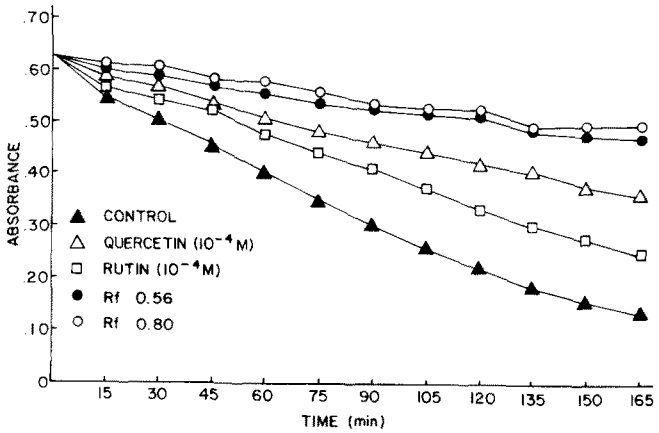

FIG. 3. Antioxidant activity of 2 unhydrolyzed bands eluted from TLC plates plus quercetin and rutin standards.

REFERENCES

1. Chang, S.S., B. Ostric-Matyasevic, O.A. Hsieh and C.J. Huang, Food Sci. 42:1102 (1977).
2. Lea, C.H., and P.A.T. Swaboda, Chem. and Ind. 1426 (1956).
3. Pratt, D.E., J. Food Sci. 37:322 (1972).
4. Marco, G.L., JAOCS 45:594 (1968).
5. Miller, H.E., Ibid. 482:91 (1971).
6. Pratt, D.E., Presentation at the Proceedings of The Am. Chem. Soc. Symposium Annual Meeting, Chicago, IL (1976).
7. Taga, M. Silvia de Lima, M.S. Thesis, Purdue University, West Lafayette, IN, 1982.
8. Philip, Flora, Ph.D. Thesis, Purdue University, West Lafayette, IN, 1974.
9. Mabry, T.J., K.R. Markham and M.B. Thomas, The Systematic Investigation of Flavonoids, Springer-Verlag, Berlin, 1970.
10. Barton, G.M., R.S. Evans and J.A.F. Gardner, Nature 170-249 (1952).
11. Reio, L., J. Chrom. 1:338 (1958).
12. Reio, L., Ibid. 4:458 (1960).
13. Seikel, M.K., The Chemistry of Flavonoid Compounds, edited by T.A. Geissman, Macmillan Company, NY, 1962.
14. Harborne, J.B., T.J. Mabry and H. Mabry, Physiology and Function of Flavonoids, in The Flavonoids, edited by J.B. Harborne, T.J. Mabry and H. Mabry, Vol. II, Academic Press Inc., New York, 1975.
15. Pratt, C., and S.H. Wender, JAOCS 36:392 (1959).
16. Struck, R.F., and M.C. Kirk, J. Agric. Food Chem. 18:548 (1970).

[Received November 24, 1983]

♣Synthesis and Polymorphism of 1,2-Dipalmitoyl-3-acyl-*sn*-glycerols[1]

DHARMA R. KODALI, DAVID ATKINSON, TREVOR G. REDGRAVE and **DONALD M. SMALL,*** Biophysics Institute, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118

ABSTRACT

Stereospecific 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dipalmitoyl-3-acyl-*sn*-glycerols with even-carbon saturated fatty acyl chains of 2-16 carbons in length were synthesized. The polymorphic behavior and packing arrangements of the most stable crystal form obtained, from the solvent of crystallization, were studied by differential scanning calorimetry and powder X-ray diffraction. Three different layered packing modes were identified: (a) double-layer diglyceride-type; (b) triple-layer triglyceride-type; (c) double-layer triglyceride-type. The first type of packing was represented by 1,2-dipalmitoyl-3-unsubstituted, 3-acetyl and 3-butyryl-*sn*-glycerols packed in a bilayer with their long hydrocarbon chains in a parallel arrangement. In the second type of packing, shown by 1,2-dipalmitoyl 3-hexanoyl and 3-octanoyl-*sn*-glycerols, the shorter acyl chains formed a middle layer interposed between 2 layers of the 1,2-palmitoyl chains of *sn*-glycerol. The third type of crystal packing was exhibited by 1,2-dipalmitoyl-3-dodecanoyl and 3-tetradecanoyl-*sn*-glycerols and tripalmitin, was analogous to trilaurin in which the acyl chains at the 1 and 2 positions of glycerol formed an extended, nearly straight line and the 3-acyl chain was folded to lie parallel and alongside the acyl chain at the 1 position. The intermediate member of the series, 1,2-dipalmitoyl-3-decanoyl-*sn*-glycerol, exhibited both the second and the third type of chain packings when obtained from different solvents of crystallization.

INTRODUCTION

In triglycerides, substitution with different fatty acids at the 1 and 3 positions of glycerol produces a chiral center at the glycerol 2-carbon, and thus, optical activity. Most naturally occurring triglycerides show optical activity because of specific distributions of different fatty acids at the 1, 2 and 3 positions of glycerol (1). Specific triacylglycerol structure has important implications for the physical properties of triglycerides and perhaps for physiological characteristics, e.g. enzymatic hydrolysis (2), and subsequent absorption or metabolism (3). Fats with 1 and 2 long-chain and 3 short-chain saturated fatty acyl *sn*-glycerols are common in nature, e.g., butter oil contains ca. 10% of 1,2-(long chain) diacyl-3-butyryl-*sn*-glycerol (4).

A characteristic feature of triglycerides in the solid state is their polymorphism, because of the possibility of different packing arrangements of similar lattice energy. This polymorphism has been the subject of numerous studies by different workers extending over almost a century (5). However in many earlier studies racemic mixtures were studied and their properties may not be the same as specific isomers (6).

In the work reported here a homologous series of optically active triglycerides was synthesized. The 1 and 2 position of *sn*-glycerol was palmitate, whereas the substitution at the 3 position was varied with the fatty acyl chain containing 2-16 carbons. Systematic thermal and X-ray diffraction studies of these compounds have been used to gain information about their complex polymorphic behavior.

MATERIALS AND METHODS

Chemicals

Tripalmitin was obtained from the Hormel Institute, Austin, MN. Fatty acids used were purchased from Sigma

*To whom correspondence should be addressed.

[1]This work was presented in part at the Indian Science Congress Association, Tirupati, India, 1983.

Chemical Company, St. Lous, MO, and were all greater than 99% purity. Sodium borohydride, periodic acid, 4-dimethylaminopyridine and N,N′-dicyclohexylcarbodiimide were from Aldrich Chemical Company. Palladium on powdered charcoal (10% catalyst) was supplied by Matheson Coleman and Bell (Norwood, OH), and palladium black was from Sigma Chemical Company. Hydrogenation apparatus, 3911 Shaker type, from Parr Instruments Company (Moline, IL), was used. Zinc chloride, D-mannitol and the solvents (HPLC grade) were from Fisher Scientific Company and the silicic acid (100-200 mesh) was from Alltech Associates.

Synthesis

Tripalmitin was recrystallized twice from acetone before use. The other compounds used were synthesized according to the synthetic scheme shown in Figure 1. The final compounds were crystallized from hexane isopropyl ether. However, because of a slight impurity, the 3-decanoyl compound was recrystallized from acetone. The key intermediate 1,2-isopropylidene-*sn*-glycerol was synthesized from D-mannitol according to Eibl (7). The 3-acyl-*sn*-glycerols II (n = 8, 10, 12) were synthesized from 1,2-isopropylidene-*sn*-glycerol by esterification with the appropriate fatty acids and subsequent acid hydrolysis of the protecting ketal group. A typical esterification procedure is described below.

1,2-Isopropylidene-3-dodecanoyl-*sn*-glycerol

Equimolar amounts of 1,2-isopropylidene-*sn*-glycerol (1.32 g), dodecanoic acid (2.0 g) and 4-dimethylaminopyridine (1.22 g) were dissolved in carbon tetrachloride (25 mL). N,N′-dicyclohexylcarbodiimide (2.06 g) in carbon tetrachloride (10 mL) was added slowly to this reaction mixture at room temperature while stirring. During the addition, the temperature of the reaction mixture was kept below 30 C. After the addition was complete, stirring was continued for 30 min and the precipitate formed was filtered and washed with carbon tetrachloride. The filtrate was concentrated by evaporation at room temperature. The product was purified

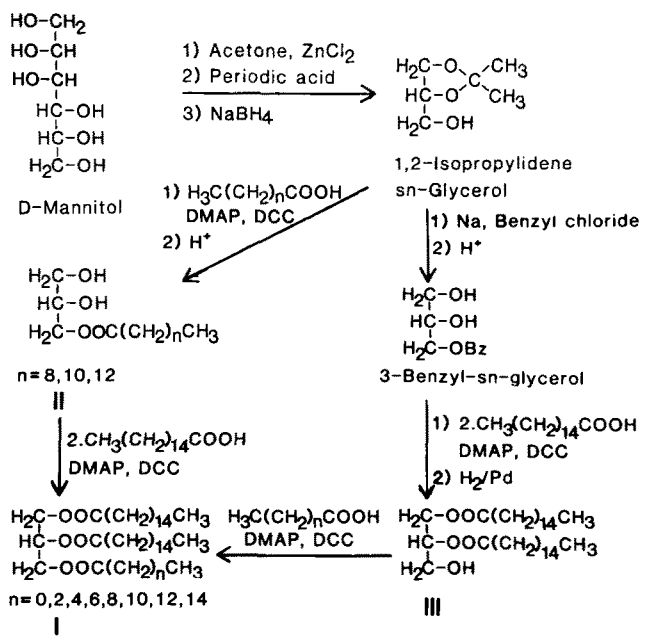

FIG. 1. Synthesis of 1,2-dipalmitoyl 3-acyl-*sn*-glycerols. DCC=N,N′-dicyclohexyl carbodiimide; DMAP=4-dimethyl-aminopyridine. I, II and III are 1,2-dipalmitoyl 3-acyl-*sn*-glycerols, 3-acyl-*sn*-glycerols and 1,2-dipalmitoyl-*sn*-glycerol. n Represents number of methylene units in the 3-acyl chain.

by silicic acid flash chromatography (8) giving 2.8 g of 1,2-isopropylidene-3-dodecanoyl-*sn*-glycerol (graded eluant from hexane to hexane/isopropyl ether, 90:10, v/v), yield 89%, m.p. 10 C.

The 3-acyl-*sn*-glycerols (II, n = 8, 10, 12) obtained after acid hydrolysis of the ketal group of 1,2-isopropylidene 3-acyl-*sn*-glycerols were purified by crystallization from ether (9). These compounds were converted to the corresponding triglycerides (I, n = 8, 10, 12) by esterification with 2 mol palmitic acid; the procedure was similar to the one described above.

Because of the possibility of facile acyl migration, the shorter 3-acyl chain compounds in this series (I, n = 0, 2, 4, 6) were prepared via 1,2-dipalmitoyl-*sn*-glycerol (III). 1,2-Isopropylidene-*sn*-glycerol on benzylation and subsequent acid hydrolysis gave 3-benzyl-*sn*-glycerol (10,11). The 3-benzyl-*sn*-glycerol was esterified first with 2 mol palmitic acid to give 1,2-dipalmitoyl-3-benzyl-*sn*-glycerol.

1,2-Dipalmitoyl-3-benzyl-*sn*-glycerol (25 g) was dissolved in tetrahydrofuran (250 mL) in the Parr hydrogenation reaction bottle. Palladium charcoal (2.5 g) and palladium black (250 mg) were added to this solution and stirred well. The reaction mixture was flushed with nitrogen and then the reaction bottle was fixed in the Parr hydrogenation apparatus. Hydrogenation was done at 30 psi/g pressure with shaking. The reaction was complete in 2 hr and 1 mol hydrogen had been absorbed. The reaction mixture was filtered, and the solvent was evaporated. The solid thus obtained was crystallized from petroleum ether to yield 20.4 g (95%) pure 1,2-dipalmitoyl-*sn*-glycerol (III), m.p. 68.5 C. Condensation of equimolar amounts of III with each appropriate fatty acid gave the final compounds I (n = 0, 2, 4, 6).

The analytical data and elemental analysis (Schwarzkopf Laboratory, New York) for the 1,2-dipalmitoyl-3-acyl-*sn*-glycerols are presented in Table I. The structures and purities of the final compounds and intermediates were checked by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). All the compounds were more than 99% pure by TLC.

TLC

TLC was performed on 250 μ thick silicagel 'H' plates from Analabs (North Haven, CT). For mono- and diglycerides, silicagel 'H' plates impregnated with 2.5% boric acid were used (12). The solvent system for mono- and diglycerides was chloroform/acetone (75:25, v/v) and for 1,2-isopropylidine-3-acyl-*sn*-glycerols and final triacylglycerols was hexane/isopropyl ether (70:30, v/v). The TLC of all the final compounds with ca. 30 μg of each compound applied to the plate and run in hexane/isopropyl ether (70:30, v/v) is shown in Figure 2.

HPLC

HPLC data were obtained on a Varian 5000 liquid chromatograph (Varian Associates, Palo Alto, CA) with UV absorption detection at 220 nm, using an Altex C18 bonded column 4.6 mm x 25 cm (Rainin Instrument Company, Inc., Woburn, MA) with a flow rate of 1.3 mL/min at room temperature. A gradient elution profile was employed from the initial composition of water/isopropanol/acetonitrile/tetrahydrofuran (3:29:63:6 by volume), changing over 30 min to a final composition of 0:55:40:4. Separate injections of each compound (ca. 200 μg) showed that only a single peak was present. When all the compounds in the series were combined and injected under the identical conditions, the separation shown in Figure 3 was obtained. The difference in the retention times of 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dipalmitoyl-3-acetyl-*sn*-glycerol was 0.5

TABLE I

Analytical Data of 1,2-Dipalmitoyl 3-Acyl-*sn*-Glycerols

3-Substituent	Yield (%)[a]	HPLC retention time[b]	Carbon (%)		Hydrogen (%)	
			Found	Required	Found	Required
Unsubstituted	95	12.0	73.84	73.94	12.15	11.97
Acetyl	90	12.5	72.82	72.78	11.75	11.48
Butyryl	87	14.5	73.20	73.35	11.82	11.60
Hexanoyl	90	16.7	73.46	73.87	11.75	11.71
Octanoyl	94	19.2	74.76	74.35	11.97	11.82
Decanoyl	85	22.0	74.95	74.79	12.07	11.91
Dodecanoyl	87	24.9	75.63	75.20	12.11	12.00
Tetradecanoyl	88	28.0	75.90	75.58	12.30	12.08
Palmitoyl	—	31.0	—	—	—	—

[a]Yield was calculated for the final esterification step based on glycerol-containing compound.

[b]The retention time of each compound is given in minutes and the other conditions and column are specified in the Materials and Methods section.

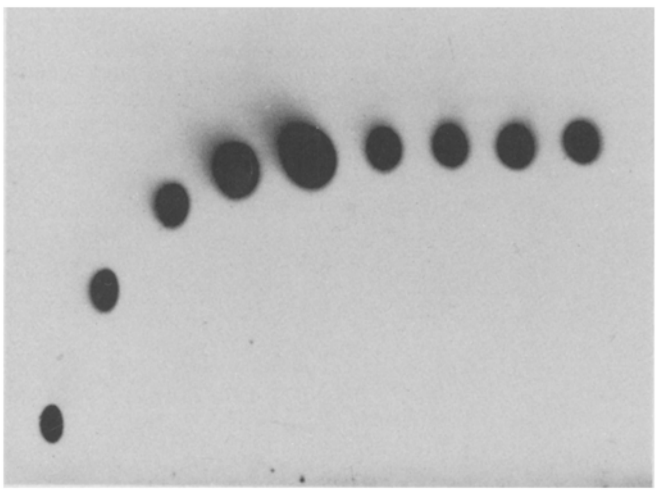

FIG. 2. TLC of 1,2-dipalmitoyl 3-acyl-*sn*-glycerols. From the left the compounds are 1,2-dipalmitoyl 3-unsubstituted, 3-acetyl, 3-butyryl, 3-hexanoyl, 3-octanoyl, 3-decanoyl, 3-dodecanoyl, 3-tetradecanoyl *sn*-glycerols and tripalmitin. Solvent system hexane/isopropyl ether (70:30, v/v) on silicagel 'H' plate. About 30 μg of each compound was spotted.

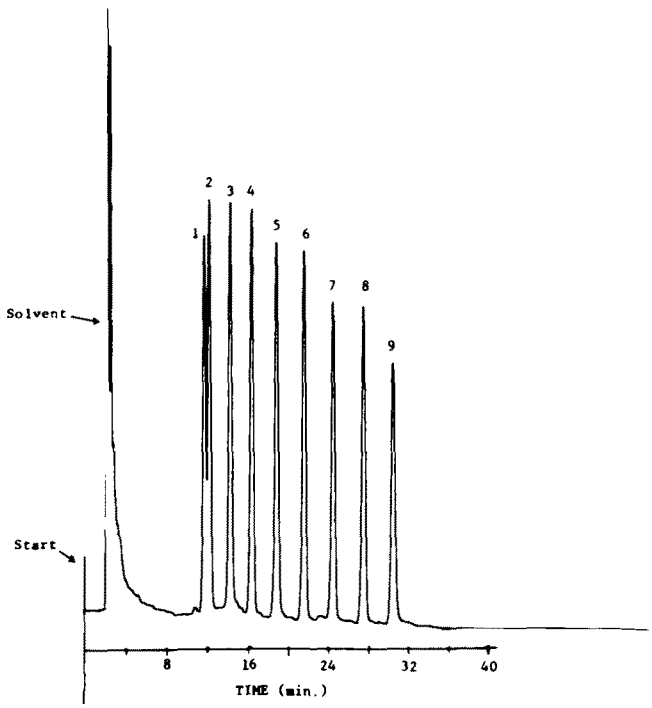

FIG. 3. HPLC separation of 1,2-dipalmitoyl 3-acyl *sn*-glycerols. Peak number 1 is 1,2-dipalmitoyl-*sn*-glycerol and peaks 2-9 correspond respectively to 1,2-dipalmitoyl 3-acyl-*sn*-glycerols with an even numbered (2-16) fatty acyl chain at the *sn*-glycerol 3-position. Conditions, column and solvent system are given in Materials and Methods.

min, but the retention times increased gradually with increasing 3-acyl chain length. The retention time for each member of the series is given in Table I.

Differential Scanning Calorimetry (DSC)

The thermal behavior of the compounds was studied using 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 hr. Samples (1.5-2.5 mg) were loaded into stainless-steel pans at room temperature and sealed. The heating and cooling rates were 5°/min. The transition temperatures taken were the peak temperatures. The areas under the transition peaks were measured by planimetry and the enthalpies (ΔH) of the transitions were calculated by comparing with a known standard enthalpy (indium). Change in entropies (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 focussing camera with Eliott torroidal mirror opticals or Frank's double mirror optics (13) with nickel filtered CuKα radiation from a rotating anode GX6 (Elliot) X-ray generator. The compounds from solvent of crystallization were packed without melting into 1 mm diameter Lindeman capillaries (Charles Supper Company, Natick, MA), which were then sealed and placed in a variable temperature sample holder.

RESULTS

Thermal Studies

Figure 4a shows the first heating scan of all members of the 1,2-dipalmitoyl-3-acyl-*sn*-glycerol series obtained from the solvent of crystallization. Each compound gave a single sharp melting transition to the isotropic liquid. When cooled from the isotropic liquid, these compounds crystallized with a single transition except in the cases of 3-hexanoyl and 3-octanoyl compounds (Fig. 4b). The cooling

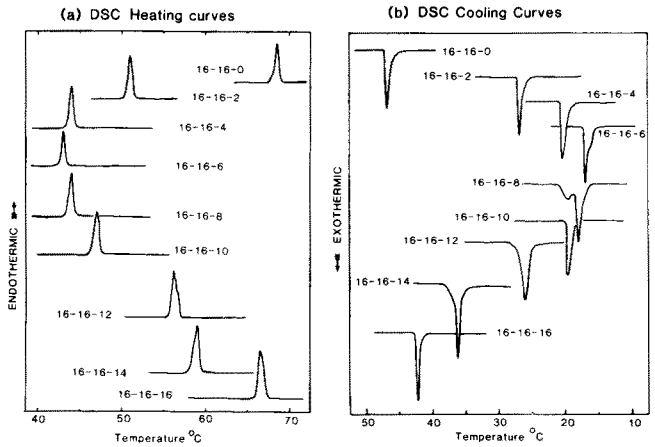

FIG. 4. DSC first heating (a) and cooling (b) curves of crystalline 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dipalmitoyl 3-acyl-*sn*-glycerols from the solvent of crystallization.

curves of these 2 compounds showed a shoulder. Undercooling by at least 21 C with respect to the first melting temperature was evident for all members of the series before the first crystallization occurred. The highest melting temperature, ΔH, and ΔS for the series are given in Table II. In most cases, the exotherm occurring on first cooling (Fig. 4b) was to the α-phase (only a 4.15 Å short spacing).

As illustrated in Figure 5, in contrast to the first heating behavior, reheating of the compounds after crystallization from the liquid showed multiple-phase transitions. Each of the compounds exhibited 2 or 3 different transitions. The lowest endotherm is the melting of the α-phase and this temperature is recorded in Table II. The melting of the α-phase is only a few degrees above the initial crystallization temperature (Table II). With the exception of the 3-dodecanoyl compound, the final melting temperature for all members of the series after recrystallization from the isotropic liquid were within 1 C of the melting temperatures of the samples from the solvent of crystallization. The second melting temperature of the dodecanoyl compound was 6 C less than the first melting. The final melting temperature and crystallization temperatures of the series show a minimum at 1,2-dipalmitoyl-3-hexanoyl-*sn*-glycerol. However, the lowest enthalpy and entropy of the first melting of the samples from the solvent of crystallization were shown by 1,2-dipalmitoyl-3-butyryl-*sn*-glycerol as illustrated in Figure 6.

X-ray Diffraction

To identify the polymorphic form obtained from solvent of crystallization, X-ray diffraction experiments were carried out. The long spacings of the compounds obtained from solvent of crystallization are given in Table III. The long spacings of 1,2-dipalmitoyl-3-unsubstituted, 3-acetyl and 3-butyryl-*sn*-glycerols were similar, ca. 43 Å. In contrast, the long spacings of the 3-hexanoyl and 3-octanoyl compounds were in the range 50-53 Å. The 3-dodecanoyl, 3-tetradecanoyl and 3-palmitoyl compounds were different again with long spacings of ca. 40 Å. 1,2-Dipalmitoyl-3-decanoyl-*sn*-glycerol crystallized from acetone gave 2 sets of diffractions corresponding to ca. 37 Å similar to the 3-dodecanoyl, 3-tetradecanoyl and 3-palmitoyl compounds and ca. 55 Å similar to 3-hexanoyl and 3-octanoyl compounds. To see if we could crystallize to a single form, the compound was further crystallized from 2 other solvents, hexane and ethanol/acetone. The first melting transitions,

TABLE II

DSC Data of 1,2-Dipalmitoyl 3-Acyl-*sn*-Glycerols

3-Substituent	Tf[a] °C	ΔH[b] Kcal/mol	ΔS[c] cal/mol/°K	Tc[d] °C	T_α[e] °C
Unsubstituted	68.5	28.1	82	47.0	51.0
Acetyl	51.0	26.1	80	27.0	32.5
Butyryl	44.0	23.7	75	20.5[f]	21.5
Hexanoyl	43.0	25.5	81	17.0[f]	–
Octanoyl	44.0	25.9	82	19.0	–
Decanoyl	46.3	29.4	90	22.0	22.5
Dodecanoyl	56.3	32.2	98	25.9	–
Tetradecanoyl	58.8	34.2	103	35.0	39.5
Palmitoyl	66.4	36.0	106	41.0	46.0

[a]Temperature of melting of the stable polymorph from the solvent of crystallization—DSC melting transition peak value at 5°/min heating.

[b]Enthalpy of melting transition of the compounds from solvent of crystallization.

[c]Entropy of the stable polymorph obtained from solvent of crystallization and calculated from ΔH of melting transitions.

[d]Temperature of crystallization of the isotropic liquid—peak value of exotherm at 5°/min cooling.

[e]Melting point of α-phase. Taken as first endothermic peak on second heating run after crystallization and immediate reheating.

[f]Two components, the peak at higher temperature.

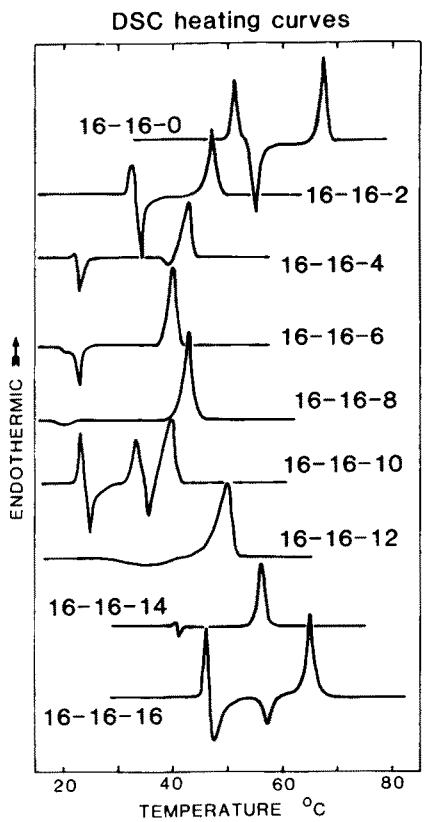

FIG. 5. DSC heating curves of 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dipalmitoyl 3-acyl-*sn*-glycerols after the first melting and recrystallization.

physical data and X-ray diffraction long spacings of this compound obtained from the different solvents of crystallization are shown in Figure 7. The form crystallized from hexane was the highest melting polymorph, with only one set of long spacings ca. 55 Å (Table III). The crystal forms obtained from either ethanol/acetone or acetone showed

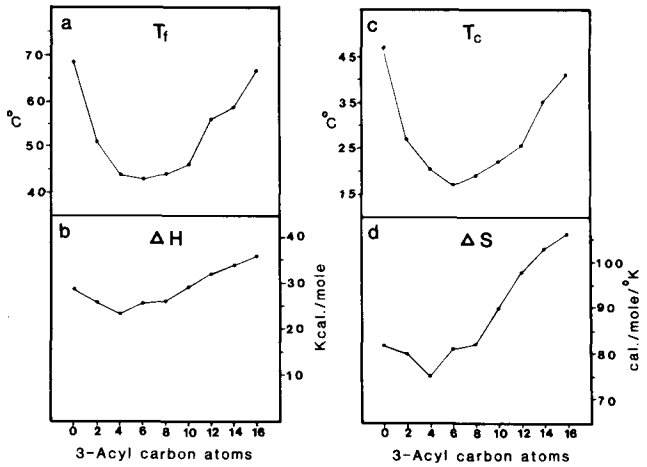

FIG. 6. Thermodynamic data for 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dipalmitoyl 3-acyl-*sn*-glycerols: a, temperature of melting (T_f); b, enthalpy (ΔH); c, temperature of crystallization (T_c); d, entropy (ΔS). Data shown are for the polymorph obtained from solvent of crystallization.

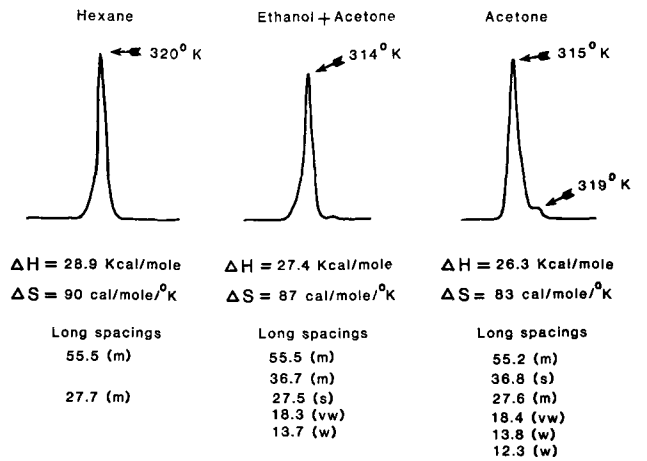

FIG. 7. DSC traces, enthalpy (ΔH), entropy (ΔS) and X-ray diffraction long spacings of 1,2-dipalmitoyl 3-decanoyl-*sn*-glycerol from different solvents of crystallization. On crystallization from hexane, a single peak (320 K) on DSC and a single set of long spacings are observed. On crystallization either from acetone or ehtanol/acetone, the major DSC peak is at 314-315 K but a minor peak at ca. 319 K is seen in both cases. Two sets of long spacing are also observed. (s)=Strong, (m)=moderate, (w)=weak, (vw)=very weak intensities.

2 sets of long spacings, indicating 2 different types of crystal packing with long spacings ca. 55 Å and ca. 37 Å. DSC also showed 2 transitions—a large melting transition for the samples obtained from ethanol/acetone or acetone at lower temperatures and a small transition at the higher melting temperature. The enthalpy and entropy of the form obtained from hexane were higher than those obtained from the crystal forms from either ethanol/acetone or acetone.

Short spacings, between 3.5 Å and 5.5 Å, corresponding to the most intense wide angle diffraction lines are given in Table III for all the compounds in the series. From these data, 1,2-dipalmitoyl-3-unsubstituted, 3-acetyl and 3-butyryl-*sn*-glycerols were obviously packed in β-subcells, whereas the 3-hexanoyl and 3-octanoyl compounds were packed in β′-subcells. In the case of 3-dodecanoyl and 3-palmitoyl compounds, the packing is typical of β-phase. In spite of recrystallization from acetone and hexane, the 3-tetradecanoyl compound consistently exhibited short spacings slightly different for those expected for the β-phase. In particular, a diffraction at 1/4.6 $Å^{-1}$ was not observed. The 3-decanoyl compound, crystallized from hexane, had short spacings probably caused by β chain packing whereas the sample crystallized from either acetone or acetone/ethanol gave a mixture of β- and β′-spacings forms consistent with the observation of 2 sets of long spacings.

The X-ray diffraction data, together with estimated intensities for the low-angle diffraction lines are summarized in Figure 8. The 3-acetyl and 3-butyryl compounds have a similar intensity profile, which showed strong 1st and 4th order diffractions. The extremely weak or missing 2nd or 3rd order diffraction line of one compound corresponded with the very weak diffraction line of the other compound. The diglyceride 1,2-dipalmitoyl-*sn*-glycerol diffraction line intensities were, however, different from 3-acetyl and 3-butyryl compounds. The X-ray diffraction intensity pattern of the 3-octanoyl compound is almost the same as the 3-hexanoyl. In the case of the 3-decanoyl compound, the intensity distribution of one set of diffraction lines coincided with that for the 3-hexanoyl and 3-octanoyl compounds and the other diffraction lines are similar to that for 3-dodecanoyl and 3-palmitoyl compounds if the intensities are considered to be proportional to the 2 types of crystals present in the sample. The intensities of the low-angle diffraction line of 3-dodecanoyl and 3-palmitoyl compounds, which exhibited a β-subcell, were similar. The 3-tetradecanoyl compound showed a slightly different

TABLE III

Powder X-ray Diffraction Long Spacings of 1,2-Dipalmitoyl 3-acyl-*sn*-Glycerols (Å)

Unsubstituted	Acetyl	Butyryl	Hexanoyl	Octanoyl	Decanoyl[a]	Dodecanoyl	Tetradecanoyl	Palmitoyl
Long spacings								
43.4 (s)	43.2 (vs)	43.7 (s)	50.6 (vs)	53.3 (s)	55.3 (s)			
					36.8 (vs)	39.7 (s)	39.7 (vs)	40.5 (vs)
21.7 (m)	(*)	21.9 (w)	25.3 (s)	26.7 (s)	27.6 (w)			
					18.4 (vw)	19.8 (w)	(*)	20.1 (w)
14.4 (s)	14.5 (w)	(*)	16.9 (w)	17.7 (w)				
					12.3 (w)	13.2 (m)	13.2 (s)	13.3 (s)
10.8 (w)	10.9 (m)	11.0 (m)	12.6 (s)	13.4 (s)	13.8 (w)			
					(*)	(*)	9.9 (vw)	(*)
Short spacings								
4.29	4.47	4.32	4.09	4.12	4.52	5.35	4.25	4.60
4.02	3.87	3.97	3.72	3.74	4.22	4.49	4.19	3.85
3.77	3.69	3.81		3.69	3.77	3.80	4.06	3.70
						3.67	3.71	

The intensities are shown in the parentheses: s = strong, m = moderate, w = weak, v = very, * = absent or extremely weak.

[a]For this compound, the long spacings are of the sample crystallized from acetone (showing both crystal forms) and the short spacings are of the sample crystallized from hexane.

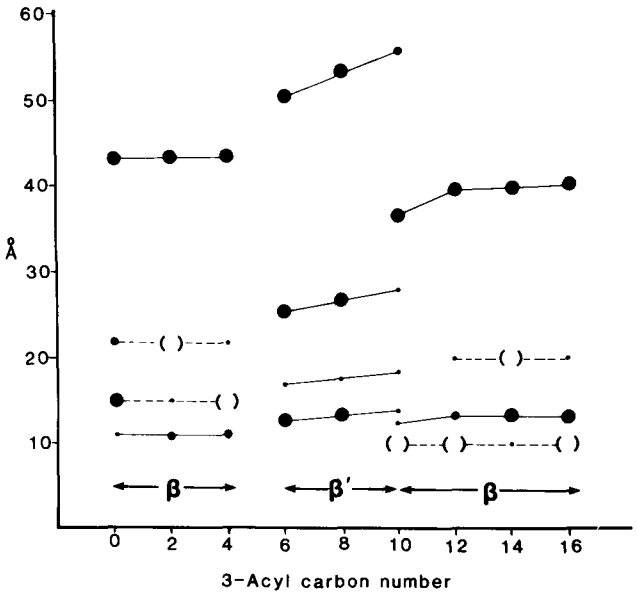

FIG. 8. X-ray long spacings of 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dipalmitoyl 3-acyl-*sn*-glycerols. The diffraction intensities are schematized by ●=strong, •=moderate, .=weak. ( ) Denotes unobserved diffractions. The subcell packing determined from the wide angle diffraction pattern is indicated at the bottom.

intensity pattern than the 3-dodecanoyl and 3-palmitoyl compounds with an absent 2nd order diffraction and weak 4th order diffraction corresponding to a weak 2nd order and absent 4th order in the 3-dodecanoyl and 3-palmitoyl compounds.

DISCUSSION

The polymorphism of glycerides has been extensively reviewed by Chapman (5) and Larsson (14). Recently, Lovegren and Gray studied the polymorphism of 1,3-dipalmito and 1,3-distearo triglycerides by using differential scanning calorimetry (15,16). An X-ray diffraction study of triglyceride polymorphism has also been reported by Zacharis et al. (17).

Each of the 3 different polymorphic forms, β, β' and α, occurring in triglycerides has characteristic infrared (IR) absorption, X-ray diffraction short spacings and melting transitions. The β-form has the highest melting transition temperature with multiple X-ray diffraction short spacings and parallel chain packing, and this is the form usually obtained from solvent of crystallization. The β' form is intermediate in melting transition temperature and also shows multiple short spacings, with perpendicular chain packing. The α form is the lowest melting polymorph with hexagonal chain packing and a single X-ray diffraction short spacing. For example, the monoacid triglycerides show characteristic short spacings at 3.7 Å, 3.9 Å, 4.6 Å and 5.3 Å for β; at 3.8 Å and 4.2 Å for β'; and a single diffraction at 4.2 Å for α forms.

X-ray diffraction and thermal studies of Howe and Malkin on racemic 1,2-diglyceride suggested a bilayer packing with double molecules (18). Recently, Pascher et al. (19) reported the crystal structure of 1,2-dilauroyl-*sn*-glycerol. According to their study, the hydrocarbon chains of the diglyceride were aligned parallel and were arranged in a bilayer structure with the glycerol group parallel to the layer plane (Fig. 9). The crystal structure of the monoacid triglyceride, trilaurin, was studied by Larsson (20) and tricaprin by Jensen and Mabis (21) who showed that the molecules were arranged in chair configuration

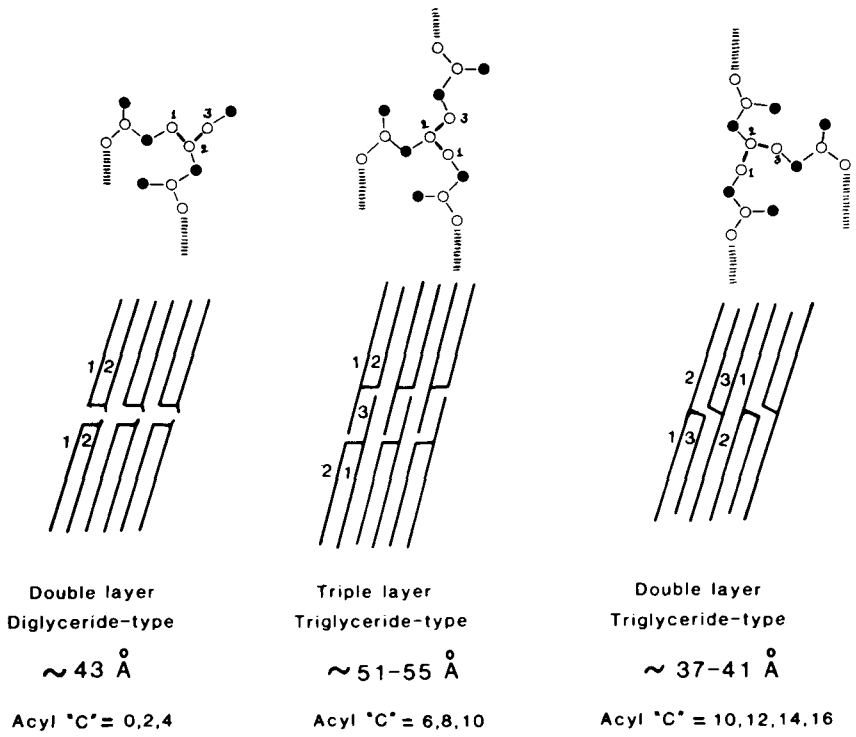

FIG. 9. Different packing arrangements of 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dipalmitoyl 3-acyl-*sn*-glycerols in the polymorphic form obtained from the solvent of crystallization. The double-layer diglyceride-type and double-layer triglyceride-type are analogous to the studies determined by Pascher et al. (19) and Larsson (20) for the dodecanoyl compounds. The trilayer packing occurs when the *sn*-3 chain is longer than 4 but shorter than 12 carbons. Note that the probable glycerol orientation is different in the 3 polymorphs.

with the 1 and 2 hydrocarbon chains extended in opposite directions and the chain at the 3-position bent at the carbonyl region to fold and lie parallel to chain 1.

Our data for the highest melting form, obtained from the solvent of crystallization (Table II) show that the series can be divided into 3 groups on the basis of different long and short spacings of X-rays. The first group comprised the 3-unsubstituted, 3-acetyl and 3-butyryl compounds with long spacings ca. 43 Å and β-subcell. The second group consisted of 3-hexanoyl and 3-octanoyl with long spacings of 51-53 Å and a β' subcell and the third group contained the 3-dodecanoyl, 3-tetradecanoyl compounds together with tripalmitin, with long spacings of 40-41 Å and probably a β-subcell. The 3-decanoyl compound crystallized from acetone (for purity reasons, see Materials and Methods) had 2 sets of long spacings, 55 Å and 37 Å and a mixture of β and β' subcell short spacings similar to the second and third group of compounds. However this compound, on crystallization from hexane, produced β-subcell short spacings but long spacings similar to the second group of compounds (55 Å).

The similarity in long spacings of the first group of compounds in the present study suggest that substitution at the 3-position of *sn*-glycerol with acetyl or butyryl chains does not increase the long spacing from 43 Å, the same as the diglyceride. Because of their small bulk and their minimal hydrophobic interactions, these chains may be accommodated in the headgroup region of the bilayer with packing similar to 1,2-dipalmitoyl-*sn*-glycerol (19).

In the third group, similar long-spacing values of 40-41 Å for 3-dodecanoyl, 3-tetradecanoyl and tripalmitin suggested that these compounds have similar packing. In analogy to the structure of trilaurin determined by Larsson (20), these compounds presumably pack in a chair conformation (Fig. 9). The acyl chains at the 1 and 2 positions of glycerol form an extended, nearly straight chain, whereas the 3-acyl chain projects out at a right angle and then folds over at the carbonyl region to lie parallel to the acyl chain at glycerol carbon 1. Variations of tilt probably account for the ca. 1 Å differences in long spacing shown by the compounds of this group.

In the second group, the sharp increase of 8-12 Å in the long-spacing values for the 3-hexanoyl and 3-octanoyl compounds was striking and must be accounted for in terms of their chain packing repetition period. The dependence of long spacings with carbon number, the relative intensity patterns and the short spacings suggest that these 2 compounds are isostructural. The diglyceride type of packing is presumably not favored by these compounds because of the increased hydrophobicity and bulk of the 3-hexanoyl and 3-octanoyl chains. Moreover, the 3-acyl chains are probably too short to pack parallel to either of the palmitoyl chains of *sn*-glycerol because of the large inequivalence in the chain lengths. Therefore, in this group the 3-acyl chains may segregate to form a middle layer. As a consequence, the glycerol backbone may be forced to take a conformation perpendicular to the layer plane as occurs in phospholipids (19). This triple layer triglyceride-type arrangement is depicted schematically in Figure 9.

The thermal behavior was also consistent with the proposed changes in the crystal packing. The melting temperatures of the compounds did not increase with increasing molecular weight of the members of the series. The melting temperatures decreased from the diglyceride to the 3-hexanoyl compound and then increased gradually up to tripalmitin, as is shown in Figure 6. The enthalpies of the melting transition for the polymorphic form from the solvent of crystallization of all the compounds in the series were ca. 25-35 kcal/mol. The enthalpy values decreased from the diglyceride to 3-butyryl-*sn*-glycerol and then increased along the members of the series to tripalmitin as shown in Figure 6. This nonlinear correlation in the thermal properties of the compounds also suggested the presence of different isostructural groups in the series.

The stable subcell packing in the first group was β, in the second group β' and in the third group, probably β. All the compounds in the series crystallized first into the α form when cooled from the isotropic liquid. Except for the 3-hexanoyl and 3-octanoyl compounds, the α-form was stable for a variable time and either by reheating or by storage the compounds slowly transformed into a more stable form. By quenching and holding the temperature at 5 C we were able to obtain a relatively stable α-form for some compounds. When characterized by X-ray diffraction, the single wide angle X-ray diffraction corresponding to 4.15 Å typical of this form was observed.

The 3 different structures, double-layer diglyceride-type, triple-layer triglyceride-type and double-layer triglyceride-type are depicted in Figure 9. These packings impose conformational changes on the glycerol backbone in relation to the layer plane, as illustrated in Figure 9. As established by Pascher et al. (19), the glycerol group is parallel to the layer plane in the double-layer diglyceride-type structure. In the triple-layer triglyceride-type structure, the glycerol moiety probably assumes a layer-perpendicular orientation. In the double-layer triglyceride-type structure, the glycerol backbone orientation is different again because of the rearrangement of the acyl chains so that the 1 and 3 chains now lie alongside each other. In this series, the packing mode depends on the length of the 3-acyl chain, with the transition 3-decanoyl compound being dimorphic and able to pack in the triple-layer and double-layer triglyceride structure.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grants HL26335 and HL07291. David Atkinson is an Established Investigator of the American Heart Association. The authors thank Ronald P. Corey and David Jackson for technical assistance and Anne M. Gibbons for preparation of the manuscript.

REFERENCES

1. Schlenk, W., Jr., JAOCS 42:945 (1965).
2. Morley, N.H., A. Kuksis and D. Buchnea, Lipids 9:481 (1974).
3. Akesson, B., and P. Michelsen, Chem. Phys. Lipids 29:341 (1981).
4. Pfeffer, P.E., J. Sampugna, D.P. Schwartz and J.N. Shoolery, Lipids 12:869 (1977).
5. Chapman, D., Chem. Rev. 62:433 (1962).
6. Larsson, K., Ark. Kemi. 23:35 (1964).
7. Eibl, H., Chem. Phys. Lipids 28:1 (1981).
8. Still, W.C., M. Kahn and A. Mitra, J. Org. Chem. 43:2923 (1978).
9. Baer, E., and H.O.L. Fischer, J. Am. Chem. Soc. 67:2031 (1945).
10. Sowden, J.C., and H.O.L. Fischer, Ibid. 63:3244 (1941).
11. Kates, M., T.H. Chan and N.Z. Stanacev, Biochemistry 2:394 (1963).
12. Thomas, A.E., III, J.E. Scharoun and H. Ralston, JAOCS 42:789 (1965).
13. Franks, A., British J. Appl. Phys. 9:349 (1958).
14. Larsson, K., Fette Seifen. Anstrichm. 74:136 (1972).
15. Lovegren, N.V., and M.S. Gray, JAOCS 55:310 (1978).
16. Gray, M.S., and N.V. Lovegren, JAOCS 55:601 (1978).
17. Zacharis, H.M., D. Mitcham, N. Lovegren and M. Gray, Advances in X-Ray Analysis, 18:535 (1975).
18. Howe, R.J., and T. Malkin, J. Chem. Soc. 2663 (1951).
19. Pascher, I., S. Sundell and H. Hauser, J. Mol. Biol. 153:791 (1981).
20. Larsson, K., Arkiv. Kemi 23:1 (1964).
21. Jensen, L.H., and A.J. Mabis, Acta Cryst. 21:770 (1966).

[Received October 6, 1983]