Preparation and Phase Behavior of Acetyl Monoglycerides

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ABSTRACT

A homologous series of 1-monoacetyl-3-monoglycerides in which the fatty acyl group varies from C_{14} to C_{22} in even C intervals has been prepared by partial acetylation of the appropriate 1-monoglycerides. Crystallization and silica gel chromatography were used to isolate the diglycerides in high purity. The isomeric monoacetyl monostearins were prepared either by application of a similar procedure to 2-monostearin or by acetylation of 1-tetrahydropyranyl-3-monostearin with subsequent removal of the blocking group to obtain 2-acetyl-1-monostearin. In the homologous series of 1-acetyl-3-monoglycerides from monomyristin through behenin the stable form at room temperature, called form I, is a tilted double chain length form, existing up to the melting point for myristin through stearin but transforming to a stable α form before melting for arachidin and behenin. The α form, low melting and entirely metastable for myristin through stearin, transforms reversibly to sub-α on cooling. Both α and sub-α are perpendicular forms and probably of single chain length structure. An interesting feature is the two-step transformation of sub-α to α on warming; the nature of this stepwise transformation, observed for all but myristin, is discussed. A nearly perpendicular double chain length β-like form occurs for palmitin and stearin and perhaps myristin. This form, which has not been well characterized thermally, though possibly entirely metastable, is conceivably a stable form at low temperatures.

INTRODUCTION

Compounds prepared by acetylating unsymmetrical monoglycerides in the 1 (or 3) position are, like propylene glycol monoesters, effective additives in cake baking. Their phase behavior, like that of the propylene glycol esters (1,2), presents the customary degree of complexity encountered with long chain compounds. An understanding of this behavior is essential to proper employment of the additives in batter systems. Accordingly, a group of substantially pure 1-acetyl-3-monoglycerides was prepared to cover the range myristin through behenin (M, P, S, A and B). In addition, the isomeric monoacetyl monostearins were prepared and studied.

EXPERIMENTAL PROCEDURES

Preparation of Monoacetyl Monoglycerides

Vicknair et al. (3) describe the preparation of 1-acetyl-3-monostearin via partial acetylation of 1-monostearin followed by fractional crystallization to purify the product. The 1,3-diglycerides of the present work were made similarly with addition of a silica gel chromatographic purification step to further eliminate low levels of mono- and triglyceride contamination (Fig. 1). The 1-AC-2-MS was prepared similarly from 2-MS. The 2-AC-1-MS is not accessible by monoglyceride acetylation. This limitation has been partially overcome by isomation of 1-THP-3-MS from reaction of MS with dihydropyran. This intermediate was purified by formation of an adduct with urea. Acetylation and removal of the blocking group followed by silica gel chromatography produced the desired 2-AC-1-MS in a somewhat less pure state than that of the other isomers.

Preparation of 1-AC-3-MS

To 358 g 1-MS (1 mole), of 95% isomeric purity, dissolved in water-washed, distilled and dried chloroform

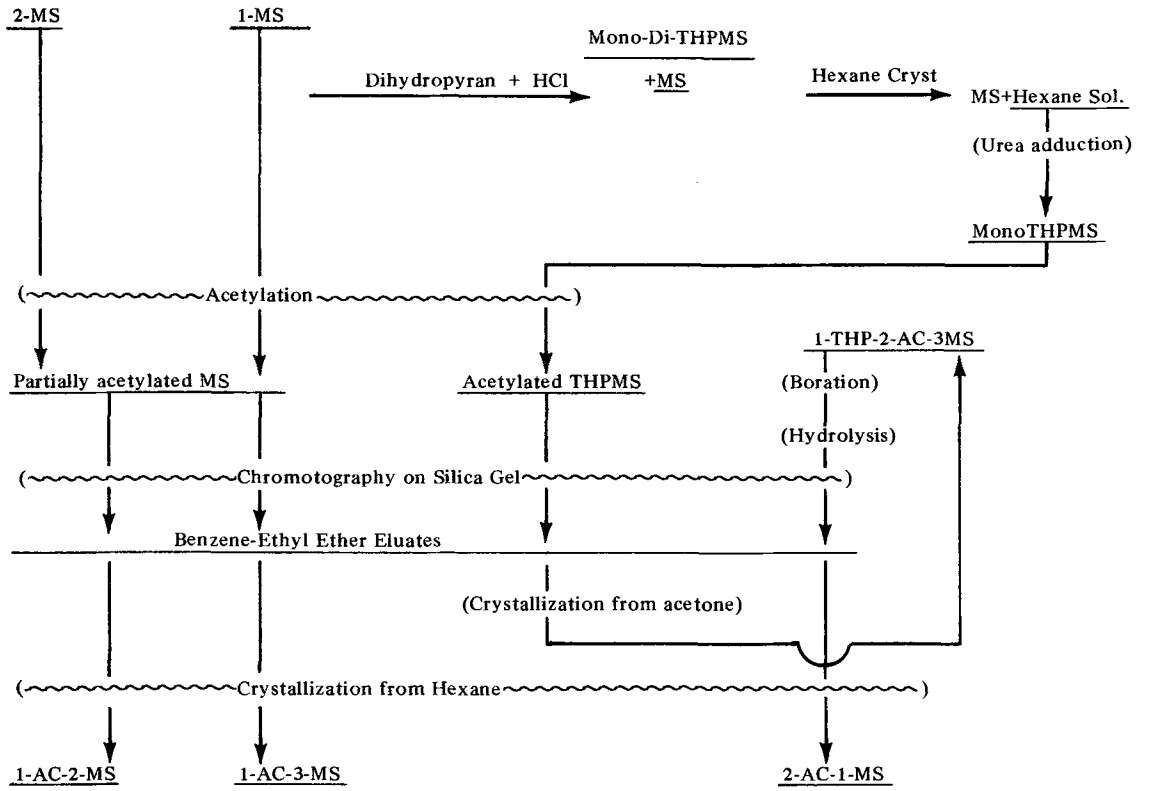

FIG. 1. Reaction scheme for preparation of isomeric monoacetyl monostearins. MS = Monostearin; THP = tetrahydropyranyl; AC = acetyl.

TABLE I

Properties and Analytical Data for the Isomeric Acetyl Monostearins, their Intermediates and the Homologous 1-Acetyl-3-Monoglycerides[a]

		Analyses							
		Saponification value		Hydroxyl value		Acid value	%1-MG	Purity by	
Compound	Yield, %	Calc.	Obs.	Calc.	Obs.	Obs.	Obs.	TLC, %	Lipase, %
1-AC-3-MS	34	280	279	140	140	0.05	0.1	99 +	93
1-MS				313	309	0.1	95.		
1-AC-2MS	43	280	279	140	142	0.3	0.3	99 +	
2-MS	28			313	311	---	5.		
2-AC-1MS	14	280	277	140	145	0	---		
1-THP-2AC-3MS[b]	51								
1-THP-3MS	35	127	130	127	126	---	---		
1-AC-3-MM	34	325	324	163	155	1.3	---		
1-AC-3-MP	31	301	300	150	149	0	0.2		
1-AC-3-MA	34	260	260	130	128	0.2	---		
1-AC-3-MB	33	246	244	123	123	0	0.2		

[a]Calc. = Calculated; obs. = observed; TLC = thin layer chromatography; MM = monomyristin; P = palmitin; S = stearin; A = arachidin; B = behenin; G = glyceride; THP = tetrahydropyranyl.

[b]This compound was not isolated in high purity but represents a mixture with considerable contamination with 1-THP-3-MS.

was added 87 g pyridine (1.1 mole). Then slowly with stirring was added 102 g acetic anhydride (1 mole) dissolved in 200 ml purified chloroform. Reaction was allowed to proceed 24 hr at room temperature, after which the preparation was washed once with 1 liter 1.3 N HCl, then thrice with water. The chloroform layer was dried over Na_2SO_4, filtered, and the solvent removed by evaporation. The residue was dissolved in 3 liters hexane and when crystallized at 27 C yielded 92 g of predominantly 1-MS. The filtrate when crystallized at 10 C yielded 154 g of predominantly 1-AC-3-MS. The crude product was purified by chromatographing a 25 g portion, dissolved in 300 ml hexane, on a 400 g silica gel + 5% H_2O column (35 x 600 mm). The column was eluted with 2 liters benzene followed by 3 liters 10% ethyl ether in benzene. The 10% ether eluate was recovered by evaporation of the solvent to yield 22 g. Recrystallization of this residue from 400 ml hexane at 16 C yielded 17.5 g of product which, converted to a total sample basis, represented a 34% yield. of purified 1-AC-3-MS.

Preparation of 1-AC-2-MS

As described for the preparation of 1-AC-3-MS, 18 g of 2-MS was acetylated and fractionated to produce 8.6 g of well purified 1-AC-2-MS.

Preparation of 2-AC-1-MS

1-THP-3-MS. was prepared by reacting 200 g 1-MS with 47 g dihydropyran and 0.2 ml of 12 N HCl in 2 liters of water-washed and dried chloroform with stirring for 1 hr. Five milliliters of 10% aqueous K_2CO_3 is added to inactivate the catalyst, and the chloroform solution was water-washed, then evaporated to remove the chloroform. The residue was dissolved in 1 liter petroleum ether and crystallized at 10 C to obtain 65 g of a MS-rich precipitate.

The filtrate was extracted twice with 300 ml portions of 85% (by volume) aqueous ethanol. The petroleum ether solution was evaporated, and the residue was dissolved in a solution of ethanol (2 liters) and water (200 ml) with 2 g Na_2HPO_4 added. Then 750 g urea was added, and the sample was stirred at 0-10 C for 4 hr. The urea adduct was filtered off and washed with cold petroleum ether. The crystals were added to a fresh portion of ethanol-water solution plus 2 g Na_2HPO_4. The sample was warmed to 60 C and then cooled with stirring to 0 C, and the stirring continued for 4 hr at 0 C. The adduct was recovered by filtration and was decomposed by addition to 3 liters water mixed with 1 liter ethyl ether. The water layer was discarded and the ether layer water-washed twice. The ether was evaporated, and the 1-THP-3-MS was recovered by crystallization from 1.5 liters hexane at -18 C. a yield of 86 g was obtained, which is a 35% yield based on the 1-MS.

Then 1-THP-2-AC-3-MS was obtained by reacting 22 g 1-THP-3-MS (0.05 mole) in 200 ml water-washed, distilled and dried chloroform and 24 g pyridine with 16 g acetyl

TABLE II

Thermal and Long Spacing Data for 1-Acetyl-3-Acyl Esters of Glycerol

Transformations and phases	M	P	S	A	B
Thermal data, C					
Trans. Pt.[a] (sub-α_1 to sub-α_2)		-9.5	3.5	12	26.5
Trans. Pt. (sub-α_2 to α)	-14	2.5	12.5	19	30
Trans. Pt. (form I to α)	---	---	---	41.5	51.5
α Melting point (rapid complete melting point)	17.0	33.5	45.0 (47.5[b])	55.2	63.0
Form I (from solvent) (regular complete melting point)	25.0	41.0	48.5 (50.3[b], 49.1[c])	55.3(α)	63.5(α)
Diffraction data long spacings, Å					
Sub-α_1	---	30.85	33.0	36.7	38.8
Sub-α_2	---	30.4	33.8	35.9	38.5
α	28.6	30.2	32.8	35.8	38.5
Form I	45.9	50.0 (50.46[d])	54.3	58.6	63
Form II (β-like)	55(?[e])	58.6	63.1	---	---

[a]Transformation point by DTA.

[b]Capillary mp (3).

[c]Dilatometric mp (3).

[d]"Beta prime" (6).

[e]Not obtained as pure form II.

TABLE III

Comparison of Thermal Data and Long Spacings for Acetyl Monostearins

Transformations and phases	2-AC-1-MS	1-AC-2-MS	1-AC-3-MS
Thermal data, C			
Trans. Pt. (sub-α_1 to sub-α_2)	0	-9.5	3.5
Trans. Pt. (sub-α_2 to α)	13.5	9.0	12.5
α melting point	45.8	42.7	45.0 (47.5[a])
Stable form mp	48.3	44.9	48.5 (50.3[a], 49.1[b])
Diffraction data (long spacings) Å			
Sub-α[c]	33.2	33.3	33.4
α	32.9	33.0	32.8
Form I	30.8	30.0	54.3
Form II (β-like)			63.1

[a]Capillary mp (3).

[b]Dilatometric mp (3).

[c]No difference observed between sub-α_1 and sub-α_2.

chloride dissolved in 50 ml purified chloroform. The sample was stirred and cooled during the acid chloride addition, after which it was held at 25 C for 72 hr. The sample was heated to reflux temperature for 1 hr, then cooled and poured onto an excess of crushed ice. The water layer was discarded and the chloroform solution diluted with 100 ml ethyl ether. The chloroform-ether solution was washed with 500 ml cold 0.5 N aqueous HCl followed by washing with 500 ml of 0.5 M K_2CO_3 solution, then washing with water. The chloroform layer was dried with Na_2SO_4, the solvent evaporated and the residue dissolved in hexane. The hexane solution of 1-THP-2-AC-3-MS was charged to a 400 g silica gel column (35 x 600 mm), and the column was eluted with 1 liter benzene followed by 3 liters 2% ethyl ether in benzene and 2 liters 10% ether in benzene. The 2% ether in benzene eluted 17.6 g of product which, when crystallized from 170 ml acetone at -18 C, yielded 12.3 g purified 1-THP-2-AC-3-MS.

2-AC-1-MS was obtained by reacting 4.3 g of 1-THP-2-AC-3-MS with 1 g boric acid dissolved in 10 ml trimethyl borate. The sample was heated in a 95 C water bath for 1 hr under 1 mm Torr after initial volatilization of the solvent was complete. The residue was dissolved in 100 ml ethyl ether, and the solution was water-washed three times. The ether solution was dried with Na_2SO_4, filtered, and evaporated to 20 ml volume. The ether solution was then diluted with 100 ml hexane and crystallized at -18 C. The crystals were recrystallized from 25 ml hexane at 4 C with recovery of 2.2 g crude 2-AC-1-MS. The crude product was purified chromatographically on a 100 g silica gel column by elution with 1 liter of 2% ether in benzene, 5 liters 5% ether in benzene and 1 liter 10% ether in benzene. The 10% ether eluate yielded 1.3 g which, on crystallization from 15 ml pentane, produced 1.0 g of well purified 2-AC-1-MS.

Purities of the Diglycerides

The monoacyl monoglycerides of this study have all been purified by chromatography on silica gel. Thin layer chromatographic (TLC) examination on Silica Gel G plates with benzene-tetrahydrofuran-acetic acid 95:5:1 indicates, for those materials examined, diglyceride purities in excess of 99%. No spots of fatty acids, monoglycerides or triglycerides are detectable.

TLC also indicates that only ca. 5% of 1,2-isomeric diglycerides occur in the 1,3-diglycerides prepared. It has not been possible to judge by TLC the purity of the 1,2-diglycerides containing an acetyl group. From TLC data on higher homologs of 2-AC-1-MS, it is probable that this compound has an isomeric purity in the range of 70-85%.

Lipase evaluation of diglyceride purities by the method of Mattson and Volpenhein (4) is applicable with difficulty to these mixed acid diglycerides because of the problem of measuring the acetyl component which is cleaved enzymatically. With the 1,3-isomer, if correction is made for loss of acetyl groups, the purity of the 1-AC-3-MS appears to be 93%. Satisfactory assays for isomeric purities were not obtained on the 1,2-isomeric structures.

Analytical data appear in Table I.

Phase Study

The experimental procedures for phase study were essentially those described in previous publications from this laboratory (5).

Thermal examination was carried out by "rapid complete melting points" (rapid cmp), "regular complete melting points" (regular cmp) and differential thermal analysis (DTA). All phases were characterized thermally except the metastable β phases.

Flat film X-ray diffraction patterns were obtained for all

TABLE IV

Short Spacings of Acetyl Monoglycerides in Å

Acetyl monoglyceride	Short spacing
1-Acetyl-3-acyl esters	
Sub-α_1	13.8 VW (diff.), 4.13 VS, 3.69 S, 2.97 VW, 2.50 W-[a]
Sub-α_2	13.8 VW (diff.), 4.13 VS-, 3.78 M+, 2.49 W
α	13.8 VW (diff.), 4.09 VS[a]
Form I (β'-like)	4.34 W+, 4.11 VS-, 3.80 W+, 3.54 M[a]
Form II (β-like)	4.50 S, 3.70 S+
Isomeric acetyl monostearins	
Approximately as above for sub-α_1, sub-α_2 and α.	
Form I (of 2-AC-1-MS)	13.1 M, 7.11 W, 6.48 VW, 4.24 M-, 4.06 VS, 3.64 S
Form I (of 1-AC-2-MS)	7.18 M, 6.60 M, 4.08 VS, 3,87 VW, 3.70 S

[a]Results in approximate agreement with previous results (3).

TABLE V

Postulated Sub-α and α Subcells for Acetyl Monoglycerides

Phase	Postulated "indices (hko)"	Spacing, Å	Calculated subcell area, per chain in Å^2	% Area (and volume) increase on transformation	Chain axis orientation
Sub-α_1	"110," "020"	4.13,3.69	18.64	---	Alternate rows[a] parallel as in O⊥
Sub-α_2	"110," "020"	4.13,3.78	18.88	1.4	No distinction[a] between alternate rows, but axes nonrandom
α	"110," "020"	4.09,4.09	19.31	3.6	Random

[a]Such an analysis receives further support from the presence of a "210" line of 2.97 Å for sub-α_1, and absence of a corresponding line for sub-α_2 (and for α).

identified polymorphic forms either as "rod pellets" or in thin-walled Pyrex glass capillaries with a General Electric XRD-1 unit employing CuKα radiation (nickel-filtered) and a 0.025 in. pinhole system. Sample-to-film distance was normally 5 cm, but was 10 cm for determination of long spacings.

The DTA apparatus was that used in a previous study (1); less refined than the more recently employed commercial apparatus (2), it was nevertheless effectively sensitive in exploring phase behavior. Approximately 0.25 g samples were measured against a reference sample of mineral oil, each in 3/16 in. diameter wells in a copper block. Thermocouples were iron-constantan, and heating rate was ca. 3 C/min. The amplified signal was applied to a Sargent recorder.

Thermal and long spacing diffraction data are listed in Table II for the homologous series of acetyl monoglycerides and in Table III for the isomeric acetyl monoglycerides; short spacings for all phases appear in Table IV.

DISCUSSION

Characterizing the Various Polymorphs of Homologous 1-Acetyl-3-Monoglycerides

The 1-acetyl-3-monoglycerides show five polymorphic states in all. The stable form at room temperature, called form I, is a tilted double chain length structure, the short spacing of which suggests a modified O⊥ (orthorhombic perpendicular) cross-sectional structure (7). This form is stable up to the mp except for arachidin and behenin which transform to a stable single chain length state below the mp.

On chilling the melt, single chain length sub-α forms are obtained. The lowest temperature form is called sub-α_1 which transforms stepwise via sub-α_2 to α. This phenomenon of stepwise transformation can be followed by both DTA and by X-ray. The higher temperature sub-α_2 differs from sub-α_1 in the absence of a weak 2.97Å spacing and in an enlarged major short spacing of 3.78 vs. 3.69Å.

A fifth type of phase, called form II (β-like, in terms of glyceride nomenclature, with strong 4.5Å short spacing reminiscent of β triglyceride phases) is formed only for the palmitic and stearic homologs (and perhaps myristic, as weakly indicated along with α phase). The long spacings, although not quite double the α values, are sufficiently long to suggest perpendicular structure. The phase is difficult to obtain in pure form, and its thermal behavior has not been characterized adequately, but by DTA it appears to melt at or slightly above the metastable α form; it is best obtained by chilling from the melt to ca. 0 C, whereupon it appears occasionally pure but usually accompanied by form I and often α or sub-α. Chilling to higher temperatures leads to α; chilling to sufficiently lower temperatures leads to sub-α. A pure form II was obtained by melting, chilling and 3 months storage at 10 C; it is just possible that β is a stable form at low temperatures.

The present data on 1-AC-3-MS are in agreement with data of Feuge et al. (3,6) on short spacings of α, sub-α and form I (their β'), and on the long spacing value of form I; our mp levels are a little lower than theirs for α and form I.

All three acetyl monostearin isomers behave similarly in showing two-step sub-α to α transformation, and all α melting points are near together. Each isomer shows a unique stable form melting a few degrees higher. Only for 1-AC-3-MS has a further form, β-like form II, been observed.

TABLE VI

Interrelationship of Subcell (or Molecule Cross Section) Areas (in Å^2) for Acetyl Monostearins vs. Docosylacetate (9)

State	Acetyl monostearin	Docosyl acetate	
		Bulk density	Film
Sub-α_1	18.6_4	18.3	18.1 (CS)
Sub-α_2	18.8_8	19.3	19.0 (S)
α	19.3_1	19.6	19.7 (LS)

Stepwise Sub-α to α Transformation

If the present sub-α and α structures can be related to established evidence on long chain structures and subcell structures, areas and volumes can be represented as in Table V.

The phenomenon of stepwise changes in crystalline long chain compounds without change in tilt angle is not new. Müller (8) observed stepwise (first order) as well as gradual (second order) changes in paraffins, notably for $C_{24}H_{50}$ which, on warming of an orthorhombic form, of O⊥ cross section, changes sharply to an intermediate form, which in turn changes gradually to a familiar hexagonal or α form, in which it remains until melting. Direct analogy to the acetyl monostearin case would seem obvious were it not for the gradual change from intermediate state to α.

More direct analogy perhaps appears in the surface film behavior of, e.g., docosyl acetate as reported by Lundquist (9) who found three vertical chain monolayer structures transforming from one to another stepwise (first order). These structures are identified with the long recognized surface states LS (superliquid) of lowest density and correlated with α bulk state, S (solid) and CS (condensed solid). Lundquist associates all three states with bulk states which, however, are not clearly shown to be stepwise distinguishable for docosyl acetate. Lundquist's correlation and the postulated relationship to the acetyl monostearin case are indicated in Table VI.

Other relevant behavior appears to be that of the 1-monoglycerides (10), three structures of which, in the case of monostearin, arachidin and behenin, undergo reversible stepwise transformation. These are tilted struc-

tures, all of the same tilt angle.

Of much interest would be a study which could correlate diffraction data especially by the DPT (diffraction pattern temperature) technique (11), thermal data by DTA and the surface film techniques of Lundquist. It might prove rare that two-stage first order vertical chain transformation (sub-α_1, to sub-α_2 to α) could be established for both bulk systems and corresponding film systems, but the demonstration of such a case would be a boon to understanding of long chain polymorphism.

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