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An investigation of the interaction of iminosulfurane transdermal penetration enhancers with model skin preparations using NMR spectroscopy

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ABSTRACT

The ^{31}P NMR resonance from the inner and outer leaflets of DMPC in unilamellar vesicle bilayers has been split by use of the slowly penetrating paramagnetic shift reagent, Pr^{3+}. The perturbing effect of subsequently added iminosulfurane transdermal penetration enhancers (TPEs) is to accelerate the collapse of this splitting, especially in the case of the bromo derivative **3**. The aforementioned acceleration of the splitting is enhanced by the addition of 16 mol% cholesterol. Conversely, 33 mol% cholesterol appears to seal the bilayer to the effect of the TPEs—even when present at 20 mol%. These observations are consistent with the deep penetration of the TPEs into the DMPC bilayer, i.e., the perturbation of the bilayer is transmembrane and supports a model in which a subset of the bromo TPE derivative **3** is kinetically trapped in the bilayer. This feature leads to an enhanced residence time of **3** in the bilayer, and by extension to the skin, and therefore to an explanation for the markedly enhanced activity of the bromo TPE derivative relative to that of other halogenated derivatives in the series of iminosulfuranes studied.

1. Introduction

A series of iminosulfuranes synthesized recently (Strekowski et al., 1999) function as potent transdermal penetration enhancers (TPEs) in *in vitro* studies based on human cadaver and hairless mouse skin samples as shown (Kim et al., 1999; Song et al., 2005). TPEs offer an alternative to drug delivery by oral or injection methodology, can be designed for controlled release of drugs, and hence can be used to administer drugs that will not survive exposure to the gastrointestinal tract and also avoids the first pass through the liver (Barry, 2006). The structure of the iminosulfuranes investigated in this work is shown in Fig. 1. The compounds differ only by the nature of the substituent at the *para* position on the phenyl ring. The parameters ER_J and Q_{24}, respectively the flux enhancement ratio and the quantity of a test drug, hydrocortisone, that appears over 24 h in the alternate chamber of a Franz cell after having penetrated the skin sample, were measured (Song et al., 2005). The unsubstituted compound **1** actually reduces ER_J to less than unity and acts as a skin sealant. Both chloro and iodo derivatives **2** and **4** exhibit modest activity; ER_J values are 1.5 and 1.6, respectively for **2** and **4**, but the bromo derivative **3** is an order of magnitude more active than the other members of this series, exhibiting an ER_J in excess of 20 and a correspondingly large value of Q_{24}. The pharmacological activity of the bromo derivative exceeds that of Azone®, a typical reference TPE. Furthermore, these iminosulfuranes are not toxic to fibroblast and kerotinocyte cell lines at the concentrations employed in previous studies and those reported herein (Song, 2004). These compounds, especially the bromo derivative **3**, are thus promising candidates for clinical use.

In order to elucidate the molecular basis of the TPE activity of these compounds, their behavior in a model system consisting of either unilamellar or multilamellar lipid bilayers has been explored in some detail (Barrow et al., 2005). With the exception of the unsubstituted compound **1**, that does not bind strongly to the lipid bilayers, the excess heat capacity profiles, obtained by differential scanning calorimetry, characterizing the pretransition in multilamellar preparations is broadened into the baseline as expected for an initial surface association of the TPE with the vesicles. In the presence of 10 mol% TPE, the main gel-to-liquid crystalline phase transition excess heat capacity profile is broadened, becomes asymmetrical about the melting temperature, T_m, and can be resolved into a number of components, corresponding to thermodynamically distinct domains, each of which can be described by a single two-state transition model. When the quantity of iminosulfurane is reduced to 1 mol%, the pretransition profile is still broadened into the baseline, and that of the gel-to-liquid crystalline phase transition is broadened but remains symmetrical and can be described

Abbreviations: DMPC, L-α-1,2-dimyristol-*sn*-glycero-3-phosphocholine; DMSO, dimethylsulfoxide; HEPES, *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]; NMR, nuclear magnetic resonance; NOESY, nuclear overhauser enhancement spectroscopy; TPE, transdermal penetration enhancer.

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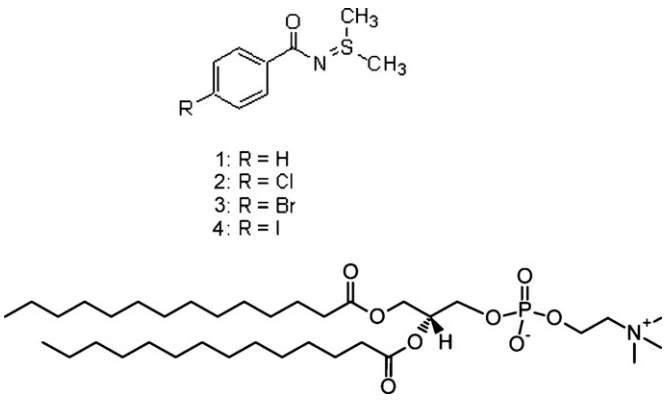

Fig. 1. The structure of iminosulfuranes **1**–**4** and DMPC.

by a single two-state model. The T_m values describing the preparations containing both 1 and 10 mol% TPE are reduced relative to that of the control preparations, i.e., the bilayer is destabilized by the TPE. The cooperative unit parameter obtained from the analysis of the thermal data from the preparations containing 1 mol% TPE is reduced by 35 and 48% by the bromo **3** and iodo **4** derivatives, respectively, indicting that these compounds reduce the cooperativity of the gel-to-liquid crystalline phase transition (Biltonen and Freire, 1978).

A strong correlation was found between the ER_J and Q_{24} parameters and the binding or partition coefficients characterizing the interaction of the iminosulfuranes **1**–**4** with unilamellar vesicle preparations as measured by isothermal titration calorimetry. However, certain characteristics of the interaction of the bromo derivative **3** with this preparation, to be subsequently described, suggest that a subpopulation of this derivative may penetrate deeply into the bilayer and become trapped. As a result, this subpopulation does not dissociate from the bilayer on the time scale of measurements based on a release protocol, nominally several hours. This hypothesis offers a justification for the assumption that only the lipids in the outer leaflet of the bilayer are interacting with **3** on the aforementioned time scale, an assumption that was necessary in order to obtain the same values for the ΔH and K (binding coefficient) from either the release or incorporation titration protocols as required by thermodynamic first principles (Barrow et al., 2005). This kinetic trap model would lead to an enhanced residence time of the bromo derivative **3** in the bilayer and by extension to the skin preparations and thus to a rationalization of the enhanced pharmacological activity of this derivative relative to the other compounds **1**, **2**, and **4**.

Additional evidence for the deep penetration of the bromo derivative **3** into the lipid bilayer was observed in the form of NOE cross-peaks between the two sets of protons of the phenyl ring of **3** and the protons of the lipid fatty acid terminal methyl groups of DMPC the structure of which is provided in Fig. 1. This cross-peak was unique to the bromo derivative **3**. Since there is an r^{-6} distance dependence on this effect, the implication is that the compound penetrates well into the bilayer perhaps to the inner leaflet and strongly supports the aforementioned entrapment model for the high activity of this compound. However, an alternative explanation of the NOE cross-peaks previously described is that the lipid fatty acid chain undergoes a large amplitude motion (Xu and Cafiso, 1986; Huster et al., 1999) that places the terminal methyl group protons in close proximity to the phenyl protons of the bromo derivative **3** on the surface of the bilayer as indicated by NOE cross-peaks between the phenyl group protons and those of the *N*-methyl moieties of the choline head group. Such a large amplitude motion by the lipid fatty acid chains is, however, a low probability event and is in the opinion of the authors not the primary explanation of the crucial NOE cross-peak to the terminal methyl group protons as previously described. Furthermore, the characteristic cross-peak between the terminal methyl group protons and those of the phenyl ring protons is not observed in the case of the iodo derivative that is also present on the surface of the vesicle bilayer. The preceding interpretation implies a mechanism based primarily on bilayer surface activity of the bromo derivative **3**. The primary mode of action leading to the high activity of this derivative thus remains uncertain.

In the present study, additional evidence is presented in support of deep penetration into the unilamellar vesicle bilayer, especially by the bromo derivative **3**. The experiments involve monitoring the effect of the TPEs shown in Fig. 1 on the splitting of the ^{31}P NMR resonances from the inner and outer leaflets of the bilayer generated by a slowly penetrating paramagnetic shift reagent, Pr^{3+}.

2. Materials and methods

Synthetic L-α-1,2-dimyristol-*sn*-glycero-3-phosphocholine (DMPC) was obtained from Avanti Polar Lipids, Alabaster, Alabama. Potassium chloride, reagent grade was purchased from Baker & Adamson Company, and *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) and cholesterol, 99% purity, were obtained from the Sigma Chemical Company, St Louis, Missouri. Methanol, Optima purity, was obtained from Fisher Scientific, Fair Lawn, New Jersey. Chloroform, OmniSolv grade, was obtained from EM Science, Gibbstown, New Jersey. Deuterium oxide was obtained from the Aldrich Chemical Company. Iminosulfuranes were synthesized as previously described (Strekowski et al., 1999).

2.1. Vesicle preparation

DMPC was rehydrated with 10 mM HEPES buffer with 160 mM potassium chloride at pH 7.5 and used to prepare small unilamellar vesicles as previously described (Bammel et al., 1987). A 9-mM suspension was premixed for 5 min and sonicated at 50 W power for 40 min (40% duty cycle) at 35 °C under a stream of nitrogen. The resulting solution was centrifuged at 130,000 × *g* at 35 °C to remove particulate matter. The supernatant was retained and stored in an oven at 35 °C. This method produces nominally 300 Å—diameter unilamellar vesicles.

Three mM solutions of iminosulfuranes were prepared in HEPES buffer (160 mM KCl and 10 mM HEPES) by adding no more than 4% ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY) to aid in dissolution of the iminosulfuranes.

DMPC and cholesterol present at various mole percentages were dissolved in chloroform/methanol 2:1 by volume. The phospholipid/cholesterol mixture was dried by rotary evaporation to a thin film. The resulting thin film was placed under high vacuum overnight to further evaporate any residual solvent. Dried films were stored at 0 °C under vacuum. The dried films were rehydrated with 10 mM HEPES buffer in 160 mM KCl, pH 7.5, and used to prepare vesicles as previously described.

2.2. Nuclear magnetic resonance

2.2.1. Phosphorous NMR

The ^{31}P NMR spectra were obtained at 202.4 MHz at 35 °C using proton decoupling and a pulse width of 16.5 μs and an acquisition time of 0.800 s. The free induction decays were obtained with 6000 transients and a relaxation delay time of 2.0 s and 150 Hz line broadening factor. The spectra were recorded using a Varian Inova Fourier Transform spectrometer. The instrument was locked to an internal deuterium standard, consisting of 10 vol% deuterium oxide that was added to the supernatant retained after vesicle preparation. Probe temperature was 35 °C. All peak shifts were

referenced to an external standard of 10 vol% of trimethylphosphate and 10 vol% of deuterium oxide in 10 mM HEPES buffer with 160 mM potassium chloride at pH 7.5. An initial ^{31}P NMR spectrum was acquired without the addition of the Pr^{3+} shift reagent. After obtaining the spectrum of the unilamellar vesicle suspension with no shift reagent present, Pr^{3+}, as the $PrCl_3$ salt was added at varying concentrations described in Section 3. The spectrum was again recorded after maximum resonance separation was achieved, nominally after 18 h, and iminosulfuranes at various mole percentages were then added to the sample, after which the spectrum was monitored over time. All spectra were recorded in triplicate unless otherwise noted.

2.2.2. Proton NMR

An aliquot of small DMPC unilamellar vesicles, suspended in a 10-mM phosphate buffer at pH 7.4, prepared in D_2O that also contained 160 mM KCl, was added to the solid iminosulfurane TPEs so that total iminosulfurane concentration was 10 mol%. This preparation was stirred for 12 h at 40 °C followed by NOESY spectroscopy studies carried out at 35 °C using an Advance 500 spectrometer manufactured by Bruker Inc. operating at 500 MHz and equipped with a triple resonance cryoprobe. The instrument was locked to the D_2O signal for stability. A standard phase-sensitive NOESY pulse sequence utilizing water proton suppression via saturation was employed with a 0.150-s mixing time; 2048 data points were collected over 1024 increments with 64 acquisitions each. A 6009-Hz spectral width was employed. Data analysis was carried out using a sinebell window function, shifted by $\pi/3$ rad, in both dimensions. Automatic baseline correction in both dimensions was employed.

3. Results and data analyses

The ^{31}P NMR signal obtained from the nominally 300-Å diameter DMPC vesicle preparations used in these investigations consists of a single symmetrical resonance with a Lorentzian-like line shape. The individual resonances from the phosphate groups on the inner and outer leaflets of the vesicle bilayer are not resolved at the 202.4 MHz frequency of the spectrometer used in these investigations primarily because of chemical shift anisotropy that increases with the square of the magnetic field strength. It is possible to achieve partial resolution of the two ^{31}P signals at low field strengths (Bammel et al., 1987). The shape of the resonance varies with vesicle size due to incomplete dipolar averaging because the phosphate group undergoes restricted motion in the bilayer (Burnell et al., 1980). In the limit of large vesicles, the spectrum approaches that expected from a powder. Addition of the slowly penetrating paramagnetic shift reagent Pr^{3+} to the DMPC vesicle suspension causes the ^{31}P resonance to split into two components due to the differential chemical shift effect of this perturbant on the two phosphorous moieties primarily due to the difference in the proximity of the Pr^{3+} to these moieties. In these investigations the differential chemical shift is dependent upon r^{-3} where r is the distance between the Pr^{3+} ion and the phosphorous species in the DMPC bilayer (Friebolin, 1991). The maximum splitting of the ^{31}P NMR resonances was achieved by addition of 4.5 mM $PrCl_3$; however, the rate of decay of this splitting increased as the Pr^{3+} concentration was incremented. In order to maintain the Pr^{3+}-induced splitting of the ^{31}P resonance over the longest time period possible, a low concentration of $PrCl_3$, 0.45 mM, was used in most of the measurements described herein. A representative splitting of the ^{31}P NMR resonance from DMPC unilamellar vesicles is illustrated in Fig. 2A.

The basic experiment performed consisted of the addition of Pr^{3+} to the DMPC suspensions followed by the monitoring of the development of the splitting over time. After maximal splitting of the two resonances was achieved, one of the TPEs **1**–**4** was added at varying mole percentages. The effect of the TPE on the splitting

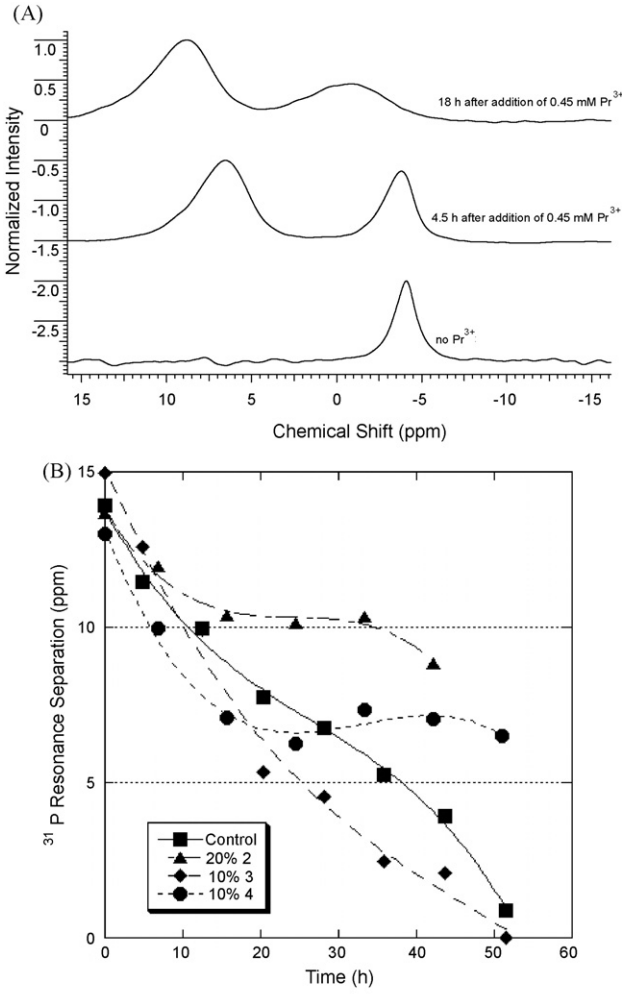

Fig. 2. (A) A representative splitting of the ^{31}P NMR resonance from DMPC unilamellar vesicles by 0.45 mM Pr^{3+}. (B). The interaction of iminosulfurane derivatives with DMPC unilamellar vesicles.

was then monitored as a function of time. In the initial measurements, vesicles consisting of pure DMPC were employed. Maximal splitting of the ^{31}P NMR resonance was observed in less than 7 h, but the splitting decayed to zero ppm in nominally 24 h (Fig. 2B). The effect of the TPEs on this splitting, however, was only marginally different from the intrinsic decay of the splitting in the control measurements with the exception of the bromo derivative **3**, present at 10 mol%, which does accelerate the collapse of the splitting. A summary of results obtained with the chloro, bromo, and iodo TPE derivatives is provided in Fig. 2B. The ^{31}P NMR resonance splitting decay does tend toward a plateau, after 20–30 h of vesicle exposure in the case of the chloro and iodo TPE derivatives **2** and **4**, indicating that these derivatives actually inhibit the collapse of the initial resonance splitting.

The duration of the Pr^{3+}-induced ^{31}P NMR resonance splitting was markedly enhanced in vesicles containing DMPC and varying mole percentages of cholesterol. At 8.8 mol% cholesterol, only a small enhancement of the duration of the splitting was observed, but at 16, 27, and 30 mol%, this splitting persisted to at least 70–92 h, the time limit over which data could be collected. Representative stack plots illustrating the time course of the Pr^{3+}-induced splitting of the ^{31}P resonances from vesicle preparations containing 16 and 30 mol% cholesterol are shown in Figs. 3 and 4. A summary of the time course of the Pr^{3+}-induced ^{31}P NMR resonance splitting is presented in Fig. 5. The time required for maximum splitting

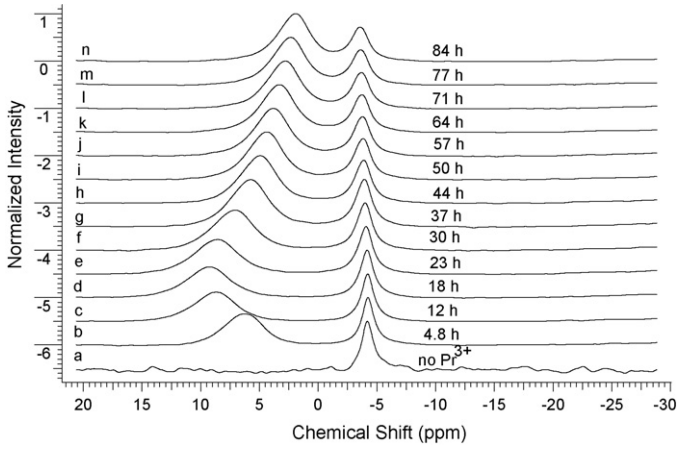

Fig. 3. A ^{31}P NMR resonance stack plot from DMPC vesicles containing 16 mol% cholesterol. Spectrum (a) is the phosphorus signal with no Pr^{3+} present. Spectra (b–n) were obtained after the addition of 0.45 mM $PrCl_3$. Resonance shifts were referenced to an external trimethylphosphate standard in HEPES buffer as described in Section 2.

also increased as the cholesterol content was increased in the vesicles. This observation is particularly evident in the case of 30 mol% cholesterol. For reasons explained in the Section 4, DMPC vesicles containing either 16 or 30 mol% cholesterol were chosen for investigations utilizing the TPE perturbants **1**–**4**. The effect of the TPEs on the ^{31}P NMR resonance splitting was markedly enhanced when cholesterol was present at 16 mol% relative to the observed perturbation of these compounds on the splitting in the absence of cholesterol. The chloro and iodo derivatives **2** and **4** accelerated the decay of the splitting by a modest amount at 40 h after the addition of these compounds to the DMPC/cholesterol vesicle system; a reduction of 20–40% in the splitting was observed. However, at the 40 h mark, the bromo compound **3** has completely collapsed the splitting and clearly is the most effective of the compounds studied in this role. A summary of the results obtained with the 16 mol% cholesterol/DMPC system is contained in Fig. 6. When the cholesterol content was increased to 30 mol%, the effect of the TPE derivatives on the Pr^{3+}-induced ^{31}P NMR resonance splitting was quite different from the effects observed at 16 mol% cholesterol. Although there is some variation in the data (due largely to the requirement that different vesicle preparations be used in each measurement), there is little if any effect of the transdermal pen-

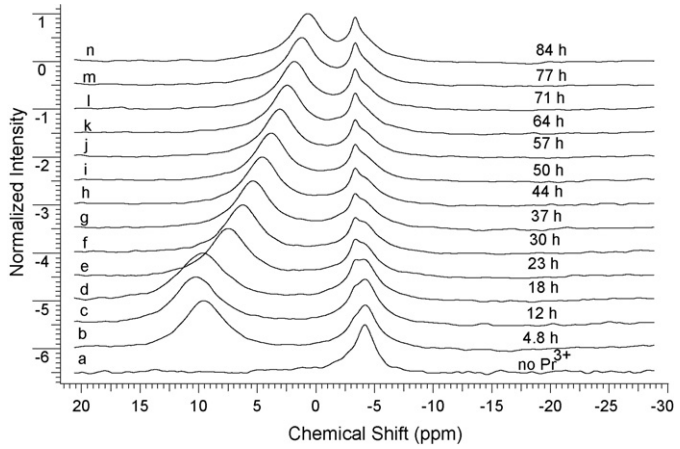

Fig. 4. A ^{31}P NMR resonance stack plot from DMPC vesicles containing 30 mol% cholesterol. Spectrum (a) is the phosphorus signal with no Pr^{3+} present. Spectra (b–n) were obtained after the addition of 0.45 mM $PrCl_3$. Resonance shifts were referenced to an external trimethylphosphate standard in HEPES buffer as described in Section 2.

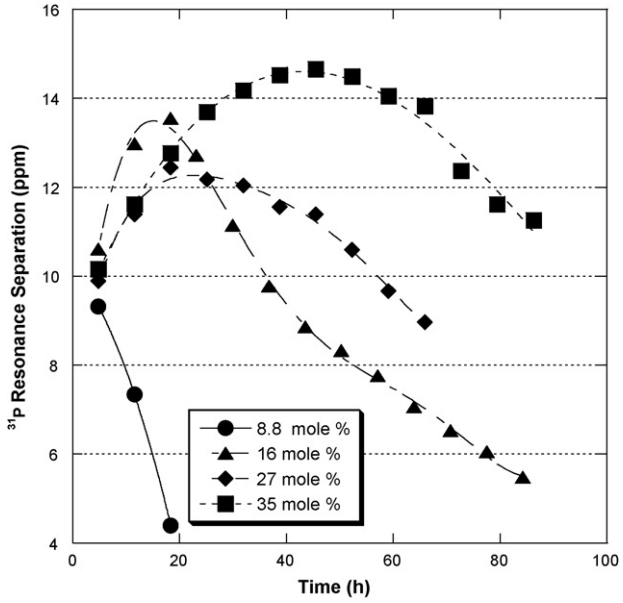

Fig. 5. The temporal development and intrinsic decay of the ^{31}P resonance separation, induced by 0.45 mM Pr^{3+}, as a function of cholesterol content in DMPC unilamellar vesicles.

etration enhancers on the ^{31}P NMR resonance splitting when the TPEs were present at 10 or 20 mol%. A summary of these observations is presented in Fig. 7. Remarkably, even if the content of the bromo derivative **3** is increased to 30 mol%, there is no increase in the reduction of the resonance splitting over 90 h (data not shown). Thus cholesterol when present at 30 mol% appears to seal the vesicle bilayer from perturbation induced by the transdermal penetration enhancers.

4. Discussion

The effect of the chloro, bromo, and iodo TPEs **2**–**4** on the ^{31}P resonance separation is modest in vesicles prepared from pure DMPC.

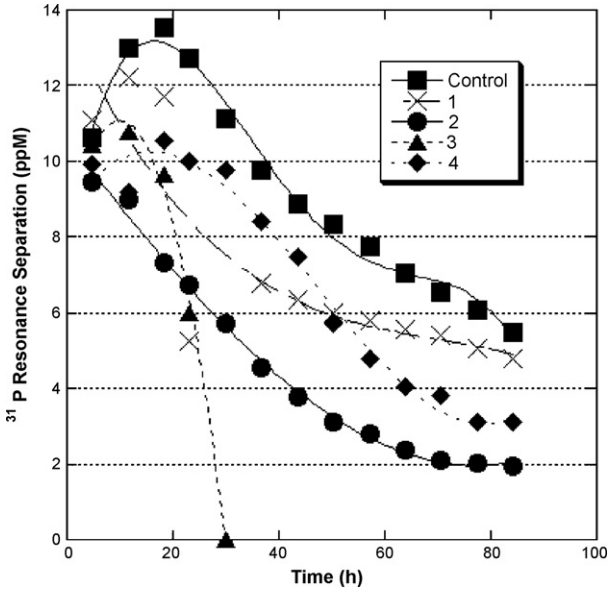

Fig. 6. A summary of the diminution of the ^{31}P resonance splitting due to the interaction of iminosulfurane derivatives with DMPC unilamellar vesicles containing 16 mol% cholesterol. ^{31}P resonance shifts were referenced to an external trimethylphosphate standard in HEPES buffer as described in Section 2.

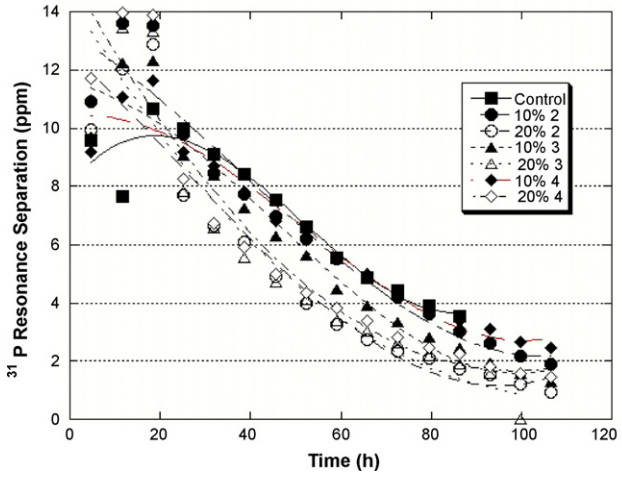

Fig. 7. A summary of the interaction of iminosulfurane derivatives with DMPC unilamellar vesicles containing 30 mol% cholesterol. ^{31}P resonance shifts were referenced to an external trimethylphosphate standard in HEPES buffer as described in Section 2.

The decay of the resonance separation is unaffected by the presence of the chloro derivative **2** over the first 20 h after it is added to the vesicle system; thereafter the splitting tends to remain essentially constant, i.e., the chloro compound is acting as a sealant in the bilayer. The iodo compound **4** does accelerate the decay rate of the ^{31}P resonance separation over the initial 20 h of bilayer exposure to it and thereafter has no additional effect and therefore tends to seal the bilayer possibly due to a rigidification of it analogous to the cholesterol effect. The latter observations also suggest that the chloro and iodo derivatives have a limited ability to penetrate the DMPC bilayer relative to that of the bromo derivative. The acceleration of the decay of the resonance splitting by the bromo derivative **3**, however, is readily apparent and develops largely monotonically over time. The perturbation of the bilayer by this derivative is thus larger and the time course of it differs from that of the chloro and iodo compounds.

The introduction of cholesterol into the DMPC vesicle preparation, however, substantially impedes the diffusion of Pr^{3+} ion in close proximity to the phosphorous moiety in the inner leaflet of the bilayer (Figs. 3 and 4). The persistence of the ^{31}P resonance splitting is accordingly increased as the mole percent of the cholesterol is increased (Fig. 5). The acceleration of the collapse of the resonance splitting induced by the perturbing iminosulfuranes, is quite apparent in the cholesterol/DMPC vesicles. Preparations containing 16 or 30 mol% cholesterol were chosen for detailed studies of the perturbations induced by the TPE derivatives. This range of cholesterol content approximates that found in human skin (Wertz et al., 1986; Hatfield and Fung, 1999). In contrast to the modest reduction in the ^{31}P resonance splitting induced by the compounds **1**, **2**, and **4**, the bromo derivative **3** is very effective in collapsing the splitting; the collapse is complete in vesicles containing 16 mol% cholesterol in less than 40 h (Fig. 6). Due to the experimental protocol used in these investigations in which the compounds were added to pre-formed vesicles, the TPE-induced perturbation of the bilayer originates at the surface of the outer leaflet but in the case of the bromo compound, and to a lesser extent for the other derivatives, the perturbation becomes trans membrane given the location of the inner-leaflet phosphate group. The Pr^{3+} species probably moves across the bilayer in a solvated form that would mitigate the large energy barrier to penetration of it into the hydrophobic region of the bilayer due to the high charge on the ion—possibly via fluctuating water channels induced by the perturbation of the transdermal agents, particularly the bromo derivatiave.

Introduction of cholesterol at 30 mol% into the DMPC vesicle preparation, however, essentially seals the bilayer. The Pr^{3+}-induced splitting of the ^{31}P resonance splitting develops more slowly in this preparation than in those containing less cholesterol but persists beyond 92 h, the time limit for data acquisition in these investigations (Figs. 4 and 5). Within the reproducibility limits dictated by the use of separate vesicle preparations for the study of each TPE derivative, no acceleration of the ^{31}P resonance splitting was observable for any of the TPE derivatives present at 10 or 20 mol% (Fig. 7). Even when the content of the bromo derivative **3** was increased to 30 mol%, no acceleration of the aforementioned resonance splitting was observed. These observations suggest that high cholesterol content may reduce or nullify the

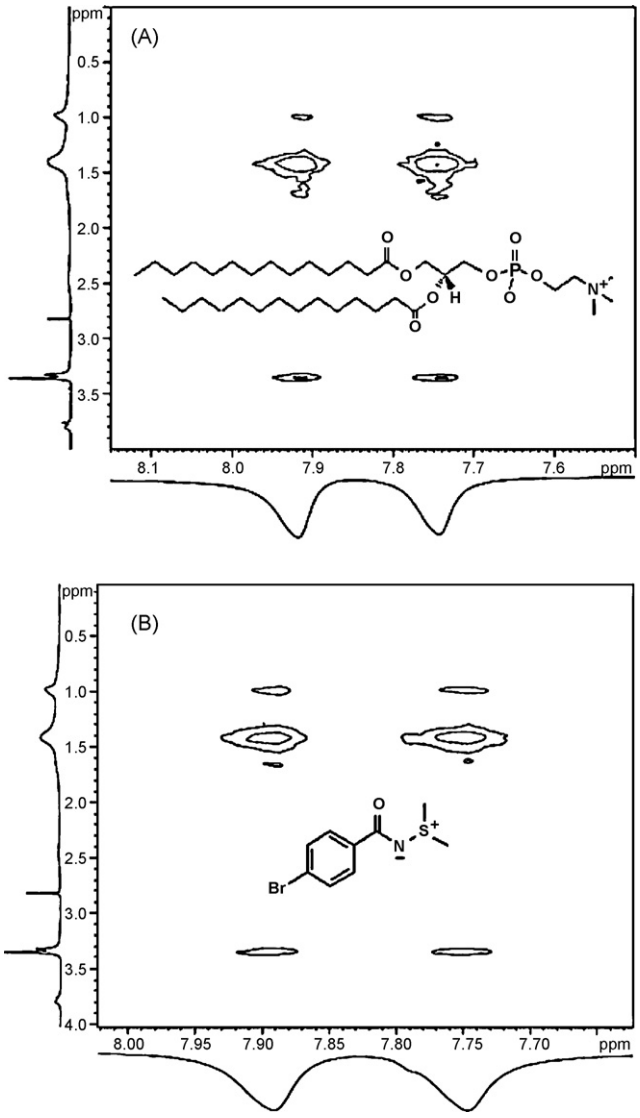

Fig. 8. (A) A contour plot, containing an overlay of the DMPC structure, derived from the NOESY spectrum of DMPC vesicles containing 16 mol% cholesterol and the bromo iminosulfurane **3** at 10 mol%. The spectrum was obtained at 35 °C with a 150-ms mixing time. Cross-peaks are observed between the two sets of equivalent phenyl protons of **3** and those of the terminal methyl group (0.95 ppm), the methylene groups at positions 4–10 (1.4 ppm), and the *N*-methyl moiety in the choline head group (3.3 ppm), a split resonance. The projections on the ordinate are resonances from the DMPC/cholesterol preparation and those on the abscissa are those from the phenyl protons of the bromo derivative **3**. (B) A contour plot, containing an overlay of the structure of the bromo iminosulfurane **3**, derived from the NOESY spectrum of DMPC vesicles containing 30 mol% cholesterol and compound **3** at 10 mol%. Others conditions are the same as in panel (A). See Section 2 for instrument parameters and preparation protocols.

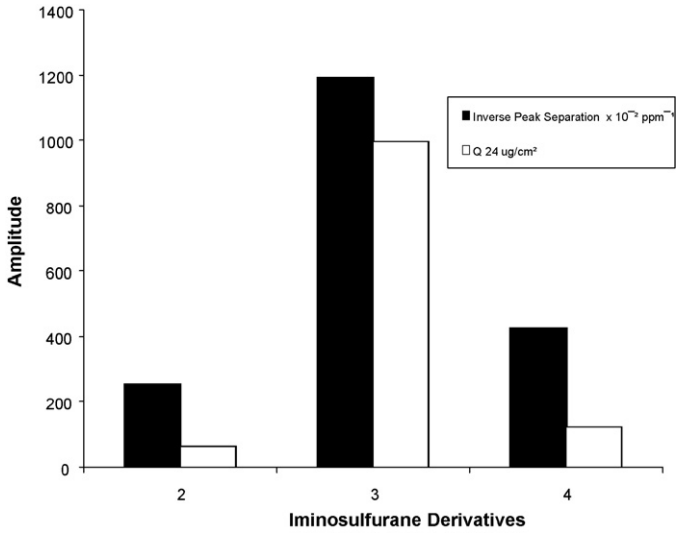

Fig. 9. A comparison of the inverse ^{31}P NMR resonance separation in DMPC vesicles containing an iminosulfurne derivative and Q_{24}, a measure of the pharmacological activity of the iminosulfurane transdermal penetration enhancers.

pharmacological activity of the iminosulfuranes; indeed, the converse relationship has previously been observed (Tsai et al., 1996). In studies based on hairless mice, the transdermal delivery of either lidocaine or caffeine induced by the closely related TPE, DMSO, was enhanced eightfold if the biosynthesis of cholesterol and fatty acids was inhibited by coapplication of fluvastatin and TOFA (5-(tetradecyloxy)-2-furancarboxylic acid).

The issue of whether cholesterol when present at 30 mol% is blocking the association of the TPEs with the DMPC bilayer is a viable explanation for the lack of any observable TPE-induced acceleration in the reduction of the ^{31}P NMR resonance splitting was addressed using NOESY NMR spectroscopy. As shown in Fig. 8, panel B, NOE cross-peaks between the phenyl protons of **3** and those of the DMPC fatty acid terminal methyl groups, the unresolved methylene groups at the fatty acid chain positions 4–10, and the *N*-methyl choline head group are observed in DMPC vesicle preparations containing cholesterol at 30 mol%; the same NOE cross-peak pattern is present as was observed in vesicles containing 16 mol% cholesterol (panel A) and no cholesterol (Barrow et al., 2005). Note that in Fig. 8, the choline head group *N*-methyl resonance and associated cross-peak to the phenyl protons is split suggesting that the bromo derivative **3** may have completely penetrated the bilayer and is populating the surface of the inner leaflet of the vesicle bilayer. The distribution of compound **3**, present at 10 mol%, is thus by these criteria similar to that in vesicles prepared from DMPC only, but this derivative is unable to render the bilayer containing 30 mol% cholesterol sufficiently permeable to the Pr^{3+} ion so that the paramagnetic ion can closely approach the phosphorous moiety in the inner leaflet—via the development of water channels or other perturbations, due to the rigidification of the bilayer by the cholesterol.

That the collapse of the ^{31}P resonance splitting induced especially by compound **3** has a major contribution from the exchange of the DMPC lipids between the inner and outer bilayer leaflets is unlikely since in phosphocholine unilamellar vesicles this spontaneous exchange occurs with a half life on the order of tens of hours to days (Kornberg and Mcconnell, 1971; Papadopulos et al., 2007). The exchange contribution to the diminution of the splitting is also included in the intrinsic decay of the splitting in the control to which data analyses described herein are referenced.

Historically, a correlation between the activity of transdermal penetration enhancers in skin samples and their behavior in model systems such as that employed in these investigations has been observed (Beastall et al., 1988; Johnson et al., 1996). Such a correlation was indeed observed in the current investigation in that the inverse splitting of the ^{31}P NMR resonance is closely correlated with the Q_{24} values measured by Song et al. (2005) for the chloro, bromo, and iodo compounds **2**–**4**. A histogram illustrating this correlation is presented in Fig. 9.

5. Conclusions

The data described in the previous section suggest that the perturbation of the bromo iminosulfurane TPE derivative **3** extends across the bilayer, i.e., it is trans membrane; this observation strongly supports a previously proposed model for the high activity of this compound that is based on deep penetration of a subpopulation of the derivative that is then entrapped in the bilayer on a time scale of tens of hours (Barrow et al., 2005). The behavior of the bromo derivative **3** in these investigations differs markedly from that of the other analogs as was also observed in previous studies (Barrow et al., 2005). The perturbation of the bilayer as measured by the reduction in the ^{31}P resonance splitting by the iminosulfurane TPE derivatives correlates, as expected, with the pharmacological activity of these compounds.

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