Chemical Synthesis and ^2H NMR Investigations of Polyisoprenols: Dynamics in Model Membranes†

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ABSTRACT: Polyisoprenols (PIs) such as dolichol and undecaprenol have been shown to play an important role as enzymatic cofactors in the synthesis of glycoconjugates of both prokaryotic and eukaryotic cells. Presented here is a synthetic route used for obtaining specifically labeled [ω,ω-$(C^2H_3)_2$]PIs that initiates with the selective oxidation of the ω-terminal double bond of the PI with *N*-bromosuccinimide. Continuation of the reaction sequence produces an ω-terminal aldehyde three carbons shorter than the original PI. A Wittig reaction with an appropriate deuterium-labeled phosphonium salt is then used to form an ω-terminal-deuterated PI identical with the starting material except for replacement of ^1H with ^2H at the two ω-terminal methyls of the PI. Deuterium NMR spectra of [ω,ω-$(C^2H_3)_2$]geraniol and -farnesol incorporated into phospholipid multilamellar vesicles show powder patterns. The quadrupole splitting of the ^2H NMR signals was interpretable in terms of the degree of orderedness of the ^2H-labeled site. The pure trans isomer geraniol gave rise to a single set of splittings for each C^2H_3 group while farnesol, a mixture of isomers, showed multiple quadrupole splittings. The quadrupole splittings of the PIs increased with increasing concentration of label and with lowering of temperature. Deuterium NMR T_1 measurements, revealing rates of motion of the ^2H-labeled site, showed fast motion for [ω,ω-$(C^2H_3)_3$]geraniol relative to [ω,ω-$(C^2H_3)_2$]cholesterol under similar conditions. A correlation time of 5×10^{-10} s was estimated for [ω,ω-$(C^2H_3)_2$]geraniol, which was 1 order of magnitude faster than for [26,27-$(C^2H_3)_2$]cholesterol. The synthetic scheme presented here also enables the selective oxidation of the ω-terminal double bond of C_{45} solanesol to a C_{42} aldehyde, which will permit the synthesis of [ω,ω-$(C^2H_3)_2$]solanesol. Synthesis of [ω,ω-$(C^2H_3)_2$]undecaprenol and -dolichol by this reaction scheme will be possible. In summary, these results demonstrate both the feasibility of synthesis of [ω,ω-$(CH_3)_2$]PIs and the utility of deuterium NMR investigations of these labeled species in revealing PI order and motions in membranes.

It has long been postulated that the phosphate esters of the polyisoprenols (PIs)[1] undecaprenol and dolichol function in the transbilayer portage of saccharide units across cell membranes in the synthesis of *N*-asparaginyl-linked glycoproteins and bacterial surface glycoconjugates [reviewed in McCloskey & Troy (1980a)]. The carbohydrate moiety, esterified at the polar end of the isoprene molecule, is believed to be translocated from one membrane surface to the other while the nonpolar hydrocarbon chain presumably remains anchored in the nonpolar region of the membrane. This postulate, though widely reported in the literature (Kanegasaki & Wright, 1970; Robyt, 1979), has not been supported by detailed experimental evidence (Johnston & Neuhaus, 1975; Weppner & Neuhaus, 1978). Indeed, recent studies have shown that the actual rate of transverse diffusion (flip-flop) for spin-labeled PI phosphate esters in unilamellar phosphatidylcholine (PC) vesicles is much too slow ($t_{1/2} > 5$ h at 25 °C) to account for the rate of polysaccharide/glycoprotein synthesis (McCloskey & Troy, 1980B). This conclusion was confirmed in microsomes isolated from hen oviduct (Hannover & Lennarz, 1979). Also, one would expect from intuitive chemical arguments that the motion of a polar head group across a nonpolar membrane region would be energetically unfavorable. It has recently been proposed that the PI does not flip-flop but instead may be involved in anchoring a growing glycan to a membranous glycosyltransferase complex (Hannover & Lennarz, 1982).

In order to investigate the ordering and motions of PIs in membranes, a study utilizing ^2H NMR of specifically deuterium-labeled PIs has been initiated. The spin-label studies cited above, though yielding information on the polar end of the molecule, did not provide information on the ω-terminus. Two points needed to be considered before these studies could be carried out. First was how to selective functionalize the ω-terminus for formation of an ω-terminal-labeled PI. Second was the nature of the label to be used. Fluorescense, ESR, and NMR measurements have all been used to probe membrane order and motions (Jahnig, 1979; Davis et al., 1979; Madden et al., 1982). ^2H NMR with isotopic replacement of ^1H by ^2H was selected since it is a nonperturbing method that has been shown to yield valuable information on membrane structure and dynamics (Oldfield et al., 1978; Jacobs & Oldfield, 1981). For example, ^2H labeling of cholesterol in PC liposomes has yielded valuable information on the motions of cholesterol in membranes and its effect on membrane order (Taylor et al., 1981, 1982). The Van Tamalen method for ω-terminal oxidation of polyisoprenes opened the route for specific ^2H labeling of the ω-terminus (Van Tamalen et al., 1982); preliminary spectra of labeled farnesol and geraniol in host liposomes are presented.

In membrane systems, deuterium-labeled lipids characteristically show a ^2H NMR powder pattern. If one assumes that the lipid undergoes axially symmetric motion, the quadropolar splitting $\Delta\nu_q$ of the spectrum is related to the orientational order of the C–D bond in question by

$$\Delta\nu_q = (3/4)(e^2qQ/h)S_{CD} \quad (1)$$

where e^2qQ/h is the static quadropolar coupling constant (168 kHz for deuterons on CD_3 segments) and S_{CD} is the bond order parameter (Seelig, 1977). S_{CD} is given by

$$S_{CD} = (1/2)\langle 3\cos^2\theta - 1\rangle \quad (2)$$

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[1] Abbreviations: PI, polyisoprenol; ESR, electron spin resonance; NMR, nuclear magnetic resonance; NBS, *N*-bromosuccinimide; THF, tetrahydrofuran; *n*-BuLi, *n*-butyllithium; HPLC, high-pressure liquid chromatography; MHz, megahertz; PC, phosphatidylcholine.

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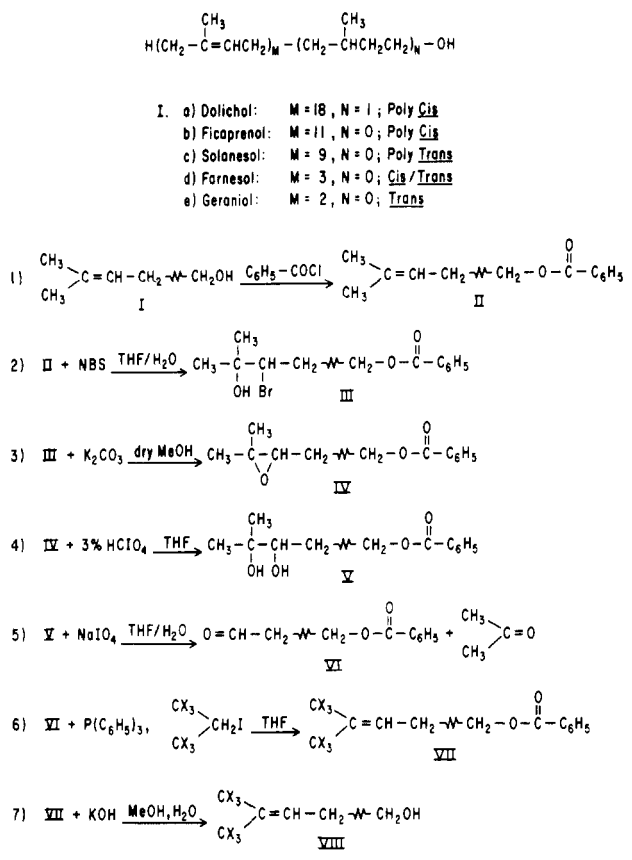

FIGURE 1: Structure and nomenclature of PIs and reaction sequence for synthesis of ω-labeled PIs. In the data presented here, X = ^{2}H.

where θ is the angle between the C–D bond vector and the principal reorientation axis of the lipid (which in most cases is not known) and the broken brackets represent a time average over all molecular conformations. If one has a rigid molecular structure and the angle θ is known, then measurements of $\Delta\nu_q$ will yield values of S_{CD} directly interpretable in terms of the orderedness of labeled lipid in the membrane (Taylor et al., 1981); however, for a flexible molecule such as a PI, S_{CD} reflects both the orderedness and also the value of the angle between the C–D bond and the principal reorientation axis. Thus, a small value of $\Delta\nu_q$ could be due to contributions of low orderedness and/or a θ value near the magic angle of 54.7°.

While $\Delta\nu_q$ and S_{CD} probe the order of lipids in membranes reflecting the amplitude of the anisotropic motions the lipids undergo, T_1 measurements yield information on the rates of the lipid motions (Smith & Jarrell, 1983). The simplest model for T_1 behavior of ^{2}H labels in membranes yields (Davis, 1979)

$$1/T_1 = (3/8)(e^2qQ/h)^2(1 - S_{CD}^2)\tau_c \qquad (3)$$

where τ_c is the molecular correlation time.

Experimental Procedures

Materials. Geraniol (C_{10}-OH) and farnesol (C_{15}-OH) were purchased from Aldrich. Geraniol was entirely trans isomer; farnesol was a mixture of cis–trans isomers. Solanesol (C_{45}-OH) was generously donated by Kuraray Co. of Japan. $(C^2H_3)_2CO$, isotopic purity 99%, was purchased from Aldrich. Deuterium-depleted water, ^1H_2O, was purchased from Aldrich with % $^2H = 5 \times 10^{-5}$. All other chemicals were of reagent grade.

Syntheses. Reaction 1 (see Figure 1). In a typical reaction, 1 equiv of PI was mixed with 1.2 equiv of benzoyl chloride in $CHCl_3$ and the mixture heated with stirring at 40 °C for 2–4 h. The reaction mixture was washed with $NaHCO_3$ (aq) 3 times and the solvent removed under reduced pressure. The ester was identified by a characteristic change in the NMR spectrum. The terminal methylene shifted from 4.15 ppm ($-CH_2-OH$) in the alcohol to 4.8 ppm ($-CH_2-OCOPh$) in the ester. Yields were >90%.

Reaction 2. One equivalent of PI ester was treated with 1.1 equiv of *N*-bromosuccinimide (NBS) in 5/1 tetrahydrofuran (THF)/H_2O at 0–4 °C for ca. 16 h. The product was extracted with ether and washed with water and the solvent removed under reduced pressure. Diagnostic NMR resonance was a single proton peak at 3.95 ppm due to the methine proton –CHBr–. Yields were ca. 75%.

Reaction 3. One equivalent of III was treated with 1.1 equiv of potassium carbonate in anhydrous methanol. The mixture was stirred 20 min at room temperature and then extracted with ether and washed with water, and the solvent was removed under reduced pressure. Diagnostic NMR signal was a triplet of area one proton at 2.7 ppm from the epoxide proton. Yield was >90%.

Reaction 4. One equivalent of IV was treated with 1 equiv of $HClO_4$ present as 3% $HClO_4$ (aq) in 2/1 THF/H_2O. The reaction mixture was stirred 30 min at room temperature and then extracted with ether and washed with water, and the solvent was removed under reduced pressure. The diagnostic NMR resonance was a one proton peak at 3.4 ppm, arising from C*H*OH. Yield was >90%.

Reaction 5. One equivalent of V was stirred with 4 equiv of $NaIO_4$ in 2/1 THF/H_2O at room temperature for 2–4 h. The product was extracted with ether and washed, and the solvent was removed under reduced pressure. Diagnostic NMR signal was the aldehyde CHO proton at 9.8 ppm.

Reaction 6. The deuterated phosphonium salt used in this reaction was formed by first treating 1 equiv of deuterium-labeled acetone with 4 equiv of sodium borohydride in aqueous solution to form the corresponding deuterium-labeled 2-propanol.[2] After a refluxing for 0.5 h with hydriiodic acid, deuterium-labeled 2-iodopropane, $(C^2H_3)_2CHI$, was formed. This was then reacted with 1.1 equiv of triphenylphosphine in xylene and refluxed for ca. 16 h. The resultant solid was isolated by filtration, washed sparingly with xylene, dried, and characterized by ^1H NMR and melting point (246–248 °C). One equivalent of this deuterated phosphonium salt was placed in a dry, N_2-purged reaction vessel in anhydrous THF. A total of 1.1 equiv of *n*-BuLi was then added with a gas-tight syringe, imparting a distinct red color to the solution. One equivalent of VI was then added, resulting in immediate loss of color. The reaction was then stirred ca. 4 h. The entire reaction sequence was conducted at room temperature. The reaction mixture was filtered, and the filtrate was extracted with ether and washed with water and the solvent removed under reduced pressure. The ^1H NMR spectrum of VII was then identical with that of II but showed loss of intensity at the methyl positions, 1.59 ppm (trans) and 1.67 ppm (cis).

Reaction 7. One equivalent of VII was treated with excess potassium hydroxide in 95/5 ethanol/H_2O. The reaction mixture was extracted with ether and washed with H_2O and the solvent removed under reduced pressure. Compared to VII, VIII shows in the ^1H NMR spectrum loss of the phenyl ring resonances (7.3–8.1 ppm) and a shift of the polar terminal methylene from 4.8 to 4.15 ppm.

[2] The reduction in aqueous solution causes a ca. 25% loss in ^2H enrichment due to exchange with solvent. The label is conserved at ca. 75% ^2H throughout all succeeding reaction steps.

Chromatography. High-pressure liquid chromatography (HPLC) was carried out on an IBM LC-9533 with UV detection at 254 or 210 nm. For routine work, a 4.5 × 250 mm analytical octadecyl reverse-phase column was used to determine the purity of isolated materials. For purification purposes, a 10 × 250 mm semipreparative octadecyl reverse-phase column was used. C_{10} and C_{15} derivatives were run in methanol; C_{45} derivatives, in 50/50 2-propanol/methanol.

NMR Measurements. All NMR measurements were carried out on Nicolet NT-360 and NT-500 instruments operating at 360.062 and 500.065 MHz for ^1H and 55.27 and 76.76 MHz for ^2H, respectively. For high-resolution ^2H NMR, the deuterium-labeled PI or PI ester was dissolved in spectrophotometric grade $CHCl_3$ (Aldrich) in 10-mm (NT-360) or 8-mm (NT-500) tubes. High-resolution ^2H spectra were acquired by the standard one-pulse sequence on bandwidths of ca. ±2 kHz, 8K points. For broad-line membrane work, the samples were prepared as follows. Measured aliquots of labeled PI and egg yolk phosphatidylcholine (Sigma, type V-E), each in chloroform, were mixed, and then the solvent was removed under reduced pressure. The sample was then placed under high vacuum (<50 μm) for ca. 16 h. The dried lipids were then suspended in minimal deuterium-depleted water and vortexed for 2–3 min before being placed in the NMR tube. ^2H spectra were acquired by use of the quadropolar echo pulse sequence, 90_x–t–90_y–t–acquire (Davis et al., 1979). Typical t values were 65 μs. The NT-360 delivered a 34-μs and the NT-500 a 27-μs 90° pulse. Though such a long 90° pulse would prove a severe problem for studying lipids deuterated at methylene or methine positions, it proved no barrier for study of C^2H_3 groups as will be discussed further below. T_1 measurements were formed by a modification of a composite-pulse T_1 experiment (Freeman et al., 1980) to include the quadropole echo at the end of the T_1 pulse sequence for data acquisition. Further experimental details of NMR spectra presented are included in the appropriate figure captions.

Results

As described above, the ω-terminus of a polyisoprenol can be selectively functionalized by the Van Tamalen method of treatment of the PI with *N*-bromosuccinimide in a mixed polar organic/aqueous solvent system. It has been shown that NBS in 5/1 THF/H_2O solvent at 0 °C is >90% specific for the ω-terminal double bond of polyisoprene systems of two to six double bonds (Van Tamalen et al., 1982).

The initial step in the reaction sequence we have developed (see Figure 1) is protection of the alcohol of the PI with acetyl chloride or benzoyl chloride (step 1). Then the PI ester is treated with NBS in 5/1 THF/H_2O in the dark for 16 h at 0–4 °C, resulting in selective addition to the ω-terminal double bond of the PI ester (step 2). Step 3 is treatment with potassium carbonate in dry methanol to produce the ω-terminal epoxide PI ester. Reaction of this product with 3% $HClO_4$ in a one-part aqueous–two-parts THF solvent opens the epoxide to produce the ω-terminal *cis*-diol PI ester (step 4). Treatment with 4 equiv of $NaIO_4$ in 2/1 THF/H_2O produces cleavage of the *cis*-diol carbon–carbon bond resulting in an ω-terminal aldehyde three carbons shorter in chain length than the original PI (step 5). The aldehyde formed at this point is the key intermediate in the reaction pathway. At this point, one can incorporate a variety of modifications to the PI structure via a Wittig reaction with the aldehyde. For the purposes of this work, ^2H labels were introduced via conversion of deuterium-labeled 2-iodopropane. This molecule was then

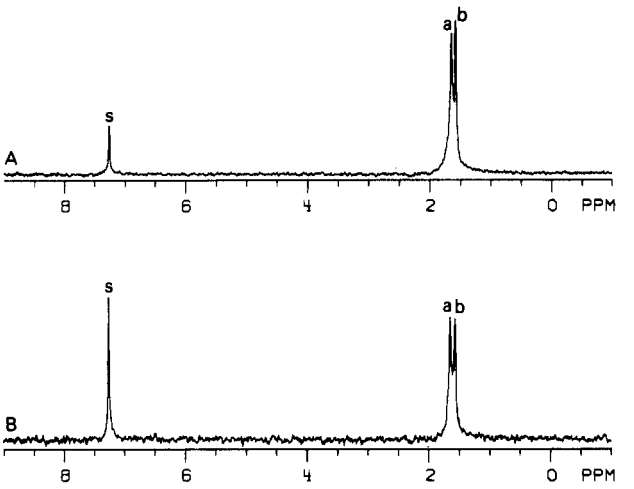

FIGURE 2: ^2H NMR spectra at 55 MHz of (A) [ω,ω-$(C^2H_3)_2$]geraniol benzyl ester and (B) [ω,ω-$(C^2H_3)_2$]farnesol benzyl ester, both in $CHCl_3$ at 25 °C. Resonance s is natural abundance $CDCl_3$ present in solvent. Peaks a and b are ω-*cis*- and *trans*-methyls, respectively.

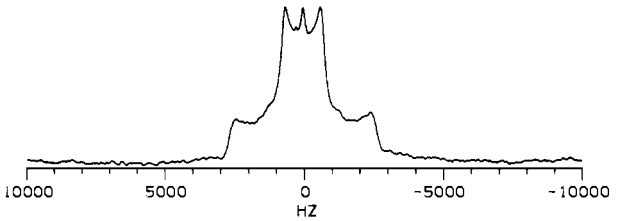

FIGURE 3: ^2H NMR spectrum at 76 MHz of 10 mol % [ω,ω-$(C^2H_3)_2$]geraniol in PC liposomes at 25 °C. The spectrum was acquired by quadrupole echo pulse sequence.

reacted with 1.1 equiv of triphenylphosphine to form the appropriate deuterated phosphonium salt for the Wittig reaction:

$$(C^2H_3)_2CO \xrightarrow{NaBH_4} (C^2H_3)_2CHOH \xrightarrow{HI} (C^2H_3)_2CHI \xrightarrow{Ph_3P} (C^2H_3)_2CHP^+Ph_3I^-$$

With this material, step 6 is then the Wittig reaction with *n*-butyllithium and aldehyde VI to form a ω-terminal-deuterated PI ester, which then can be hydrolyzed (step 7) to form a PI identical with the starting material except for the replacement of ^2H for ^1H at the two terminal methyl groups. Steps 1, 3–5, and 7 all go in yield of >90% with steps 2 and 7 in yields of ca. 75 and 50%, respectively. All of the PI derivatives formed have characteristic differentiating NMR signals and are identifiable on analytical reverse-phase HPLC. Generally, it was not necessary to purify any of the intermediates III–VII; semipreparative-scale reverse-phase HPLC of VII or VIII could be utilized to obtain pure final product for ^2H NMR studies. Figure 2 shows the ^2H NMR spectra of the benzyl esters of [ω,ω-$(C^2H_3)_2$]geraniol (A) and [ω,ω-$(C_2H_3)_2$]farnesol (B) in $CHCl_3$ at 55 MHz. The deuterium-labeled PIs in $CHCl_3$ solution give rise to narrow-line isotropic spectra. Two peaks arise (a and b) in each spectrum due to the differing chemical shifts of the *cis*- and *trans*-methyls.

Incorporation of [ω,ω-$(C^2H_3)_2$]geraniol into egg yolk PC liposomes gives rise to a powder pattern spectrum typical of ^2H-labeled lipids in multilayers. Figure 3 shows 10 mol % ^2H-labeled geraniol in egg yolk PC at 25 °C, 76 MHz. Two separate quadropolar splittings are observed, a narrow component of 1.3 kHz and a broad component of 4.8 kHz. Variable-temperature studies showed that the splittings decrease with increasing temperature, Figure 4. The plots of $\Delta\nu_q$ vs. temperature are not linear, the change in $\Delta\nu_q$ slowing at higher temperature. The effect on $\Delta\nu_q$ of varying the

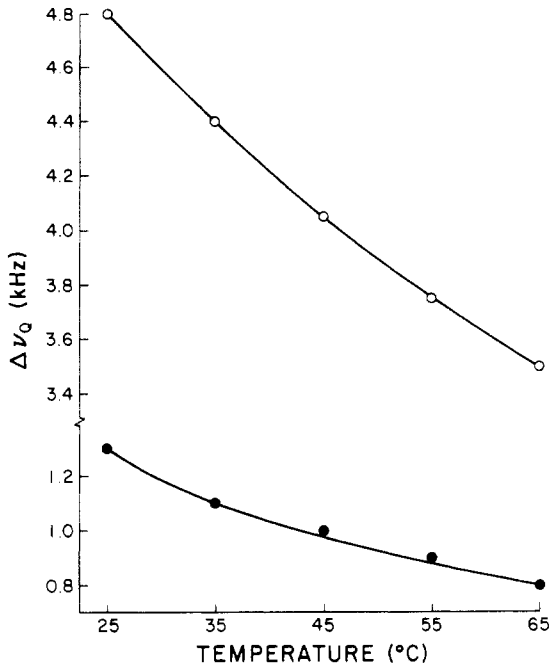

FIGURE 4: $\Delta\nu_q$ vs. temperature for 10 mol % [ω,ω-(C^2H_3)$_2$]geraniol in host PC. The $\Delta\nu_q$ values of the broad component of the spectrum are represented by (O) and those of the narrow component by (●).

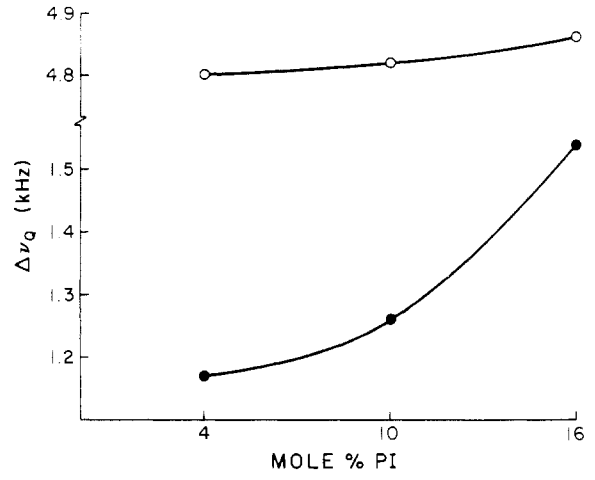

FIGURE 5: $\Delta\nu_q$ vs. mole percent [ω,ω-(C^2H_3)$_2$]geraniol in host PC at 25 °C. The $\Delta\nu_q$ values of the broad component of the spectrum are represented by (O) and those of the narrow component by (●).

concentration of PI to host PC was studied with results shown in Figure 5. In comparison with an analogous labeled lipid, cholesterol ^2H labeled at the 26- and 27-methyls, the change in $\Delta\nu_q$ with concentration for [ω,ω-(C^2H_3)$_2$]geraniol is small, as the cholesterol splittings change >0.5 kHz with increasing concentration. However, the trend is similar to ^2H-labeled cholesterol with increasing concentration of incorporated label resulting in larger quadrupole splittings indicative of more ordering.

T_1 measurements were conducted on 10 mol % [ω,ω-(C^2H_3)$_2$]geraniol at 25 °C and 76 MHz. Plots of peak intensity vs. τ were fitted with a three-parameter equation (Levy & Peat, 1975). The narrow component and broad component have T_1 values of 220 and 140 ms, respectively.[3] These are significantly longer than the T_1 values of ca. 90 ms recorded for [26,27-(C^2H_3)$_2$]cholesterol under similar conditions of label

[3] The T_1 values were found to be independent of field strength for results obtained at 55 and 76 MHz.

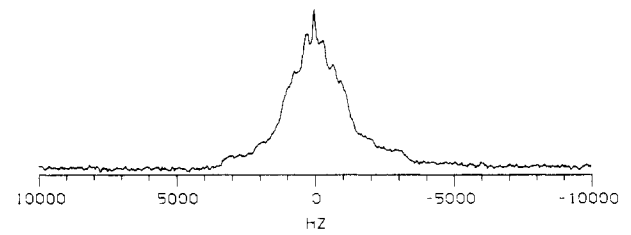

FIGURE 6: ^2H NMR spectrum at 76 MHz of 10 mol % [ω,ω-(C^2H_3)$_2$]farnesol in host PC at 25 °C acquired by quadrupole echo pulse sequence.

mole percent and temperature.

The ^2H NMR spectrum of [ω,ω-(C^2H_3)$_2$]farnesol in egg yolk PC is shown in Figure 6. In contrast to labeled geraniol, which shows two well-defined quadropole splittings, farnesol shows five discernible sets of quadrupole splittings ranging from 0.6 to 5.9 kHz.

Discussion

The magnitude of the quadrupole splitting, $\Delta\nu_q$, is related both to the degree of orderedness of a carbon–deuterium bond and the angle that the C–D bond makes with the principle reorientation axis. In the case of a freely rotating methyl group, C^2H_3, the average carbon–deuterium bond vector extends from the carbon to a point equidistant between the three deuterons. The free rotation of the methyl group causes a 3-fold reduction in the quadrupole splittings relative to a fixed methylene or methine deuteron (Taylor et al., 1982). Thus, the $\Delta\nu_q$ values for the C^2H_3 groups in farnesol and geraniol will be one-third the magnitude of a methine C^2H at the same carbon. The reduced quadrupole splittings for a methyl group compared to methylene or methine labels was advantageous for our studies. The relatively low pulse power of the NT-500, resulting in a 90° pulse width of 27–30 μs, would have been a severe barrier to studying the splittings of more rigid labels but posed no problems for C^2H_3 groups where $\Delta\nu_q$ was always <7 kHz.

Consider first geraniol; the two separate quadrupole splittings most likely arise due to the *cis*- and *trans*-C^2H_3 groups possessing different orientations (θ) with respect to the principle orientation axis. This has been argued for the two splittings observed in [26,27-(C^2H_3)$_2$]cholesterol. Thus, in farnesol, which is a mixture of cis and trans isomers about the two internal double bonds, one observes several quadrupole splittings produced by the different combinations of cis–trans isomers. One can predict that pure geometric isomers of farnesol would give rise to only two splittings as observed for geraniol. Experiments to demonstrate this are in progress.

The ω-terminal double bond constrains the nonpolar terminus of the PI to act as a single rigid unit within a longer flexible molecule. Thus, both *cis*- and *trans*-C^2H_3 would be expected to have the same degree of order, and the difference in $\Delta\nu_q$ in geraniol or any pure geometric isomer would be due to a difference in θ between *cis*-C^2H_3 and *trans*-C^2H_3.

The observation of a decrease in $\Delta\nu_q$ with increasing temperature parallels that made for ^2H-labeled cholesterol in host PC (Smith & Jarrell, 1983). This can be attributed to less order arising from larger motions of the label about its average position. The small increase in $\Delta\nu_q$ with increasing mol % PI also corresponds to observations made for ^2H-labeled cholesterol, with a higher concentration of label resulting in more ordering. The variation in $\Delta\nu_q$ over the PI concentration range studied was not large. This study was not extended to higher PI concentrations since the concentration of PI in any biological membrane is <10%.

The observed T_1 values for [ω,ω-(C^2H_3)$_2$]geraniol are longer than those observed for [26,27-(C^2H_3)$_2$]cholesterol in egg PC bilayers (at 27 °C) (Taylor et al., 1982). This would indicate more rapid motion of the ω-terminal of the PI relative to the similar region of cholesterol. The narrow $\Delta\nu_q$ component of [ω,ω-(C^2H_3)$_2$]geraniol shows a T_1 closer to that of the terminal methyl of [$^2H_{62}$]dipalmitoylphosphatidylcholine in bilayers (Davis, 1979). The difference in T_1 between the broad and narrow components could arise from the dependence on S_{CD} in the T_1 equation (3), since τ_c for the two methyls should be the same. From eq 3, the average value of τ_c for the C^2H_3 groups was 5×10^{-10} s, approximately 1 order of magnitude smaller than that for [26,27-(C^2H_3)$_2$]cholesterol under similar conditions.

These model studies are being extended to longer chain polyisoprenols of biological interest in the synthesis of glycoconjugates, specifically C_{55} undecaprenol and C_{95} dolichol. Preliminary work has shown the ability to form the bromohydrin at the terminal isoprene of C_{45} solanesol. The primary fraction of solanesol acetate treated with NBS shows ω-terminal specificity of the addition by a pair of methy signals at 1.20–1.25 ppm in the ^1H NMR spectrum (not shown). These arise from the two ω-terminal methyl groups, which, being no longer allylic, shift upfield by ca. 0.4 ppm as in the C_{10} and C_{15} compounds. Use of the reaction sequences outlined in the introduction has permitted synthesis of a C_{42} aldehyde analogous to structure VI in Figure 1. This will then serve as a precursor to [ω,ω-(C^2H_3)$_2$]solanesol. Further extensions of the work will permit the synthesis of [ω,ω-(C^2H_3)$_2$]undecaprenol and -dolichol. Use of the methods outlined here will be pursued to investigate the ordering and motions of these long-chain PIs in model and biological membranes and to shed light on their structural and dynamic roles in membrane structure and reactions.

Acknowledgments

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Registry No. NBS, 128-08-5; geraniol, 106-24-1; farnesol, 4602-84-0; solanesol, 13190-97-1; geraniol benzoyl ester, 94-48-4; farnesol benzoyl ester, 89637-63-8; solanesol acetate ester, 58000-93-4; benzoyl chloride, 98-88-4; acetyl chloride, 75-36-5; geraniol (bromohydrin) benzoyl ester, 89637-64-9; farnesol (bromohydrin) benzoyl ester, 89637-65-0; solanesol (bromohydrin) acetate ester, 89637-66-1; geraniol (epoxide) benzoyl ester, 89637-67-2; farnesol (epoxide) benzoyl ester, 89637-68-3; geraniol (diol) benzoyl ester, 89637-69-4; farnesol (diol) benzoyl ester, 89637-70-7; geraniol (aldehyde) benzoyl ester, 89637-71-8; farnesol (aldehyde) benzoyl ester, 89637-72-9; 2-propanone, 67-64-1; geraniol-*d* benzoyl ester, 89637-73-0; farnesol-*d* benzoyl ester, 89637-74-1; 2-iodopropane-*d* phosphonium salt, 89637-75-2; 2-iodopropane-*d*, 39091-64-0; triphenylphosphine, 603-35-0; geraniol-*d*, 66063-44-3; farnesol-*d*, 89637-76-3.

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