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Location of the toxic molecule abietic acid in model membranes by MAS–NMR

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Abstract

Abietic acid, the major component of conifer oleoresin, is an environmental toxic molecule with potential hazard to animal and plant life. Being amphipatic, the study of its location and the interaction with membrane components is important to get insight into the mechanism of its toxic action. High resolution magic angle spinning natural abundance ^{13}C nuclear magnetic resonance studies have been undertaken in order to assess its location in egg yolk phosphatidylcholine multilamellar vesicles model membranes. ^{13}C spin-lattice relaxation times in the presence of Gd^{3+}, a paramagnetic agent, of both the phospholipid and abietic acid molecules have been measured in order to obtain information on molecular distances (see J. Villalaín, Eur. J. Biochem. 241 (1996) 586–593). The molecule of abietic acid is placed in the upper part of the palisade structure of the membrane, its carboxyl group is in close proximity to the phospholipid ester groups and it does not extend beyond the C4/C7 carbons of the phospholipid molecule. © 1997 Elsevier Science B.V.

Keywords: Abietic acid; Magic angle spinning NMR; Model membranes

1. Introduction

Conifer oleoresin, synthesized as a defensive secretion against insect and pathogen attack, is a complex mixture of roughly equal proportions of turpentine (cyclic monoterpenes) and rosin (diterpenoid resin acids) [1,2]. Resin acids are tricyclic diterpenes, which are divided into two classes, abietanes, having a isopropyl substituent at C13, and pimaranes, having both methyl and vinyl substituents at C13. Resin acids, of which the main component is abietic acid (Scheme 1), are also widely used in adhesives, paints, printing inks, soldering fluxes and paper sizing, and as an emulsifying agent in the polymerization of synthetic rubber and they are also present in hot-melt glues, hair-sprays, paints, pine-essence cleaners and even cigarette smoke [3–5].

Although not detailed toxicological studies involving abietic acid appear to have been carried out in mammals, abietic acid may contribute to neurotoxicity by membrane depolarization and release of neurotransmitters [6], diterpene acids inhibit platelet aggregation in a non-specific way [7] and exposure to pine-wood dust or colophony derived from pine wood

Abbreviations: MAS: Magic angle sample spinning; EYL: Egg yolk phosphatidylcholine; T_1: Spin-lattice relaxation time; T_{1M}: Paramagnetic contribution to the spin-lattice relaxation time T_1

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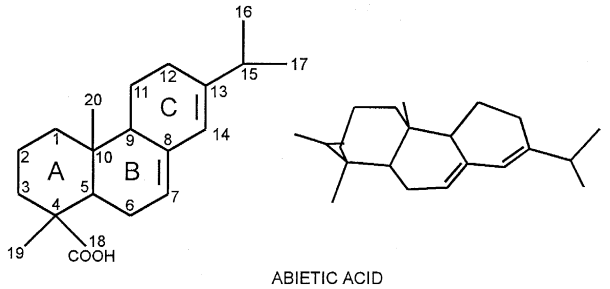

ABIETIC ACID

Scheme 1. Chemical structure and numbering of abietic acid.

can produce acute and chronic lung disease as well as eczema [3,4,8,9]. Furthermore, abietic acid is nearly as toxic as pentachlorophenol as indicated by the inhibition of methane production in different methanogens [10], it causes dose- and time-dependent lysis of alveolar, tracheal and bronchial epithelial cells [11] and the dehydro derivative of abietic acid affects the physical state of cytoskeletal proteins and the lipid bilayer of erythrocyte membranes [12]. The release of these compounds into the environment is also of a great concern because of their potential health hazard to animal, including humans, and plant life [13]. Resin acids are believed to cause much of the toxicity of pulp mill effluents to fish [14] and the poisoning symptoms described indicate an action on the nervous system [15]. Recently, supporting the idea that abietic acid may affect natural membranes by interacting with its components, we have found that abietic acid greatly affects the phase transition of dipalmitoylphosphatidylcholine and dielaidylphosphatidylethanolamine model membranes [16]. All of these data favour the hypothesis that abietic acid, an amphipathic molecule, incorporates into biological membranes, being one of the main target site for its toxic action. Therefore, the knowledge of the location of the molecule of abietic acid in the membrane and its interaction with the components of the membrane may help to get insight into the mechanism of action of this toxic in particular and resin acids in general.

The aim of this work is to ascertain the location of the abietic acid molecule inside non-perturbed model multilamellar vesicles by the application of magic angle spinning (MAS) ^{13}C nuclear magnetic resonance (NMR). The use of intrinsic probes to obtain distance information is always a matter of controversy due to the possibility of any perturbation introduced into the system. At the same time, X-ray crystallography is hampered by the fact that the system is far from being in its natural hydrated state. High-resolution ^{13}C-NMR has been used to study the interaction of hydrophobic molecules inserted in small unilamellar vesicles. However, multilamellar vesicles, which resemble natural membranes much better than small unilamellar vesicles, do not have rapid isotropic tumbling, and give rise to broad NMR lines because the angular dependence of the local magnetic field is not efficiently averaged [17]. Recent work on multilamellar model and natural membranes using solid-state NMR has opened the way to obtain direct information on membrane structure and dynamics, and it has made possible to determine specific properties of lipids and peptides incorporated into the membrane lamellar phase [17–25]. In this study, we have obtained information on the location of abietic acid in egg yolk phosphatidylcholine (EYL) multilamellar vesicles model membranes from the variation of the spin-lattice relaxation times (T_1) in the absence and in the presence of a paramagnetic agent, Gd^{3+} [25]. The effect of Gd^{3+} on the spin-lattice relaxation times has an explicit distance dependence, allowing us to use it to evaluate relative distances on a molecular scale.

2. Material and methods

2.1. Materials

EYL was obtained from Lipid Products (Surrey, Great Britain), abietic acid and $GdCl_3 \cdot 6H_2O$ from Aldrich (Madrid). The fatty acid composition of EYL was the following: 16:0 (32.1%), 16:1 (2.1%), 18:0 (11.7%), 18:1 (36.2%), 18:2 (12.5%) and 20:4 (5.5%). All other reagents used were of analytical grade from Merck (Darmstadt, Germany). Water was deionized, twice distilled and passed through a Milli-Q apparatus (Millipore Ibérica, Madrid) to a resistivity better than 18 MΩ cm. The purity of EYL before and after the measurements were checked by thin-layer chromatography on silica-gel plates (Merck, Darmstadt, Germany) in chloroform/methanol/water (65:25:5 by vol) where it showed only one spot.

2.2. Sample preparation

Samples were prepared as already described [25]. Briefly, 100 mg of EYL in chloroform and, if required, the appropriate amounts of abietic acid in chloroform:methanol 1:1 (by vol) in order to obtain a phospholipid to abietic acid molar ratio of 3:1, were dried under a stream of O_2-free N_2 in the dark and further desiccated under high vacuum in the dark for more than 3 h. The samples were dispersed and hydrated in 1 ml of H_2O to form multilamellar vesicles and kept for 15 min at 35–40°C, with occasional mixing in order to obtain a homogeneous and uniform suspension. To assure sample homogeneity, lipid dispersions were subjected to five freeze–thaw cycles from −80 to 35°C with occasional vortexing. When required, the multilamellar vesicles were formed in the presence of a final concentration of 1 mM Gd^{3+}.

2.3. Nuclear magnetic resonance

Magic angle spinning (MAS) ^{13}C-NMR spectra were obtained using the same spectrometer and parameters described before [25]. ^{13}C-NMR T_1 measurements were carried out by the fast inversion-recovery method [26]. Since long T_1 experiments are particularly sensitive to long-term changes in magnet homogeneity, short and long τ values were alternated and interleaved by the spectrometer computer. In order to check lipid polymorphism in the presence of Gd^{3+}, ^{31}P-NMR was used with the following parameters: a gated-broad band decoupling of 10 W, 25 KHz of spectral width, 32 K data points, a 120 s interpulse time and a 90° radio frequency pulse. A sample containing EYL and abietic acid at a 3:1 molar ratio in the presence of Gd^{3+} showed a broad asymmetrical signal with a high-field peak and a low field shoulder, characteristic of bilayer structures (not shown for brevity).

3. Results

MAS-^{13}C-NMR spectra of multilayers formed by pure EYL yielded very well resolved spectra (Fig. 1A) in which four main spectral regions could be discerned: Carbonyl groups, olefinic carbons, glycerol backbone carbons and acyl-chain aliphatic carbons. The same resolution and regions were obtained

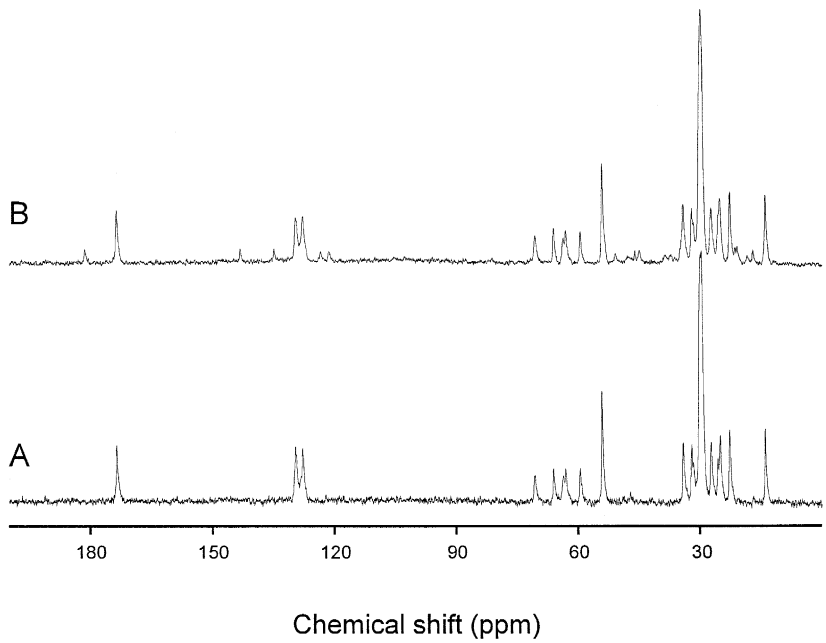

Fig. 1. Proton-decoupled MAS-^{13}C-NMR spectra of fully hydrated multilayers of (A) pure EYL and (B) EYL and abietic acid at a molar ratio of 3:1. The spectra have been obtained at room temperature and processed with 10 Hz line broadening.

for the sample containing EYL and abietic acid at a molar fraction of 3:1 (Fig. 1B). Additionally, some small resonances, which belonged to the abietic acid molecule, were distinguished and resolved (compare Fig. 1A and B). The assignment of the different resonances of the phospholipid was made according to [18] whereas those pertaining to abietic acid were assigned according to [27]. Because of the highly resolved nature of the MAS-^{13}C-NMR spectra, it was possible to determine the T_1 of all carbon atom sites of EYL, both in pure form and in the presence of abietic acid, as well as the T_1 values of the abietic acid molecule.

Since the relaxation rate is proportional to the number of directly bonded hydrogens (n), the relaxation parameter T_1 has been scaled by the number of attached hydrogen atoms to produce nT_1. The nT_1 values for the EYL resonances are plotted in Fig. 2A as a function of their position in the phospholipid molecule. The nT_1 values of EYL (in the absence of Gd^{3+}) were quite similar to other previously reported nT_1 values [21,25,28,29]: They are smaller for the glycerol backbone carbons and increase towards the head-group and the terminal methyl group of the hydrocarbon acyl chain (Fig. 2A). These and other studies have allowed a well defined picture of the phospholipid molecule in the bilayer: The glycerol backbone is the most rigid section of the molecule while the head group and fatty acyl chains are capable of increased motion [30]. The nT_1 values of the glycerol backbone carbons of EYL were very similar either in pure EYL or in the presence of abietic acid (Fig. 2A). However, the nT_1 values of the acyl chain carbon atoms of EYL in the presence of abietic acid, and to a lesser extent those of the head-group also, were shorter than in pure EYL, suggesting that these regions were more ordered in the presence of abietic acid (Fig. 2A). The nT_1 values of the different carbons of abietic acid are shown in Fig. 2B (in order to avoid any confusion between carbons of abietic acid and EYL, carbon atoms from abietic acid will be named with a dash, i.e., C-1, whilst carbon atoms from EYL will be named without, i.e., C1). Except non-protonated carbons and methyl groups of the abietic acid molecule, the nT_1 values of abietic acid carbon atoms were similar to the nT_1 values of the glycerol backbone carbons of EYL, which is the most rigid part of the phospholipid molecule. The peculiar

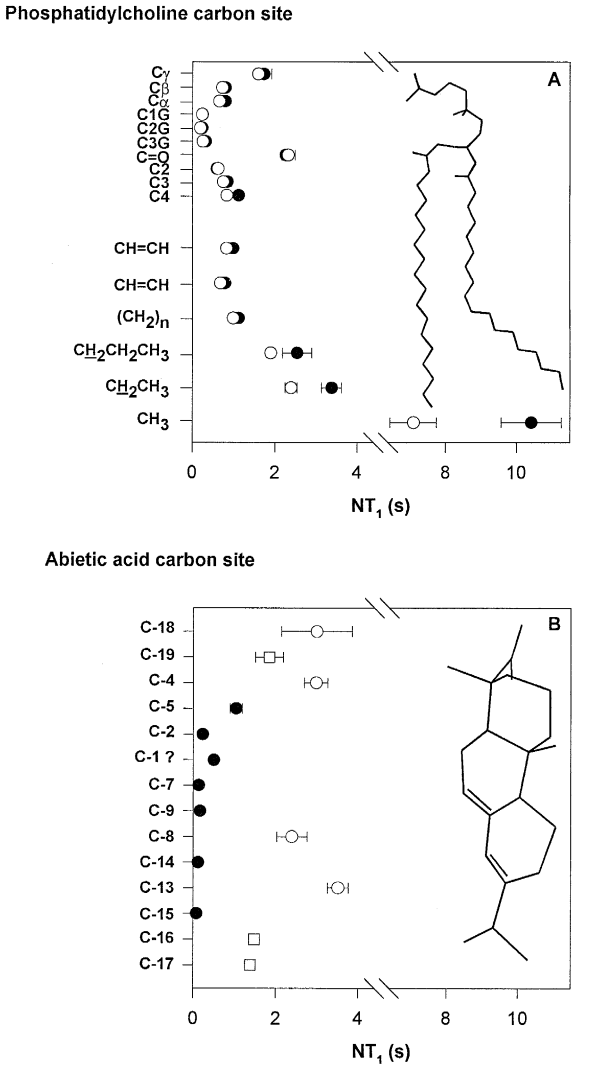

Fig. 2. ^{13}C-NMR spin-lattice relaxation times (nT_1) in s for (A) EYL and (B) abietic acid resonances. (A) EYL, either in pure form (●) or in the presence of abietic acid at a EYL/abietic acid molar ratio of 3:1 (○). (B) Abietic acid in the sample EYL/abietic acid at a molar ratio of 3:1, where the methyl groups are denoted as (□), non-protonated carbons as (○) and all the other as (●).

motion of the methyl groups (carbons C-16, C-17 and C-19) may explain their increased nT_1 values [25]. However, their nT_1 values were smaller than the nT_1 values of the acyl methyl groups of EYL but similar to the nT_1 value of the Cγ head-group methyl carbons of EYL. On the other hand, the T_1 values of the non-protonated carbons of abietic acid (carbons C-4, C-8, C-13 and C-18) were greater than the other

ones because they are not directly bound to any hydrogen atom [31].

In order to evaluate distances on a molecular scale, we have used the relative paramagnetic contribution to the spin-lattice relaxation time in the presence of Gd^{3+}. This parameter is derived as follows [see Ref. [25]]: since $(T_{1M})^{-1}$, the paramagnetic contribution to the spin-lattice relaxation time, is proportional to r^{-6}, where r is the distance between Gd^{3+} and the nucleus in question [31], the ratio of the $(T_{1M})^{-1}$ values of any two nuclei allows the calculation of the ratio of their r values. Therefore, the relative paramagnetic contribution to the spin-lattice relaxation time is obtained from the ratio of the $(T_{1M})^{-1}$ value of any carbon X, i.e., $(T_{1M}^{X})^{-1}$, either from EYL or from abietic acid, and, in our case, the $(T_{1M})^{-1}$ value of the C3G carbon of EYL, i.e., $(T_{1M}^{C3G})^{-1}$. The $(T_{1M}^{C3G})^{-1}$ value has been used to normalize the data, since the relaxation values for the different carbons of both EYL and abietic acid in samples containing both molecules can be obtained from the same spectra, and therefore the phospholipid data can then be used as an internal control. In samples containing pure EYL, the $(T_{1M}^{C3G})^{-1}$ value refers to the C3G carbon of pure EYL, whereas for samples containing both EYL and abietic acid, the $(T_{1M}^{C3G})^{-1}$ value refers to the C3G carbon of EYL in the presence of abietic acid.

The relative paramagnetic contribution to the spin-lattice relaxation time is plotted in Fig. 3 for the resonances of EYL, either in pure form or in the presence of abietic acid at a molar fraction of 3:1. For pure EYL, the values of the relative paramagnetic contribution to the spin-lattice relaxation times corresponding to the Cα head-group and the C3G glycerol backbone carbons were higher than those found for the Cβ head-group carbon and the C2G and C1G glycerol backbone carbons and much higher than those observed for the Cγ head-group and C=O carbons of the phospholipid (see Fig. 3). These data confirms that the Gd^{3+} ion is effectively bound to the phosphate group of EYL in a definite position [30]. Smaller effects were observed for the other carbons of EYL, but significantly, all carbons of EYL sensed the presence of Gd^{3+}. Since the glycerol backbone carbons and the carbonyl groups of EYL are nearer to the surface of the membrane than the acyl chain carbons, and, at the same time, the CH_3 terminal

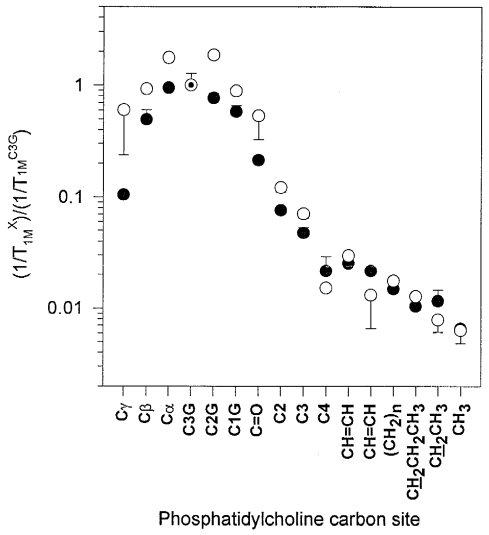

Fig. 3. Logarithmic plot of the relative paramagnetic contribution to the spin-lattice relaxation time of the resonances of EYL. Pure EYL (●) and EYL in the presence of abietic acid at a molar fraction of 3:1 (○). See text for details.

methyl group and the $CH_2(\omega\text{-}1)$ and $CH_2(\omega\text{-}2)$ methylene carbons are nearer to the middle of the bilayer than the $(CH_2)_n$ carbons and olefinic groups of the acyl chain, the observed effect on the T_1 values for the different carbons of pure EYL decreased as the distance from the surface of the membrane increased (Fig. 3).

In the presence of abietic acid, the relative paramagnetic contribution to the spin-lattice relaxation time for the EYL carbons presented the same trend as in the case of pure EYL: The nearer to the phosphate, the greater the observed effect is (Fig. 3). This trend was observed for both regions of the phospholipid, the head-group and the hydrocarbon chain regions. For EYL in the presence of abietic acid, the values of the relative paramagnetic contribution to the spin-lattice relaxation times corresponding to the Cα head-group and the C2G glycerol backbone carbons were higher than those found for the Cβ head-group and the C1G and C3G glycerol backbone carbons, and much higher than those observed for the Cγ head-group and C=O carbons of the phospholipid (see Fig. 3). These data confirmed, as noted above for pure EYL, that the Gd^{3+} ion is bound in a definite position at the phosphate group of EYL in the sample containing EYL and abietic acid at a molar fraction of 3:1. Since the relaxation measure-

ments of the different carbons of both EYL and abietic acid can be obtained from the same spectra, the phospholipid data can then be used as an internal unperturbed control. Therefore, the paramagnetic contribution to the spin-lattice relaxation time, $(T_{1M})^{-1}$, obtained for the different carbons of EYL and abietic acid in the EYL/abietic acid mixture should allow us to obtain relative distances of all carbons of both molecules to the membrane surface where the Gd^{3+} ion is bound.

We were not able to obtain the T_1 values for all carbons of abietic acid in the presence of Gd^{3+}. Nevertheless, there were several resonances which were resolved in the spectra and permitted us to obtain the $(T_{1M})^{-1}$ values of several carbons along the whole molecule of abietic acid, allowing us to compare the relative paramagnetic contribution to the spin-lattice relaxation time of the carbons of both molecules, EYL and abietic acid, in the same model membrane system. In order to calculate distances from abietic acid to the surface, some assumptions must be made. First, the hydrocarbon chains of the phospholipid molecule are considered as a single fragment despite the fact that they are positionally inequivalent in crystalline, gel and liquid phases [32,33] and second, the distances from the different carbons to the phosphate may depend also on phospholipid composition and temperature [33,35]. Taking into account the fatty acid composition of EYL (see Section 2) and assuming an average hydrophobic thickness of approximately 3 nm [34–37], we obtained Fig. 4A, where we have plotted the average distance of the different carbon atoms of EYL (in the sample containing EYL and abietic acid at a molar fraction of 3:1) to the membrane surface with respect to the relative paramagnetic contribution to the spin-lattice relaxation time. In Fig. 4A, it is possible to discern a very good correlation between the distance to the membrane surface of the different carbon atoms of EYL and the relative paramagnetic contribution to the spin-lattice relaxation time observed for each particular atom. The similarity of the $(T_{1M})^{-1}$ data for EYL, both in pure form and in the presence of abietic acid (see above), permitted us to compare the $(T_{1M})^{-1}$ values of the EYL carbons (in the presence of abietic acid) with the $(T_{1M})^{-1}$ values of the abietic acid carbons in the mixture. Therefore, from the $(T_{1M})^{-1}$ values of abietic acid, the distance of the different carbons of the abietic acid molecule to the surface of the membrane can be obtained (Fig. 4B).

By comparing Fig. 4A and B, it can be observed that the carboxyl group of the abietic acid molecule would then be in the vicinity of the carbonyl groups of the phospholipid (as well as ring A, see Scheme 1), whereas rings B and C and the isopropyl substituent would be located in the upper part of the palisade structure of the membrane (the relative paramagnetic contribution to the spin-lattice relaxation time for carbons C-2/C-19, C-4 and C-5 of abietic

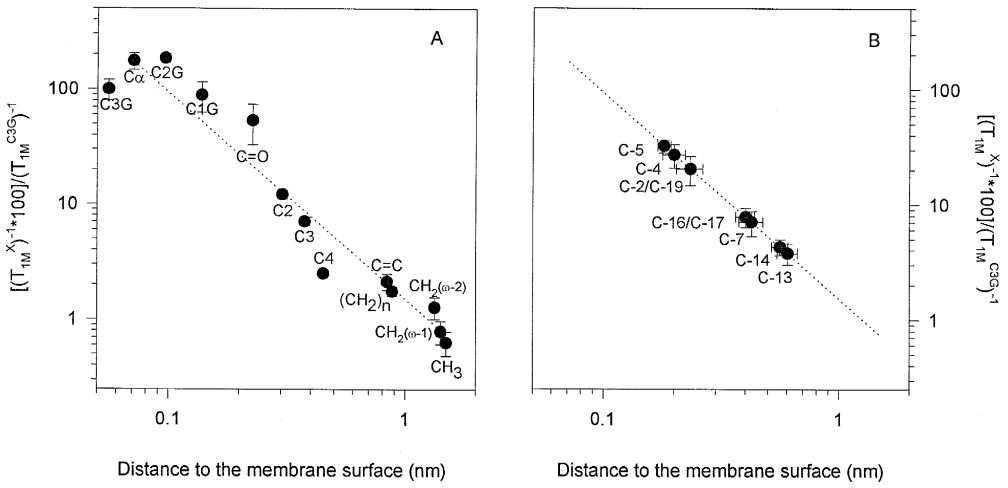

Fig. 4. Mean relative distance to the membrane surface (in nm) for the different carbon atoms of EYL (A) and abietic acid (B) in the sample containing EYL/abietic acid at a molar fraction of 3:1. See text for details.

acid are similar to those of the C=O carbon of EYL whereas those of carbons C-7, C-13, C-14 and C-16/C-17 of the abietic acid molecule are similar to those of the C2–C4 carbons of EYL). No carbon of abietic acid (at least of those observed here) were placed below C4 carbon of the phospholipid molecule (compare Fig. 4A and B. The length of abietic acid, in its completely extended conformation, is ca. 1.3 nm and its carboxyl group is very close to the carbonyl groups of the phospholipid. Therefore, if abietic acid were perpendicular respect to the membrane surface and intercalated between the phospholipid molecules, the extreme of the molecule, i.e., the isopropyl substituent, would extend till the C7 carbon group of the phospholipid.

4. Discussion

Abietic acid is a tricyclic diterpene with an isopropyl substituent. This toxic molecule is amphipathic, with a polar carboxyl group, a hydrophobic tail and an intervening rigid part composed by three rings. Therefore, biological membranes might be one of its most likely sites of action. In this study, we have aimed to examine the precise location of this toxic molecule inside a model membrane by using MAS-^{13}C-NMR in the presence of the paramagnetic ion Gd^{3+}. The effect of the paramagnetic ion on the spin-lattice relaxation times of the lipid resonances has an explicit distance dependence, allowing it to be used to evaluate relative distance on a molecular scale [25].

Before discussing the experimental data in the presence of Gd^{3+}, it is interesting to analyze the nT_1 values of the phospholipid in the absence of the paramagnetic ion. We can compare the phospholipid nT_1 values in the presence of abietic acid (Fig. 2A) with previously published data of the nT_1 values of EYL in the presence of other amphipathic molecules, such as α-tocopherol, coenzyme Q, both in the oxidized and in the reduced forms, and cholesterol [21,25]. In these previous works it was found that, whereas cholesterol and coenzyme Q did not significantly changed the nT_1 values of EYL, α-tocopherol decreased the nT_1 values of the head-group and the acyl chain carbons of the phospholipid. These results were related to the different location of these molecules in the bilayer palisade, the molecule of α-tocopherol being the nearest to the membrane surface. Comparing those results with the ones obtained in this work (Fig. 2A), abietic acid would then be located nearer to the membrane surface than to the centre of the bilayer, similarly to α-tocopherol, but distinctly as cholesterol or coenzyme Q in both its oxidized and reduced forms.

It is also possible to obtain interesting information about the dynamics of the abietic acid molecule when inserted in the membrane by comparing the nT_1 values of abietic acid and the nT_1 values of the phospholipid in the samples containing both types of molecules (Fig. 2A and B). The nT_1 values of the abietic acid carbons (except the non-protonated carbons and the methyl groups, see above) are similar to those of the glycerol backbone carbons of EYL (both in the pure form and in the presence of abietic acid), whereas the nT_1 values of the methyl groups of abietic acid are similar to the nT_1 value of the Cγ head-group of EYL. Therefore, the degree of motional ordering of the whole abietic acid molecule should be very similar to the glycerol backbone portion of EYL, the most rigid part of the phospholipid molecule.

In order to obtain specific distance information, we have used the effect produced on the spin-lattice relaxation time of the resonances by the presence of the paramagnetic Gd^{3+} ion. Phospholipid resonances are either shifted or broadened [38] by the adsorption of lanthanide ions onto the membrane surface [39] and the effect of the lanthanide ions on the T_1 of the phospholipid resonances, $(T_{1M})^{-1}$, has an explicit distance dependence, r^{-6} [31]. This distance dependence can allow us to evaluate distances on a molecular scale by using the relative paramagnetic contribution to the relaxation time [25], since the T_1 of the nuclei of the molecules inserted in the membrane would be affected depending on their proximity to the surface where the ion is located. However, this approximation is only valid if Gd^{3+} binds exclusively to the phosphate group without perturbing the phospholipid, either in pure form or when mixed with another type of molecule (in our case abietic acid), and all carbons of EYL sense the presence of the Gd^{3+} ion. Moreover, since the $(T_{1M})^{-1}$ values obtained for the different carbons of both EYL and abietic acid can be obtained from the same spectra,

the phospholipid data can then be used as an internal unperturbed control. The data presented in Fig. 3 clearly confirm that Gd^{3+} is bound to the polar head group of the phospholipid in a specific position without perturbing the phospholipid molecule in samples containing both EYL and abietic acid, and therefore, it is possible to estimate relative distances between the phospholipid molecule and the molecule of abietic acid.

Another interesting information can be obtained by observing Fig. 3. Recently it has been found that cholesterol increased the $(T_{1M})^{-1}$ values of the head-group carbons of EYL and decreased the $(T_{1M})^{-1}$ values of the hydrocarbon chain carbons, i.e., cholesterol increased the membrane thickness [25]. In our case the $(T_{1M})^{-1}$ values for all carbons of EYL in the presence of abietic acid were similar to those of pure EYL (Fig. 3). Therefore, abietic acid, apparently, did not increase the membrane thickness of the membrane. The increase of the thickness of the membrane by cholesterol was due to its special disposition in the palisade structure of the membrane, being arranged in an interdigitated form with the phospholipid molecules [25]. Hence, the molecule of abietic acid must intercalate between the phospholipid molecules but it does not extend into the other monolayer of the membrane.

The measurement of precise distances is limited by the absence of information on the lateral distance from the abietic acid molecule to the Gd^{3+} ion, since Gd^{3+} is preferably attached over the EYL molecule and also on the precise mechanism of the relaxation effect. Data presented in Fig. 4A represents the distance from the different carbons of EYL to the surface of the membrane (i.e., the Gd^{3+} ion adsorbed to the phosphate group). Therefore, data in Fig. 4B represents the distance from the abietic acid molecule to the paramagnetic ion, and not the actual distance to the membrane surface, which should be slightly different. Another uncertainties which might arise in the measurement of an exact distance from the molecule of abietic acid to the membrane surface come from the fact that, due to the proximity of the abietic acid molecule to the membrane surface, different Gd^{3+} ions might affect the same abietic acid molecule so that the effect of the Gd^{3+} ion on the carbon resonances of abietic acid would not depend exactly according to their position along the molecule. At the same time, it would be possible that the molecule of abietic acid might oscillate in the membrane between an oblique and a perpendicular position in the membrane. The data presented in Fig. 4, where C-16/C-17 carbons of abietic acid appear to be nearer to the membrane surface than carbons C-7, C-13 or C-14, might be explained by these effects. Nevertheless, this method give us a very good estimate of the relative position of the molecule of abietic acid with respect to the molecule of EYL in the membrane. Therefore, abietic acid, as found in this work, should then be located near the surface of the membrane with its carboxyl group in close proximity to the phospholipid C=O groups (as well as ring A) whereas rings B and C and the isopropyl substituent should be located in the upper part of the palisade structure of the membrane but not extending beyond the C4/C7 carbon of the phospholipid molecule.

Recently, it has been shown that dehydroabietic acid alters the physical state of cytoskeletal proteins and the motion and order of the lipid bilayer [12] whereas abietic acid depolarizes isolated mammalian synaptosomes and provokes the release of acetylcholine [6]. We have recently shown that abietic acid significantly perturbs the packing of membrane lipids, affects lipid polymorphism and induces the presence of immiscibilities in the fluid phase [16]. Some of the specific toxic effects of abietic acid in natural systems might be exerted through the alteration of membrane function by the interaction with its components, either lipids or proteins, this interaction modulated by the specific location of abietic acid in the membrane.

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