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Differential effects of plant sterols on water permeability and on acyl chain ordering of soybean phosphatidylcholine bilayers

(Δ⁵-sterols/9β,19-cyclopropylsterols/14α-methylsterols/osmotic swelling/²H NMR)

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ABSTRACT **To gain some insight into the structural and functional roles of sterols in higher plant cells, various plant sterols have been incorporated into soybean phosphatidylcholine (PtdCho) bilayers and tested for their ability to regulate water permeability and acyl chain ordering. Sitosterol was the most efficient sterol in reducing the water permeability of these vesicles and stigmasterol appeared to have no significant effect. Vesicles containing 24ξ-methylcholesterol exhibited an intermediate behavior, similar to that of vesicles containing cholesterol. Cycloartenol, the first cyclic biosynthetic precursor of plant sterols, reduced the water permeability in a very effective way. Of two unusual plant sterols, 24ξ-methylpollinastanol and 14α,24ξ-dimethylcholest-8-en-3β-ol, the former was found to be functionally equivalent to sitosterol and the latter was found to be relatively inefficient. ²H NMR experiments have been performed with oriented bilayers consisting of soybean PtdCho with sitosterol, stigmasterol, or 24ξ-methylpollinastanol. The results provided clear evidence that sitosterol and 24ξ-methylpollinastanol exhibit a high efficiency to order PtdCho acyl chains that closely parallels their ability to reduce water permeability. By contrast, stigmasterol shows a low efficiency for both functions. These results show that sitosterol and stigmasterol, two major 24-ethylsterols differing only by the absence or presence of the Δ²² double bond in the side chain, probably play different roles in regulating plant membrane properties; they also may explain why 9β,19-cyclopropylsterols behave as good surrogates of sitosterol.**

In mammalian cells, the bulk of free cholesterol is localized in the plasma membrane where it participates in the regulation of membrane fluidity and in the activity of many membrane-bound enzymes (1). Changes in cholesterol content have been shown to induce modifications in cellular functions, which depend on the dynamics of the cell-surface membrane such as the recognition of an external signal (1). In higher plant cells, the three typical phytosterols: sitosterol [(24*R*)-ethylcholest-5-en-3β-ol], stigmasterol [(24*S*)-ethylcholesta-5*E*,22-dien-3β-ol], and 24ξ-methylcholesterol [the mixture of 24*R* (i.e., campesterol) and 24*S* (i.e., 22-dihydrobrassicasterol) epimers] are also mainly concentrated in the plasma membrane (2). To investigate whether the different plant sterols play similar roles in higher plant cells, we started a study with well-defined model membrane systems prepared from soybean phosphatidylcholine (PtdCho), a major representative plant phospholipid, and various sterols in different molar ratios. Because of their high content in polyunsaturated fatty acyl chains (3), soybean PtdCho bilayers can be considered a valuable model of higher plant membrane. The present work deals with the effect of some plant sterols on water permeability and acyl chain ordering of these bilayers. Permeability changes were monitored by measuring the swelling rates of large unilamellar vesicles (LUVs) following an osmotic shock in a stopped-flow spectrophotometer. This method had been developed to investigate the role of some prokaryotic terpenoids as membrane reinforcers (4–8). The kinetics of swelling was shown to be controlled by the water permeability of bilayers and to depend on the lipid composition of vesicles (5). We have selected the following sterols (Fig. 1): the three typical plant Δ⁵-sterols (sitosterol, stigmasterol, and 24ξ-methylcholesterol); cholesterol (cholest-5-en-3β-ol), the standard of reference for sterol effects on mammalian membranes; cycloartenol (4,4,14α-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol), the first cyclic biosynthetic precursor of plant sterols; and two unusual sterols, 24ξ-methylpollinastanol (14α,24ξ-dimethyl-9β,19-cyclo-5α-cholestan-3β-ol) and 14α,24ξ-dimethylcholest-8-en-3β-ol, which had been shown to accumulate in plants treated with sterol biosynthesis inhibitors belonging to two important classes of fungicides, N-substituted morpholines and azoles, respectively (9). To investigate in more detail phospholipid–sterol interactions, we used a complementary ²H NMR approach, a method that provides a precise description of acyl chain dynamics in a bilayer (see ref. 10 for a review). By using oriented bilayers consisting of soybean PtdCho with sitosterol, stigmasterol, or 24ξ-methylpollinastanol, we increased considerably the sensitivity of this technique, thus allowing the use of 1-myristoyl-2-(perdeuterio)-myristoyl-*sn*-glycero-3-phosphocholine (DMPC-d_{27}) as a probe of the molecular order of unsaturated hydrocarbon chains. The results show that the structural features of the sterol molecule needed for a good interaction with phospholipid acyl chains, pictured for instance by an ability to increase the order parameter of acyl chains, are probably the same as those involved in the regulation of membrane permeability.

MATERIALS AND METHODS

Materials. Soybean and egg PtdCho and phosphatidylglycerol were purchased from Sigma and used without further purification. Sitosterol and stigmasterol were from Fluka and 24ξ-methylcholesterol and cholesterol were from Sigma; 24ξ-methylpollinastanol and 14α,24ξ-dimethylcholest-8-en-3β-ol were isolated from corn roots treated with fenpropimorph and Lab 170250F, respectively, as described (3). All the

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Abbreviations: PtdCho, phosphatidylcholine; DMPC-d_{27}, 1-myristoyl-2-[$^2H_{27}$]myristoyl-*sn*-glycero-3-phosphocholine; LUV, large unilamellar vesicle.

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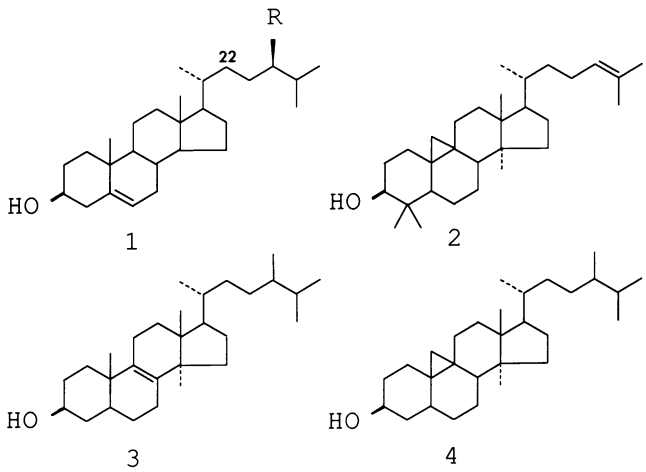

FIG. 1. Structures of sterols used in this study. 1: Δ^5-sterols; R = H, cholesterol; R = CH_3, campesterol; R = C_2H_5, sitosterol; Δ^{22} and R = C_2H_5, stigmasterol; 2: cycloartenol; 3: 14α,24ξ-dimethylcholesta-8-en-3β-ol; 4: 24ξ-methylpollinastanol.

sterols were checked for purity by GC (3). The deuterated probe, DMPC-d_{27}, was prepared by acylation of 1-myristoyl-*sn*-glycero-3-phosphocholine with deuterated myristic anhydride according to Perly *et al.* (11), with 4-piperidinopyridine as a catalyst. Deuterated myristic anhydride was synthesized according to ref. 12 and 1-myristoyl-*sn*-glycero-3-phosphocholine was prepared from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine by using phospholipase A2 as described by Chakrabarti and Khorana (13), except that the borate buffer was replaced by water. DMPC-d_{27} was purified first by column chromatography on Biosil A from Bio-Rad and then by HPLC.

Preparation of Vesicles. LUVs were prepared in 350 mM NaCl buffered with 10 mM Tris·HCl (pH 8.0) and containing 5 mM NaN_3 by the reverse-phase evaporation procedure of Szoka and Papahadjopoulos (14) from soybean PtdCho, phosphatidylglycerol, and different sterols. Sterol-free vesicles were also prepared from soybean or egg PtdCho. To avoid the oxidation of fatty acyl chains, which is shown by the increase in absorbance at 233 nm relative to that at 215 nm (15), 1 mol % of butylated hydroxytoluene was systematically introduced in the assays and all the steps involved in the preparation of vesicles were performed under argon. This point is important, because the presence of hydroxyl groups in the acyl chains has been reported to increase membrane permeability (16, 17).

The liposome suspensions were successively filtered twice through 0.8- and twice through 0.4-μm polycarbonate filters (Nuclepore). The size of vesicles was determined by light scattering and estimated by the dissymmetry value Z corresponding to the $I(45°)/I(135°)$ ratio, where $I(45°)$ is the intensity of light scattered at a 45° angle (5). The measurements were run at 20°C. Lipid phosphorus as well as sterol contents of vesicles were determined after LUV recovery as described (3). Thus, values given in the text correspond to phospholipid and sterol amounts actually incorporated into vesicles and may differ considerably from the amounts added initially.

Stopped-Flow Measurements. The osmotic swelling experiments were performed as described (5). The reliability of our permeability measurements has been well demonstrated (5–8). In particular, because of the high sensitivity of light scattering, we could use dilute suspensions of vesicles, thus avoiding vesicle aggregation. We compared the kinetics of osmotic swelling for vesicles calibrated in size and with a well-defined lipid composition.

Liposomal preparations were degassed under vacuum at 40°C and filtered through 0.4-μm filters just before the measurements to limit the noise due to dust and bubbles. Typical lipid concentrations were between 0.1 and 0.2 mg/ml. Vesicles in 350 mM NaCl were rapidly (3 ms) mixed at 20°C with the same vol of 50 mM NaCl buffer. Results were expressed as the half-time of the kinetics ($t_{1/2}$ in ms). Each experimental value given corresponds to the average of four to six kinetic measurements.

^{2}H NMR Experiments. Lipid mixtures were prepared with 16 mol % sterol, 10 mol % DMPC-d_{27}, 1 mol % butylated hydroxytoluene, and 73 mol % soybean PtdCho in 1 ml of isopropanol and deposited onto the surface of 40–50 glass plates (22 × 0.15 × 6–9 mm). The plates were placed under vacuum overnight and stacked into glass tubes (10 mm o.d.). ^{2}H-depleted water (50 μl) (Aldrich) was added. NMR samples typically consisted of 10 mg of DMPC-d_{27}, except for samples containing 24ξ-methylpollinastanol for which only 2 mg was used. ^{2}H NMR experiments were performed at 46 MHz with a Bruker MSL 300 spectrometer. Quadrupolar echos were obtained by using two 90° pulses of 7.5 μs width spaced 25 μs apart and 500-ms repetition rates. The sweep width was 250 kHz and the digital resolution after zero filling was 30 Hz per point. Spectra were recorded at 30°C. Temperature dependence was performed with sitosterol-containing samples from 23°C to 60°C. The orientation with the plane of glass plates orthogonal to the external magnetic field was defined as the 0° orientation. The accuracy of the angular settings was determined by multiple settings, especially at 90° and was found to be ±2°. We checked by changing the plate orientation that the axis of rotation of DMPC-d_{27} was the normal to the bilayer (18). Quadrupolar splittings ($\Delta\nu_Q$) for all the positions along the *sn*-2 acyl chain of dimyristoylphosphatidylcholine can be measured directly from the NMR spectra recorded at 90° (Fig. 2). The segmental order parameters (S_{mol}) were calculated according to the following relationships (19, 20):

$$\Delta\nu_Q\,(90°) = -{}^3/_4\, e^2qQ/h \times S_{C-^2H}$$

$$S_{mol} = -2\, S_{C-^2H} \text{ for a methylene group}$$

$$S_{mol} = -6\, S_{C-^2H} \text{ for a methyl group}$$

where $\Delta\nu_Q$ (90°) is the quadrupolar splitting for the 90° orientation, e^2qQ/h is the quadrupolar coupling constant (167 kHz), and S_{C-^2H} is the orientational order parameter of the C—^{2}H bond. Overall, the accuracy of the S_{mol} values was estimated to be ±0.5%.

RESULTS

Water Permeability Measurements. A rapid mixing of a suspension of vesicles prepared in 350 mM NaCl with a diluted buffer (50 mM NaCl) leads to a monoexponential decrease in scattered light corresponding to the influx of water into the vesicles and to their swelling (Fig. 3). It has been shown that the water transport through the membrane is the limiting factor of this first-order kinetics (5). The half-time $t_{1/2}$ is inversely proportional to membrane permeability. The swelling rate is slightly affected by the vesicle size estimated by the parameter Z in such a way that $t_{1/2}$ increases with increasing Z values (5). In Table 1 are listed the $t_{1/2}$ and Z values for vesicles containing different amounts of various sterols compared to those obtained with sterol-free vesicles prepared from soybean or egg PtdCho. Most of the preparations exhibit similar Z values, thus allowing a direct comparison of $t_{1/2}$ values.

The water permeability of sterol-free soybean PtdCho vesicles is twice that exhibited by sterol-free egg PtdCho vesicles (Table 1), clearly illustrating the influence of the

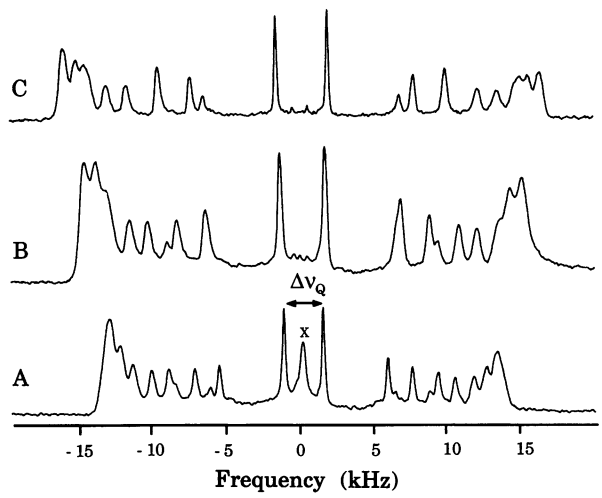

FIG. 2. ^{2}H NMR spectra of 90°-oriented multibilayers consisting of 10 mol % DMPC-d_{27}/soybean PtdCho mixtures with or without sitosterol or stigmasterol. Trace A, sterol-free soybean PtdCho; trace B, soybean PtdCho/16 mol % stigmasterol; trace C, soybean PtdCho/16 mol % sitosterol. Number of scans, 10,000; recycle time, 500 ms. In trace A, a small amount of residual deuterated water was present, giving the isotopic signal indicated by X.

unsaturation degree of phospholipid acyl chains on this process, in agreement with the literature (21–23). The incorporation of any sterol into the vesicles leads to a decrease in water permeability, except for stigmasterol. Surprisingly, this sterol exhibits no ability in regulating membrane permeability even at 15 mol %—i.e., at its solubility limit in soybean PtdCho LUVs (3). Among typical plant sterols, sitosterol appears to be the most efficient in reducing water permeability, inducing a 3-fold decrease relative to sterol-free vesicles. This effect is comparable in amplitude to that previously observed for DMPC/cholesterol vesicles containing 30 mol % sterol (5), but it is higher than that found by the same method for the egg PtdCho/cholesterol (30 mol %) system (6). Cholesterol and 24ξ-methylcholesterol exhibit similar behaviors with a 2-fold decrease in water permeability. Cycloartenol is even more efficient than sitosterol. Concerning the effects induced by the two unusual sterols, results of Table 1 indicate that whereas 24ξ-methylpollinastanol is as active as sitosterol, 14α,24ξ-dimethylcholest-8-en-3β-ol is not able to reduce significantly the water permeability of soybean PtdCho vesicles.

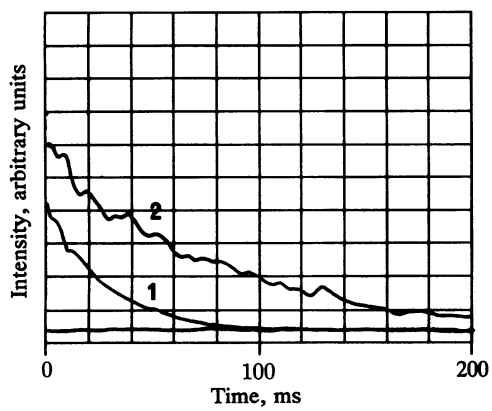

FIG. 3. Oscilloscope traces corresponding to changes in scattered light intensity at 20°C as a function of time for vesicles prepared in 350 mM NaCl from soybean PtdCho with or without sitosterol upon exposure to 50 mM NaCl. The two traces were recorded separately and then superimposed: 1, sterol-free soybean PtdCho; 2, soybean PtdCho with 29 mol % sitosterol.

Table 1. Effect of different plant sterols on the osmotic swelling rate of soybean PtdCho vesicles at 20°C

LUV composition	Sterol, mol %	Z, $I(45°)/I(135°)$	$t_{1/2}$, ms	n
Egg PtdCho	0	9.5	45	4
Soybean PtdCho	0	10.4	24	4
		10.7	22	4
		10.8	22	7
Soybean PtdCho/sitosterol	26	14	65	6
	29	11.3	60	6
Soybean PtdCho/ 24ξ-methylcholesterol	24	10	50	5
Soybean PtdCho/stigmasterol	8	9.6	23	5
	11	10.8	23	5
	15	10	20	5
Soybean PtdCho/cholesterol	19	11.5	45	5
	23	9	55	5
Soybean PtdCho/cycloartenol	22	9	65	5
	30	11.4	67	5
Soybean PtdCho/ 14α,24ξ-dimethylcholest-8-en-3β-ol	15	8.5	29	6
Soybean PtdCho/ 24ξ-methylpollinastanol	22	14.7	65	4
	29	12	60	5

n, Number of experiments. SDs for $t_{1/2}$ values and sterol contents are estimated to be ±10%.

Among the results described above, the most striking one is the large difference observed between sitosterol and stigmasterol as well as between 24ξ-methylpollinastanol and 14α,24ξ-dimethylcholest-8-en-3β-ol in their ability to decrease water permeability of soybean PtdCho bilayers. Stigmasterol differs from sitosterol only by the presence of an additional double bond at C-22 in the side chain; the two other compounds are isomeric, 24ξ-methylpollinastanol containing a 9β,19-cyclopropane ring instead of the angular methyl group at C-10 and the $\Delta^{8(9)}$ double bond. It appeared therefore of considerable importance to investigate sterol–phospholipid interactions at the molecular level, especially to determine whether the ability of sitosterol and 24ξ-methylpollinastanol to reduce water permeability could be related to their ability to order PtdCho acyl chains. For this purpose, we chose ^{2}H NMR as a reliable technique to obtain molecular information on the dynamics of lipid molecules (10).

^{2}H NMR Experiments. Samples consisted of 16 mol % sterol, because this value corresponds to the solubility limit of stigmasterol in soybean PtdCho. Fig. 4 shows the S_{mol} profiles for the four lipid mixtures: pure PtdCho, PtdCho/sitosterol, PtdCho/stigmasterol, and PtdCho/24ξ-methylpollinastanol. All the S_{mol} profiles exhibit a characteristic shape, with a plateau from position 3′ to position 8′ of the DMPC *sn*-2 acyl chain and a gradual decrease when going toward position 14′, which indicates the highly dynamic character (large amplitude of motions) of the center of the bilayer (10). S_{mol} values for pure PtdCho correspond to those of a phospholipid with relatively disordered acyl chains as expected for this highly unsaturated plant lipid (3). For a comparison, at the same temperature, S_{mol} values at the plateau for pure DMPC and soybean PtdCho are 0.46 and 0.42, respectively; the corresponding values for the terminal methyl groups are 0.17 and 0.13, respectively. Thus, DMPC embedded into soybean PtdCho molecules behaves as a probe of the overall behavior of all the lipid components, in agreement with the fact that it is perfectly miscible with them.

In the presence of 16 mol % sterol, the S_{mol} profile is shifted toward higher values for all the positions along the DMPC

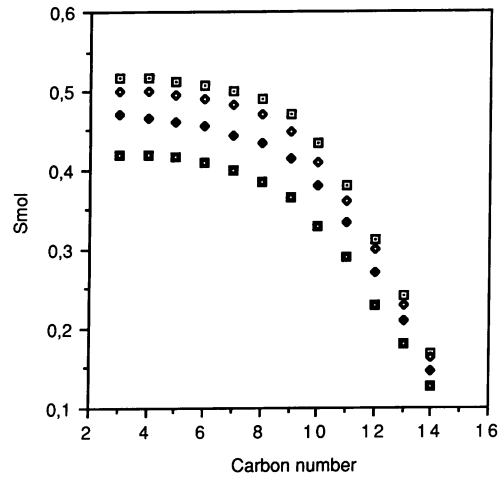

FIG. 4. Effect of different plant sterols on the segmental order parameter S_{mol} as observed by ^2H NMR of a DMPC-d_{27} probe in soybean PtdCho multibilayers oriented at 90°. Sterol was present at 16 mol %. ■, Sterol-free soybean PtdCho; ◆, soybean PtdCho/stigmasterol; ◆, soybean PtdCho/24ξ-methylpollinastanol; ⊡, soybean PtdCho/sitosterol.

sn-2 chain (Fig. 4). The most significant effect is observed with sitosterol, for which the order parameter varies from 0.51 at the plateau (21% increase) to 0.17 at position 14′ (35% increase). The replacement of sitosterol by 24ξ-methylpollinastanol in the lipid mixture produces essentially the same effects: 18% and 28% increase, respectively. Although stigmasterol also increases the S_{mol} value at the plateau (13% increase), it clearly has a much weaker ordering effect. The spectra recorded at temperatures ranging from 23°C to 60°C for lipid mixtures containing sitosterol showed a corresponding regular decrease in the S_{mol} values for all the positions (data not shown). As expected, no break point or discontinuity occurred in that temperature range for which acyl chains are in a liquid–crystalline state. Arrhenius plots [ln $S_{mol} = f(1/T)$ (K^{-1})] give straight lines, with activation energies varying from 10.0 kJ (position 14′) to 8.2 kJ (plateau), indicating that the order parameter is more sensitive to the temperature as one goes toward the inner positions along the acyl chain. Similarly, these positions are also the most sensitive to the presence of sterol.

DISCUSSION

The first attempts to investigate the ability of cholesterol to regulate membrane permeability were performed about 20 years ago (21–24). These early studies showed that the osmotically induced swelling rate of lecithin liposomes or cell membranes from *Acholeplasma laidlawii* decreased considerably in the presence of cholesterol, indicating a reduced permeability to water (21) or other small molecules such as erythritol or glucose (22–24). Permeability changes were found to be directly dependent on the sterol concentration for vesicles prepared with lipids in the liquid–crystalline state (21). These investigations led to an identification of several structural features of the sterol molecule important for its role in membrane permeability. A planar ring system, a 3β-hydroxyl group, and an isooctyl side-chain all appear required for cholesterol-like behavior. A more recent study dealing with the behavior of several double bond isomers of cholesterol indicates that the Δ^5 double bond is the most effective feature for optimal sterol–phospholipid interactions and regulation of membrane permeability (25).

The typical plant sterols (sitosterol, stigmasterol, and 24ξ-methylcholesterol) all satisfy the above requirements. However, when their behavior in egg PtdCho or DMPC vesicles was tested, they were found to be less efficient than cholesterol (22, 23). They also were less efficient than cholesterol in reducing the methanol-induced β-cyanine efflux from red beet disks (26). Cholesterol has therefore been considered for a long time to be the standard reference for sterol effects on membranes (1, 27, 28). However, many studies have emphasized the importance of the nature of lipid species in sterol–phospholipid interactions. Phospholipids from plant membranes are generally characterized by a higher amount of unsaturated acyl chains than those from animal or fungal membranes. It was therefore of crucial importance to reinvestigate the behavior of plant sterols with lipids of plant origin.

The high efficiency of sitosterol and 24ξ-methylcholesterol in reducing membrane permeability to water (Table 1) is remarkable and can be related to recent results reported by Stillwell *et al.* (29). These authors have shown that as little as 10 mol % of a sitosterol/24ξ-methylcholesterol (60:40) mixture is enough to reverse by >75% the abscisic acid-induced permeability to carboxyfluorescein of various model membranes including both synthetic and natural, saturated and unsaturated PtdCho/phosphatidylethanolamine mixed bilayers. Taken together, these results indicate a stabilization of lipid bilayers by the two sterols, although the mechanisms of permeability to water and carboxyfluorescein are probably different.

In contrast, stigmasterol, another major sterol in higher plant membranes, was found to have no significant effect on the swelling rate of soybean PtdCho vesicles (Table 1). In egg PtdCho vesicles, this sterol has been shown to be far less efficient than cholesterol in reducing the membrane permeability to glycerol and erythritol (22). In *A. laidlawii* cell membranes rich in palmitic acid (16:0) and oleic acid (18:1), stigmasterol also has only a slight effect on the permeability to the same solutes (23).

Stigmasterol differs from sitosterol only by the Δ^{22} double bond in the side chain. This double bond can influence either the preferred conformation or the dynamics of the side chain.

The ability of two unusual sterols, 24ξ-methylpollinastanol and 14α,24ξ-dimethylcholest-8-en-3β-ol, in regulating water permeability of soybean PtdCho vesicles was also investigated. As these sterols accumulate in plants treated with some fungicides used in agriculture, it was of interest to check the functional consequences of their incorporation in the membranes from such plants. These two isomers behave in a completely different way. Whereas the cyclopropylsterol was found to be as efficient as sitosterol in reducing water permeability, the second compound was inefficient (Table 1). Cycloartenol, another cyclopropylsterol, was found to be the most efficient compound tested (Table 1).

A correspondence between sterol-induced changes in phospholipid acyl chain ordering monitored by fluorescence polarization or electron spin resonance and in membrane permeability to various solutes has been reported for different model systems (1, 25, 28, 30–33). We report here the results of a study dealing with plant sterol effects on water permeability and on acyl chain ordering of soybean PtdCho bilayers by using two nonperturbing methods. Our experiments clearly show the ability of sitosterol to increase the order parameter S_{mol} for each position of the *sn*-2 acyl chain of DMPC-d_{27}, an effect that can be correlated with the well known condensing effect of cholesterol on lipids in the liquid–crystalline state (1). In contrast, stigmasterol was found to have a much weaker ordering effect (Fig. 4). These data are in agreement with our previous results of steady-state fluorescence anisotropy measurements with diphenylhexatriene as a probe (3). ^2H NMR spectroscopy gives valuable information on local order parameters that can never be matched by fluorescence depolarization experiments, which senses only an average order parameter of the adjacent

acyl chain. However, there is a good agreement between the S_{mol} value for the position 11′ of the DMPC *sn*-2 acyl chain in ^2H NMR experiments and the order parameter S given by fluorescence measurements, in agreement with the literature (34, 35). As an example, for similar lipid mixtures containing 16 mol % sitosterol and measurements carried out at 20°C, the S_{mol} value for the position 11′ (0.425), estimated from S_{mol} Arrhenius plots, is very close to the S value (0.406) calculated from anisotropy data (3). Therefore, whatever the experimental approach used to evaluate the ordering effect, sitosterol and stigmasterol exhibit completely different behaviors. The ability of sitosterol to order soybean PtdCho bilayers is correlated with its capacity to significantly reduce membrane permeability to water. The opposite correlation holds true for stigmasterol. Thus, the introduction of the double bond at position 22 in the side chain is accompanied by a striking reduction in the ability of the sterol to fulfill these two functions.

^2H NMR experiments have also shown that 24ξ-methylpollinastanol is almost as efficient as sitosterol to order soybean PtdCho bilayers (Fig. 4). This result can be correlated with the fact that cycloartenol was found to have an ordering effect on DMPC acyl chains similar to that of cholesterol (A.M. and E. J. Dufourc, unpublished work). The ability of these 9β,19-cyclopropylsterols both to order acyl chains and to reduce water permeability of soybean PtdCho bilayers makes these compounds efficient membrane reinforcers. In contrast, for 14α,24ξ-dimethylcholest-8-en-3β-ol, the isomer of 24ξ-methylpollinastanol, its low ordering effect, demonstrated by fluorescence anisotropy experiments (3), parallels its inefficiency to regulate membrane permeability (Table 1). The presence of the $\Delta^{8(9)}$ double bond instead of the cyclopropane ring makes the molecule more rigid and planar. As a consequence, the 14α-methyl group projects from the sterol α face and hinders the interaction with the acyl chain of the adjacent phospholipid (28). In the case of cyclopropylsterols, it has been established that the cyclopropane ring gives some flexibility to these molecules at the level of ring C, affecting somewhat the angular orientation of the 14α-methyl group (36). Several lines of experiments give some support to the fact that 9β,19-cyclopropylsterols are relatively good surrogates of Δ^5-sterols. Thus, whole plants or cell suspension cultures treated with N-substituted morpholines or azadecalines are able to grow even when cyclopropylsterols replace almost completely the normally occurring Δ^5-sterols (37–41). Fenpropimorph-resistant tobacco calli have also been isolated, and their growth appears not to be impaired by the complete replacement of Δ^5-sterols by cyclopropylsterols (42).

Taken together these structural and functional data clearly indicate that sitosterol and 24ξ-methylcholesterol probably play roles in membranes of higher plants similar to that of cholesterol in mammalian membranes. The specific role of stigmasterol may be different and remains to be investigated.

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