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The dependence of glyceroglycolipid orientation and dynamics on head-group structure *

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The head-group orientations and molecular dynamics of three glyceroglycolipids in aqueous dispersions, as determined by ^2H-NMR, are compared. 1,2-Di-*O*-tetradecyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (α-DTGL) and 1,2-di-*O*-tetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-glycerol (α-DTML), selectively ^2H-labelled on the pyranose ring, at the exocyclic hydroxymethyl group, and at C3 of glycerol, have been studied by ^2H-NMR and the results compared with those reported earlier for 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL). The α-glucolipid exhibits a gel-to-liquid crystal phase transition and a lamellar to hexagonal mesophase transition at temperatures which are similar to those of the β-anomer, β-DTGL. However, α-DTGL exhibits head group orientations and molecular ordering in the lamellar and hexagonal phases which differ strikingly with those reported for the corresponding β-glucolipid. Whereas the head group of β-DTGL is extended away from the bilayer surface into the aqueous phase, that of α-DTGL is almost parallel to the bilayer surface. α-DTGL exhibits a molecular order parameter of 0.56 which is substantially greater than that of its anomer, β-DTGL, 0.45. The latter indicates that the head group region of the α-glyceroglucolipid is characterized by smaller angular fluctuations than that of β-DTGL. On entering the hexagonal mesophase the pyranose ring of the β-glucolipid undergoes a large reorientation relative to the motional axis of the head group, whereas the α-anomer exhibits only a small orientational change. 1,2-Di-*O*-tetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-glycerol (α-DTML) undergoes a phase transition at 47°C, attributed to the unusual lamellar gel to hexagonal phase transition. The pyranose ring of α-DTML, in a mixture with dimyristoylphosphatidylcholine (1:9 mol ratio) to give a lamellar liquid crystalline phase, is oriented away from the bilayer surface into the aqueous environment and has an S_{mol} of 0.75. The results for α-DTML, ^2H-labelled at the C3 position of glycerol, suggest that this segment also has high molecular ordering. α-DTML in a lamellar environment has the least flexible membrane surface of the glyceroglycolipids investigated to date. ^2H-NMR spin lattice relaxation times have been used to probe the head group motions of the glycolipids. The results indicate that the rate of head group motion increases in the order α-DTML < α-DTGL < β-DTGL. The glycolipid head group has lower motional rates than the corresponding motion of phospholipids, with the possible exception of cardiolipin.

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Abbreviations: DTGL, 1,2-di-*O*-tetradecyl-3-*O*-(D-glucopyranosyl)-*sn*-glycerol; DTML, 1,2-di-*O*-tetradecyl-3-*O*(D-mannopyranosyl)-*sn*-glycerol.

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Introduction

Glyceroglycolipids, although known for some time to occur in plants [1,2], bacteria [3,4] and mycoplasma [4,5], have only recently received more intensive investigation with regard to their physical properties in biomembranes and in model membrane systems [6–14]. Much of this effort has focussed on the phase behaviour of various glyceroglycolipids and the thermodynamic parameters associated with transitions between phases [10,13,15]. The majority of the studies have concentrated on the effects of the alkyl or acyl chain composition. One study examining the correlation between the saccharide moiety and the physicochemical properties of glyceroglycolipid membranes concluded that head group flexibility is an important factor in determining the physical properties of these glycolipid systems [11]. The head groups of glyceroglycolipids have been suggested to form strong direct intermolecular hydrogen bonds between the hydroxyl groups of the sugar moieties [15], to bind divalent cations [6,16], and to affect, through their molecular shapes, the ability of glycolipids to form non-lamellar structures [8,11]. Until recently the contribution of the glycolipid head group to the membrane surface properties has been inferred through indirect measurements.

^{2}H-NMR has been used to probe directly the surface of glyceroglycolipid membranes [6,7,17,38]. Earlier studies from this laboratory established that in aqueous dispersions of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (Fig. 1) the orientation of the carbohydrate head group is sensitive to the aggregate structure of the lipid: essentially extended away from the plane of the bilayer into the aqueous environment for lipid in the lamellar phase and less extended (more compact) in the hexagonal phase [17]. The glycerol backbone was found to have motional properties similar to those of phospholipids, but to be more ordered than the carbohydrate moiety [38]. The present study extends these studies to examine the effects of the type of saccharide (glucose vs. mannose, epimerization) in the head group, and the nature of the glycerol-carbohydrate linkage (anomerization) on the head group orientation and motion of the glyceroglycolipid head group. It demonstrates that relatively minor changes in the head group can lead to important effects on head group orientation and dynamics which are reflected in other physical properties of the membrane systems.

Materials and Methods

Dimyristoylphosphatidylcholine (DMPC) was obtained from Sigma, St. Louis, MO.

1,2-Di-*O*-tetradecyl-3-*O*-(α-D-glucopyranosyl)-*rac*-glycerol (α-DTGL) ^{2}H-labelled at C1′ and C6′ of the glucose moiety were chromatographically isolated as the minor product in the synthesis of the corresponding β-anomer [17]. The ^{2}H-labelled α-DTGL had correct elemental analysis, ^{13}C-NMR spectra and the same mobility on TLC ($CHCl_3$/MeOH, 10:1, v/v) as α-DTGL prepared by a different procedure (vide infra).

α-DTGL ^{2}H-labelled to different degrees at the C2′, C4′ and C6′ positions was prepared as follows. 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (11 mmol) was acetylated in a mixture of pyridine (50 ml) and acetic anhydride (20 mmol) at room temperature overnight. Excess acetic anhydride was destroyed with ethanol and the resulting mixture concentrated. The residue was coevaporated with toluene to give a colourless oil which was homogeneous to TLC (ethyl acetate/hexane, 1:3, v/v). The crude acetylated derivative (11 mmol) was dissolved in dichloromethane (200 ml), saturated with HBr (approx. 0.43 M), and HBr was bubbled through the solution at 0°C for 15 min after which TLC showed complete reaction. The solution was concentrated in vacuo and the residue dissolved in dichloromethane. The organic solution was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulphate and concentrated to give the bromide as a colourless oil which was used without further purification.

Crude bromide and tetraethylammonium bromide (11 mmol) in dry dichloromethane (50 ml) were stirred at room temperature for 1 h. 1,2-Di-*O*-tetradecyl-*sn*-glycerol [17] (5 mmol) and ethyldiisopropylamine (11 mmol) were added and the mixture stirred at room temperature. After 24 and 48 h, a further 1 mmol of the glycerol derivative was added. After 144 h total reaction time, the reaction was terminated by the addition of 5 ml of

methanol, diluted with dichloromethane and the resulting mixture washed with 1 M HCl, dried over magnesium sulphate, and concentrated. The residue was chromatographed on silica gel with ethyl acetate/hexane (5 : 95, v/v) as eluant to give the blocked glycoside. The benzylated compound in acetic acid (200 ml) was hydrogenolyzed with 10% Pd/C (600 mg) at room temperature for 24 h. The catalyst was removed by filtration and washed with methanol. The combined filtrates were concentrated. The product was purified by chromatography on silica gel with a step-gradient (2–5%) of methanol in chloroform to give after crystallization from methanol at −10°C, 2.7 g (60% based on 1,2-di-*O*-tetradecy-*sn*-glycerol) of α-DTGL; m.p. 113.5–114°C; elemental analysis : calc. C 68.69%; H 11.53%; found C 68.59%; H 11.68%. ^{13}C-NMR revealed the presence of β-DTGL (less than 10%) which could not be removed by recrystallization.

α-DTGL (100 mg), pre-exchanged with methan[^{2}H]ol and dried, in dry 1,2-dimethoxyethane (15 ml) was refluxed with ^2H_2O-equilibrated Raney nickel (approx. 1 ml settled volume). At 1, 1.5 and 2 h additional catalyst (1 ml) was added. After 3 h the catalyst was removed by filtration, washed with methanol and the combined filtrates concentrated. Recrystallization from methanol afforded the labelled DTGL (30.5 mg). ^{13}C-NMR revealed complete deuteration at C2′, C4′ and 20% deuteration at C6′.

^{2}H-labelled 1,2-di-*O*-tetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-glycerol was prepared according to published procedures [19] using D-[2-$^2H_1$]mannose (Jarrell, H.C., unpublished results), D-[5,6-$^2H_3$]mannose [22] (100% ^{2}H at C6, approx. 30% ^{2}H at C5), D-[2,3,4,6,6′-$^2H_5$]mannose [20] (100% ^{2}H at C2, approx. 80% ^{2}H at C3 and 80% C4, and 50% ^{2}H at C6), and 1,2-di-*O*-tetradecyl-*sn*-[3-$^2H_2$]glycerol [38]. The products had satisfactory elemental analyses, ^{13}C-NMR spectra, m.p. 111–113°C (lit. 113–114°C [19]), and were homogeneous to TLC (chloroform/methanol, 10 : 1, v/v).

NMR samples were prepared by hydrating the lipid with a three-fold excess of ^{2}H-depleted water (Aldrich Chem. Co., Milwaukee, WI). Hydrated samples were cyclicly heated to 55°C with vortexing and freeze-thawed until homogeneous. Lipid mixtures were prepared by evaporating a chloroform/methanol solution of the glycolipid and DMPC (1/9 mol ratio) under a flow of argon followed by 3 h under high vacuum and hydration as described above.

^{2}H-NMR spectra were obtained at 30.7 MHz with data treatment as described previously [17,38]. Spectra were acquired with a 90° pulse width of 3.6 μs (10 mm solenoid coil) or 2.3 μs (5 mm solenoid coil) and a recycle time of 75–100 ms (over $5T_1$). Longitudinal relaxation times (T_1) were obtained as described previously [23]. Spectral de-Paking to give the 90° oriented-sample spectrum was achieved according to the procedure of Bloom and coworkers [24] as described previously [23]. Head-group orientations of the glycolipids were calculated from ^{2}H-NMR data as described previously [17]. Atom positions for the head group were obtained from neutron and X-ray diffraction data on methyl α-D-gluco- and mannopyranosides [21].

Results and Discussion

The structure of the three glyceroglycolipids used in the present study are shown in Fig. 1. The α-glucolipid (α-DTGL) (Fig. 1a) and its corresponding anomer β-DTGL (Fig. 1b) differ only in the orientation of the glucose-glycerol linkage relative to the plane of the glucose ring. The α-mannolipid (α-DTML) differs from its C2′ epimer, α-DTGL, only by the orientation of the C2′ hydroxyl group relative to the sugar ring; axial in α-DTML and equatorial in α-DTGL. The three lipids are models of naturally occurring glycosyldiacylglycerols [1–5]. Previous studies have shown that the C2–C3 bond of the glycerol backbone in phospholipids [25–28] and in β-DTGL [38] is oriented nearly parallel to the bilayer normal. Inspection of the structures in Fig. 1 suggests that if this is true for the α-glycolipids, the orientation of the sugar ring relative to the bilayer surface may be very different for the β-amomer.

Effect of the anomeric linkage

A previous study from this laboratory established that β-DTGL exhibited a transition from a gel- to a liquid-crystalline phase at 52°C and from a lamellar to an hexagonal phase at 58°C [17]. Temperature dependent ^{2}H-NMR spectra of [2′,4′,6′,6″-$^2H_4$]α-DTGL are shown in Fig. 2. At

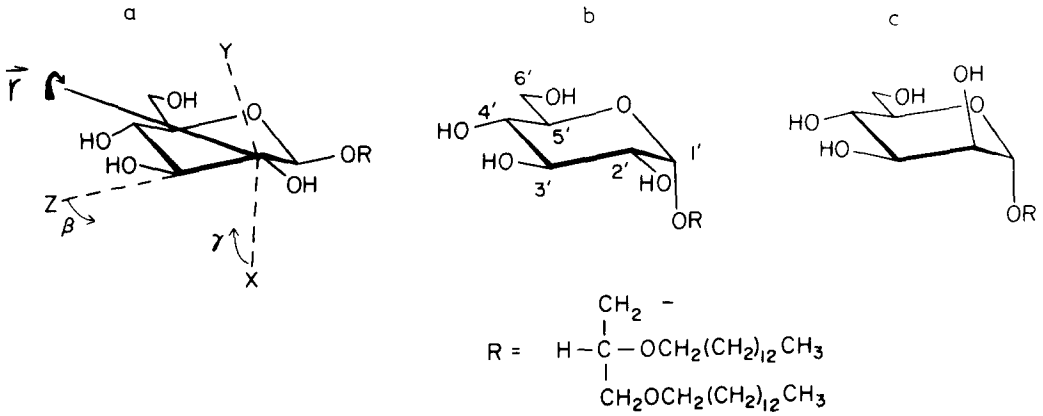

Fig. 1. Structure of 1,2-di-*O*-tetradecyl-3-*O*-(glycopyranosyl)-*sn*-glycerols: (A) β-D-glucopyranosyl; (B) α-D-glucopyranosyl; (C) α-D-mannopyranosyl. Definition of the angles relating the motional axis of the sugar ring, r, to the molecule-fixed axis system: $\vec{r}$ has direction cosines of (cos γ sin β, sin γ sin β, cos β). For the mannose derivative the molecule-fixed axis system is rotated by 90° about z relative to that of (A) and (B).

45°C the spectrum is broad and of low signal to noise ratio (reflecting a short transverse relaxation time, T_{2c}). At 52°C the spectra indicate the onset of rapid motion, attributable to the lamellar liquid crystalline phase which persists to 58°C. At 60°C the spectrum exhibits much smaller quadrupolar splittings, which suggests a hexagonal aggregate structure for the lipid. If all motional and orientational parameters remain the same as in the lamellar phase, and if the lipid is diffusing rapidly about the cylindrical long axis, the quadrupolar splitting is expected to be reduced by a factor of 2 relative to that observed in the lamellar phase [27]. At 60°C the quadrupolar splittings are less than one-half the values at 58°C, suggesting that the orientation of the head group has changed relative to its motional axis (vide infra). A similar result was obtained for β-DTGL [17].

^{2}H-NMR spectra for the various labelled α-DTGL systems are shown in Fig. 3. The ^{2}H spectra of [1′-$^2H_1$]α-DTGL (Fig. 3C) clearly reflect the axial symmetry of the head group motion. In order to describe the motion and orientation of the glucose residue more quantitatively, the motional description used for β-DTGL [17,38] may be adopted. The motion of the head group is represented as a rapid rotation of the rigid ring about an axis which is fluctuating about the bilayer normal. The observed quadrupolar splittings are given by [30,31].

$$\Delta\nu_Q = \frac{3}{4}\frac{e^2qQ}{h}\frac{(3\cos^2\alpha_i - 1)}{2}\cdot S_{mol} \quad (1)$$

where e^2qQ/h is the quadrupolar coupling constant (158 kHz for the ^{2}HCOH group and 164 kHz for the $^2H_2$COH group [17]), S_{mol} is the segmental order parameter describing the motion of the sugar ring, and α_i is the angle between the ith C–^{2}H bond and the motional axis of the sugar ring. The orientation of the motional axis of the pyranose ring may be obtained from the ratio of the quadrupolar splittings given by [32]

$$R_j = \frac{3\cos^2\alpha_j - 1}{3\cos^2\alpha_i - 1}$$

where

$$\cos\alpha_i = a_x\cos\gamma\sin\beta + a_y\sin\gamma\sin\beta + a_z\cos\beta$$

(a_x, a_y, and a_z) and cos γ sin β, sin γ sin β, cos β) are the direction cosines of the ith C–^{2}H bond and the motional axis, respectively, in the molecule-fixed axis system (Fig. 1). Details of the calculations are given elsewhere [17,31,32]. The assignment of the splittings for deuterons at the C2′ and C4′ positions is ambiguous. In the fitting of the ^{2}H-NMR data the resonance assignments of the two deuterons were interchanged and the solutions examined. Only one of the two possible assignments gave a satisfactory fit of the calcu-

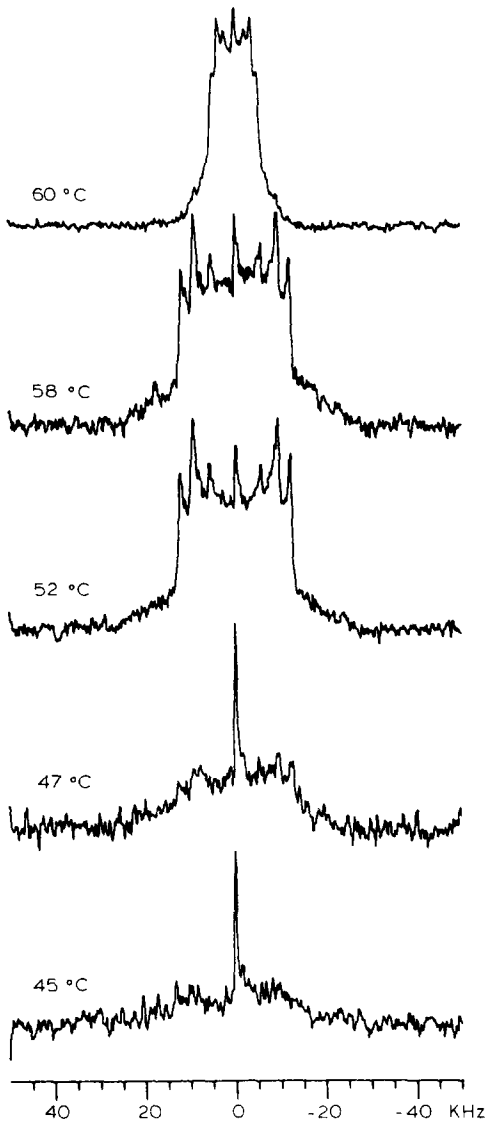

Fig. 2. Temperature dependence of ^{2}H-NMR spectra (30.7 MHz) of α-DTGL labelled at the C2′, C4′ and C6′ positions of the carbohydrate head group.

lated splittings to the experimental values. The results are shown in Table I, as are those for β-DTGL. In Table I, the angles γ and β refer to the orientation of motional axis of the sugar relative to the pyranose ring where Z is along the C2′–C3′ bond and the oxygen atom at C2′ lies in the X–Z plane (Fig. 1). In order to visualize more readily the head group orientations, the results in Table I are represented pictorially in Fig. 4. If S_{mol} were 1.0 (i.e., no wobbling about the bilayer normal), Fig. 4 represents the orientation of the sugar ring relative to the axis of motional averaging. Fig. 4 reveals that the orientation of the pyranose ring differs dramatically for the two anomers. The sugar ring of the β-glucolipid is essentially fully extended away from the bilayer surface, while the pyranose ring of the α-anomer is almost parallel to the bilayer surface. In addition, the amplitudes of the head-group fluctuations away from the bilayer normal, as reflected by S_{mol}, differ for the two anomers (Table I). The α-anomer is more ordered ($S_{mol} = 0.56$) than the β-glycolipid ($S_{mol} = 0.45$). A previous study established that the glycerol C3 segment of β-DTGL has an S_{mol} value of 0.65, indicating that for the sugar ring to have an order parameter of 0.45, there is some motion about the C1′-*O*-C3 glycosidic bond [38]. If the glycerol C3 segment of α-DTGL has similar ordering, an S_{mol} value of 0.56 for the pyranose ring reflects that the corresponding motion about the glycosidic bond is of a smaller amplitude than that occurring in the β-anomer.

On entering the hexagonal phase, β-DTGL undergoes a reorientation of the pyranose ring relative to its motional axis [17] which leads to a more compact head group (smaller surface area per molecule) [38]. In the case of the α-glycolipid, there is no correspondingly large reorientation of the head group relative to its motional axis (Table I; Fig. 4). Whereas the segmental order parameter of the sugar ring in β-DTGL decreases from 0.45 to 0.38 on going from the lamellar to the hexagonal phase [17], α-DTGL exhibits similar ring order parameters for both the lamellar (0.56) and hexagonal (0.60) phases, with that in the hexagonal phase slightly larger.

The hydroxymethyl group of β-DTGL was shown to have two conformations relative to the sugar ring, which were in slow exchange on the ^{2}H-NMR time scale [17]. ^{2}H-NMR spectra of α-DTGL labelled at the C6′ position exhibits four quadrupolar splittings (Fig. 5). The data were analysed as described for the β-anomer [17] to determine the preferred orientations of the C6′ group relative to the pyranose ring. Since the integrated intensities of each of the resonances, as measured from the dePaked spectra (Fig. 5B), were equal, the assignment of the resonances to

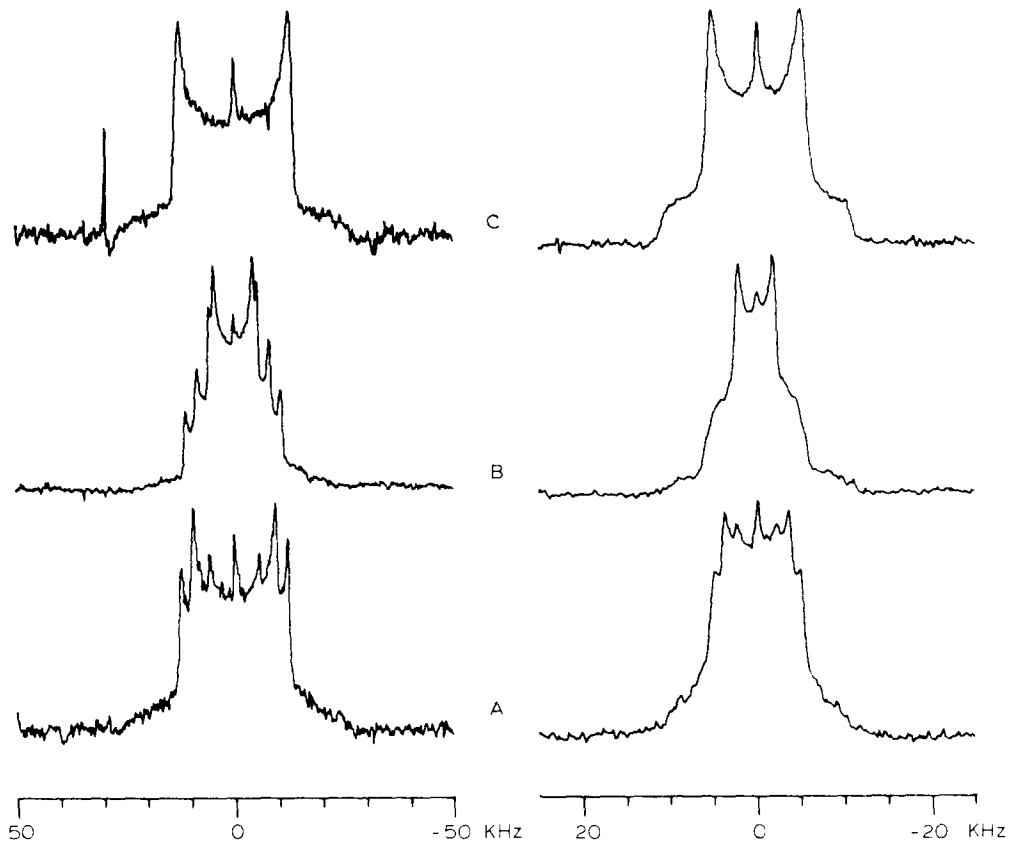

Fig. 3. ^{2}H-NMR spectra (30.7 MHz) of α-DTGL as a function of position of labelling of the lipid head group: (A) [2′,4′,6′,6″-$^2H_4$]α-DTGL; (B) [6,′6″-$^2H_2$]α-DTGL; (C) [1′-$^2H_1$]α-DTGL. Left at 52°C and right 60°C.

TABLE I

CALCULATED ORIENTATIONS OF GLYCEROGLYCOLIPID HEADGROUPS AND QUADRUPOLAR SPLITTINGS ($\Delta\nu_Q$)

Lipid (°C)	Temperature	Orientation [a]		$\Delta\nu_Q$ [b] (kHz)					S_{mol}
		β	γ	C1′	C2′	C3′	C4′	C5′	
α-DTGL	52 [c]	73	91	26.3	19.0	–	24.5	–	0.56
				(26.5)	(19.2)	–	(24.7)	–	
	58 [d]	67	96	11.0	7.9	–	10.6	–	0.60
				(11.0)	(7.9)	–	(10.7)	–	
β-DTGL [e]	52 [c]	10	137	23.1	22.7	24.1	26.5	–	0.45
				(23.1)	(22.8)	(24.0)	(26.6)	–	
α-DTML	52 [d]	72	7	–	4.5	11.0	14.8	11.2	0.41
					(4.5)	(11.1)	(14.6)	(11.2)	
α-DTML-DMPC	45 [c]	11	95	–	15.5	30.5	38.1	30.7	0.75
				–	(15.4)	(30.3)	(38.5)	(30.8)	

[a] Two orientations are obtained, which differ only by 180° rotation about the motional axis.
[b] Values in parentheses are experimental $\Delta\nu_Q$ values.
[c] Lamellar phase.
[d] Hexagonal phase.
[e] Data from Ref. 17.

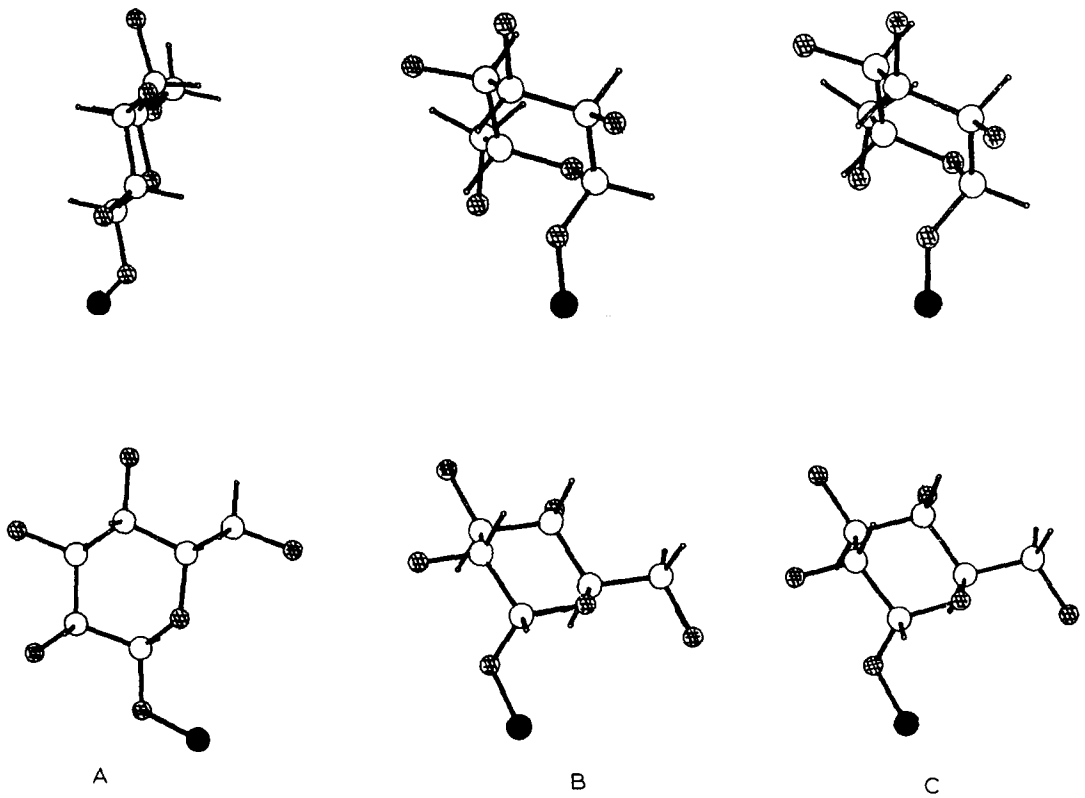

Fig. 4. Orientation of glycolipid head group relative to the motional axis of the sugar ring as given in Table I for: (A) β-DTGL (lamellar) 52°C; (B) α-DTGL (lamellar) 52°C; (C) α-DTGL (hexagonal). If S_{mol} of 1.0, $\bar{r}$ is coincident with the bilayer normal for (A) and (B) and is perpendicular to the long axis of the hexagonal cylinders for (C). The top view represents a rotation of the bottom view by 90° about the bilayer normal (A) and (B) or about the local rotation axis (C). (●) represents the point of attachment of the head group to the glycerol moiety.

either of the pro R or pro S deuterons at C6′ was not unambiguous using only the data for the C6′-labelled lipid. Inspection of ^2H spectra of α-DTGL, labelled at C2′ and C4′, also showed a small percentage of labelling at C6′ which gave $\Delta\nu_Q$ value of 11.7 kHz suggesting that the partial labelling at C6′ had occurred only in the pro R or pro S position. Studies of the ^2H exchange of simple monosaccharides with Raney nickel catalyst have demonstrated that the pro S position of C6 is more rapidly labelled than is the pro R position [22]. It is reasonable to expect that the partial labelling at C6′ of α-DTGL is in the pro S position. The quadrupolar splittings of 11.0 and 22.0 kHz are therefore assigned to the pro S deuteron and those of 9.0 and 16.6 kHz are associated with the pro R deuteron at C6′. The two orientations of the hydroxymethyl group relative to the pyranose ring are shown in Fig. 6. High resolution NMR studies of simple α-glucopyranosides have concluded that there are two major rotamers about the C5–C6 bond which are similar to those shown in Fig. 6 and which have relative populations of 1.5 : 1 [33]. The present study indicates that the two rotamers are in a ratio of 1 : 1. In the hexagonal phase, the C6′ position gives three quadrupolar splittings (data not shown) with integrated area of 2 : 1 : 1, a result which is consistent with two rotamers having essentially equal populations which are in slow exchange. The data available are not sufficient to allow the determination of the orientation of the rotamers as was done for lipid in the lamellar phase because of the uncertainty in the spectral assignments.

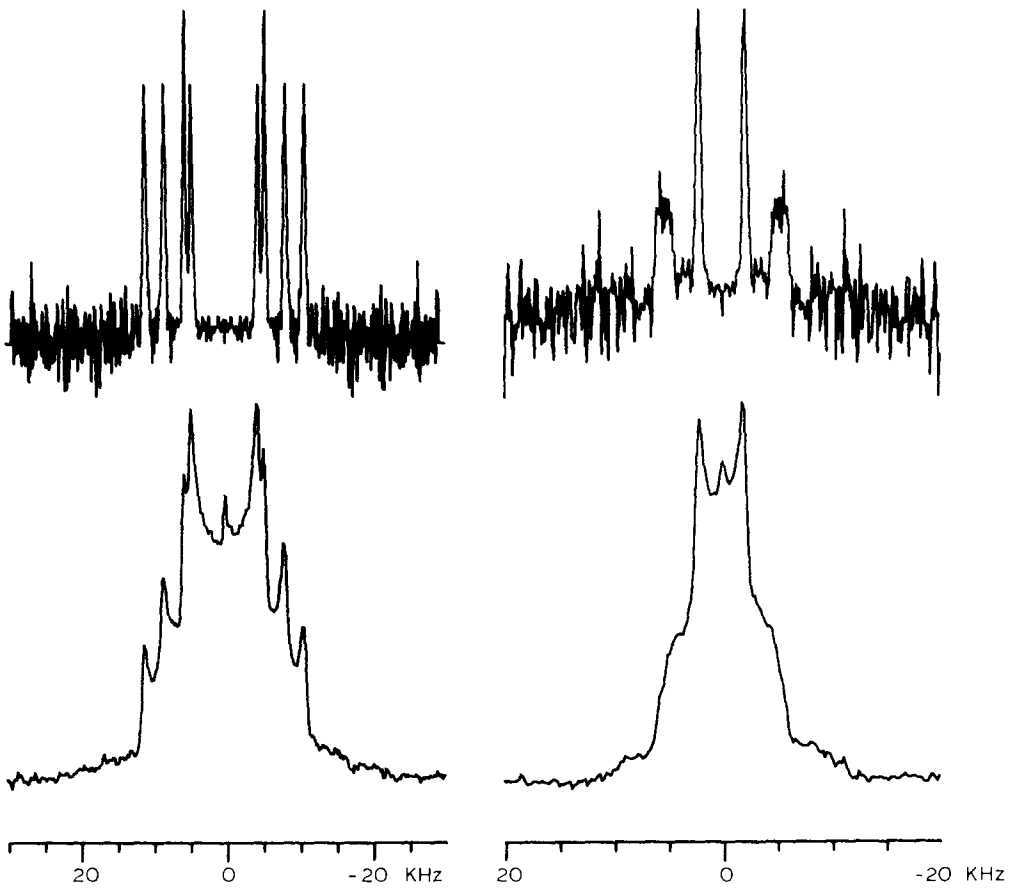

Fig. 5. ^{2}H-NMR spectra, obtained at 30.7 MHz, of α-DTGL labelled at the C6′ position: at 52°C, powder spectrum (bottom left), dePaked spectrum (top left); at 58°C, powder (bottom right), dePaked spectrum (top right).

Effect of epimerization

In addition to the orientation of the glycerol-carbohydrate linkage relative to the pyranose ring the orientation of the saccharide hydroxyl groups

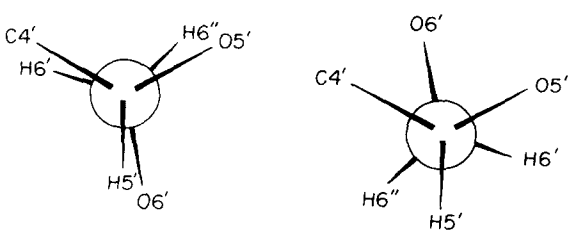

Fig. 6. Calculated orientations of the hydroxymethyl group relative to the pyranose ring in α-DTGL. View is along the C5′–C6′ bond from C5′ toward C6′ with the C4′–C5′–O5′ segment being part of the rigid sugar ring.

may differ in glyceroglycolipids. Thus epimerization at C2 of glucose gives mannose (Fig. 1). Glyceroglycolipids containing two mannose residues occur in *Micrococcus luteus* [4,34]. In order to assess the effect of epimerization at a position of the pyranose ring (other than at C1′) on the head group properties, 1,2-di-*O*-tetradecyl-3-*O*-(α-D-mannopyranosyl)-*sn*-glycerol (α-DTML) labelled at several positions of the sugar ring, on the hydroxymethyl group (C6′), and at C3 of the glycerol backbone were examined. Temperature-dependent ^{2}H-NMR spectra of α-DTML labelled at C2′, C4′ and C6′ of the pyranose ring (Fig. 7) reveal a transition from a broad axially asymmetric spectrum to a sharp axially symmetric powder spectrum above 47°C. These spectral features are attributed to transition from a gel- to a liquid-

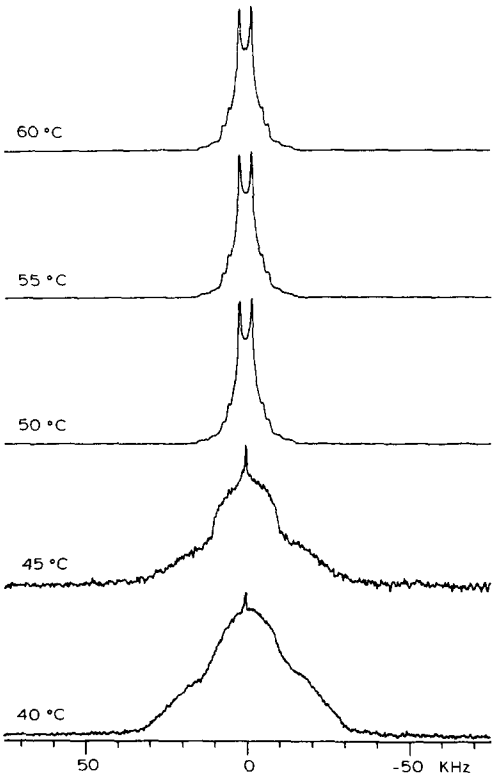

Fig. 7. Temperature dependence of the H-NMR spectra (30.7 MHz) of α-DTML ^{2}H-labelled at C2′, C3′, C4′, and C6′.

crystalline phase. On heating from 49°C to 60°C, no large spectral changes are observed. Similar results were obtained for the other labelled α-DTML lipids (data not shown). The quadrupolar splittings for deuterons at the glycerol and ring positions (Table I), relative to those of the corresponding positions of α-DTGL and β-DTGL, suggest that the mannolipid may have a hexagonal structure above 47°C. In order to confirm the latter speculation, and to examine the lipid in a lamellar environment, mixtures of α-DTML (10 mol%) in DMPC were investigated. Above 45°C the ^{2}H-NMR spectra (Fig. 8) are axially symmetric and exhibit quadrupolar splittings which are substantially larger than those of the pure lipid in the liquid crystalline phase (Table III). These results suggest that pure α-DTML undergoes a transition from the gel state directly to the hexagonal phase. Such a transition, although rare, has been observed in other systems [10,35]. α-DTML has a gel-to-liquid crystal transition temperature similar

TABLE II

^{2}H SPIN LATTICE RELAXATION TIMES OF LIPID HEAD GROUPS

Lipid	Temperature (°C)	T_1 (ms)
α-DTGL [a]	52	4.8
	58	4.8 [b]
β-DTGL [a]	52	5.3– 5.6
	58	5.3– 5.6 [b]
	70	6.7– 7.3 [b]
α-DTML [a,b]	51	3.4– 3.6
	55	3.7– 4.9
	70	5.4– 6.9
DPPC [c]	50	29.7–38.4
POPG [d]	5	5.6– 5.8
Escherichia coli cardiolipin [e]		3 – 9

[a] At 30.7 MHz for: [1′-$^2H_1$]α-DTGL; [2′,3′,4′,6′,6″-$^2H_5$[β-DTGL and [2′,3′,4′,6′,6″-$^2H_5$]α-DTML.

[b] For lipid in the hexagonal phase.

[c] Data from Ref. 36 at 46.1 MHz.

[d] Data from Ref. 37 at 46.1 MHz. Sample is approx. 10°C above its gel-to-liquid crystalline phase transition temperature.

[e] Data from Ref. 18.

to those of the two glucolipids, but does not form a stable lamellar phase. This suggests that replacement of the equatorial hydroxyl group at C2′ of α-DTGL with an axial moiety to give α-DTML destabilizes the lamellar structure.

The orientation of the pyranose ring of α-DTML in the lamellar phase was calculated from the quadrupolar splittings obtained for the α-DTML-DMPC mixture (Table I) (Fig. 9) and the calculated $\Delta\nu_Q$ values are shown in Table I. The orientation of the pyranose ring of α-DTML relative to the bilayer normal is similar to that of β-DTGL (Fig. 4) and differs strikingly from that of α-DTGL. However, the amplitude of the angular fluctuations of the rigid sugar ring of α-DTML as reflected by the segmental order parameter, S_{mol} (0.75), is dramatically smaller than that of β-DTGL (0.45) and α-DTGL (0.56). A previous study showed that β-DTGL (10 mol%) in DMPC had a slightly smaller S_{mol} value (0.38) than pure β-DTGL at 52°C. The latter result indicates that the large S_{mol} value of α-DTML does not result from the phospholipid matrix in which the glycolipid is dispersed. In addition, the head group of

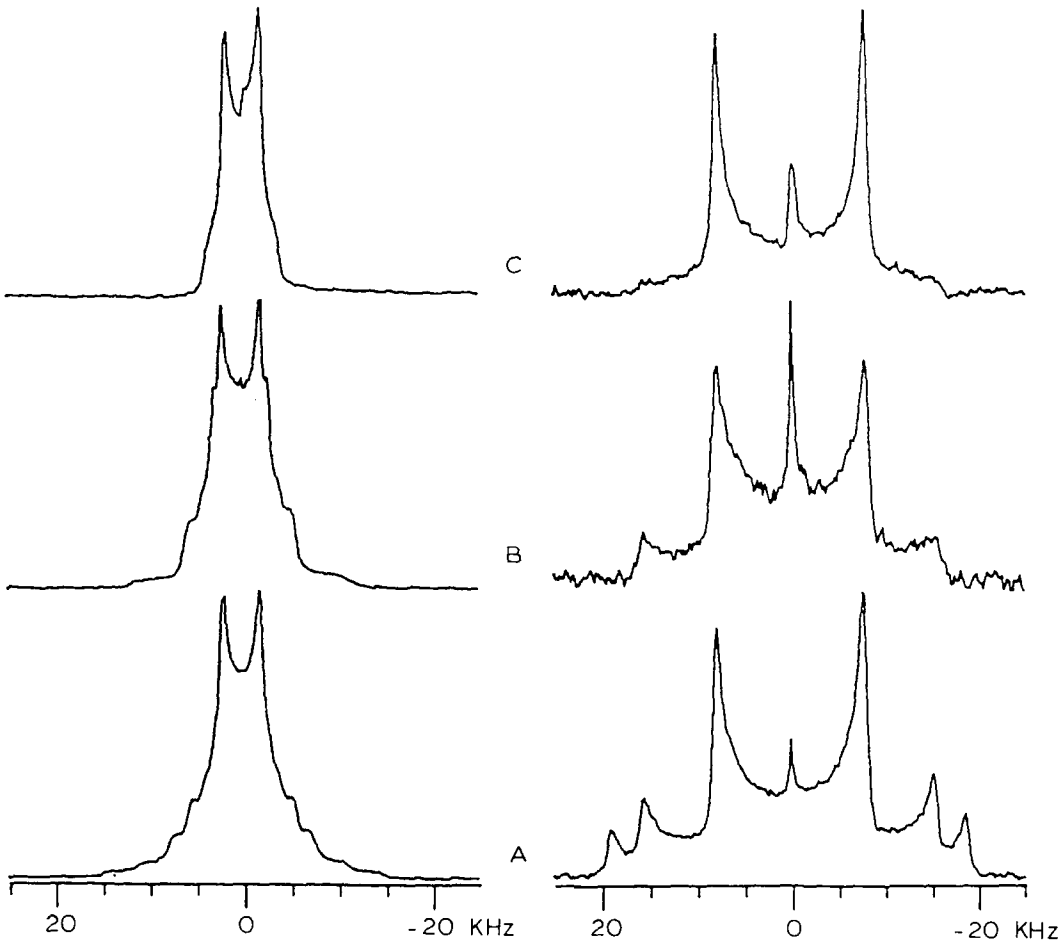

Fig. 8. ^{2}H-NMR spectra (30.7 MHz) as a function of position of labelling: pure α-DTML 52°C (left), α-DTML (10 mol%) in DMPC 45°C (right); (A) labelled at C2′, C3′, C4′ and C6′; (B) labelled at C5′ and C6′; and (C) labelled at C2′.

the β-glucolipid underwent a small reorientation relative to the bilayer normal on incorporation into a DMPC matrix. Since β-DTGL and α-DTML have similar head group orientations relative to the bilayer normal and the amplitude of the wobble of the pyranose ring about the bilayer normal is smaller for the mannolipid (S_{mol} 0.75, semicone angle * of 24° for the wobble) than for the β-glucolipid (S_{mol} 0.45, semicone angle of 37°), the former lipid is expected to have the smaller head group area/molecule. The ability of lipids to form non-lamellar structures has been related to the shape of the lipid molecue, with those lipids having a smaller head group area relative to the acyl chain volume being more predisposed to forming non-lamellar phases [7]. Both β-DTGL and α-DTML have the same alkyl chains so that they differ primarily in the area associated with the head group. Hence, the inability of pure α-DTML to form a stable bilayer can be correlated with its small head group area.

On entering the hexagonal phase the head group of the mannolipid undergoes a reorientation relative to the local motional axis (Fig. 9). The segmental order parameter for the sugar ring is re-

* The semicone angle is calculated simply from S_{mol} via $P_2(\beta)$; it is an empirical measure and does not correspond to the exact limit of motional amplitude for the motional axis.

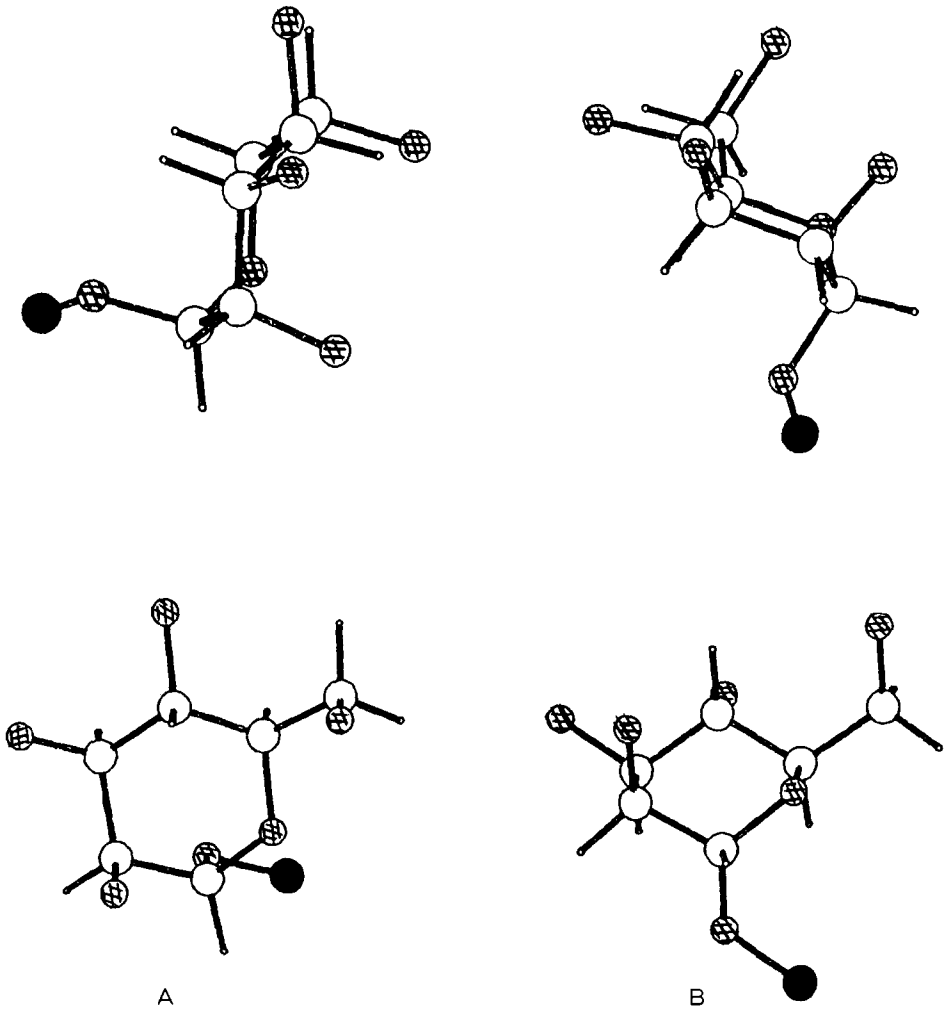

Fig. 9. Calculated head group orientation of α-DTML: lamellar phase (left); hexagonal phase (right). Top view is 90° rotation of the bottom view about the motional axis of the sugar ring. (●) represents the C3 carbon of glycerol.

duced from 0.75 (lamellar) to 0.41 (hexagonal) indicating that the head group is undergoing wobbling of larger amplitude in the hexagonal phase. Interestingly, although α-DTGL and α-DTML differ considerably in orientation of the pyranose ring relative to its motional axis in the lamellar phase, they have similar orientations in the hexagonal phase (Fig. 4 and Fig. 9).

The large segmental order parameter (0.75) obtained for α-DTML in DMPC multibilayers requires that its anchor to the membrane surface, the glycerol backbone segment C2–C3, have the same or higher degree of ordering. For phospholipids [25–28] and β-DTGL [38] the glycerol C3 segment was found to have an S_{mol} value of 0.65 and the C2-C3 bond to be tilted by a few degrees away from the bilayer normal. ^{2}H-NMR spectra of α-DTML ^{2}H-labelled at the C3 moiety of glycerol in a mixture (10 mol%) with DMPC at 45°C and pure lipid at 52°C are shown in Fig. 10, as are spectra for β-DTGL in the corresponding bilayer and hexagonal phases. If the two lipids had the same orientiation of the glycerol C3 segment, the ratio of the two quadrupolar splittings for each of the two deuterons at C3 would be the same, but the $\Delta\nu_Q$ values would be at least 1.63 (the ratio of the S_{mol} values) -times greater for DTML. Inspection of Table I indicates that the

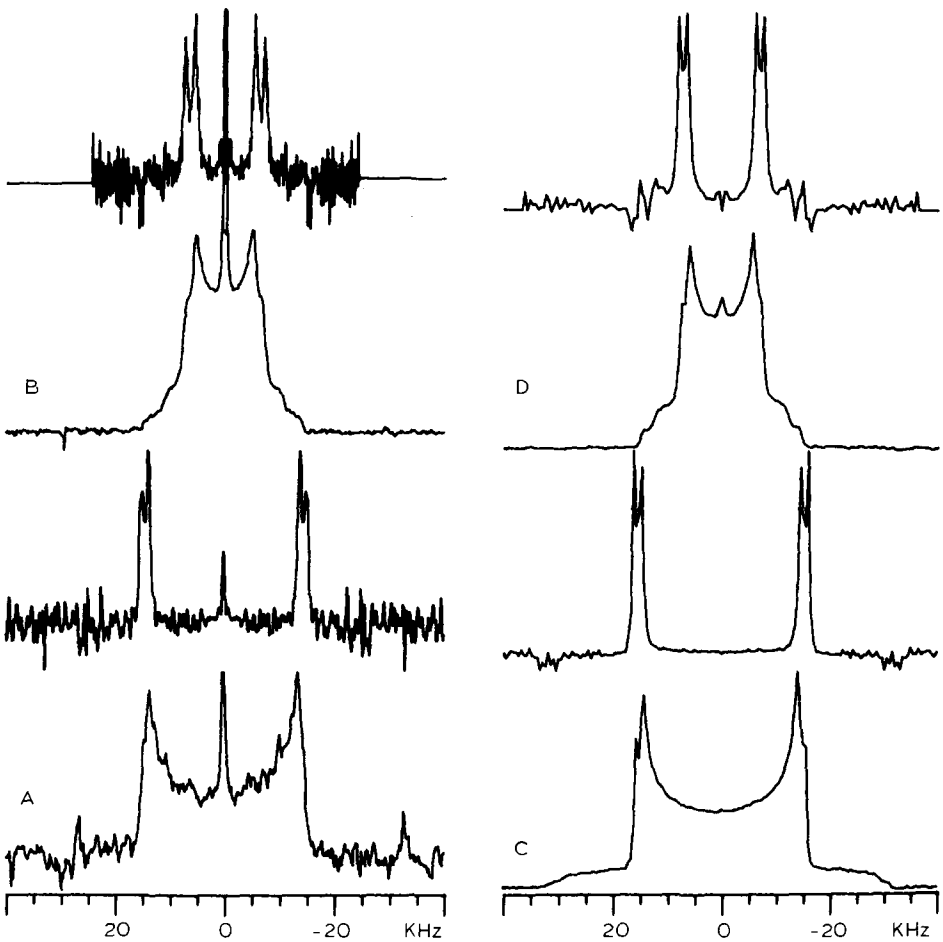

Fig. 10. ^{2}H-NMR spectra (30.7 MHz) of glyceroglycolipids ^{2}H-labelled at C3 of glycerol (A) α-DTML (10 mol%) in DMPC at 45°C; (B) pure α-DTML at 52°C; (C) β-DTGL at 52°C; and (D) β-DTGL at 58°C. Top spectrum in each figure is the 90° oriented sample spectrum (dePaked) calculated from the corresponding powder spectrum.

ratio of the $\Delta\nu_Q$ values is essentially the same for the two lipids, but that the $\Delta\nu_Q$ values associated with the C3 group of β-DTGL are larger than the corresponding values for α-DTML. The results suggest that the orientation of the C3 group of glycerol differs for the two lipids. In addition, inspection of the dePaked spectra for the two lipids labelled at C3 (Fig. 10) reveals that for the glucolipid the resonances associated with the smaller $\Delta\nu_Q$ value are broader than those associated with the larger splitting. α-DTML shows the opposite behaviour with the outer lines being broadened more than the inner resonances. A previous study demonstrated that for β-DTGL the greater broadening of the resonances associated with the smaller $\Delta\nu_Q$ value arises from the greater ^{1}H–^{2}H dipolar interaction between the pro S deuteron at C3 and the proton at C2 and reflects a preferred conformation about the C2–C3 bond of the glycerol backbone [38]. Although there are insufficient data to interpret fully the results for α-DTML, some comments may be made.

The segmental order parameter for the glycerol C3 segment must be 0.75 or more. Orientations of this segment relative to the bilayer normal were examined, by a previously reported procedure [38], for those orientations of the glycerol segment which reproduced the observed $\Delta\nu_Q$ values and corresponded to $S_{mol} \geq 0.75$. Since the assignment of the resonances to the pro S and pro R deuterons

on C is not known, both combinations of assignments were considered. Only two possible orientations of the C2–C3 bond were consistent with the experimental results; one has the bond almost along the bilayer surface, which is deemed unlikely, and the other has the C2–C3 bond tilted relative to the bilayer normal by approx. 8°. For this orientation an S_{mol} of 0.85 was obtained. For β-DTGL the C2–C3 bond of glycerol was found to be tilted away from the bilayer normal by 2–4° [38]. In addition, the calculated orientation of α-DTML differs from that of β-DTGL by a 40° rotation about the C2–C3 bond (i.e., the two lipids have different conformations of the glycerol backbone). For α-DTML this orientation causes the pro R deuteron to be in closer proximity to H2 than the pro S deuteron, which would lead to a greater dipolar broadening for the pro R deuteron resonance in accord with the experimental results (Fig. 10). If the C2–C3 bond of glycerol is almost parallel to the bilayer normal, the orientation of the pyranose ring of the mannolipid as shown in Fig. 9 requires that the conformation about the glycosidic bond (C1′–O3–C3) differ dramatically from that of β-DTGL, and most likely from that of the α-glucolipid.

In both α-DTGL and β-DTGL the exocyclic hydroxymethyl group has been established to exist in two rotameric forms which are in slow exchange on the ^{2}H-NMR timescale. Inspection of ^{2}H spectra of α-DTML labelled at C5′ and C6′ (approx. 30% labelling at C5′ and 100% at C6′) reveals that in addition to the resonance of the deuteron at C5′ only two quadrupolar splittings are observed for the C6′ deuterons, indicating that there is only one preferred rotamer about the C5′–C6′ bond or that there are two or more rotamers in fast exchange. Neutron diffraction studies of methyl α-D-mannopyranoside [21] indicate that the C6–O6 bond is antiperiplaner to the C5–H5 bond, while ^{1}H-NMR studies indicate that in addition to the latter rotamer a second one is present in which the C6–O6 bond is *gauche* (+) to the C5–O5 bond. For these rotamers, quadrupolar splittings of 33.7 and 92 kHz, and 92 and 37.9 kHz, respectively, are calculated for the molecular ordering and orientation of the mannose head group determined for the mannolipid in DMPC. Fast exchange between equal populations of these rotamers would give $\Delta\nu_Q$ values of 29 and 27 kHz for the pro S and pro R deuterons, respectively. The results indicate that the orientation of the hydroxymethyl group relative to the pyranose ring must differ from those reported for simple mannopyranosides but the present results do not permit a quantitative elucidation of these orientations.

Head-group dynamics

^{2}H-NMR spin-lattice relaxation times (T_1) were measured by the inversion-recovery technique for the three glycolipid head groups (Table II). Inspection of Table II reveals that the shortest relaxation times are associated with α-DTML while the longest T_1 values are shown by β-DTGL. α-DTGL exhibits intermediate T_1 values. Included in Table II for comparison are the relaxation times which have been reported for several phospholipid head groups [36,37,18]. The relaxation times for the glycolipids increase with temperature indicating that the motion is in the short correlation time regime. For β-DTGL the T_1 values are also greater when measured at a higher magnetic field (Jarrell, H.C., unpublished results) indicating that the relaxation times are near the minimum value for T_1 and hence the molecular motion is approaching a correlation time, τ_c, of 0.65 ω_0^{-1} (for 30.7 MHz, $\tau_c \approx 3 \cdot 10^{-9}$ s) at 52°C. The rates of head group motion for the three glycolipids can be concluded to increase in the order α-DTML < α-DTGL < β-DTGL. The head-group segments of phosphatidylcholine and phosphatidylglycerol exhibit longer relaxation times corresponding, in the fast motional regime, to correlation times of 0.05–0.5 ns [36,37]. In contrast, the head group of cardiolipin, which is constrained between two diacylglycerophosphate residues, exhibits short relaxation times for the three glycerol segments (Table II), reflecting rather long correlation times of 0.5–5 ns [18]. The three glycolipids of the present study have slower motion at the membrane surfaces than do the phospholipids, with the possible exception of cardiolipin.

Conclusions

Relatively minor changes in glyceroglycolipid head groups have been shown to lead to dramatic

differences in the physical properties of glycolipid membranes. Replacement of an equatorial linkage of the glycerol backbone to the head group results in a less dynamic membrane surface, as reflected in the greater orientational order parameter of the head group and its slower rate of molecular motion. Altering the lipid head group by the replacement of an equatorial hydroxyl group with an axial one can lead to a destabilization of the bilayer structure and formation of non-lamellar structures. The latter effect most likely reflects the smaller area occupied by the head group relative to that of its epimer.

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