Synthesis and Polymorphism of 3-Acyl-*sn*-glycerols†

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ABSTRACT: 3-Acyl-*sn*-glycerols with even-numbered saturated fatty acyl chains from decanoate to lignocerate were synthesized. Successful hydrolysis of the long acyl chain intermediate 1,2-isopropylidene-3-acyl-*sn*-glycerols from stearate to lignocerate was accomplished by applying the compounds to silica gel and exposing them to hydrogen chloride gas at −75 °C. The purity of the compounds was checked by boric acid impregnated thin-layer chromatography, ^{13}C NMR, and reverse-phase high-pressure liquid chromatography. Differential scanning calorimetry and X-ray diffraction techniques were used to study the polymorphism of the compounds. In the β phase obtained from solvent of crystallization, the acyl chain packing was in a two-dimensional oblique lattice with specific chain–chain interactions with a tilt angle of 55.4° from the bilayer plane. The thickness of the region containing two glycerol head groups was 12.7 Å. The phase transition enthalpy of melting for the β phase was 1.06 kcal/mol of CH_2. On being cooled these compounds crystallized reversibly to an unstable α phase, which on being further cooled underwent a second crystallization to a β or β' phase. The thermodynamic parameters and long spacings of these compounds in both β and α phases were linear, indicating isostructural packing in each phase. The enthalpy of the melting transition of the α phase was 0.69 kcal/mol of CH_2. In this phase, the chains were packed in a hexagonal lattice with nonspecific chain–chain interactions. The thickness of the head-group region (12.2 Å) and the tilt angle (55°) of the acyl chains in the α phase were very similar to those in the β phase.

Monoacylglycerols are amphipathic molecules of importance in both industrial and biological contexts. In the food industry racemic mixtures of 1- and 3-acylglycerols and 2-acylglycerol are employed as emulsifying agents and they also membranes as the starting point for synthesis of more complex emulsifiers and detergents. In biology monoacylglycerols are important products and intermediates of many lipid reactions. For example, 2-acyl-*sn*-glycerols are one of the final products of lipid digestion in the intestine (Carey et al., 1983), intermediates of lipoprotein lipase triglyceride lipolysis in the plasma (Redgrave, 1983), and also intermediates of cellular triglyceride lipase reactions.

During the metabolism of glycerolipids, monoacylglycerols may be present transiently in fairly high concentrations in the intestinal lumen and cell during fat absorption, in lipoproteins, in plasma membranes, in the plasma bound to albumin, and perhaps within cells of liver and adipose tissue. In view of their wide distribution it is important to understand the physical properties of monoacylglycerols and their interactions with membranes and lipoproteins.

2-Lauroyl-*sn*-glycerol (β-monolaurin) was crystallized by Larsson (1964a) and found to form a triclinic crystal lattice in which the glycerol moieties are in a hydrogen-bonded lattice roughly parallel to the plane of the bilayer. The chains were packed in a triclinic subcell and tilted at approximately 44°, with respect to the plane of the bilayer.

Most of the previous studies on monoacylglycerols have used racemic mixtures of 1- and 3-acyl-*sn*-glycerols. Larsson (1964b) studied a racemic mixture of 1- and 3-stearoyl-*sn*-glycerols and found two crystal forms based on a large complex monoclinic lattice containing eight molecules in the unit cell. In both forms the fatty acyl chains were tilted at 55° with respect to the bilayer plane. An enantiomeric analogue of 3-lauroyl-*sn*-glycerol, 11-bromo-3-undecanoyl-*sn*-glycerol, packs in a monoclinic cell with the glycerol roughly perpendicular to the plane of the bilayer and the chains tilted at about 63° to the bilayer plane (Larsson, 1966a). Thus, the position of the chain and/or the particular enantiomer of the monoacylglycerol may determine not only the chain packing but also the orientation and conformation of the glycerol moiety in the crystal lattice.

Monoacylglycerols and other substituted aliphatic hydrocarbons have many polymorphic forms that are generally classified as α, β, or β' on the basis of their chain-packing mode (Larsson, 1966b). In monoacylglycerol systems, the α form is in general the least stable. However, small amounts of water may stabilize the α form so that it becomes more stable than other crystalline states (Lawrence & McDonald, 1966).

Because enantiomeric monoacylglycerols have not been investigated in detail, we have begun a series of studies to delineate differences in the physical behavior of different isomers of monoacylglycerols. In this paper we describe the synthesis of a homologous series of 3-acyl-*sn*-glycerols and determine their physical characteristics by calorimetry and X-ray diffraction. Future studies will examine the interaction of these molecules with water and other lipids.

Materials and Methods

Chemicals. Fatty acids (99% purity) were purchased from Sigma Chemical Co. Sodium borohydride, periodic acid, 4-(dimethylamino)pyridine and *N*,*N*′-dicyclohexylcarbodiimide were from Aldrich Chemical Co. Zinc chloride, D-mannitol, and the solvents (HPLC grade) were from Fisher Scientific Co. 2-Palmitoylglycerol was purchased from Serdary Research Labs (Canada). Silicic acid (100–200 mesh) from Alltech Associates was used.

Synthesis. The synthetic scheme adopted in the synthesis of 3-acyl-*sn*-glycerols is shown in Figure 1. 1,2-Iso-

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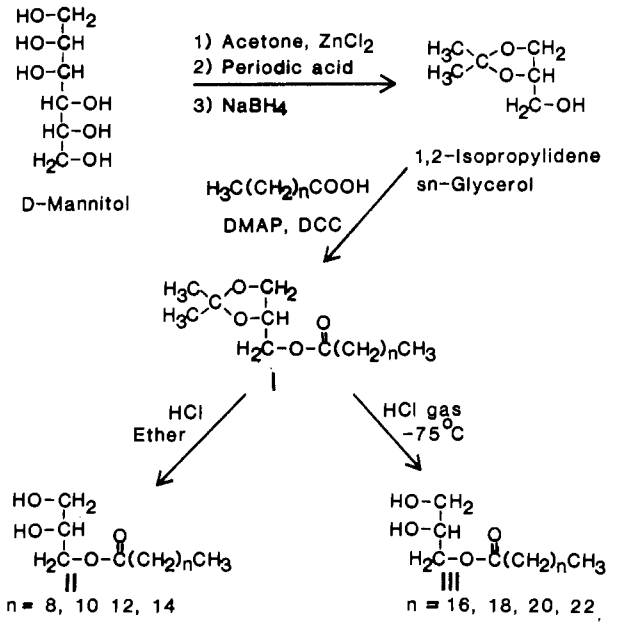

FIGURE 1: Synthesis of 3-acyl-*sn*-glycerols. Compounds I are 1,2-isopropylidene-3-acyl-*sn*-glycerols. *n* represents the number of methylene units in the 3-acyl chain. DCC, *N,N'*-dicyclohexyl carbodiimide; DMAP, 4-(dimethylamino)pyridine. II and III are 3-acyl-*sn*-glycerols.

propylidene-*sn*-glycerol was synthesized from D-mannitol by the procedure of Baer (1939) as modified and improved by Eibl (1981). This compound was esterified with the appropriate fatty acids in the presence of 4-(dimethylamino)pyridine and *N,N'*-dicyclohexylcarbodiimide to give 1,2-isopropylidene-3-acyl-*sn*-glycerols (I) (Kodali et al., 1984). These compounds were purified by column chromatography done under pressure (Still et al., 1978) on silica gel with gradient elution from hexane to hexane/diisopropyl ether, 90/10 (v/v).

The intermediates 1,2-isopropylidene-3-acyl-*sn*-glycerols (I) were hydrolyzed to the 3-acyl-*sn*-glycerols (II) by adopting the procedure of Baer & Fischer (1945), which uses hydrochloric acid in diethyl ether. However, this procedure was effective only for those compounds with a 3-acyl chain length of less than 18 carbons. Hydrolysis of the protecting ketal group of the 1,2-isopropylidene-3-acyl-*sn*-glycerols became increasingly difficult as the 3-acyl chain length increased beyond 16 carbons, because the longer acyl chain compounds were poorly soluble in ether or in other common organic solvents at low temperatures. Higher reaction temperatures are known to increase acyl migration (Martin, 1953). When boric acid hydrolysis (Hartman, 1959) of 1,2-isopropylidene-3-behenoyl-*sn*-glycerol was attempted, the hydrolysis did not proceed, and the starting material was quantitatively recovered. However, the long-chain 1,2-isopropylidene-3-acyl-*sn*-glycerols (I, n = 16, 18, 20, and 22) were successfully hydrolyzed by applying the compounds to silicic acid and exposing them to hydrogen chloride gas at −75 °C for about 5–10 min. A typical hydrolysis procedure adopted for these compounds is described below.

3-Behenoyl-sn-glycerol (III, n = 20). 1,2-Isopropylidene-3-behenoyl-*sn*-glycerol (I, n = 20), 4.54 g (0.01 mole), was dissolved in chloroform/methanol/water (25/15/1 by volume) and applied to silica gel (30 g). The solvents were evaporated by passing nitrogen gas over them with stirring so that the compound formed a thin film on the silica gel. Then, the silica gel was placed in a three-necked flask and cooled to −75 °C before hydrogen chloride gas was passed with stirring. After 10 min the flow of hydrogen chloride was stopped, and the flask was flushed with nitrogen. Then the reaction mixture was extracted 3 times with 50-mL portions of chloroform/methanol (70/30 v/v) at 0 °C. The organic solvent extracts were combined and washed with 50 mL of water. The solvents were evaporated, and the solid obtained was crystallized from ether to give pure 3-behenoyl-*sn*-glycerol: yield 2.5 g (60%); mp 85.5 °C.

The other long-chain monoacylglycerols were synthesized by similar procedures except for the length of time of exposure to hydrogen chloride gas. The time of hydrolysis for the 3-stearoyl- and 3-arachidoyl-*sn*-glycerols was 5–7 min and for 3-lignoceroyl compound was 10 min. All the monoacylglycerols were crystallized from diethyl ether except 3-lignoceryl-*sn*-glycerol, which was crystallized from hexane.

The structures and purities of the intermediates and the final compounds were checked by thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC), and NMR. The ^{13}C NMR spectra of the 3-acyl-*sn*-glycerols showed only one carbonyl peak at ~174.35 ppm downfield from tetramethylsilane, assigned to the carbonyl carbon of the fatty acyl chain esterified at the primary hydroxyl of the glycerol. In contrast, the carbonyl carbon of 2-acyl-*sn*-glycerols was shifted upfield by 1 ppm. For example, in 2-palmitoyl-*sn*-glycerol the carbonyl carbon peak was 173.30 ppm.

The purity of the 3-acyl-*sn*-glycerols was checked on silica gel H plates (250 μm thick) (Analabs, North Haven, CT) impregnated with 2.5% boric acid (Thomas et al., 1965) with the solvent system chloroform/acetone, 75/25 (v/v). All the 3-acyl-*sn*-glycerols showed a single spot without any impurity of 2-acyl-*sn*-glycerols and were >99% pure.

HPLC data were obtained on a Varian 5000 liquid chromatograph (Varian Associates, Palo Alto, CA) with UV absorption detection at 220 nm. The column used was a 4.6 mm × 25 cm Altex C_{18} bonded (Rainin Instrument Co., Inc., Woburn, MA) with a flow rate of 1.21 mL/min. A gradient elution profile was employed from the initial composition of tetrahydrofuran/water/2-propanol/acetonitrile, 7.6/23/5/64.4 (by volume), changing over 30 min to a final composition of 3.2/0/70/26.8.

Separate injections of each monoacylglycerol (~150 μg) showed that only a single peak was present in the elution pattern. When all the compounds in the series were combined and injected under identical conditions, the individual compounds were well separated, as shown in Figure 2a. The retention times increased with the molecular weight and are given in the legend to Figure 2.

Under the same conditions, the 2- and 3-positional isomers of monoacylglycerols were well separated, as shown for 2-palmitoyl- and 3-palmitoyl-*sn*-glycerols in Figure 2b. The difference in retention time between these two compounds was approximately 1 min with the 2-palmitoyl-*sn*-glycerols being eluted first.

Acyl migration, which yields 2-monoacylglycerols during the racemization has been reported (Serdarevich, 1967; Gronowitz et al., 1975) during the synthesis of enantiomeric monoacylglycerols. However the HPLC data show no evidence of 2-isomers. Thus HPLC, ^{13}C NMR spectra, and boric acid impregnated TLC confirmed the absence of 2-acyl-*sn*-glycerols in the final 3-acyl-*sn*-glycerols (II and III). Optical rotation measurements on the compounds in pyridine gave levo rotations for all members of the series with absolute values comparable to previously published data (Gronowitz et al., 1975; Baer & Fischer, 1945). Therefore, under the conditions employed in this study, acyl migration appeared to be insignificant.

Table I: DSC Data of 3-Acyl-*sn*-glycerols[a]

	β phase				α phase			
3-substituent[b]	T_f (°C)[c]	ΔH (kcal/mol)[d]	ΔS (cal mol^{-1} K^{-1})[e]	T_{c_1} (°C)[f]	T_f (°C)[g]	ΔH (kcal/mol)[h]	ΔS (cal mol^{-1} K^{-1})[i]	T_{c_2} (°C)[j]
decanoyl (10)	56.0	10.0	30.4	26.0	29.2	4.5	14.9	1.0
lauroyl (12)	62.0	12.3	35.8	42.0	45.0	6.25	19.7	13.5
myristoyl (14)	64.0	15.1	44.5	55.0	58.7	7.75	23.4	46.0
palmitoyl (16)	70.5	17.1	49.8	63.8	67.2	8.8	25.9	51.2
stearoyl (18)	77.0	19.4	55.4	72.0	75.3	10.0	28.7	59.0
arachidoyl (20)	81.6	20.4	57.5	77.0	80.4	11.7	33.1	53.9
behenoyl (22)	85.5	22.6	63.0	83.0	84.8	13.0	36.3	62.0
lignoceroyl (24)	89.8	24.9	68.6	85.8	89.0	14.1	39.0	67.4

[a] All the heating and cooling rates were 5 K/min. [b] Acyl carbon number is given in parentheses. [c] Temperature of first melting of the β phase from solvent of crystallization. [d] Enthalpy of the melting transition of the β phase from solvent of crystallization. [e] Entropy of the β phase calculated from ΔH of the β-phase melting transition. [f] Temperature of first crystallization from the isotropic liquid to the α phase. [g] Temperature of melting of the α phase to the isotropic liquid. [h] Enthalpy of the melting transition of the α phase to isotropic liquid. [i] Entropy of the α phase calculated from the ΔH of the α-phase melting transition. [j] Temperature of second crystallization.

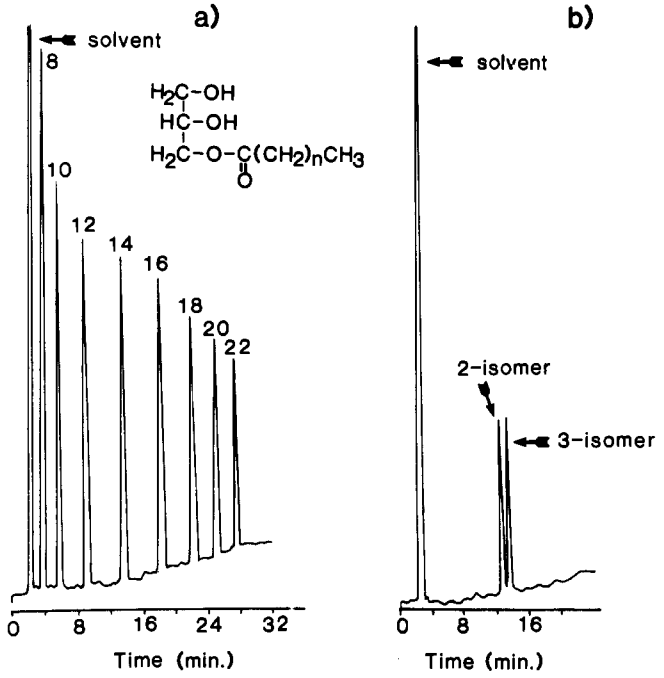

FIGURE 2: (a) HPLC separation of 3-acyl-*sn*-glycerols. The number on each peak represents *n*, the number of methylene units in the acyl chain. The retention times of these compounds are 3.5, 5.2, 8.5, 13.3, 17.9, 21.8, 24.8, and 27.2 min in the same order as is shown in the chromatogram. (b) Separation of 2-palmitoylglycerol and 3-palmitoyl-*sn*-glycerol; the retention times are 12.5 and 13.4 min, respectively. The conditions, column, and solvent system for both (a) and (b) are identical and are given under Materials and Methods.

Differential Scanning Calorimetry. The thermal behavior of the compounds was studied on a Perkin–Elmer Model DSC-2 (Norwalk, CT) differential scanning calorimeter. The compounds from the solvent of crystallization were dried under vacuum for 3–4 h. Samples (1.5–2.5 mg) were loaded into stainless steel pans at room temperature and sealed. The heating and cooling rates were 5 °C/min. The transition temperatures reported were the peak temperatures at 5 °C/min heating or cooling rate. The areas under the transition peaks were measured by planimetry, and the enthalpies (ΔH) of the transitions were calculated by comparison with a known standard enthalpy (indium). The changes in entropy (ΔS) of the compounds at the transition were calculated from the Gibb's equation, $\Delta S = \Delta H/T$.

X-ray Diffraction. Powder X-ray diffraction data were obtained on a focusing camera with Eliott toroidal mirror optics or Frank's double mirror optics (Franks, 1958) with nickel-filtered Cu Kα radiation from a rotating anode Elliott GX6 (Marconi Avionics, Hertfordshire, England) X-ray generator. The compounds from solvent of crystallization were packed without melting into 1 mm diameter Lindeman capillaries (Charles Supper Co., Natick, MA), which were then sealed and placed in a variable-temperature sample holder. The α phases of the monoacylglycerols were characterized on a Luzzati-Baro camera modified to include single mirror focusing collimation. Diffraction patterns were collected and analyzed with a position-sensitive detector (PSD 1100 Tennelec, TN) coupled to a computer-based analysis system (TN1710, Tracor Northern, WI).

Results

The general phase behavior of the 3-acyl-*sn*-glycerols was studied by DSC and X-ray diffraction. The 3-acyl-*sn*-glycerols obtained from solvent of crystallization had the most stable crystal forms whose X-ray diffraction pattern showed multiple short spacings in the wide-angle region between 1/3.5 and 1/4.6 Å^{-1}. This pattern is characteristic of the β phase and is illustrated for 3-stearoyl-*sn*-glycerol in Figure 3a. For all of the compounds, on being heated this β phase underwent a single sharp transition to the isotropic liquid at a characteristic temperature as illustrated in Figure 4 for 3-stearoyl-*sn*-glycerol. The sole exception was the 3-decanoyl compound, which showed a small pretransition. The enthalpies of the transition from the β phase to the isotropic liquid were in the range of 10–25 kcal/mol, and the entropies of the transition were in the range of 30–69 cal mol^{-1} K^{-1}. The melting temperatures (T_f), enthalpies (ΔH), and entropies (ΔS) of the β phase are given in Table I.

On being cooled from the isotropic liquid (Figure 4), all of the compounds first crystallized to an unstable polymorphic form, which showed an intense short spacing in the wide-angle region of the diffraction pattern around 1/4.1 Å^{-1} (Figure 3b), characteristic of the α phase.

With further cooling, the α phase crystallized to a more stable β or β′ phase. For the 3-myristoyl-, 3-palmitoyl-, and 3-stearoyl-*sn*-glycerols, the second crystallization was to the β phase. Therefore, subsequent reheating showed only a single melting transition to the isotropic liquid identical with the first melting transition of the sample from solvent of crystallization as illustrated in Figure 4 for the 3-stearoyl compound. The thermal behavior of the rest of the monoacylglycerols was more complicated. Reheating of 3-decanoyl-*sn*-glycerol showed a small pretransition at 17 °C, then melting to an isotropic liquid at 51.5 °C. 3-Lauroyl-*sn*-glycerol showed multiple transitions, the first (30 °C) and second (46 °C) being endothermic and

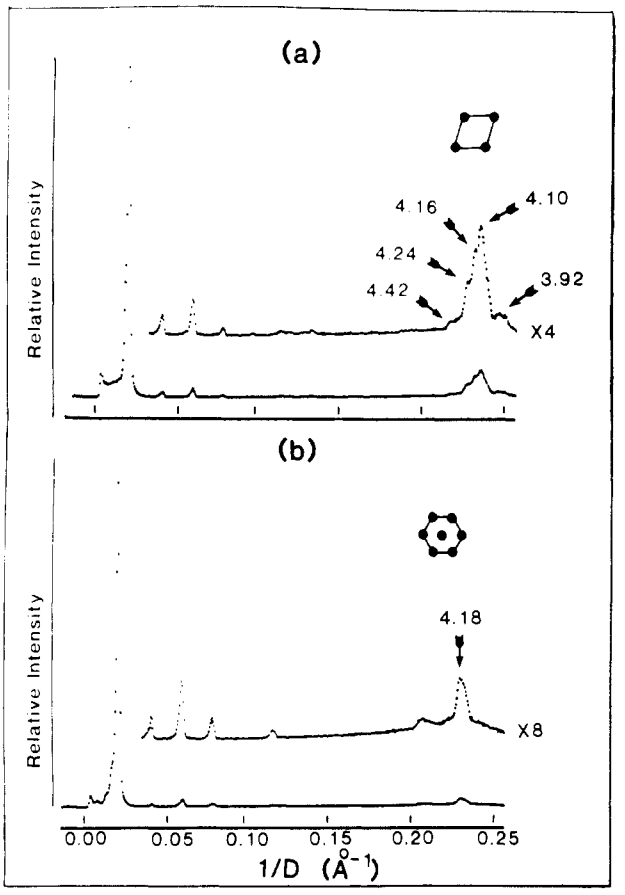

FIGURE 3: X-ray diffraction patterns of (a) β phase and (b) α phase of 3-stearoyl-*sn*-glycerol. The short spacing region is expanded by 4 in (a) and 8 in (b). The oblique subcell lattice for the β phase and hexagonal subcell lattice for the α phase are shown schematically.

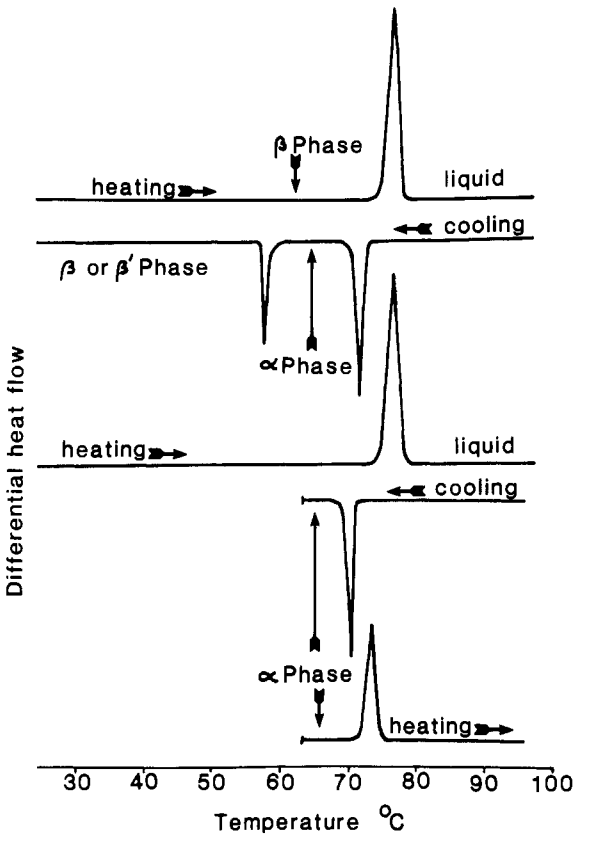

FIGURE 4: General thermal behavior of 3-acyl-*sn*-glycerols, represented by 3-stearoyl-*sn*-glycerol. (From the top downwards) Consecutive DSC runs of the compound from the solvent of crystallization and subsequent phase behavior are shown.

the third (54 °C) being exothermic before finally melting to an isotropic liquid at 61 °C. The 3-arachidoyl-, 3-behenoyl-, and 3-lignoceroyl-*sn*-glycerols showed two endothermic transitions on reheating. The first transition that occurred at 58.8, 82.3, and 71.2 °C, respectively, probably represents a polymorphic β' to β transition. The second transition was the melting of the β form to an isotropic liquid and occurred at the same temperature (within the limits of 1 °C) and with the same enthalpy as that of the form obtained from solvent of crystallization.

The relative stability of the α phase depended on the chain length and the temperature of equilibration and varied from a few minutes to many hours. For instance, if the α phase was held at a temperture 5–10 °C below its crystallization point (see Table I) 3-decanoyl-*sn*-glycerol converted almost completely to the β' phase in 30 min whereas 3-palmitoyl-*sn*-glycerol was stable in the α phase for at least 12 h. The transition from the isotropic liquid to the α phase was always reversible if the compound was reheated immediately after the first crystallization. The melting temperature and enthalpy of the transition from the α phase to the isotropic liquid were always less than those of the β phase to isotropic liquid transition. The enthalpies of the α-phase melting transitions were in the range from 4.5 to 14 kcal/mol, and the entropies were from 15 to 39 cal mol^{-1} K^{-1}. The thermodynamic data are summarized in Table I.

The X-ray diffraction long spacings of the monoacylglycerols both in the β phase and in the α phase increased as the acyl chain length increased. The long spacings in both phases were very similar, but the short spacings were quite different and consistent with the different subcell packing. The X-ray diffraction long and short spacings are summarized in Table II.

Discussion

The thermal properties of 2-acyl-*sn*-glycerols and racemic mixtures of 1- and 3-acyl-*sn*-glycerols have been studied by many workers (Larsson, 1964a–c; 1966a,b; Rewadikar & Watson, 1930; Malkin & Shurbagy, 1936). Racemic 1- and 3-acyl-*sn*-glycerols with acyl chains ranging from decanoyl to stearoyl show three polymorphic forms: α, β', and β (Malkin & Shurbagy, 1936).

In the present study of 3-acyl-*sn*-glycerols, the highest melting transition temperature for all the compounds obtained from the solvent of crystallization was that of the β form. The melting temperature of this form increased linearly with increasing acyl chain length (Figure 5a).

On cooling from the isotropic liquid, the monoacylglycerols crystallized to an unstable α phase. The stability of this phase greatly depended on acyl chain length, the shorter chain compounds being least stable. As shown in Figure 5a, the difference between the melting temperatures of the α phase and the β phase was wide in the case of the lower members of the series. In the higher members of the series the melting temperatures of the α phase approached the melting temperatures of the β phase.

The melting temperature of the α phase is only about 3 °C above its crystallization temperature (Table I). Thus, nucleation of the α phase from the melt occurs with very little undercooling. This appears to be a general feature in many lipids such as triacylglycerols (Kodali et al., 1984), alkanes, and phosphatidylcholines (Small, 1984). The very slight undercooling necessary to produce crystallization suggests that

Table II: Powder X-ray Diffraction Data of 3-Acyl-*sn*-glycerols[a]

decanoyl	lauroyl	myristoyl	palmitoyl	stearoyl	arachidoyl	behenoyl	lignoceroyl
			β-Phase Long Spacings[b]				
33.0 (s)	37.8 (s)	42.5 (s)	46.1 (s)	50.8 (s)	54.5 (s)	59.0 (s)	62.3 (s)
16.6 (w)	19.0 (vw)	21.3 (w)	23.2 (w)	25.4 (w)	27.2 (w)	29.5 (w)	31.2 (w)
11.7 (w)	12.4 (w)	14.1 (w)	15.5 (w)	17.0 (w)	18.1 (w)	19.7 (w)	20.7 (w)
8.3 (m)	9.4 (m)	10.6 (w)	11.6 (w)	12.8 (w)	13.5 (vw)	14.9 (vw)	15.6 (vw)
			β-Phase Short Spacings[b]				
4.61	4.60	4.29	4.24	4.24	4.46	4.23	4.46
4.35	4.39	4.24	4.15	4.16	4.35	4.15	4.23
4.25	4.31	4.13	4.10	4.10	4.26	4.11	4.06
4.11	4.13	4.10	3.92	3.93	4.13	4.06	3.83
3.94	3.93	3.93	3.67	3.86	4.08	3.94	3.66
3.87	3.82	3.66	3.59	3.71	3.93	3.88	
3.78	3.08	3.57		3.64	3.86	3.69	
3.59	3.61			3.56	3.65	3.61	
					3.57		
			α-Phase Long Spacings[b,c]				
32.7	37.0	43.0	44.9	48.5	*d*	58.5	*d*
(-)	(-)	(-)	22.9	24.3		29.3	
(-)	(-)	(-)	15.3	16.3		19.7	
8.0	9.3	(-)	(-)	12.2		14.4	

[a] The estimated intensities are shown in parentheses: s = strong; m = moderate; w = weak; vw = very weak; (-) = absent or extremely weak. [b] All data are given in angstroms. [c] A single short spacing in the α phase at ~1/4.15 Å^{-1} was seen for all compounds studied. [d] X-ray diffraction experiments on the α phase of these two compounds could not be performed because of insufficient amounts.

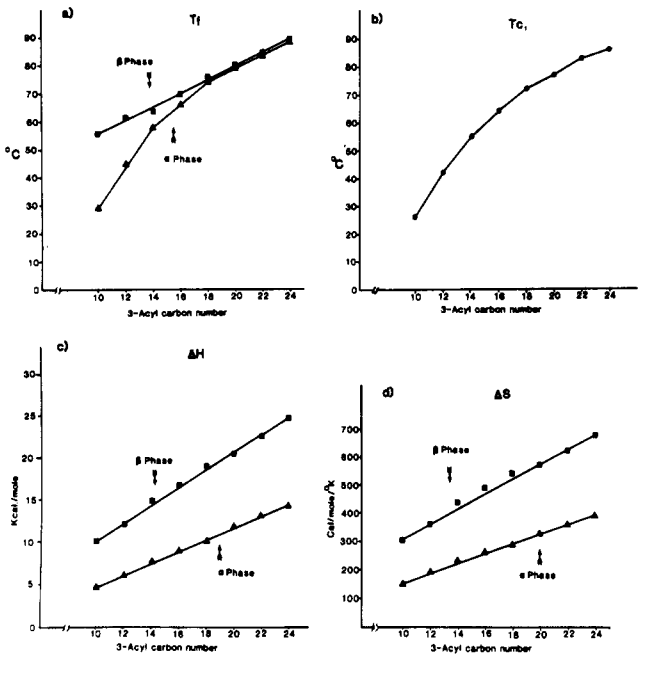

FIGURE 5: Thermodynamic data of 3-acyl-*sn*-glycerols: (a) temperatures of melting (T_f) of β phase (■) and α phase (▲); (b) temperatures of first crystallization (T_{c1}) from isotropic liquid; (c) enthalpies (ΔH) of β phase (■) and α phase (▲); (d) entropies (ΔS) of β phase (■) and α phase (▲). Note the 3-acyl carbon number and not (n) the number of methylene units is used in contrast to Figures 1 and 2.

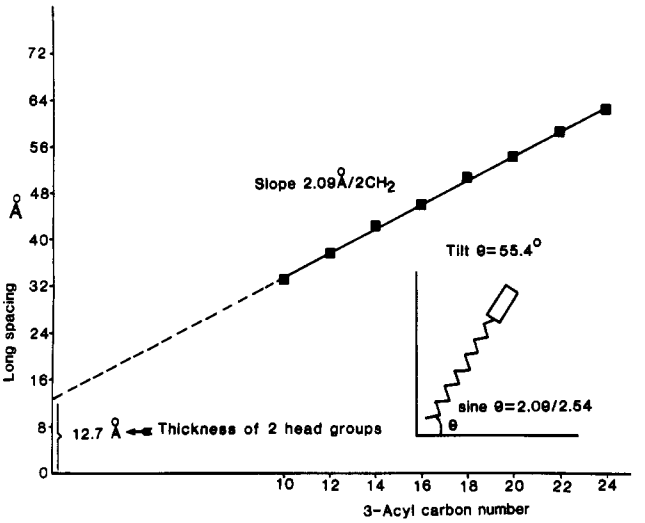

FIGURE 6: X-ray diffraction long spacings for the β phase of 3-acyl-*sn*-glycerols as a function of acyl chain length. The insert shows that the angle of tilt from the bilayer plane = 55.4°. The intercept at zero acyl carbon number = 12.7 Å.

effective nuclei for the α phase are present in the melt just below the melting point.

As the acyl chain length of the compounds increased, the temperature of the first crystallization (to the α phase) increased as shown in Figure 5b. The enthalpy of the first crystallization of each compound in the series was almost equivalent to the enthalpy of melting of that compound from α phase to isotropic liquid. The enthalpies and entropies for the transition from the α phase to the isotropic liquid of all the compounds were much lower than those of the β phase to liquid transition (Figure 5c,d). The enthalpies of both the β phase and α phase to liquid transition were linear with the chain length and gave enthalpies of 1.06 and 0.69 kcal/mol of CH_2, respectively.

The β-phase X-ray diffraction long spacings of the monoacylglycerols correspond to the thickness of a bilayer with the molecules packed head to head. Accordingly, the long spacings showed a linear increase with increasing chain length as shown in Figure 6. The slope of the plot of the long spacings gives an increment per two CH_2's of 2.09 Å. Carbon–carbon bond lengths and angles require this distance in the acyl chain to be 2.54 Å. Therefore, the molecules are tilted from the bilayer plane by a calculated angle of 55.4°. On extrapolation of this linear relationship to an acyl carbon number of zero, the intercept with the y axis is 12.7 Å, which is equivalent to the thickness of the region containing two glycerol head groups in the bilayer.

According to Malkin & Shurbagy (1936), the long spacings of racemic mixtures of 1- and 3-acyl-*sn*-glycerols from decanoyl to stearoyl in the β and β′ phases were similar. However, the long spacings of the α phase were always greater than those

Table III: Projected Length of CH_2 Group, Angle of Chain Tilt, and Thickness of Head-Group Region of Monoglycerols

series	crystalline form	slope of long spacing vs. n (Å/2 CH_2)[a]	length of CH_2 perpendicular to bilayer plane[b]	angle of tilt from bilayer plane[c]	thickness of polar region perpendicular to bilayer plane[d]	ref
3-acyl-*sn*-glycerols	β	2.09	1.05	55.4	12.7	this work
n = 10–24 (even)	α	2.08	1.04	55.0	12.2	this work
racemic α-monoglycerides	β	2.14	1.07	57.4	11.6	Malkin & Shurbagy (1936)
n = 10–18 (odd and even)	α	2.6	1.3	90	11.4	Malkin & Shurbagy (1936)

[a] The slopes and intercepts of diffraction long spacing vs. the number of carbons in the acyl chain were calculated by the least-squares method. The r values for all equations were better than $r = 0.994$. [b] Slope/2. [c] The angle of tilt (θ) is calculated from the equation $\sin\theta$ = slope/2.54, where 2.54 is the theoretical length of two CH_2 groups perpendicular to the bilayer plane. [d] Intercept of long spacing vs. carbon number.

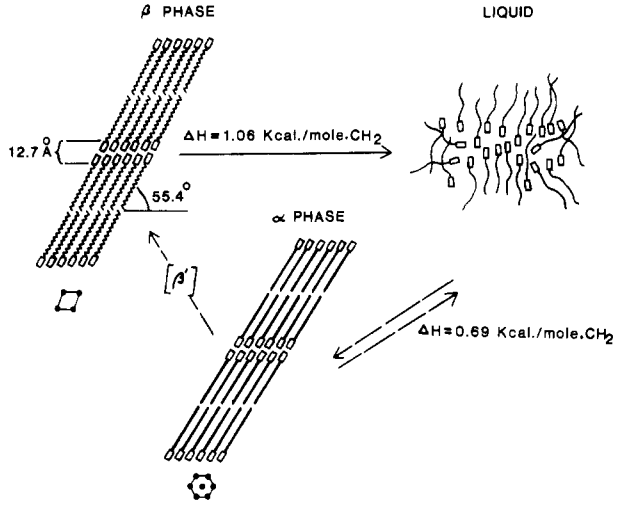

FIGURE 7: Schematic representation of the general phase behavior of 3-acyl-*sn*-glycerols.

of β and β' phases. Larsson (1964b) has characterized two stable β-polymorphic forms of racemic 1- and 3-stearoyl-*sn*-glycerol (β_1 and β_2). In both these forms the molecules were tilted 55° with respect to the bilayer plane, the difference being the chain tilt alternated in successive double layers in β_1 and single layers in β_2. The D and L isomers were distributed alternately in the unit cell of both forms. In a later study, Larsson (1964c) also showed that although differences in crystal form may exist between the optically active and racemic compounds, the tilt angle was always 55° from the bilayer plane.

The enantiomeric compounds employed in this study showed very similar long spacing values for both the α and β phases (Table III). For the α phase the slope of the linear relationship between long spacing and 3-acyl carbon number was 2.08 per two CH_2, which gives a tilt of 55.0° from the bilayer plane. The intercept corresponding to the thickness of two head groups was 12.2 Å. These data are compared to the results of Malkin & Shurbagy (1936) on racemic compounds in Table III. Thus, in contrast to the racemic monoacylglycerols, the 3-acyl-*sn*-glycerols exhibit a similar angle of tilt of the molecules from the bilayer plane and thickness of the head-group region in the α and β phases. This difference in tilt between racemic and optically active compounds in the α phase probably results from conformational constraints in the packing of the head-group regions in racemic compounds compared to the optically active compounds. A similar observation has been made by Larsson for 3-stearoyl-*sn*-glycerol (Larsson, 1964c). Recently, Persson (1984) has shown that racemic mono-*O*-alkylglycerols exhibit very similar phase behavior to that of the monoacylglycerols. However, the main difference, being the α-phase long repeat distance values of mono-*O*-alkylglycerols, shows a variation with temperature, which presumably reflects a change in tilt angle with temperature in the α phase.

The findings of this study are summarized in Figure 7. The 3-acyl-*sn*-glycerols from the solvent of crystallization are packed with the fatty acyl chains in a two-dimensional oblique lattice with specific chain–chain interactions. In this "β phase" the thickness of two head groups is 12.7 Å, and the tilt angle of the molecules from the bilayer plane is 55.4°. On being heated, this phase melts to an isotropic liquid with an enthalpy of 1.06 kcal/mol of CH_2. This value is very similar to most other aliphatic lipids (Small, 1984). When cooled, the liquid crystallizes reversibly to an α phase with a transition enthalpy of 0.69 kcal/mol of CH_2. In the α phase the chains are packed in an hexagonal lattice with nonspecific chain–chain interactions. The thickness of the head groups and the tilt angle are very similar to those in the β phase. When the α phase is left for adequate time just below its melting point or if it is cooled well below the crystallization temperature, conversion to a more stable β' or β phase will occur.

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Registry No. I (n = 8), 57416-00-9; I (n = 10), 57416-01-0; I (n = 12), 57416-02-1; I (n = 14), 57416-03-2; I (n = 16), 10567-18-7; I (n = 18), 93862-81-8; I (n = 20), 93862-82-9; I (n = 22), 93862-83-0; II (n = 8), 57457-67-7; II (n = 10), 5309-44-4; II (n = 12), 5309-45-5; II (n = 14), 5309-46-6; III (n = 16), 14811-92-8; III (n = 18), 93922-53-3; III (n = 20), 93922-54-4; III (n = 22), 93894-92-9.

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Synthesis of Lysogangliosides†

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ABSTRACT: The synthesis of gangliosides G_{M3}, G_{M2}, G_{M1}, and G_{D1a} solely lacking the fatty acid moiety, and thus called lysogangliosides in analogy to lysophospholipids, is described. Since a selective elimination of the fatty acid residue has not been achieved as yet, the gangliosides were first subjected to alkaline hydrolysis. By this procedure the fatty acyl as well as the acetyl groups of the sialic acid residue(s) were completely removed. The acetamido group of the *N*-acetylgalactosamine moiety of the gangliosides G_{M2}, G_{M1}, and G_{D1a} was very little ($\simeq$10%) hydrolyzed. In a two-phase system composed of water and ether, the selective protection of the sphingoid amino group was accomplished with a hydrophobic protective group (9-fluorenylmethoxycarbonyl). Lysogangliosides were obtained after re-N-acetylation of the sialooligosaccharide amino group(s) followed by removal of the protecting group. The overall yield was about 30%. The structures of the lysogangliosides were confirmed by chemical analysis as well as negative ion FAB mass spectrometry and ^{1}H NMR spectroscopy. By simple re-N-acylation of lysogangliosides with any labeled fatty acid, labeled gangliosides are now obtainable that are identical with their parent gangliosides except for their labeled fatty acid residue. This has been demonstrated by the synthesis of G_{M1} with a [1-^{13}C]palmitic acid moiety in its ceramide portion. If desired, double-labeled gangliosides may be obtained by use of labeled acetic anhydride in the synthesis of the lysogangliosides.

Gangliosides are characteristic components of mammalian plasma membranes in which they are located asymetrically with their sialooligosaccharide moiety facing the extracellular matrix. Though the ganglioside pattern observed on the cell surface seems to be differentiation- and cell-specific and is altered in a characteristic way by viral cell transformation, little is known about the physiological function of gangliosides and their influence on membrane properties [for a review, see Hakomori, (1981)]. Previously, individual gangliosides have been implicated as receptors for bacterial toxins (e.g., G_{M1}[1] for cholera toxin) and viruses (e.g., gangliosides G_{D1a}, G_{T1b}, and G_{Q1b} for binding of Sendai virus) [for reviews, see Fishman & Brady (1976) and Markwell et al. (1981)]. In addition, gangliosides are assumed to form clusters around certain membrane proteins and thus may regulate receptor function and influence the dynamic state of membrane lipids (Sharom & Grant, 1978).

To study the role of gangliosides in these crucial cellular phenomena, ganglioside derivatives carrying special probes in their ceramide portion that render them suitable for biophysical and biochemical analysis are needed. A prerequisite for these studies is the insertion of the appropriate ganglioside derivatives into plasma membranes. This is possible at least for nitroxide-labeled ganglioside analogues and for ganglioside G_{M3}, as has been shown previously (Schwarzmann et al., 1981, 1983; Schwarzmann & Sandhoff, 1983).

Thus, gangliosides with a nitroxide group at various positions in the *N*-acyl chain are suitable for the investigation of protein–ganglioside interactions, as well as for the study of the dynamic state of gangliosides in the lipid environment of the cell membrane. Likewise, gangliosides bearing a photoactivatable azido group in their hydrophobic moiety may be used to study their nearest neighbors. Further examples of the application of labeled gangliosides are their use in studying ganglioside metabolism as well as the lateral diffusion of gangliosides when radio- and fluorescent-labeled ganglioside derivatives are used, respectively.

The best way to prepare the above-mentioned ganglioside derivatives is the replacement of their fatty acid residue by an appropriately labeled fatty acid moiety. Thus, lyso-

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[1] Abbreviations: G_{M3}, II^{3}Neu5Ac-LacCer; G_{M2}, II^{3}Neu5Ac-GgOse$_3$Cer; G_{M1}, II^{3}Neu5Ac-GgOse$_4$Cer; G_{D1a}, IV^{3}Neu5Ac,-II^{3}Neu5Ac-GgOse$_4$Cer; lyso-G_{M3}, II^{3}Neu5Ac-LacSph; lyso-G_{M2}, II^{3}Neu5Ac-GgOse$_3$Sph; lyso-G_{M1}, II^{3}Neu5Ac-GgOse$_4$Sph; lyso-G_{D1a}, IV^{3}Neu5Ac,II^{3}Neu5Ac-GgOse$_4$Sph; Fmoc, 9-fluorenylmethoxycarbonyl; Cbz, benzyloxycarbonyl; FAB, fast atom bombardment; NMR, nuclear magnetic resonance; Me_2SO-d_6, dimethyl-d_6 sulfoxide; D_2O, deuterium oxide; Me_4Si, tetramethylsilane; TLC, thin-layer chromatography.

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