Motional Anisotropy-Induced Unequal Deuterium NMR Spin–Lattice Relaxation of 3α-Deuteriocholesterol and 3β-Deuterioepicholesterol in Solution as a Measure of Sterol Motion about the Molecular Axis

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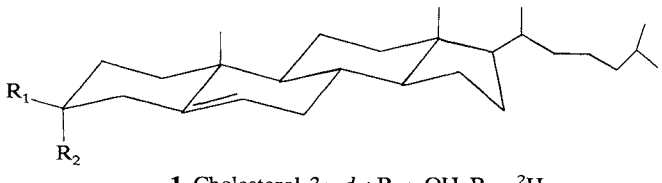
Deuterium spin–lattice relaxation (T_1) data for 3α-deuteriocholesterol and 3β-deuterioepicholesterol in solution were obtained at 12.21 MHz as a function of solvent viscosity (η). The correlation times (τ_c) for both 3-deuteriosterols varied in a linear fashion over the range of viscosities studied. The data showed that although both 3α-deuteriocholesterol and 3β-deuterioepicholesterol undergo rapid reorientations ($\tau_c \approx 10^{-11}$ s) in solution, the relaxation of the deuterium in 3α-deuteriocholesterol was approximately 2.5 times faster than in 3β-deuterioepicholesterol. These unequal rates of relaxation are due to the difference in the angles between the C—D bond and the molecular axis for the two sterol isomers. An equation containing an angular-dependent term was derived to analyze the T_1 data further. The effective correlation times (τ_{eff}), thus calculated, converged for identical viscosities. In the case of 3α-deuteriocholesterol, it was determined that rotation about the molecular axis comprised up to 60% of the motions relaxing the deuteron. The results show that for molecules having an inherent anisotropy and undergoing isotropic motions, it is possible to separate the portion of motion due to rotation about the molecular axis from other motions which contribute to the relaxation processes at a specific center.

INTRODUCTION

Spin–lattice relaxation times of carbons in selected steroids have revealed that the side-chain and methyl carbons undergo internal rotational motions in addition to the overall tumbling motions of the sterol molecule.[1,2] The motional characteristics of the ring carbons in these molecules are due primarily to the overall tumbling motions, since there is no internal reorientation within the rigid sterol ring system.[3] Thus, the contribution of internal motions to the relaxation is eliminated, thereby simplifying the analysis of experimental data. However, the spin–lattice relaxation times for C-3 of several sterols studied by ApSimon *et al.*[2] were found to be shorter than those of the other ring carbons, and the anisotropic rotation of sterols about the long molecular axis (C_∞) was suggested as the cause for decreased motion of the ^{13}C–H bond vector. Interpretation of relaxation data in these ring systems, therefore, requires the analysis of the more complex anisotropic motion if the bond vector of the nucleus causing relaxation is affected to a different degree by the motion of a preferred axis of rotation.

To date, the description of this phenomenon has remained qualitative in nature, since the theory of anisotropic motion invokes high symmetry restrictions which cannot be applied to steroids.[4] However, according to a recent study of the anisotropic motion of cholesterol in phospholipid vesicles, the molecular shape of steroids can be approximated to that of an axially symmetric ellipse.[5]

This paper reports a quantitative explanation of the anisotropic motions of cholesterol and epicholesterol in dilute solutions based on a modification of the theory developed by Brainard and Szabo.[5] These authors illustrated that it is the orientation of a ^{13}C–H interaction vector with respect to C_∞ that is critically important in determining the relaxation rates, even in systems wherein the molecular order parameter, which describes the orientation of C_∞ with respect to the bilayer normal of a membrane, is fairly small ($S = 0.2$). We have investigated an extreme case of this theory. Our results show that the geometry of a C–^{2}H interaction vector at C-3 significantly affects the observed quadrupolar relaxation rates of inherently anisotropic steroids undergoing isotropic motion, wherein the molecular order parameter vanishes ($S = 0$). We measured deuterium spin–lattice relaxation times (T_1) of



1 Cholesterol-3α-d_1; $R_1 = OH$, $R_2 = {}^2H$
2 Epicholesterol-3β-d_1; $R_1 = {}^2H$, $R_2 = OH$

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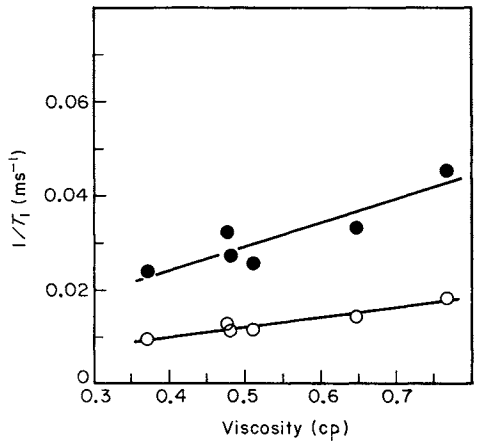

Figure 1. Reciprocal deuterium spin–lattice relaxation times in 3α-deuteriocholesterol (○) (correlation coefficient = 0.971) and 3β-deuterioepicholesterol (●) (correlation coefficient = 0.906) plotted versus viscosity.

3α-deuteriocholesterol (**1**) and 3β-deuterioepicholesterol (**2**) as a function of solvent viscosity, and analyzed the data in the light of the inherent motional anisotropy of these molecules. We also demonstrate the feasibility of using the unequal rates of relaxation found for the two sterols to separate the motion due to rotation about the molecular axis from other molecular motions.

RESULTS AND DISCUSSION

The deuterium spin–lattice relaxation times (T_1) for both 3α-deuteriocholesterol and 3β-deuterioepicholesterol are shown in Fig. 1 as a plot of $1/T_1$ versus the viscosity (η) of the solvents used. For both 3-deuteriosterols, a linear relationship holds over the range of viscosities studied, reflecting the expected decrease in overall molecular motion with an increase in solvent viscosity. The deuterium quadrupolar correlation times (τ_c) given in Table 1 were calculated using Eqn (1)[6] for a molecule undergoing isotropic reorientation, with e^2Qq/h being the electric quadrupole coupling constant.

$$1/T_1 = \frac{3\pi^2}{2}\left(\frac{e^2Qq}{h}\right)^2 \tau_c \qquad (1)$$

The relaxation rates of 3α-deuteriocholesterol and 3β-deuterioepicholesterol indicate that both sterols undergo rapid reorientations ($\tau_c \approx 10^{-11}$ s) in solution. However, the relaxation of the 3α-deuteron in cholesterol was found to be about 2.5 times faster than that of the 3β-deuteron in epicholesterol. This finding is consistent with the earlier observations made on androstane derivatives,[2] which suggested a possible dependence of the ^{13}C relaxation on the orientation of the ^{13}C-3–H bond vector. Our data were further analyzed in terms of the difference in the angle of the C-3—^2H bond in cholesterol and epicholesterol with respect to the molecular axis. If a term accounting for this angle is introduced into Eqn (1) to nullify the angular dependence, then the calculated relaxation rates should become equal for cholesterol and epicholesterol undergoing isotropic reorientations.

We now derive such an equation using as the starting point a function established by Brainard and Szabo.[5] The equation

$$1/\pi T_1 = \frac{3}{8\pi}\left(\frac{e^2Qq}{\hbar}\right)^2 [P_2(\cos\beta)]^2(1-S^2)\tau_\perp \qquad (2)$$

was used to analyze the correlation time of a deuterium attached to cholesterol in the case where there is rapid rotation about the molecular axis but restricted wobbling or off-axis motion of cholesterol in a membrane. The term e^2Qq/h is the quadrupole coupling constant; $\hbar$ is $h/2\pi$; β is the angle between the C—^2H bond and the molecular axis (C_∞); $P_2(\cos\beta)$ is S_β, which is the order parameter of the C—^2H bond with respect to C_∞ and is given by $S_\beta = 1/2(3\cos^2\beta - 1)$; S is the order parameter defining the off-axis or wobble motion of the sterol; and $\tau_\perp$ is the correlation time describing the motion of the molecular axis in a membrane. In using Eqn (2), we stipulate that in addition to the rapid rotation about the molecular axis, the off-axis motion is also rapid so that the molecular order parameter S becomes zero. Thus, Eqn (2) reduces to

$$1/\pi T_1 = \frac{3}{8\pi}\left(\frac{e^2Qq}{\hbar}\right)^2 [P_2(\cos\beta)]^2\tau_{\text{eff}} \qquad (3)$$

which describes the isotropic motion of a molecule

Table 1. Deuterium correlation times[a] and effective correlation times[b] of 3α-deuteriocholesterol (chol) and 3β-deuterioepicholesterol (epi)

Solvent	η[c]	T_1^{chol}[d]	$\tau_c^{chol}\times10^{11}$	$\tau_{eff}^{chol}\times10^{10}$	T_1^{epi}[d]	$\tau_c^{epi}\times10^{11}$	$\tau_{eff}^{epi}\times10^{10}$
Methylene chloride	0.372	104	2.3	1.08	42	5.6	0.93
Chloroform	0.477	81	2.9	1.39	31	7.5	1.27
Carbon tetrachloride	0.767	54	4.3	2.09	22	10.6	1.79
Benzene	0.511	87	2.7	1.30	39	6.0	1.01
Toluene	0.481	88	2.7	1.28	37	6.3	1.06
o-Xylene	0.647	70	3.3	1.61	30	7.8	1.31

[a] Calculated using Eqn (1)[6] and a quadrupole coupling constant of 170 kHz.[7]
[b] Calculated using Eqn (4) and an angle β of 80° for 3α-deuteriocholesterol[8] and 23° for 3β-deuterioepicholesterol based on the value for cholesterol.
[c] Solvent viscosity in centipoise at 37 °C.
[d] Deuterium T_1 values (ms) were measured at 37 ± 1 °C for 0.025 M solutions.

with an inherent anisotropy. Equation (3) can be rewritten as

$$1/T_1 = \frac{3\pi^2}{2}\left(\frac{e^2Qq}{h}\right)^2\left(\frac{3\cos^2\beta - 1}{2}\right)^2 \tau_{\text{eff}} \qquad (4)$$

For molecular systems having no inherent anisotropy and undergoing isotropic reorientations, Eqn (4) reduces to Eqn (1), the familiar form relating deuterium T_1 values to τ_c.

The τ_{eff} values listed in Table 1 were calculated using Eqn (4). As can be seen, the incorporation of an angular-dependent term gives rise to convergent correlation times for both cholesterol and epicholesterol undergoing isotropic motions in solution. Further, the rotation about the molecular axis contributes up to 60% of the relaxation of the 3-deuteron in cholesterol. We believe that these results will help in separating the motional components of inherently anisotropic sterols in anisotropic membrane environments.

EXPERIMENTAL

Proton decoupled deuterium NMR spectra were recorded at 12.21 MHz on a Varian FT-80A pulse Fourier transform spectrometer using a broadband probe, 4K data points and a spectral width of 2 kHz. An external D_2O lock was used for field frequency locking purposes, and the residual leakage was reduced by a nulling procedure. Deuterium T_1 values (±10%) were measured on 0.025 M undegassed samples at 37 ± 1 °C using the Varian T_1 Calc and Analyzer Programs. Solvents (Gold Label grade) were purchased from Aldrich Chemical Company and were used without further purification. Solvent viscosities at 37 °C were obtained from the International Critical Tables.[9]

3α-Deuteriocholesterol and 3β-deuterioepicholesterol were synthesized by reducing cholest-5-en-3-one with sodium borodeuteride in absolute ethanol. The isomers were separated by thin-layer chromatography (TLC) using light petroleum (b.p. 30–60 °C)–ethyl acetate (85:15, v/v) as the developing solvent. The pure compounds were characterized by TLC, melting point, mass spectrometry and ^{13}C NMR.

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