.
[24] C.J.A. van Echteld, R. van Stigt, B. de Kruijff, J. Leunnissen-Bijvelt, A.J. Verkleij, J. de Gier, Biochim. Biophys. Acta 648 (1981) 287–291.
[25] J. Seelig, Biochim. Biophys. Acta 507 (1978) 207–218.
[26] M.K. Jain, N.M. Wu, J. Membr. Biol. 34 (1977) 157–201.
[27] A. Ortiz, J. Villalaín, J.C. Gómez-Fernández, Biochemistry 27 (1988) 9030–9036.

[28] A. Ortiz, F.J. Aranda, J.C. Gómez-Fernández, Biochim. Biophys. Acta 1106 (1992) 282–290.
[29] F. De Verteuil, D.A. Pink, E.B. Vadas, A. Zuckerman, Biochim. Biophys. Acta 640 (1981) 207–222.
[30] V. Micol, F.J. Aranda, J. Villalaín, J.C. Gómez-Fernández, Biochim. Biophys. Acta 1022 (1990) 194–202.
[31] J. Gagné, L. Stamatatos, T. Diacovo, S.W. Hui, P.L. Yeagle, J.R. Silvius, Biochemistry 24 (1985) 4400–4408.
[32] S.M. Gruner, M.W. Tate, G.L. Kirk, P.T.C. So, D.C. Turner, D.T. Keane, C.P.S. Tilcock, P.R. Cullis, Biochemistry 27 (1988) 2853–2866.