Biochimica et Biophysica Acta, 1105 (1992) 213–220

BBAMEM 75576

The interaction of various cholesterol 'ancestors' with lipid membranes: a ^{2}H-NMR study on oriented bilayers

Marie-Anne Krajewski-Bertrand, Alain Milon [1], Yoichi Nakatani and Guy Ourisson

Laboratoire de Chimie Organique des Substances Naturelles, associé au CNRS, Université Louis Pasteur, Centre de Neurochimie, Strasbourg (France)

(Received 12 November 1991)

Key words: Molecular evolution; NMR, ^{2}H-; Lipid membrane; Tricyclohexaprenol; Octaprenediol; Isoarborinol

The effect of putative cholesterol 'precursors' on model membranes has been studied by deuterium nuclear magnetic resonance (^{2}H-NMR) spectroscopy. Oriented bilayers were prepared from 1-myristoyl-2-[$^2H_{27}$]myristoyl-*sn*-glycero-3-phosphocholine (DMPC-d_{27}) and tricyclohexaprenols or octaprenediols. Order parameter profiles were determined and showed that tricyclohexaprenols and octaprenediols increase the acyl chain order in DMPC bilayers, but to a smaller extent than cholesterol. The order parameter increases, depending on the chain position, from 5% to 7% in the presence of ditertiary octaprenediol, and from 16% to 21% in the presence of tricyclohexaprenol-*Z*,*Z*. Aqueous multilamellar dispersions of DMPC-d_{27} and of DMPC-d_{27} containing 30 mol% tricyclohexaprenol-*E*,*E* were prepared, and the first moments calculated from ^{2}H-NMR spectra over the temperature range 5–55°C. Tricyclohexaprenol-*E*,*E* almost abolishes the phase transition of DMPC. Thus, as predicted, tricyclohexaprenols and octaprenediols have a cholesterol-like behaviour in lipid membranes; however their effect on the model DMPC system is weak. On the contrary, isoarborinol has no effect on the lipid chain order in the liquid-crystalline phase of DMPC bilayers. ^{2}H-NMR spectra of aqueous dispersions of DMPC-d_{27} and 30 mol% isoarborinol between 25 and 60°C showed the coexistence of two lamellar phases over a wide temperature range, which was confirmed by differential scanning calorimetry (DSC) and ^{31}P-NMR spectroscopy. This absence of ordering effect of isoarborinol might be related to some inherent structural features.

Introduction

A general theory of the molecular evolution of biomembrane constituents was developed in 1979 by Ourisson and co-workers [1]. It was postulated that various polyterpenoids could be reinforcers of bacterial membranes, and could have the same mechanical role in lipid bilayers as cholesterol in eucaryotes. We have developed methodologies to study the incorporation and the reinforcing effect of these molecules in lipid vesicles; the osmotic swelling of vesicles allows the evaluation of the elasticity of bilayers and their water permeability [2,3]. We have thus demonstrated that α,ω-dihydroxylated carotenoids do stabilize DMPC model membranes [3,4], as well as bilayers composed of the bacterial lipids of *Halobacterium* [5]. The reinforcing effect of hopanoids has also been shown [6–9].

We are now interested in studying the effect of other molecules postulated to be phylogenetic precursors of cholesterol [10] (Fig. 1). They are all polyisoprenoids, i.e. they are built from C_5 isoprenic units. Tricyclohexaprenols **1** and **2** and isoarborinol **5** have the molecular dimensions, amphiphilic nature and partial rigidity which could make them good surrogates of cholesterol. Tricyclohexaprenol-*E*,*E* **1** and tricyclohexaprenol-*Z*,*Z* **2** could be the products of a simple cyclization of hexaprenols. Isoarborinol **5** may be viewed as an intermediate structure between hopanoids and lanosterol, a precursor of sterols. Octaprenediols **3** and **4** could be α,ω-dihydroxylated carotenoid surrogates, with their molecular dimensions and amphiphilic nature. The ditertiary octaprenediol **4** may be the product of an acid-catalyzed hydration of the tail-to-tail

[1] Present address: Centre de Recherche de Biochimie et de Génétique Cellulaires, CNRS, UPR 8201, 118 route de Narbonne, 31062 Toulouse, France.

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPC-d_{27}; 1-myristoyl-2-[$^2H_{27}$]myristoyl-*sn*-glycero-3-phosphocholine; ^{2}H-NMR, deuterium nuclear magnetic resonance; ^{31}P-NMR, phosphorus-31 nuclear magnetic resonance; DSC, differential scanning calorimetry; HPLC, high performance liquid chromatography; TLC, thin-layer chromatography.

Correspondence: G. Ourisson, Université Louis Pasteur, Centre de Neurochimie, 5 rue Blaise Pascal, 67084 Strasbourg, France.

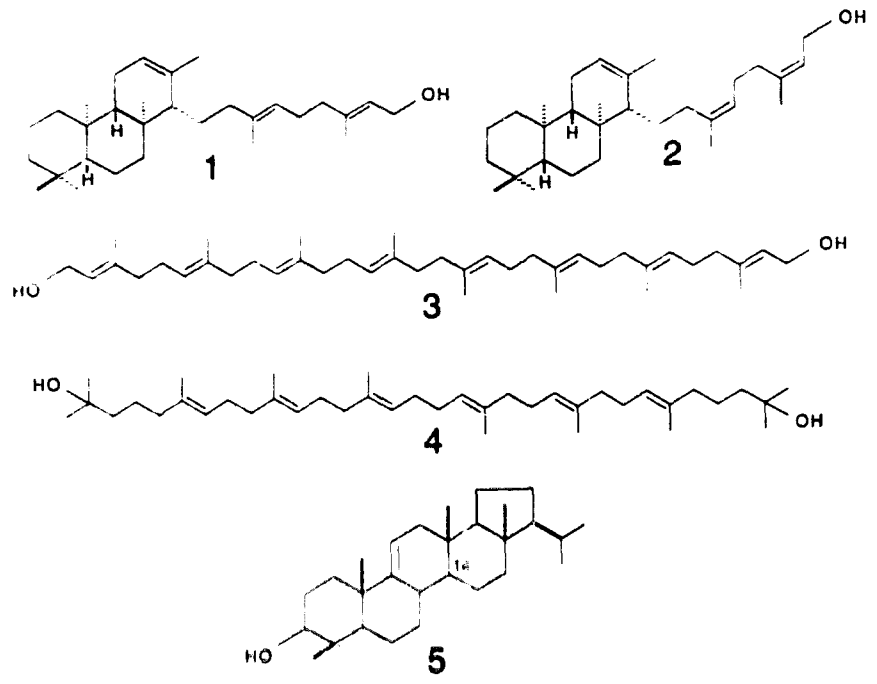

Fig. 1. Structure of the 'cholesterol precursors' studied: **1**, tricyclohexaprenol-*E*,*E*; **2**, tricyclohexaprenol-*Z*,*Z*; **3**, diprimary octaprenediol; **4**, ditertiary octaprenediol; **5**, isoarborinol.

dimerization product of geranylgeraniol. The diprimary octaprenediol **3** could be obtained by the head-to-head dimerization of geranylgeraniol, a reaction that could be the first step of the biosynthesis leading to archaebacterial C_{40} lipids. Compound **4** would still require dehydrogenations to become a carotenoid, and compound **3** would require hydrogenations to become a fragment of an archaebacterial lipid.

Recently, we have shown that two of these molecules, tricyclohexaprenol-*E*,*E* **1** and the ditertiary octaprenediol **4**, can act as weak membrane reinforcers [11]. They are well incorporated in lipid membranes; they do not have a marked effect on the elasticity of the bilayer but they do lower the water permeability through the membrane. We now intend to gain new insights into the effect of such substances on a lipid membrane and to compare in more detail all these postulated cholesterol precursors. ^{2}H-NMR provides an excellent method to investigate lipid chain dynamics in bilayers [12,13]. By using oriented bilayers, the sensitivity of this technique is greatly increased [14].

The hydrocarbon skeletons of tricyclohexaprenols and isoarborinol have been found in sediments, where they might originate from microorganisms [15]. However, tricyclohexaprenols and octaprenediols are still unknown in living organisms. Isoarborinol is present in small amounts in a few higher plants [16], but has never been identified in bacteria. Thus, the choice of phospholipids to be used for membrane studies is largely arbitrary. We decided to study all these 'cholesterol precursors' in 1-myristoyl-2-[$^2H_{27}$]myristoyl-*sn*-glycero-3-phosphocholine (DMPC-d_{27}); we have already used extensively DMPC with various membrane reinforcers, which will allow us to compare all these substances in the same model system.

Experimental

Materials

The tricyclohexaprenols were a gift from Dr. D. Heissler [15]. Octaprenediols were a gift from Dr. B. Chappe [17]. Isoarborinol acetate was a gift from Prof. S. Natori. It was hydrolyzed with potassium hydroxide in 1-propanol/water (10:1, v/v). Isoarborinol was recrystallized from chloroform/ethanol.

2-Lysomyristoylphosphatidylcholine was purchased from Sigma. Deuterium-depleted water and 4-pyrrolidinopyridine were purchased from Aldrich.

Synthesis of 1-myristoyl-2-[$^2H_{27}$]myristoyl-sn-glycero-3-phosphocholine (DMPC-d_{27})

Perdeuterated myristic acid was prepared by catalytic exchange with D_2O as previously described [18]. The deuteration level was 98% as measured by mass spectroscopy. DMPC-d_{27} was synthesized by acylation of 2-lysomyristoylphosphatidylcholine by [$^2H_{54}$]myristic anhydride in presence of 4-pyrrolidinopyridine as described by Perly et al. [19]. DMPC-d_{27} was chromatographed on silica gel and further purified by HPLC (Waters) on a silica gel column packed with Partisil 10 (250 × 9.4 mm) from Whatman. The mobile phase consisted of a gradient of hexane/2-propanol/water, from 39:52:6 (by vol.) to 39:52:8.5 (by vol.). The flow rate was 3 ml/min. The purity of DMPC-d_{27} was checked by TLC on F_{254} silica gel plates (0.25 mm thick from Merck; eluent: chloroform/methanol/water (6:8:0.4, v/v).

^{1}H-NMR (200 MHz, $CDCl_3$): δ 5.19 (m, 1H, glycerol CH), 4.40 and 4.12 (m, 2H, glycerol CH_2OCO), 4.36 (m, 2H, choline CH_2OP), 3.97 (m, 2H, glycerol CH_2OP), 3.88 (m, 2H, CH_2N), 3.39 (s, 9H, $N(CH_3)_3$), 3.11 (broad, residual H_2O), 2.28 (m, 2H, CH_2CO), 1.57 (m, 2H, CH_2CH_2CO), 1.25 (m, 20H, $(CH_2)_n$), 0.87 (t, 3H, CH_3).

MS (FAB$^+$): *m/z*, 705.6 (MH^+), 224.1; (FAB$^-$) *m/z*, 689.6 ($M - CH_3$), 644.5, 618.5, 254.3 (perdeuterated acyl chain), 227.2 (non-deuterated acyl chain).

Preparation of NMR samples

To prepare oriented samples, 10 mg of DMPC-d_{27} and the appropriate amount of the additive were dissolved in 2-propanol, or in chloroform in the case of isoarborinol. The organic solution was applied dropwise on microscope cover glasses (45 to 50 plates, 20 × (6–9) × 0.15 mm) and dried under vacuum overnight. The plates were stacked in a 10-mm (o.d.) NMR tube. To hydrate the lipids, about 50 μl of deuterium-depleted water was added, and the samples were allowed to stay at 35°C at least for 3 h; then the tube was sealed. It was shown that a preliminary hydration with water vapour at 40°C did not improve the quality of the samples.

Aqueous multilamellar dispersions for ^{2}H- or ^{31}P-NMR were prepared as follows: DMPC-d_{27} and the additive were dissolved in chloroform and the solvent was evaporated. The lipid film was hydrated with 300 μl of deuterium-depleted water and heated to 40°C with vortex-mixing to homogeneity. The sample was then lyophilized and rehydrated. This procedure was repeated twice in the case of DMPC-d_{27} + 30 mol% isoarborinol to obtain a homogeneous dispersion. The liposomes were transferred to a 5-mm (o.d.) NMR tube.

NMR spectroscopy

All NMR measurements were carried out on a Bruker MSL-300 spectrometer; ^{2}H spectra were recorded at 46.053 MHz with the quadrupolar echo sequence: 90_y-t-90_y-τ-acquire, with t = 25 μs and a 90° pulse of about 7 μs (10 mm coil) or 4 μs (5 mm coil). The recycle time was 1 s unless otherwise stated. A spectral width of 250 kHz was used for oriented samples, and of 500 kHz for liposomes.

The observed quadrupolar splitting of a C-D bond having an axially symmetric motion is given by:

$$\Delta\nu_Q = (3/2)\cdot(e^2qQ/h)\cdot S_{C\text{-}D}\cdot(3\cos^2\theta - 1)/2$$

with: e^2qQ/h the static quadrupolar coupling constant (168 kHz for aliphatic C-D bond), $S_{C\text{-}D}$ the C-D bond order parameter and θ the angle between the symmetry axis for the motion and the magnetic field direction.

It has been shown that the bilayer normal is the director of motional averaging for lipids in various membranes, especially in DMPC bilayers [20]. In these experiments the bilayer normal was set perpendicular to the magnetic field (θ = 90°), by positioning manually the NMR-tube into the probe. The estimated accuracy of the angular settings was ±2° as determined by multiple settings of the 90° orientation.

A segmental order parameter S_{mol} can be assigned to each labelled position and is expressed as [12,21]:
$S_{mol} = -2\ S_{C\text{-}D}$ for the methylene groups,
$S_{mol} = -6\ S_{C\text{-}D}$ for the terminal methyl group.

First moments (M_1) of the ^{2}H-NMR spectra were calculated using the expression:

$$M_1 = \int_0^\infty \omega f(\omega)\,d\omega \Big/ \int_0^\infty f(\omega)\,d\omega$$

where $f(\omega)$ is the spatial intensity at frequency ω.

^{31}P spectra were obtained at 121.496 MHz, using a Hahn echo pulse sequence and inverse gated broad-band proton decoupling (decoupling power: 7 W). The pulse spacings in the echo sequence were 50 ms, with a 90° pulse of 10.7 μs; the recycle time was 3 s. A spectral width of 50 kHz was used.

Differential scanning calorimetry (DSC)

DSC measurements were performed on a Perkin-Elmer DSC-4 instrument. The heating rate was 0.2 C°/min over the temperature range of 10°C to 60°C.

Results

^{2}H-NMR studies of tricyclohexaprenols and octaprenediols in DMPC-d_{27} bilayers

The tricyclohexaprenol-*E,E* and the ditertiary octaprenediol can be incorporated into lipid bilayers in high concentration: respectively 30 mol% in DMPC and 25 mol% in the polar lipids of *Halobacterium halobium* [11]. These incorporation ratios were used to prepare the oriented samples, i.e. to DMPC-d_{27} was added either 30 mol% of tricyclohexaprenol-*E,E*, or -*Z,Z*, or 25 mol% of the ditertiary or diprimary octaprenediol. Fig. 2 shows the ^{2}H spectra of these systems at 40°C, compared to the spectrum of pure DMPC-d_{27} bilayers. It must be pointed out that oriented samples allow a good resolution of the labelled positions along the phospholipid chain, and that a good signal-to-noise ratio is obtained with a moderate number of transients, although the amount of DMPC-d_{27} is small.

Order parameter profiles are plotted in Fig. 3. The peak assigments of the oriented spectra were made on the basis of peak intensity and on the assumption that S_{mol} decreases monotonically from position 3 to the terminal methyl group as is the case in pure DMPC

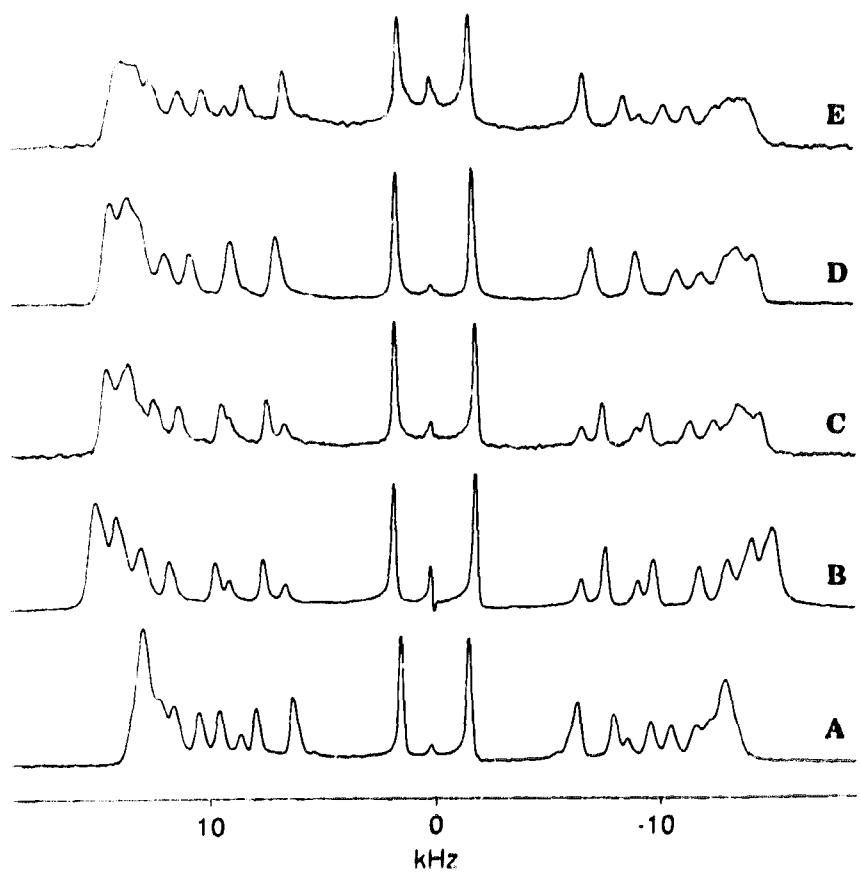

Fig. 2. ^{2}H-NMR spectra of 90° oriented multibilayers of: A, DMPC-d_{27}; B, DMPC-d_{27} + 30 mol% tricyclohexaprenol-*Z,Z*; C, DMPC-d_{27} + 30 mol% tricyclohexaprenol-*E,E*; D, DMPC-d_{27} + 25 mol% diprimary octaprenediol; E, DMPC-d_{27} + 25 mol% ditertiary octaprenediol. Number of scans, 12000; recycle time, 1 s; temperature, 40°C.

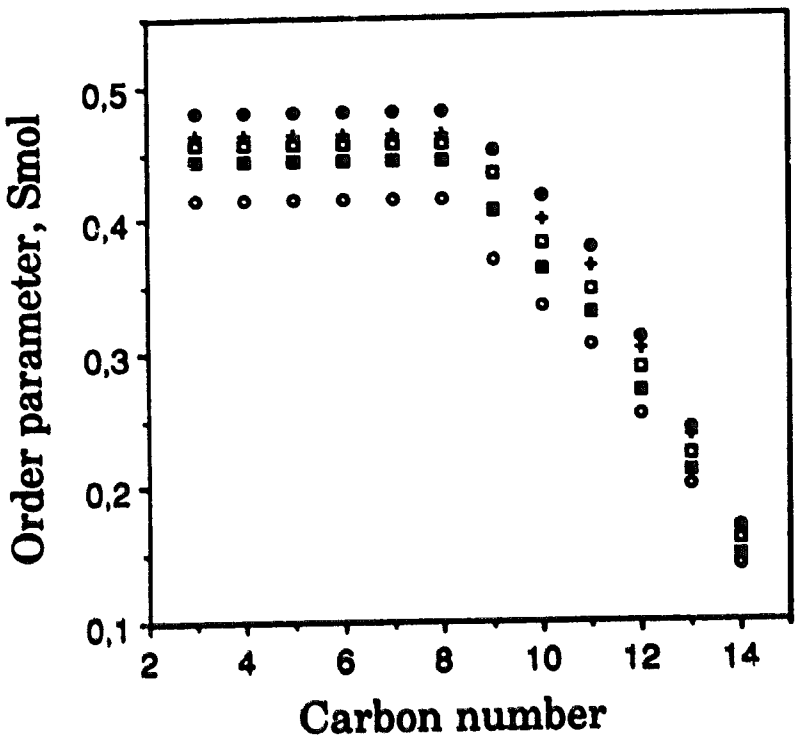

Fig. 3. Order parameter profiles for the various systems: ●, DMPC-d_{27} + 30 mol% tricyclohexaprenol-*Z,Z*; +, DMPC-d_{27} + 30 mol% tricyclohexaprenol-*E,E*; □, DMPC-d_{27} + 25 mol% diprimary octaprenediol; ■, DMPC-d_{27} + 25 mol% ditertiary octaprenediol; ○, DMPC-d_{27}. The segmental order parameter S_{mol} was calculated from the spectra of the 90° oriented multibilayers at 40°C.

bilayers [12,22]. All the products studied have a weak ordering effect on DMPC bilayers : the presence of any of these 'cholesterol precursors' increases S_{mol} for all the positions along the chain, in the sequence tricyclohexaprenol-*Z,Z* > tricyclohexaprenol-*E,E* > diprimary octaprenediol > ditertiary octaprenediol. Nevertheless, their effect is much smaller than that of cholesterol [22,23]: the order of the plateau region is increased by 7% by the ditertiary octaprenediol, and by 16% by the tricyclohexaprenol-*Z,Z* whereas S_{mol} of the terminal methyl group increases by 5% to 21% for the same products. When incorporated at 30 mol%, cholesterol increases S_{mol} by 78% to 130% for the plateau region and the terminal methyl group, respectively [23].

Multilamellar liposomes were prepared from either pure DMPC-d_{27} or DMPC-d_{27} + 30 mol% tricyclohexaprenol-*E,E* in excess deuterium-depleted water, and their ^{2}H-NMR spectra were recorded at various temperatures between 5°C and 60°C. Fig. 4 shows the thermal variation of the spectral first moment M_1 for the two systems. DMPC-d_{27} presents a marked phase transition at T_c = 23°C. Below 22°C, spectra are characteristic of a gel phase whereas, above 25°C, axially symmetric powder spectra are obtained, which indicates that the DMPC-d_{27} bilayers are in a liquid-crystalline state. M_1 follows this course: it decreases slightly from 5 to 22°C then drops during the phase transition by about a factor 2, and finally decreases with a weak slope. The presence of tricyclohexaprenol-*E,E* in the bilayers almost abolishes the phase transition and induces a gradual gel to liquid-crystal evolution. The liquid-crystalline bilayers show higher values of M_1 than the pure DMPC-d_{27} bilayers: this indicates that tricyclohexaprenol-*E,E* increases the order of lipid chains, as M_1 of an axially symmetric ^{2}H-NMR powder pattern spectrum is proportional to the mean quadrupolar splitting [13]. In the gel phase, the M_1 value for DMPC-d_{27} + 30 mol% tricyclohexaprenol-*E,E* is smaller than for pure DMPC-d_{27}; this indicates a perturbation of the lipid chain packing, which results in a very gradual transition toward the liquid-crystalline state with increasing temperature. This behaviour is similar to that observed for various phospholipid-cholesterol systems (see for example, Ref. 24, for a detailed study of DPPC-d_{62}-cholesterol mixtures) with the already mentioned difference that cholesterol increases much more efficiently the order of lipid chains in the fluid phase, and abolishes the phase transition to a greater extent.

DMPC-d_{27} + 30 mol% isoarborinol

Because of the small amount of this compound available to us (15 mg), we studied directly systems containing high molar ratio (30 mol%) of isoarborinol in DMPC-d_{27}, with the intent to observe the maximum effect, if any, of this product on bilayers. Oriented bilayers were prepared following the procedure already described, and a ^{2}H-NMR spectrum was recorded (data not shown). It appeared that the phospholipids were poorly oriented, and the powder type spectrum presented nearly the same quadrupolar splittings as pure DMPC-d_{27}.

As it could be possible that isoarborinol formed precipitates excluded from the phospholipid bilayers, aqueous multilamellar dispersions containing 30 mol%

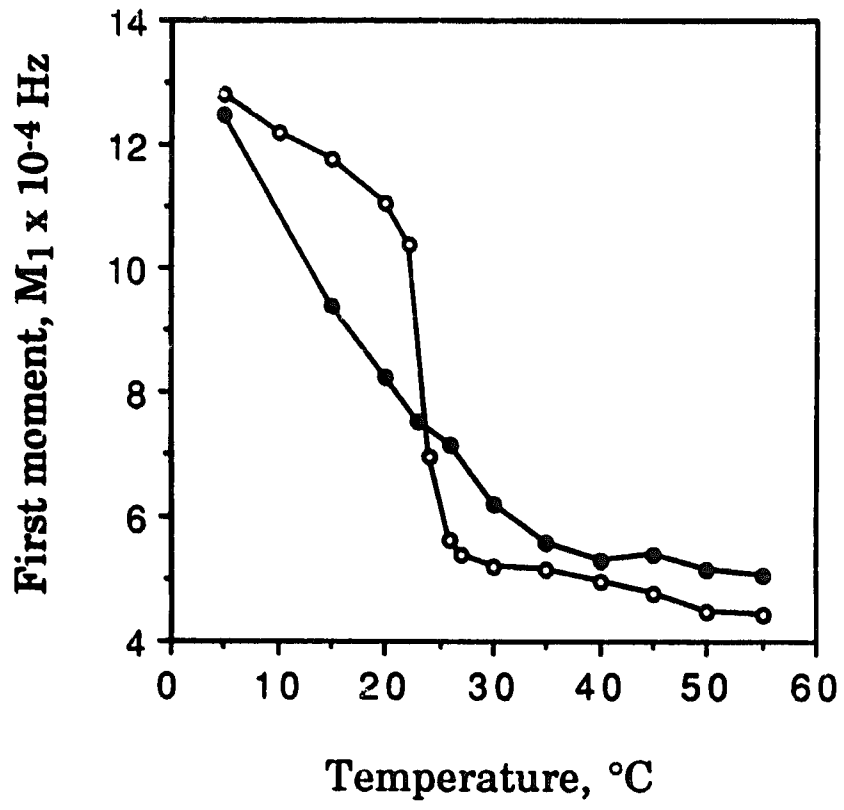

Fig. 4. Variation of the first moment M_1 with temperature for liposomes of: ○, DMPC-d_{27}; ●, DMPC-d_{27} + 30 mol% tricyclohexaprenol-*E,E*.

isoarborinol in DMPC-d_{27} were prepared in an attempt to optimize the mixing of both products. DSC measurements done with these liposomes showed a very broad and dissymmetrical transition, with an onset temperature of 14°C, a maximum at 20°C, and a completion temperature of about 42°C. No sharp peak at 23°C could be detected. This demonstrates that there is an effective mixing between isoarborinol and DMPC-d_{27} and at least a partial miscibility below the phase transition.

^{31}P-NMR spectra were obtained as a function of temperature. Fig. 5B shows the proton-decoupled ^{31}P spectrum of DMPC-d_{27} + 30 mol% isoarborinol at 25°C. It is identical with the spectrum of pure DMPC at 30°C (Fig. 5A), i.e. it is an axially symmetrical spectrum characteristic of a lamellar organization of phospholipids, with a chemical shift anisotropy $\Delta\sigma = 46$ ppm (measured as the frequency separation between the main peak and the low-field shoulder) and an estimated linewidth at half-height of 500 Hz. Similar spectra were obtained at 40°C and 60°C for the DMPC-isoarborinol liposomes, with $\Delta\sigma$ slightly decreasing to 42 ppm at 60°C.

Deuterium NMR spectra of the same liposomes were recorded as a function of temperature between 25°C and 60°C, and are shown in Fig. 6. The most striking feature of the spectrum at 25°C (Fig. 6a) is the occurrence of two distinct superimposed patterns. The

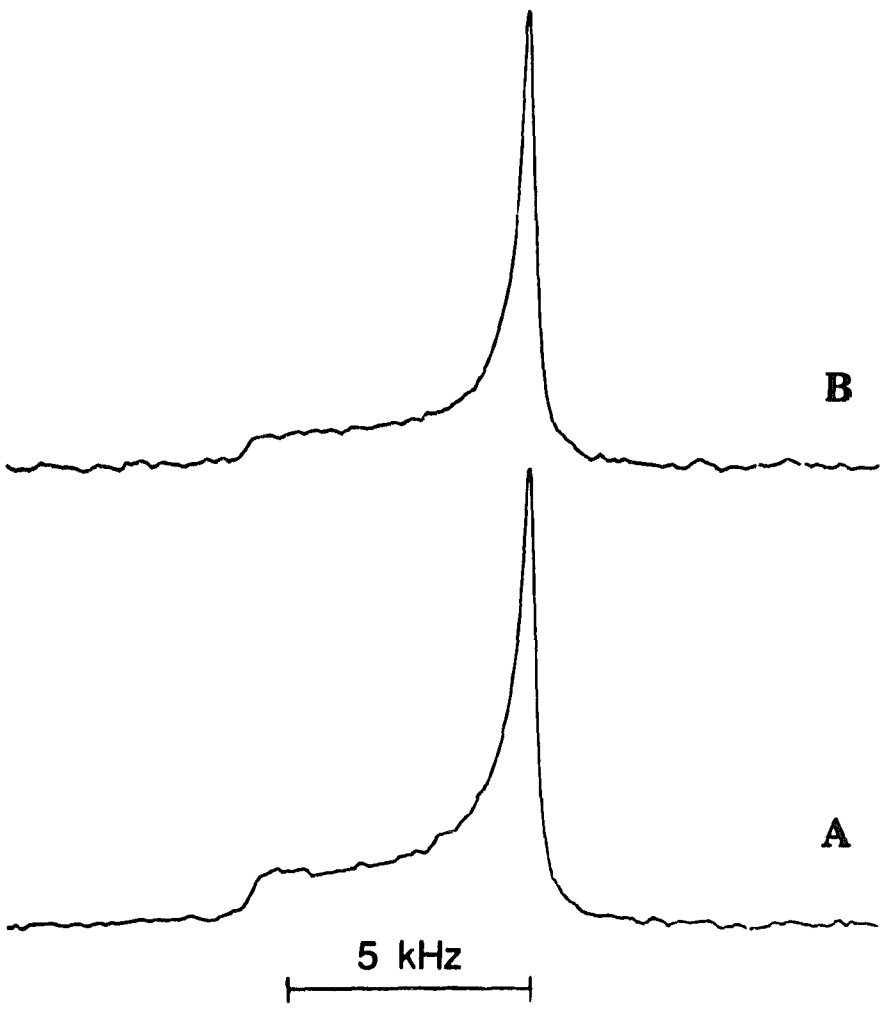

Fig. 5. Proton-decoupled ^{31}P-NMR spectra of multilamellar dispersions of: A, DMPC-d_{27} (250 mg) at 30°C; B, DMPC + 30 mol% isoarborinol (25 mg DMPC-d_{27}) at 25°C. Number of scans, 300 for (A), 1200 for (B); recycle time, 3 s, line broadening, 150 Hz.

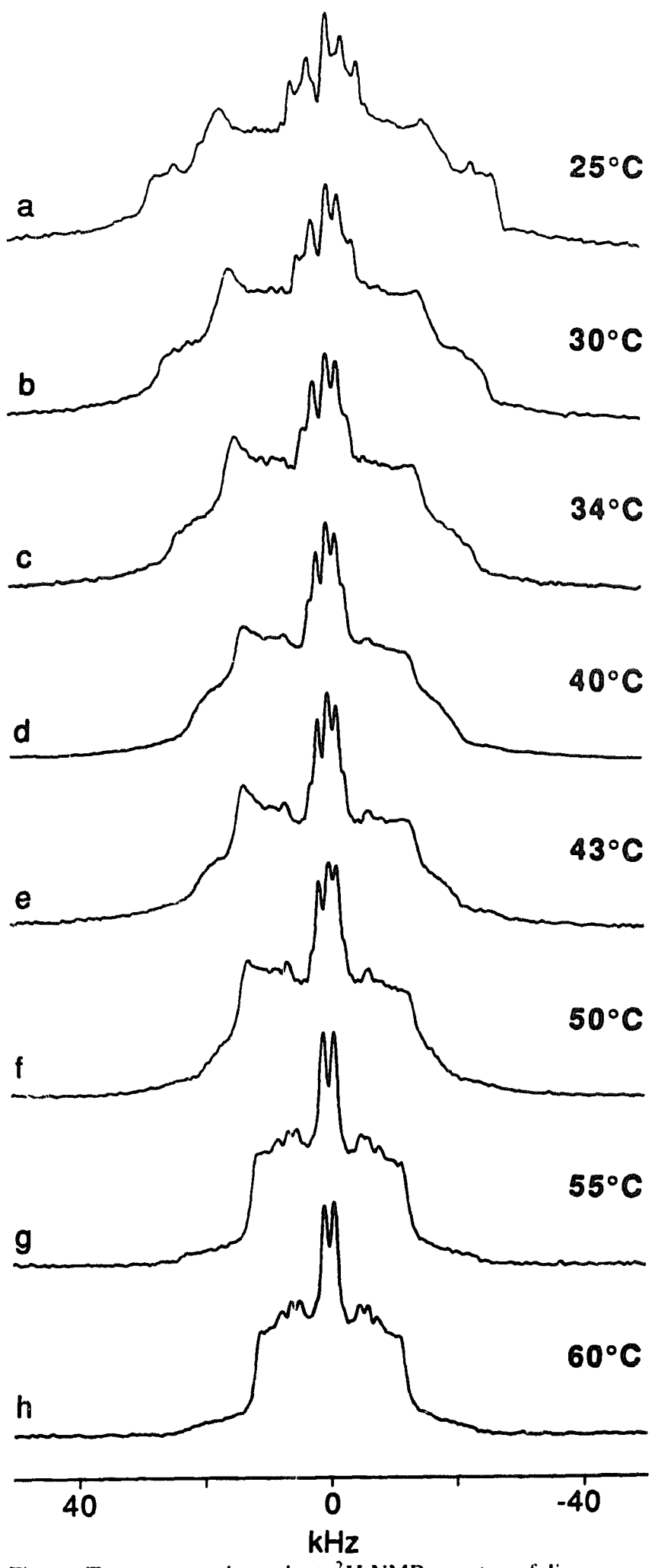

Fig. 6. Temperature-dependent ^{2}H-NMR spectra of liposomes of DMPC-d_{27} + 30 mol% isoarborinol. Number of scans: 9600 for a, b, c, e, f, 58000 for d, 4600 for g and 2100 for h; recycle time, 500 ms; line broadening, 150 Hz.

first one has a narrow central doublet (splitting = 5.2 kHz) and edges split by 32.5 kHz. The wider pattern shows a central doublet (splitting = 10.3 kHz) and edges split apart by 55 kHz. This wide pattern progressively disappears with increasing temperatures: at 30°C the

position of the central doublet and of the edges can still be clearly identified (splittings 8.7 and 50 kHz, respectively), but from 34°C to 50°C these components appear as shoulders in the main spectrum. At 55°C and 60°C, the spectra are characteristic of a single liquid crystalline phase.

This complex thermal behaviour of isoarborinol-DMPC-d_{27} mixtures will be discussed in more detail in the next section.

Discussion

The results obtained from ^{2}H-NMR spectra of oriented bilayers show that tricyclohexaprenols and octaprenediols are completely miscible with DMPC, and have an ordering effect on the lipid chains. This effect is very weak for the ditertiary octaprenediol, as S_{mol} increases only by 5 to 8% depending on the carbon atom position, whereas it is slightly higher for the diprimary octaprenediol, as S_{mol} increases by 10 to 13%. Octaprenediols could be considered as weak surrogates of α,ω-dihydroxylated carotenoids, which have been shown to reinforce membranes like transmembrane 'rivets' [2,3]. However, octaprenediols do not have a rigid structure, contrary to carotenoids. Our previous study of the ditertiary octaprenediol [11] as well as results obtained with the diprimary diol (unpublished results) indicate that both compounds lower the water permeability of bilayers, but less efficiently than carotenoids in the same bacterial lipid membrane. The present ^{2}H-NMR study shows that octaprenediols do not order lipid chains significantly more than C_{20} related molecules, phytol and phytanic acid. Cushley and co-workers have indeed demonstrated that phytol or phytanic acid, incorporated at 20 mol% in a mixture of 1-palmitoyl-2-[$^2H_{31}$]palmitoylphosphatidylcholine and 1-stearoyl-2-[$^2H_{31}$]palmitoylphosphatidylcholine, increase the mean order parameter by 9% [25].

The weak effect of octaprenediols could indicate that they do not act as transmembrane 'rivets'. On the contrary, because of their flexibility they could adopt various conformations in bilayers. For instance both of their hydroxyl groups could be on the same side of the membrane (U-shaped conformation). The hydrophobic parts of octaprenediols are either primary or tertiary alcohols, i.e. groups of weak polarity compared to usual phospholipid polar heads. Such groups could even be embedded in part in the hydrophobic core of the lipid bilayer. The chain could then adopt a helical conformation, which seems to be of low energy, as seen by molecular modelling (Milon, A., unpublished data). It has indeed been shown that in C_{40} archaebacterial lipids which possess glycerol at one or both polar heads (like di(bisphytanyl) diglyceryl tetraether), the glycerol head groups are partly soluble in the hydrocarbon matrix [26].

The occurrence of various conformations in the membrane could account for the observed difference between the two diols. As a tertiary alcohol is less polar than a primary alcohol, arrangements with one or both heads in the lipidic phase could be made easier, thus leading to the very weak ordering effect of the ditertiary diol, which is smaller than that of the diprimary diol.

In contrast, tricyclohexaprenols are molecules that possess a rigid tricyclic part and dimensions similar to those of cholesterol. However, the hydroxyl group is attached to the flexible part of these molecules, whereas it is attached to the rigid tetracyclic part of cholesterol. This situation can be compared with that of hopanoids. The present work shows that tricyclohexaprenols increase significantly the order of lipid chains, though to a smaller extent than cholesterol. Their effect can be compared to that of α-tocopherol [27]; when incorporated at 20 mol% in lecithin bilayers, this substance increases the mean order parameter by 17%. Depending on the carbon atom position, the segmental order parameter of DMPC-d_{27} bilayers containing 30 mol% of tricyclohexaprenol-*E*,*E* increases by 12 to 19%, and with 30 mol% of the *Z*,*Z* isomer it increases by 16 to 24%.

The influence of tricyclohexaprenol-*E*,*E* on the phase transition of DMPC-d_{27} is similar not only to that of cholesterol (vide infra) but also to what is observed in phytol-, phytanic acid- or α-tocopherol-lecithin mixtures [25,27]. All these substances, because of their bulky structure, perturb the phospholipid packing in the gel phase, but they do increase the order of the acyl chains in the fluid phase.

Such a behaviour of polycyclic or branched amphiphiles is radically different from what is observed for linear molecules like palmitic acid or tetradecanol [28,29]. Both have been shown to broaden and shift the phase transition of the host phospholipids to markedly higher temperatures. This demonstrates that these molecules stabilize the gel phase of phospholipids. In the liquid crystalline phase, tetradecanol at 33 mol% has no ordering effect on DMPC bilayers [29], whereas palmitic acid, incorporated at 20 mol%,increases the average order parameter of DPPC-d_{62} by 10% [28]. Finally, it must be mentioned that long-chain polyisoprenols, i.e. C_{55} undecaprenol or C_{95} dolichol, seem to induce non-bilayer organization of phospholipids when incorporated into egg lecithin vesicles, as monitored by ^{2}H-NMR and ^{31}P-NMR [30].

As a conclusion, it appears that tricyclohexaprenols and octaprenediols can be considered as genuine 'cholesterol surrogates', as their behaviour toward the water permeability of vesicles [11], the order parameter of lipid chains and the phase transition is cholesterol-like. However, it is now clear that these substances are not very efficient in ordering the lipid chains, at least

in our model systems. If tricyclohexaprenols are constituents of very primitive bacteria, they are adapted to the (unknown) structures of the polar lipids of these bacteria; their low efficiency with DMPC bilayers may then be due to a misfit with this eukaryotic phospholipid. This illustrates the difficulty in quantifying the structural requirements that make – or not – a molecule a good membrane reinforcer.

The case of isoarborinol differs radically from the previous molecules, as it shows a complex thermal behaviour when mixed with DMPC-d_{27}. The existence of two different ^{2}H-NMR patterns at 25°C (Fig. 6a) must originate from at least two phases which differ in their isoarborinol: DMPC ratio but which are both lamellar, as seen by the ^{31}P-NMR spectrum at 25°C (Fig. 5A). However, it is not possible to know whether both phases are in a liquid-crystalline state or not on the basis of ^{31}P-NMR alone, as Cullis et al. have clearly shown that the effective chemical shift anisotropy $\Delta\sigma$ is relatively insensitive to the occurrence of a phase transition between gel and liquid crystal [31,32]. These two phases, that coexist from 25°C to 50°C, are in slow exchange with respect to the ^{2}H-NMR time-scale, and thus lead to distinct NMR signals. The wide NMR pattern presents splittings similar to pure DMPC-d_{27} in the gel phase just below the phase transition: the terminal methyl groups give a well-resolved doublet (splitting: 10 kHz in pure DMPC-d_{27} at 22°C), whereas the broad shoulders are characteristic of an asymmetric powder pattern (data not shown). In the DMPC-isoarborinol mixture this gel phase, which has probably a low content of isoarborinol, progressively disappears with increasing temperature in favour of an isoarborinol-rich liquid crystalline phase, that shows a typical axially symmetric powder pattern. The existence of a single phase above 50°C leads to an increased sensitivity of the NMR signal, as can be seen by the signal-to-noise ratio obtained at 60°C (Fig. 6b) with 2100 transients which is similar to the one obtained at 25°C (Fig. 6a) with 9600 transients. The low sensitivity below 50°C is probably responsible for the appearance of an isotropic signal (due to residual HDO). However, the existence of a small amount of another phase which could explain this signal cannot be excluded.

The stabilization of the gel phase well above the phase transition temperature of DMPC-d_{27} was completely unexpected from such a bulky molecule. Such a behaviour has been observed for DPPC-d_{31}/palmitic acid mixtures, for which very similar spectra have been reported [28].

The isoarborinol-rich fluid phase has the same mean quadrupolar splitting as pure DMPC-d_{27}, as the first moments of both systems are similar above 50°C ($M_1 \approx 4.5 \cdot 10^4$ Hz for both at 55°C). This demonstrates that, even at high temperature where it is fully miscible with DMPC, isoarborinol has no ordering effect on the lipid chains and thus cannot be considered as a cholesterol surrogate, at least in DMPC membranes.

The example of isoarborinol clearly demonstrates that a rigid substance, the structure of which is close to sterols, may not order at all lipid chains in a membrane. In this case, the numerous studies concerning sterols are very helpful in understanding such a result (see Ref. 33 for a review). It has been well established that the presence of the 14α-methyl group in the lanosterol structure is the most crucial point that makes this molecule inefficient in membranes. This 14α-methyl protrudes from the sterol α-face and hinders the interaction with the adjacent acyl chains of phospholipids. Although isoarborinol is a pentacyclic molecule, its general shape is comparable to that of lanosterol, as seen by X-ray crystallography [34]. Therefore, the same arguments concerning the 14α-methyl group may apply to this molecule. Moreover, isoarborinol is shorter than cholesterol or lanosterol [34], which could also explain its absence of effect on chains mobility.

Acknowledgement

We thank Dr. E.J. Dufourc who provided us with a copy of his program for the calculation of spectral moments and Prof. S. Natori, Dr. B. Chappe and Dr. D. Heissler for gifts of additives.

References

1 Rohmer, M., Bouvier, P. and Ourisson, G. (1979) Proc. Natl. Acad. Sci. USA 76, 847–851.
2 Milon, A., Wolff, G., Ourisson, G. and Nakatani, Y. (1986) Helv. Chim. Acta 69, 12–24.
3 Milon, A., Lazrak, T., Albrecht, A.M., Wolff, G., Weill, G., Ourisson, G. and Nakatani, Y. (1986) Biochim. Biophys. Acta 859, 1–9.
4 Lazrak, T., Milon, A., Wolff, G., Albrecht, A.M., Miehé, M., Ourisson, G. and Nakatani, Y. (1987) Biochim. Biophys. Acta 903, 132–141.
5 Lazrak, T., Wolff, G., Albrecht, A.M., Nakatani, Y., Ourisson, G. and Kates, M. (1988) Biochim. Biophys. Acta 939, 160–162.
6 Poralla, K., Kannenberg, E. and Blume, A. (1980) FEBS Lett. 113, 107–110.
7 Bisseret, P., Wolff, G., Albrecht, A.M., Tanaka, T., Nakatani, Y. and Ourisson, G. (1983) Biochem. Biophys. Res. Commun. 110, 320–324.
8 Kannenberg, E., Blume, A., McElhaney, R.N. and Poralla, K. (1983) Biochim. Biophys. Acta 733, 111–116.
9 Kannenberg, E., Blume, A., Geckeler, K. and Poralla, K. (1985) Biochim. Biophys. Acta 814, 179–185.
10 Ourisson, G., Albrecht, P. and Rohmer, M. (1982) Trends Biochem. Sci. 7, 236–239.
11 Krajewski-Bertrand, M.-A., Hayer, M., Wolff, G., Milon, A., Albrecht, A.M., Heissler, D., Nakatani, Y. and Ourisson, G. (1990) Tetrahedron 46, 3143–3154.
12 Seelig, J. (1977) Q. Rev. Biophys. 10, 353–418.
13 Davis, J.H. (1983) Biochim. Biophys. Acta 737, 117–171.

14 Schuler, I., Milon, A., Nakatani, Y., Ourisson, G., Albrecht, A.M., Benveniste, P. and Hartmann, M.A. (1991) Proc. Natl. Acad. Sci. USA 88, 6926–6930.
15 Heissler, D. and Ladenburger, C. (1988) Tetrahedron 44, 2513–2521; Heissler, D., Jenn, T. and Nagano, H. (1991) Tetrahedron Lett. 47, 7587–7590; Ladenburger, C. and Heissler, D. (1991) J. Chem. Res. (S), 330..
16 Vorbrüggen, H., Pakrashi, S.C. and Djerassi, C. (1963) Liebigs Ann. Chem. Bd. 668, 57–76.
17 Chappe, B., Musikas, H., Marie, D. and Ourisson, G. (1988) Bull. Chem. Soc. Jpn. 61, 141–148.
18 Dinh-Nguyên, N., Raal, A. and Stenhagen, E. (1972) Chem. Scr. 2, 171–178.
19 Perly, B., Dufoure, E.J. and Jarrell, H.C. (1984) J. Labelled Compd. Radiopharm. 21, 1–13.
20 Jarrell, H.C., Jovall, P.A., Giziewicz, J.B., Turner, L.A. and Smith, I.C.P. (1987) Biochemistry 26, 1805–1811.
21 Stockton, G.W., Polnaszek, C.F., Tulloch, A.P., Hasan, F. and Smith, I.C.P. (1976) Biochemistry 15, 954–966.
22 Oldfield, E., Meadows, M., Rice, D. and Jacobs, R. (1978) Biochemistry 17, 2727–2740.
23 Krajewski-Bertrand, M.-A., Nakatani, Y., Ourisson, G., Dufoure, E.J. and Milon, A. (1992) J. Chim. Phys., in press.
24 Vist, M.R. and Davis, J.H. (1990) Biochemistry 29, 451–464.
25 Yue, J., Thewalt, J.L. and Cushley, R.J. (1988) Chem. Phys. Lipids 49, 205–213.
26 Gulik, A., Luzzati, V., De Rosa, M. and Gambacorta, A. (1985) J. Mol. Biol. 182, 131–149.
27 Wassal, S.R., Thewalt, J.L., Wong, L., Gorrissen, H. and Cushley, R.J. (1986) Biochemistry 25, 319–326.
28 Pauls, K.P., MacKay, A.L. and Bloom, M. (1983) Biochemistry 22, 6101–6109.
29 Westerman, P.W., Pope, J.M., Phonphok, N., Doane, J.W. and Dubro, D.W. (1988) Biochim Biophys. Acta 939, 64–78.
30 Knudsen, M.J. and Troy, F.A. (1989) Chem. Phys. Lipids 51, 205–212.
31 Cullis, P.R., De Kruyff, B. and Richards, R.E. (1976) Biochim. Biophys. Acta 426, 433–446.
32 Hemminga, M.A. and Cullis, P.R. (1982) J. Magn. Reson. 47, 307–323.
33 Bloch, K.E. (1983) CRC Crit. Rev. Biochem. 14, 47–92.
34 Nes, W.N., Wong, R.Y., Griffin, J.F. and Duax, W.L. (1991) Lipids 26, 649–655.