Sterol–Phospholipid Interaction in Model Membranes: Role of C_5–C_6 Double Bond in Cholesterol†

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ABSTRACT: Several double-bond isomers of cholesterol where the normal C_5–C_6 double bond (Δ^5) has been moved to different positions in the ring skeleton, i.e., Δ^1, Δ^4, Δ^7, $\Delta^{8(9)}$, $\Delta^{8(14)}$, and Δ^{14}, have been synthesized and incorporated in phosphotidylcholine vesicles. In addition, dienes like $\Delta^{5,7}$, $\Delta^{7,14}$, and $\Delta^{8,14}$ have also been studied. Many of these cholesterol analogues are intermediates in the sterol biosynthesis in different organisms. The incorporation studies indicated that more than 90% of the sterol was present in the vesicles. The effect of these cholesterol analogues was studied by glucose permeability, electron spin resonance, and fluorescence polarization spectroscopy. These studies indicated that Δ^{14}-cholesten-3β-ol was most effective in restricting glucose permeability or in increasing the order parameter but was still not as effective as cholesterol. This was followed by $\Delta^{8(14)}$- and $\Delta^{8(9)}$-cholesten-3β-ol. The Δ^1, Δ^4, and Δ^7 analogues and the dienols were relatively less effective in condensing the membrane. These studies indicate that the double bond at C_5–C_6 in cholesterol is most effective for optimal sterol–phospholipid interaction and may have formed the basis of the migration of the double bond from rings C and D in sterols to C_5–C_6 during the evolution of cholesterol.

Cholesterol forms an integral component of various biological membranes and is usually found in larger amounts in plasma membrane rather than in intracellular membranes. It is also known to regulate the fluidity of membranes and is involved in the control of various membrane-associated properties like permeability, activity of membrane-bound enzymes and receptors, immune response, and endocytosis (Demel & Dekruyff, 1976; Yeagle, 1985). The interaction of cholesterol with phospholipids (Demel & Dekruyff, 1976) and more recently with proteins is also well documented (Yeagle, 1985). A planar ring system, a 3β-hydroxyl group, and the isooctyl side chain were initially believed to be essential for interaction with lipids and proteins in membranes (Demel et al., 1972; Demel & Dekruyff, 1976) though recent data indicate that a blocked hydroxyl group, i.e., methyl ether of cholesterol, is equally compatible both in artificial and in natural membranes (Lala et al., 1979; Demel et al., 1984). Similarly, a detailed study of the side-chain analogues has shown that a four carbon atom side chain at C_{17} is minimally required and longer or shorter chains cannot achieve the condensing effect of cholesterol (Suckling et al., 1979; Nakamura et al., 1980; Bloch, 1983). All these studies point to a rather stringent structural requirement of membranes vis-à-vis the cholesterol molecule. Unlike the structural components mentioned above, the C_5–C_6 double bond of cholesterol has not been reported to be essential, and its absence only makes a marginal difference to the condensing effect of cholesterol (Demel et al., 1972; Demel & Dekruyff, 1976). We report here a study on the effect of various double-bond isomers of cholesterol in artificial membranes and observe that none of these isomers can condense or order the membranes as effectively as cholesterol.

Materials and Methods

All chemicals and solvents used were of reagent grade. Cholesterol was obtained from SRL, Bombay, and was crystallized twice from methanol before use, mp 149 °C. Dihydrocholesterol and Sephadex G-50 were obtained from Sigma Chemicals Co., and 7-dehydrocholesteryl benzoate was from ICN Chemicals. UV spectra were recorded on a Varian Superscan-3 in methanol. The infrared spectra were recorded on a Perkin-Elmer 237B spectrometer. The NMR and mass spectra were recorded on Varian XL-100 and MAT-112 spectrometers, respectively. HPLC analysis was carried out in a Du Pont 8800 pumping system connected to a Du Pont RI detector or a Gilson Spectrochrom-M UV detector. Sterols were purified in a Zorbax ODS column with methanol–water (98:2) as the mobile phase. The ESR[1] spectrum was recorded on a Varian E-112 spectrometer. Fluorescence polarization studies were carried out on a Perkin-Elmer MPF-44A fluorescence spectrometer. Radioactivity (^{14}C) was determined on an LKB Rac Beta 1217 liquid scintillation counter with xylene-based aqueous scintillant.

All sterols were prepared by known procedures, and details are provided only where any modifications specially during purification were involved. The NMR values for $C_{3\alpha}$-H and olefinic protons for samples tested for homogeneity by HPLC (>99% pure) are given in ppm. All NMR were recorded in $CDCl_3$.

Δ^1-Cholesten-3β-ol. Cholestanone was brominated with 3% bromine in acetic acid to obtain 2α-bromocholestanone in 70% yield, mp 168 °C, (lit. mp 169 °C) according to the procedure of Cookson (1954). This was followed by dehydrobromination according to the procedure of Djerassi and Scholz (1947) to obtain cholest-1-en-3-one in 55% yield: mp 99 °C (lit. mp 99 °C); UV λ_{max} 230 nm. Cholest-1-en-3-one was then reduced with lithium aluminum hydride in ether at 0–5 °C following the procedure of Kita et al. (1954). The crude product after workup was chromatographed on a column of alumina where the desired compound was eluted with 5% ethyl acetate in benzene. Traces of the 3α-isomer were removed by preparative TLC on silica gel. It was then crystallized from methanol to

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[1] Abbreviations: PC, egg phosphatidylcholine; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; ESR, electron spin resonance.

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obtain Δ^1-cholesten-3β-ol in 52% yield, mp 131 °C (lit. mp 131 °C). The NMR spectrum showed the C_1-H and C_2-H at 5.92 and 5.48 ppm with J = 10 Hz, the $C_{3\alpha}$-H appearing as a broad multiplet at 4.3 ppm.

Δ^4-Cholesten-3β-ol. Cholest-4-en-3-one, prepared by Oppenauer oxidation of cholesterol (Oppenauer, 1955), was reduced with lithium aluminum hydride in ether according to procedure of Collins and Hobb (1963) but had to be carefully purified to remove the dihydro compound and the 3α-isomer impurities by preparative TLC. Chloroform–methanol (99:1) proved to be the best solvent for developing the TLC plate in our hands. The TLC-purified material on crystallization from methanol gave Δ^4-cholesten-3β-ol in 80% yield, mp 131 °C (lit. mp 131 °C). The NMR spectrum showed the olefinic proton C_4-H at 5.26 ppm; the $C_{3\alpha}$-H appeared as a multiplet at 4.15 ppm.

Δ^7-Cholesten-3β-ol. This compound was prepared by reduction of 7-dehydrocholesteryl benzoate with Raney Nickel followed by hydrolysis essentially according to the procedure of Palmer et al. (1976) in 70% yield, mp 123 °C. The NMR spectrum showed the olefinic proton C_7-H at 5.18 ppm, the $C_{3\alpha}$-H appearing as a broad multiplet at 3.6 ppm.

$\Delta^{8(14)}$-Cholesten-3β-ol. This compound was obtained readily by isomerization of Δ^7-cholesten-3β-ol with platinum oxide in acetic acid according to the procedure of Cornforth and Popjak (1957) in 87% yield, mp 119 °C (lit. mp 120 °C). The NMR spectrum indicated the disappearance of olefinic proton. The $C_{3\alpha}$-H appeared at 3.62 ppm as before as a broad multiplet.

Δ^{14}-Cholesten-3β-ol. This compound was obtained by isomerization of $\Delta^{8(14)}$-cholesten-3β-ol in chloroform solution by passing dry HCl gas through it at −40 °C according to the procedure of Cohen et al. (1967). The final compound was obtained in 63% yield, mp 130 °C (lit. mp 131 °C). The NMR spectrum showed the olefinic proton at 5.18 ppm and $C_{3\alpha}$-H as a broad multiplet at 3.64 ppm.

$\Delta^{8,14}$-Cholestadien-3β-ol. $\Delta^{8,14}$-Cholestadien-3β-ol benzoate was prepared from 7-dehydrocholesteryl benzoate according to the procedure of Lee et al. (1969) by refluxing in ethanol with HCl for 2 h to give $\Delta^{8,14}$-cholestadiene 3β-benzoate in 97% yield, mp 136 °C (lit. mp 139 °C).

$\Delta^{8,14}$-Cholestadien-3β-ol was obtained by subsequent hydrolysis of the corresponding benzoate with 5% KOH in methanol. The compound obtained had to be purified by HPLC. It was obtained in 68% yield, mp 115 °C (lit. mp 115 °C). UV spectrum of the compound showed λ_{max} at 250 nm (lit. λ_{max} 250 nm). The NMR spectrum of the compound showed the olefinic proton at 5.2 ppm and $C_{3\alpha}$-H as a broad multiplet at 3.6 ppm.

$\Delta^{7,14}$-Cholestadien-3β-ol. $\Delta^{5,7}$-Cholestadien-3β-ol benzoate was isomerized to $\Delta^{7,14}$-cholestadien-3β-ol benzoate according to the procedure of Taylor and Djerassi (1977) by passing dry HCl gas through its chloroform solution at −42 °C. The benzoate was obtained in 72.5% yield, mp 144 °C (lit. mp 144 °C). $\Delta^{7,14}$-Cholestadien-3β-ol was obtained by the hydrolysis of the corresponding benzoate with 5% KOH in methanol. The alcohol was further purified by HPLC. It was obtained in 51% yield, mp 104 °C (lit. mp 104 °C). The UV spectrum of the compound showed λ_{max} at 242 nm (lit. λ_{max} 242 nm). The NMR spectrum of the compound showed the C_7 and C_{15} olefinic protons at 5.5 and 5.8 ppm, respectively, and $C_{3\alpha}$-H as a broad multiplet at 3.6 ppm.

$\Delta^{8(9)}$-Cholesten-3β-ol. $\Delta^{8(9)}$-Cholesten-3β-ol benzoate was obtained by the reduction of $\Delta^{8,14}$-cholestadien-3β-ol benzoate according to the procedure of Lee et al. (1969). $\Delta^{8,14}$-Cholestadien-3β-ol benzoate in ethyl acetate was reduced in the presence of hydrogen over Raney Nickel (W2) catalyst in 90% yield, mp 138 °C (lit. mp 140 °C). The corresponding alcohol was obtained by hydrolysis of the benzoate with 5% KOH in methanol. It was further purified by HPLC to give $\Delta^{8(9)}$-cholesten-3β-ol in 65% yield, mp 128 °C (lit. mp 129 °C). The NMR spectrum of the compound showed disappearance of the olefinic proton at 5.2 ppm. The $C_{3\alpha}$-H appeared as a broad multiplet at 3.6 ppm.

Preparation of Vesicles, Determination of Sterol Incorporated, and Glucose Entrapment. Egg PC was isolated from fresh egg yolk according to the procedure of Singleton et al. (1965). The purity of PC was established by TLC analysis, NMR spectroscopy, and determination of the A_{233}/A_{215} ratio spectrophotometrically to ensure that fatty acyl chains in PC are not oxidized (Klein, 1970). PC–sterol vesicles were prepared by taking appropriate amounts of sterol and PC in dry chloroform and evaporating under a stream of nitrogen to get a thin film, which was dried under vacuum for 6 h. To this film, 2 mL of 1 mM glucose (6×10^6 cpm) in 10 mM NaCl was added and vortexed for 2 min. It was then sonicated at 4 °C with a $^1/_4$-in. microtip on a Branson sonicator B-30 for 20 min under argon. The output control was set at 2, and a 50% duty cycle was used for pulse sonication. This preparation was then centrifuged at 105000g for 30 min at 4 °C. To determine the sterol incorporation, the supernatant was analyzed for sterol and PC by extraction with $CHCl_3$–methanol (2:1). Sterol analysis was carried out by HPLC; a standard curve was drawn in the case of each sterol for quantitation. Phosphate assay for estimating PC was carried out according to Ames (1966). The final concentration of PC in the vesicle preparation was 10 mM. A 1.5-mL aliquot of this vesicle preparation was loaded on a 50-mL Sephadex G-50 column preequilibrated with 1 mM glucose in 10 mM NaCl. The vesicles appearing in the void volume were counted to determine the amount of glucose entrapped. An average vesicle size determination was computed by the method of Rhoden and Goldin (1979). The vesicle diameter by this method for PC was 384 Å, and for PC–cholesterol (2:1) vesicles it was 388 Å. At this molar ratio the PC–sterol vesicles had diameters ranging from 350 to 450 Å.

Glucose Permeability Assay. The void volume referred to above was used for the glucose permeability assay according to the procedure of Lala et al. (1978). Glucose release per hour is defined as $100(1 - G_A/G_B)$, where G_B and G_A are amounts of glucose retained in vesicles before and after 1 h of incubation, respectively.

ESR Studies. 12-Doxylstearic acid was prepared from 12-ketostearic acid according to the procedure of Waggoner et al. (1969). ESR experiments were carried out at different sterol concentrations (25, 33, 40, and 50 mol %) in PC with 12-doxylstearic acid as probe.

In a typical ESR experiment, 2.5 μmol of PC and the desired quantity of sterol and 12-doxylstearic acid (25 nmol) were taken in chloroform. A dry film was formed with a stream of nitrogen and the film further dried under vacuum (0.1 mmHg) for 6 h. To the dried film 150 mM NaCl (250 μL) was added and vortexed for 2 min and then added to a 200-μL aqueous quartz cell. The ESR spectrum was then recorded. The order parameter S was calculated as

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - A_{xx}}$$

where $2A_{\parallel}$ is the distance between low-field maximum and high-field minimum and $2A_{\perp}$ is the distance between low-field

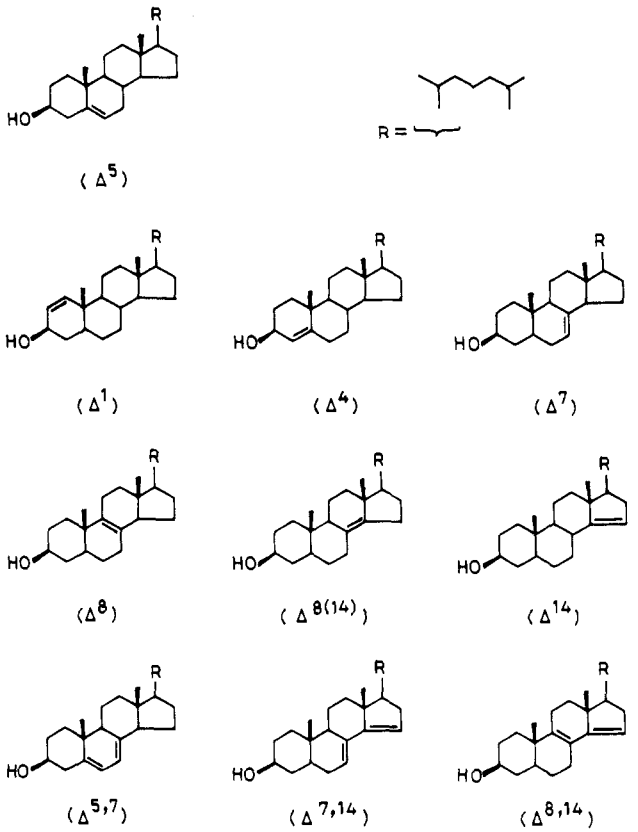

FIGURE 1: Structure of cholesterols and cholestadienols used in this study: Δ^5-cholesten-3β-ol (cholesterol), Δ^1-cholesten-3β-ol, Δ^4-cholesten-3β-ol, Δ^7-cholesten-3β-ol, Δ^8-cholesten-3β-ol, $\Delta^{8(14)}$-cholesten-3β-ol, Δ^{14}-cholesten-3β-ol, $\Delta^{5,7}$-cholestadien-3β-ol, $\Delta^{7,14}$-cholestadien-3β-ol, and $\Delta^{8,14}$-cholestadien-3β-ol.

minimum and high-field maximum. A_{zz} and A_{xx} are the principle values of hyperfine splitting, which correspond to maximum and minimum extent of hyperfine splitting for the probe.

Fluorescence Polarization Measurements. Fluorescence polarization studies were carried out according to Lala et al. (1978), at 20, 33, and 50 mol % sterol concentration. The steady-state fluorescence polarization P was calculated as

$$P = \frac{I_{\parallel}^{c} - I_{\perp}^{c}\sigma}{I_{\parallel}^{c} + I_{\perp}^{c}\sigma}$$

where $I_{\parallel}^{c}$ and $I_{\perp}^{c}$ are the scattering corrected fluorescence intensities with the pair of excitation and emission polarizers parallel and perpendicular to each other and σ is the grating correction factor. Microviscosity or order parameter from these steady-state polarization values was determined as given in the literature (Shinitzky & Barenholz, 1978; Van Blitterswijk et al., 1981).

Results

Several cholesterol isomers wherein the C_5–C_6 double bond has been moved were synthesized. The ring A and B analogues included Δ^1-cholesten-3β-ol, Δ^4-cholesten-3β-ol, and Δ^7-cholesten-3β-ol. In addition, ring C and D analogues like Δ^8-cholesten-3β-ol, $\Delta^{8(14)}$-cholesten-3β-ol, and Δ^{14}-cholesten-3β-ol were prepared to see the effect of double-bond movement. Finally, three dienes, $\Delta^{5,7}$-cholestadien-3β-ol, $\Delta^{7,14}$-cholestadien-3β-ol, and $\Delta^{8,14}$-cholestadien-3β-ol were prepared (Figure 1). These compounds were rigorously purified and characterized. Even though these compounds have been reported in the past, the NMR spectral data of all compounds are not available. This has been incorporated under Materials and Methods as these sets of compounds are likely to prove useful for studying the role of cholesterol in various biological systems.

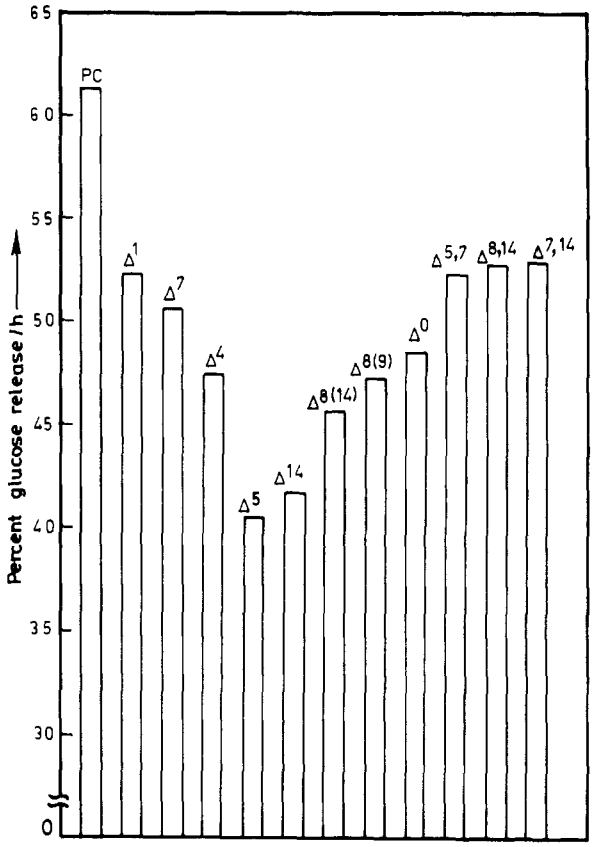

FIGURE 2: Percent glucose release per hour in PC–sterol vesicles at 2:1 molar ratio. Δ^0 refers to 5α-cholesten-3β-ol, and other signs to refer the position of the double bond in the sterol; thus, Δ^5 corresponds to cholesterol.

Incorporation of Sterols in Egg PC Vesicles. The ability of a sterol to be incorporated into vesicles can depend upon the structure of sterol (Demel et al., 1972; Suckling et al., 1979). Single bilayer vesicles from a 2:1 molar ratio of egg PC and sterols were prepared. In all cases, it was observed that more than 90% of the added sterol was incorporated into vesicles except Δ^4-cholesten-3β-ol, which could be incorporated to a maximum of 86%. These results clearly indicated that, at 33 mol % sterol, vesicle properties would reflect the structural variation involved, i.e., the movement of the C_5–C_6 double bond in the cholesterol skeleton.

Glucose Permeability. The entrapment of glucose in PC–sterol vesicles and its permeability across the membrane has been used in the past to assess the condensing effect of sterols. Useful information has been obtained with this technique, which is reasonably sensitive to structural modification in cholesterol (Demel et al., 1972, 1984; Lala et al., 1978). The amount of glucose entrapped in PC–sterol vesicles prepared with sterols used in this work was found to be quite similar, again indicating a similar vesicle preparation in all cases. Glucose release per hour at 33 mol % sterol is given in Figure 2. These data clearly indicate that most of these sterols are reasonably effective in restricting the permeability of glucose unlike C_3-hydroxyl epimers of sterols, e.g., cholestan-3α-ol, which under the present experimental conditions was found to be no better than PC itself. Nevertheless, a relative comparison of the various double-bond isomers of cholesterol indicated that Δ^{14}-cholesten-3β-ol was most effective and almost comparable to cholesterol. On the other hand, Δ^1-cholesten-3β-ol was found to be least effective. The absence of double bond, i.e., cholestan-3β-ol, gives a glucose permeability value

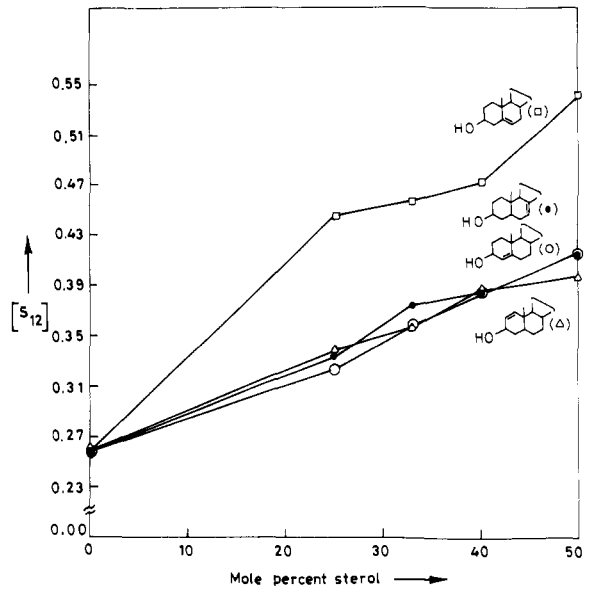

FIGURE 3: Increase in membrane order parameter (S_{12DS}) as determined with 12-doxylstearic acid for PC–sterol vesicles with increasing concentration of sterol; cholesterol (□), Δ^1-cholesten-3β-ol (△), Δ^4-cholesten-3β-ol (○), and Δ^7-cholesten-3β-ol (●).

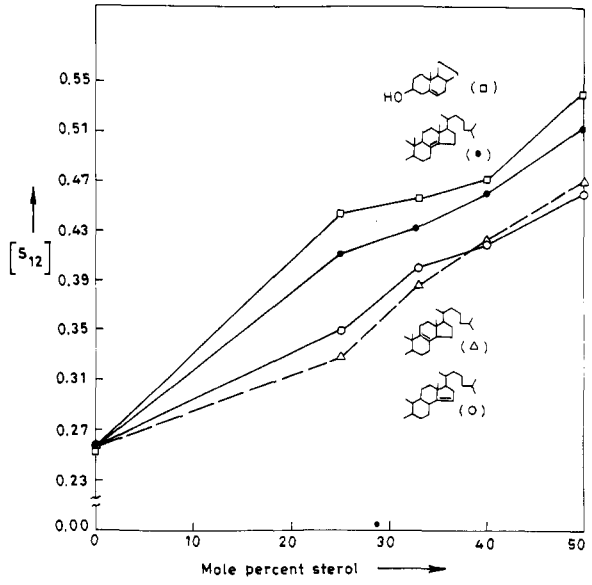

FIGURE 4: Increase in S_{12DS} for PC–sterol vesicles with increasing concentration of sterol: cholesterol (□), Δ^{14}-cholesten-3β-ol (○), Δ^8-cholesten-3β-ol (△), and $\Delta^{8(14)}$-cholesten-3β-ol (●).

that is intermediate between those of Δ^1-cholesten-3β-ol and Δ^{14}-cholesten-3β-ol. The three dienes tried out in our hands gave a response that is quite similar to that of Δ^1-cholesten-3β-ol.

Electron Spin Resonance Studies. Fatty acid spin-labels like 12-doxylstearic acid have been used to determine the degree of order in membranes (Schrier et al., 1978). Increasing concentration of cholesterol gives rise to an increase in order parameter, indicating the condensing effect of cholesterol (Schreier-Mucillo et al., 1973). This technique has also proved quite useful in assessing the effect of structural variations in cholesterol (Dahl, 1981). The effect of sterols with double bonds in rings A and B (Δ^1, Δ^4, and Δ^7) on the membrane order parameter (S) with increasing concentration of these sterols in egg PC liposomes as determined by 12-doxylstearic acid is given in Figure 5. It is clear from these data that compounds with double bonds in rings C and D (Δ^8, $\Delta^{8(14)}$, and Δ^{14}) are more effective in condensing membranes

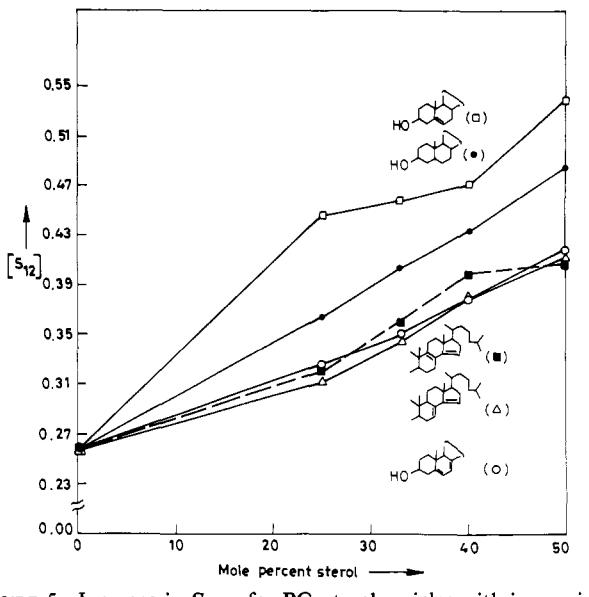

FIGURE 5: Increase in S_{12DS} for PC–sterol vesicles with increasing concentration of sterol: cholesterol (□), cholestan-3β-ol (●), $\Delta^{5,7}$-cholestadien-3β-ol (○), $\Delta^{7,14}$-cholestadien-3β-ol (△), and $\Delta^{8,14}$-cholestadien-3β-ol (■).

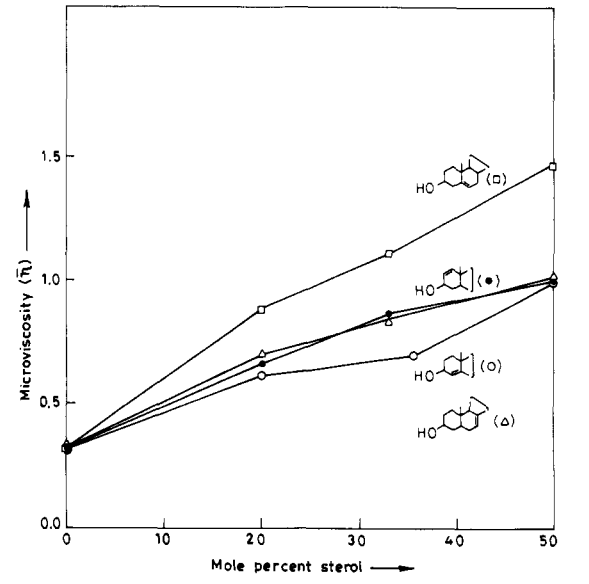

FIGURE 6: Increase in microviscosity η of PC–sterol vesicles with increasing concentration of sterol: cholesterol (□), Δ^1-cholesten-3β-ol (●), Δ^4-cholesten-3β-ol (○), and Δ^7-cholesten-3β-ol (△).

relative to sterols with double bonds in rings A and B (Δ^1, Δ^4, Δ^7), with the exception of cholesterol. Similarly, all the dienes used were found to be relatively less effective in increasing the membrane order parameter.

Fluorescence Polarization Studies. Fluorescence polarization studies using diphenylhexatriene as a probe have been useful in assessing the ability of cholesterol to condense the membrane. An increase in fluorescence polarization with increasing concentration of cholesterol and its analogues has been observed and related to membrane microviscosity η (Shinitzky & Barenholz, 1978; Lala et al., 1978) or more recently to the order parameter (S) (Van Blitterswijk et al., 1981). By use of this technique, it was observed that all sterols show similar behavior until 33 mol % and only at 50 mol % do the sterols with double bonds in rings C and D (Δ^8, $\Delta^{8(14)}$, and Δ^{14}; Figure 7) appear to be more closer to cholesterol compared to sterols with double bonds in rings A and B (Δ^1, Δ^4, and Δ^7; Figure 6). The effect of the position of the double bond in the cholesterol nucleus on the order parameter and

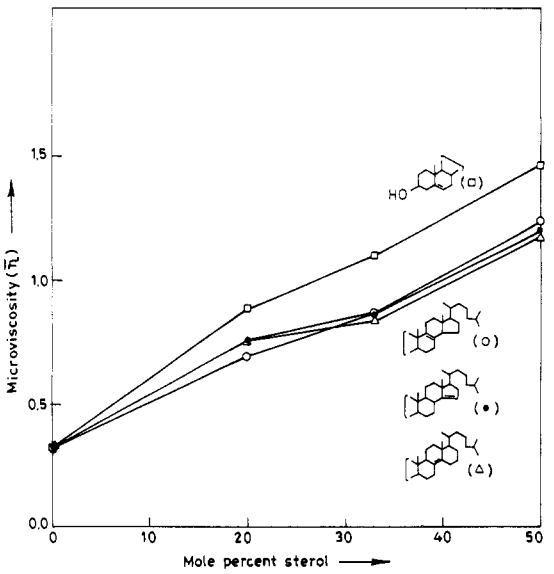

FIGURE 7: Increase in microviscosity η of PC–sterol vesicles with increasing concentration of cholesterol (□), Δ^8-cholesten-3β-ol (○), $\Delta^{8(14)}$-cholesten-3β-ol (●), and Δ^{14}-cholesten-3β-ol (△).

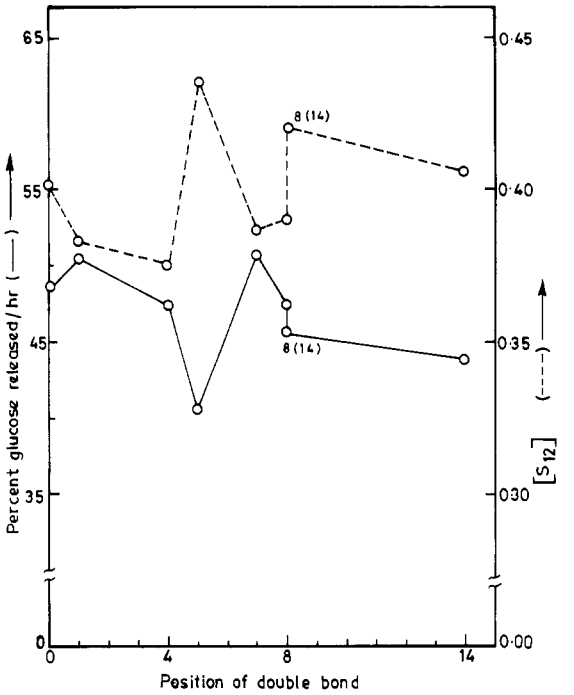

FIGURE 8: Effect of position of double bond in cholesterol ring skeleton on S_{12DS} and glucose permeability in corresponding PC–sterol vesicles at 33 mol % sterol. The C_8–C_{14} double bond bearing sterol is shown separately as at C_8 two double bonds, $\Delta^{8(9)}$ and $\Delta^{8(14)}$, can occur. The zero position corresponds to no double bond, i.e., 5α-cholestan-3β-ol.

the glucose permeability at 33 mol % sterol is given in Figure 8, clearly indicating the similarity in data obtained with the two different techniques.

Discussion

The use of artificial membranes to study the role of sterols in membranes has added considerably to our current understanding of crucial role played by cholesterol (Demel & Dekruyff, 1976). In addition, the use of structural analogues of cholesterol in these studies has greatly helped in understanding the minimal structural requirement for sterol–phospholipid interactions. The results obtained from artificial membranes have also been found to be valid in natural membranes, i.e., in sterol auxotrophs (Nes & McKean, 1977; Bloch, 1983). Thus the 3α-hydroxy isomer of cholesterol, i.e., epicholesterol, is found to be ineffective in both artificial and natural membranes (Demel & Dekruyff, 1976; Nanda Kumari et al., 1982). Unfortunately, due to the very similar behavior of cholesterol and its 5,6-dihydro analogue, cholestan-3β-ol, in artificial membranes (Demel et al., 1972), relatively little attention has been paid to the role of the C_5–C_6 double bond in cholesterol. We have approached this problem by synthesizing and evaluating various double-bond isomers of cholesterol, i.e., Δ^1-, Δ^4-, Δ^7-, Δ^8-, $\Delta^{8(14)}$, and Δ^{14}-cholesten-3β-ols. In addition, $\Delta^{5,7}$-, $\Delta^{7,14}$-, and $\Delta^{8,14}$-cholestadien-3β-ols have also been tried out. Many of these sterols are found to be intermediates in the biosynthesis of cholesterol and in some cases the end products. Thus, Δ^7-cholesten-3β-ol (lathosterol) has been detected in mammalian skin and cuburbitaceac (pumpkin), which biosynthesize only Δ^7-cholesten-3β-ol (Nes et al., 1977; Nes & McKean, 1977). Similarly, Δ^8-cholesten-3β-ol, $\Delta^{8(14)}$-cholesten-3β-ol, and Δ^{14}-cholesten-3β-ol have been detected in the $\Delta^{8(14)}$-monoene route for removal of the 14α-methyl group (Nes & McKean, 1977). In the conjugated diene route for removal of the 14α-methyl group (Nes & McKean, 1977), one finds that $\Delta^{7,14}$- and $\Delta^{8,14}$-cholestadiene-3β-ol besides Δ^8-cholesten-3β-ol are essential intermediates, so it was decided also to synthesize and evaluate these compounds. Δ^1- and Δ^4-cholesten-3β-ol were synthesized to see the effect of double-bond migration to ring A.

It is clear from the results obtained that sterols with double bonds in rings A and B (Δ^1, Δ^4, and Δ^7), except cholesterol (Δ^5), are relatively inefficient in simulating the effect of cholesterol as observed by three different techniques. On the other hand, the Δ^8-, $\Delta^{8(14)}$-, and Δ^{14}-cholesten-3β-ols are much more effective, especially $\Delta^{8(14)}$- and Δ^{14}-cholesten-3β-ols. Figure 8 indicates the effect of double-bond movement in cholesterol on the order parameter and glucose permeability. These two independent techniques give rise to similar conclusions. It is interesting to note that though many of these double-bond isomers of cholesterol get close to simulating cholesterol, none of them including dihydrocholesterol (Δ^0) and the dienes are as effective as cholesterol. Thus the structural requirement of cholesterol for optimal interaction with phospholipids is so stringent that even movement of the double bond in cholesterol is not desirable. It is tempting to speculate here that this could be one of the reasons why during biosynthesis of cholesterol the double bonds from rings C and D are moved to the C_5–C_6 position by a series of enzymes (Nes & McKean, 1977).

The use of molecular models indicates that in the cholesten-3β-ols used here the presence of double bonds at C_1–C_2 and C_4–C_5 severely bends ring A, affecting the orientation of hydroxyl group of cholesterol, and this might affect the usual phospholipid–cholesterol interaction. This phenomenon has been observed recently in the case of the 3α-hydroxyl isomer of cholesterol with ^{2}H NMR, where in the differential orientation of the 3α-isomer vis-à-vis cholesterol has been attributed to its inability to favorably interact with phospholipid and thereby condense membranes (Dufourc et al., 1984; Murari et al., 1986). From molecular models we can only make such an empirical conclusion. The availability of X-ray diffraction data of these sterols and molecular graphics might provide a more comprehensive explanation.

It would be interesting to see the effect of these cholesterol analogues in natural membranes. We tried to use yeast mutant GL-7, which is a sterol auxotroph, to test these compounds, but unfortunately, most of them metabolized with the exception of Δ^1-cholesten-3β-ol and Δ^4-cholesten-3β-ol. Both these compounds were found to be not as effective as chole-

sterol in supporting the growth of GL-7 with the latter compound supporting the growth very poorly (Nanda Kumari et al., 1982).

In two interesting recent papers (Rodruiguez et al., 1982; Rodruiguez & Parks, 1983) it was observed that cholesterol plays a dual role in the membrane, one being the usual bulk membrane fluidity modulation and the second one, which is a nonfluidity-based function, possibly involving sterol–protein interaction. Their results were based on the inability of 5α-cholestan-3β-ol to support the growth of a sterol auxotroph (yeast mutant) unless supplemented with a very low concentration of cholesterol, which by itself was not capable of supporting growth at that concentration. They have called this effect the "sparking effect" of cholesterol. These results point to the significance of the double bond of C_5–C_6 of cholesterol. Similar results have been obtained in the case of the fungus *Phytophthora cactorum*. This fungus lacks the ability to synthesize sterol, and it does not require sterol for vegetative growth but interestingly shows a strong auxotrophic dependence for sterols, e.g., cholesterol, in sexual reproduction. Elliot and Sansone (1977) demonstrated with *P. cactorum* that with 5α-cholestan-3β-ol, while oogonia will form, abortion occurs before maturation takes place to the oospore stage. These studies again demonstrate the importances of the C_5–C_6 double bond of cholesterol.

Maruyoma et al. (1982) have recently reported the ability of some cholesterol analogues, many of which are double-bond isomers of cholesterol, to sustain the growth of the silkworm *Bombyx mori*. These results permit us to compare our results with similar sterols in artificial membranes to results obtained in an in vivo system. Thus, moderate growth was found with larva reared on 5α-cholestan-3β-ol. It was further observed that Δ^{14}-cholesten-3β-ol was very effective in supporting growth though not as effective as cholesterol. This is an interesting observation in view of the fact that our studies also show Δ^{14}-cholesten-3β-ol as the most effective sterol next to cholesterol in regulating membrane fluidity. Δ^{7}-Cholesten-3β-ol was also found to be effective in supporting growth though it was relatively ineffective when compared to cholesterol. It would be interesting to study the effect of various sterols reported here in in vivo systems of the type discussed above to get better insight of the crucial role played by the C_5–C_6 double bond of cholesterol.

Registry No. Cholesterol, 57-88-5; Δ^{1}-cholesten-3β-ol, 72880-82-1; Δ^{4}-cholesten-3β-ol, 517-10-2; Δ^{7}-cholesten-3β-ol, 6036-58-4; Δ^{8}-cholesten-3β-ol, 7199-91-9; $\Delta^{8(14)}$-cholesten-3β-ol, 15147-62-3; Δ^{14}-cholesten-3β-ol, 53368-65-3; $\Delta^{5,7}$-cholestadien-3β-ol, 434-16-2; $\Delta^{7,14}$-cholestadien-3β-ol, 27751-96-8; $\Delta^{8,14}$-cholestadien-3β-ol, 17608-73-0.

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