Isolation and Melting Properties of Branched-Chain Esters from Lanolin

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ABSTRACT: Saturated branched FA and their derivatives are both biodegradable and stable to oxidation. Their m.p. are expected to be enough lower than their straight-chain counterparts to make them ideal as biolubricants. But physical property data for branched fatty esters are limited. In this study, a complex mixture of branched methyl esters was obtained from lanolin through saponification, extraction of unsaponifiables, and methylation. Hydroxy compounds were removed by chromatography on alumina. Vacuum spinning-band distillation separated the mixture roughly by chain length. Countercurrent urea complex formation and low-temperature crystallization separated even-chain iso- and odd-chain anteiso-methyl esters of chain lengths 14 through 18 at >95% purity. Transesterification was used to convert methyl esters to isopropyl esters. The m.p. and heat of fusion of each ester were determined by DSC.NMR was used to verify the structure of branched esters.

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KEY WORDS: Branched-chain fatty acid, differential scanning calorimetry, isopropyl ester, lanolin, melting point, methyl ester, nuclear magnetic resonance, urea.

There is growing interest in using the derivatives of iso- and anteiso-methyl-branched FA in lubricants and industrial fluids to replace petroleum-based products. Methyl esters (ME) of normal saturated FA are biodegradable and have excellent oxidative stability, but their m.p. are too high for many applications. The m.p. of FA are affected by chain length, molecular structure, and polymorphism. Branches on the carbon chain prevent close chain packing and greatly lower the m.p. Thus, utilization of branched FA can expand the use of stable saturated FA in biodiesel and biolubricants. The m.p. of some branched FFA were reported by Weitkamp in 1945 (1), but melting data for the branched fatty esters are limited.

Branched FA occur widely in nature in bacteria, wool wax, and animal surface fats. The most common types of branched acids are the iso-acids, usually with an even number of total carbon atoms, and the anteiso-acids, usually with an odd number of carbon atoms (Scheme 1). They usually are present as minor constituents in complex lipid mixtures, so available sources of branched FA are quite limited. Other researchers at Iowa State University are exploring the expression of these branched FA in oilseed crops. In our study, pure individual branched fatty esters were isolated from lanolin, and their m.p. and heats of fusion were determined.

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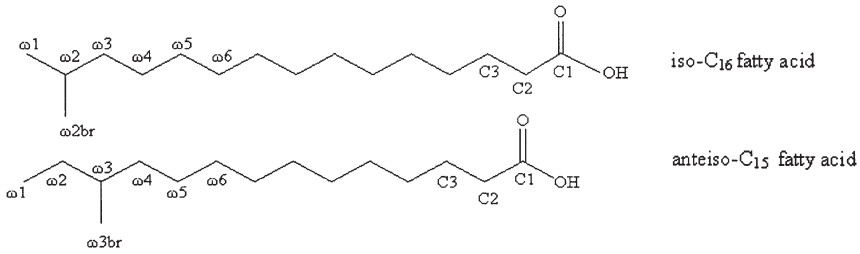
EXPERIMENTAL PROCEDURES

Preparation of lanolin ME. Saponification of lanolin (Sigma-Aldrich Inc., St. Louis, MO) and extraction of unsaponifiables followed the procedure of Barnes *et al*. (2). To get good separation of unsaponifiables, it was important to adjust for the saponification value of the particular batch of lanolin so that the residual sodium hydroxide concentration was correct according to the phase diagram of Barnes *et al*. (2). Unsaponifiables were removed by seven or more extractions with heptane at 70°C. Ethanol evaporation was controlled during extraction to keep the ratio of water to ethanol equal to 2. The sodium soaps in the extracted aqueous layer were converted to calcium soaps by the method of Downing *et al*. (3) by treating them with 10% (wt/vol) aqueous calcium chloride and heating on a steam bath for 4 h. The calcium salts were dried, powdered, washed with distilled water, and converted to ME by refluxing with sulfuric acid, methanol, and benzene (1:50:50, by vol). The benzene solution of the ME was washed with water, and the solvent was evaporated.

GC identification. ME were analyzed by using an HP 5890 Series II gas chromatograph (Hewelett-Packard) with an SPB-5 column that was 30 m × 0.25 mm with a 0.25 μm film thickness (Supelco, Bellefonte, PA). The injector and FID were at 300°C, and the oven temperature was programmed from 100 to 300°C at a rate of 10°C/min. Chromatographic peaks of normal saturated FAME were identified by comparing the retention time with (i) a C10:0 to C18:0 normal even-numbered chain FAME standard (Nu-Chek-Prep, Elysian, MN) and (ii) a C17:0 FAME (Sigma-Aldrich). The peaks of unsaturated FAME were determined in a similar GC instrument with an SP-2330 fused-silica column (15 m × 0.25 μm and a 0.20 mm film thickness; Supelco). The cholesterol peak was identified with a commercial standard (Sigma-Aldrich). The tentative identification of branched FAME was according to the chromatographic characteristics reported by Pelick and Shigley (4) and Rankin (5). Moldovan *et al*. (6) have reviewed earlier work on lanolin FAME and carefully documented their chromatographic properties.

Purification by alumina chromatography. ME, 16 g, in 400 mL of hexane/diethyl ether (94:6 vol/vol) were passed through



SCHEME 1

100 g of alumina in a 25-mm diameter column (Sigma-Aldrich Inc.). The eluted FAME were examined by GC and TLC on silica plates developed with hexane/diethyl ether/acetic acid (80:20:1, by vol). Spots were visualized by spraying with 0.01% (wt/vol) 2′,7′-dichlorofluorescein in methanol.

Spinning band distillation. The purified FAME were separated by vacuum distillation of two 50-g batches through a B/R spinning band distillation system (B/R Instrument) at 0.8 Torr. Because of the complexity of the mixture and the small amount of each fraction, b.p. were not reliable guides to chain length. The temperature was raised slowly until a fraction began to distill; when this fraction was completed and distillation stopped, the temperature rise was continued, and another fraction was taken. Eighteen fractions were collected from two distillations.

Countercurrent distribution of ME with urea. Following Sumerwell's method (7), the distribution was accomplished in a series of 50-mL test tubes, each of which contained 25 mL of a solution of 0.116 g urea/mL of methanol and 0.4 g crystalline urea, except for Tube 1. In addition to the 25 mL of urea solution, Tube 1 contained 1 g of the ME to be fractionated and 3 g crystalline urea/g normal saturated ME in the sample. This amount of urea was found to be sufficient to complex and retain most of the saturated FA in Tube 1 (8,9). The sealed Tube 1 was heated to 60°C until the urea and FAME dissolved. The tube was cooled to room temperature and held for at least 2 h for equilibration. The liquid portion in Tube 1 was transferred to Tube 2, and 25 mL of the urea solution was added to Tube 1. The mixtures in both Tubes 1 and 2 were heated and cooled as before. The liquid in Tube 2 was transferred to Tube 3, the liquid in Tube 1 to Tube 2, and 25 mL urea solution was added to Tube 1. This process was repeated until the ME were distributed over 10 to 15 tubes. In instances where urea complexes were slow to form, shaking, blowing air on the liquid surface, or temporary cooling to −8°C was used to induce crystallization.

Urea complex decomposition and GC analysis. When Tube 1 was observed not to form crystals easily, a 1-mL sample was taken from the liquid phase of the first and last tubes to determine whether enough equilibrations had been used to give good distribution of the components. These samples were treated with 10 mL of water at 60°C, 3 drops of concentrated hydrochloric acid, and 1 mL of hexane. One microliter of the hexane was injected into the gas chromatograph. If the first tube consisted primarily of the normal saturated FAME and the last tube contained a short-chain, unsaturated, or anteiso FAME, the distribution was ended and the FAME were recovered. To release the FAME, the liquid in each tube was treated with 100 mL of water at 60°C and 2 mL of concentrated hydrochloric acid. The solids left in the test tube were treated with 40 mL of water at 60°C and 1 mL of concentrated hydrochloric acid. The ME were recovered by extraction with hexane. If short-chain FAME (chain length < 14) were being recovered, diethyl ether was used to extract the FAME to avoid losses during solvent evaporation. The diethyl ether could be evaporated with minimal losses of FAME by heating the solution in 40°C water while keeping the diethyl ether level above that of the water. The ME obtained separately from the solids and liquids of each tube in the countercurrent distribution were examined by GC. Samples having similar compositions by GC were combined.

Low-temperature crystallization. About 100 mg of anteiso-C_{17} sample at 85% purity obtained from one urea countercurrent distribution fraction was dissolved in ~5 mL of ethyl acetate and slowly cooled with constant shaking in a dry ice/acetone bath to −73°C and held for ~20 min. The liquid was removed with a previously cooled glass filter stick. The precipitate was dissolved in an additional 5 mL of ethyl acetate, cooled to −73°C and again filtered. The same procedure was repeated three more times, so four filtrates and one precipitate were obtained. Anteiso-C_{13} and -C_{17} were crystallized in the same way except the crystallization solvent for anteiso-C_{13} was acetone instead of ethyl acetate.

Preparation of isopropyl esters (IE) from ME. Isopropanol was freed of dissolved carbon dioxide by boiling and cooling while sparging with air treated with Drierite and soda lime. The isopropanol was reacted with sufficient sodium metal to give a 1 N sodium isopropoxide solution. Two hundred milligrams of sodium isopropoxide solution was used for each milligram of FAME, and the reaction mixture was stirred vigorously for 3 h at room temperature. On the completion of reaction, sufficient acetic acid was added to neutralize the catalyst, and the IE was extracted with hexane. The excess isopropanol and acetic acid were removed by washing with 5% (wt/vol) aqueous sodium bicarbonate solution. The IE were analyzed by GC.

Melting points and heats of fusion of esters by DSC. The m.p. and heat of fusion of individual branched-chain ME and IE were measured with a DSC7 equipped with an Intracooler System II (PerkinElmer). Indium and *n*-decane were used for calibration. Samples of 5–7 mg were weighed to 0.01 mg ac-

curacy in stainless steel pans, and an empty pan was used as a reference. In our standard procedure the sample and the reference were heated from 25 to 80°C at 40°C/min, held at 80°C for 10 min to melt the sample completely, and cooled to −80°C at 10°C/min, a rate recommended by the AOCS Method Cj 1-94 (10). The sample was held isothermally for 15 min at −80°C and then reheated to 80°C at 5°C/min, a rate recommended for measuring the melting properties of IE (11). Replicates were determined for each sample. The calorimetric parameters obtained from heat flow (mW) vs. temperature (°C) curves in the melting scan were melting onset temperature (T_{on}), temperature of the completion of melting (T_{com}), temperature of maximum heat flow (T_p), and heat of fusion (D*H*).

NMR of branched-chain esters. The branched-chain esters were examined by ^{13}C NMR and ^{1}H NMR, except for ante-C_{13}-ME, ante-C_{17}-ME, and iso-C_{18}-IE, which were not examined because of insufficient material. The ^{13}C NMR spectra were measured with a Varian VXR-400 NMR and the ^{1}H NMR spectra with a Varian VXR-300 NMR spectrometer. A small amount of the ester was dissolved in 0.6 mL $CDCl_3$ using 5-mm NMR tubes. For the ^{13}C NMR spectra, the assignment of chemical shifts of important carbon atoms of the esters was done with ACD/ChemSketch Predictor software from Advanced Chemistry Development Inc. (Toronto, Canada).

RESULTS AND DISCUSSION

Preparation of ME. The lanolin FAME were yellow, solid at room temperature, and included more than 100 compounds, as shown in Figure 1a. Normal saturated and unsaturated FAME as well as iso- and anteiso-branched FAME from chain length 8 to 31 were present. There were minor amounts of sterols and hydroxy FAME as well as other polar compounds.

The alumina treatment removed most of the hydroxy and polar compounds. After the alumina treatment the FAME did not give a polar band on TLC plates. The peaks marked with arrows in Figure 1a were those that were removed or reduced after alumina column purification. By using commercial standards and previous GC results (4–6), we tentatively identified the iso- and anteiso-branched and normal FAME with chain length 8 to 31 as shown in Figure 1b. Our results confirmed Pelick and Shigley's observation that "iso-compounds almost always give a smaller peak in company with the normal even carbon acids whereas the anteiso-compounds show just the opposite in company with the odd carbon acids" (4). Small peaks eluting about 0.1 min before ante-C_{15}, ante-C_{17}, and ante-C_{19} (Figure 1b) were the corresponding odd iso-FAME (6).

Spinning-band distillation. During distillation, for uncertain reasons, the head temperature did not correspond to the real vapor temperature, so we were not able to collect the fractions by their b.p. The fractions we collected had considerable ranges of chain length. Most of the FAME with chain lengths >19 were left as the pot residue as shown in Figure 1c. The first and second distillations gave 10 and 8 fractions, respectively. The major components of each fraction differed by 1 or 2 carbon numbers.

Urea and low-temperature purifications. Our countercurrent urea distribution method differed from Sumerwell's (7) in the use of a urea/methanol solution that was only 50% saturated with urea at room temperature instead of a fully saturated solution. This resulted in a more effective separation of the FAME in our samples. Table 1 shows a typical countercurrent urea separation for one of the distillation fractions. If the greatest concentrations of a particular FAME occurred in an early fraction, these FAME formed complexes with urea easily. If the purity of a FAME peaked in a late tube, that FAME formed a urea complex with more difficulty. Based on several urea countercurrent distributions using different starting materials, we concluded that the ease of forming urea complexes is: normal C_{n+1} > normal C_n > normal C_{n-1} > iso-C_{n+1} > iso-C_n > anteiso-C_{n+1} > unsaturated C_n > anteiso-C_{n-1}, where n is a particular chain length between 11 and 18 carbons. The solid phase behaved similarly to the liquid phase except the maximal concentration for a FAME tended to occur later in solid phase than in liquid phase as shown in Table 1.

Countercurrent distribution of ME with urea was an effective method of isolating and concentrating branched-chain esters from the distillation fractions. Using this technique, we obtained 95–98% pure iso-C_{18}, iso-C_{16}, iso-C_{14}, and anteiso-C_{15}, but the anteiso-C_{17} and anteiso-C_{13} were only 88 and 80% pure, respectively (Table 2). After several low-temperature crystallizations, purities of ~97.6% anteiso-C_{17} and 93.7% anteiso-C_{13} were obtained in the final precipitates. The purity of the precipitate increased with the number of crystallizations, and the major impurities were collected in the filtrates. Transesterification products were almost as pure as the corresponding ME from which they came. The IE purities are listed in Table 2. Anteiso-C_{13}-IE was not prepared because of insufficient ME.

DSC. The melting onset (T_{on}), completion (T_{com}), and peak value (T_p) of melting were obtained from the DSC melting curve. T_{on}, generated by Pyris software (version 3.80; PerkinElmer Instruments, Wellesley, MA), is an extrapolation to the baseline of the steepest slope of the low-temperature side of the peak, and T_{com} is generated in the same way using the high-temperature side of the peak. This is in accordance with AOCS Recommended Method Cj 1-94 (10).

According to the AOCS recommended method for the DSC measurement, methyl stearate is to be used as a secondary standard. We found the T_{on} and T_{com} of methyl stearate were 35.5 and 39.6°C, respectively. But the official method reported that its onset temperature should be 39 ± 2°C. Values deviating from the standard method persisted in spite of testing several commercial samples of methyl stearate and several DSC instruments.

Since the m.p. by the traditional capillary tube measurement is defined as the temperature at which the solid fat becomes a clear liquid, one should use T_{com} for comparison with earlier m.p. data. Also, the completion of melt is meaningful when evaluating the low-temperature performance of potential lubricating oils.

The T_{on}, T_{com}, and T_p of ME and IE of various branched FA are given in Table 2. The m.p. increase with chain length. The

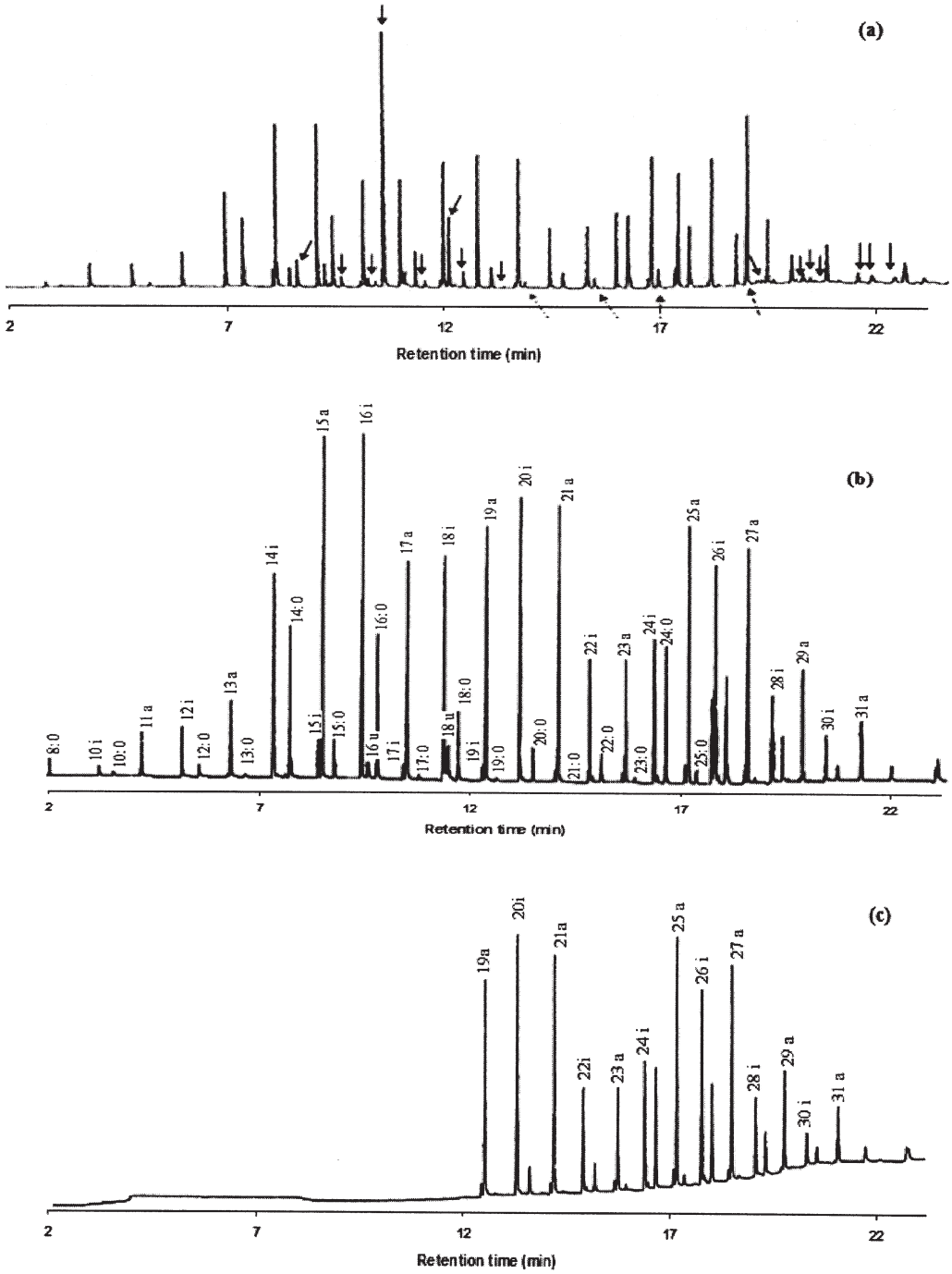


FIG. 1. Gas chromatography of lanolin FAME: (a) after methylation; (b) after alumina treatment; (c) pot residual after distillation. The solid arrows in (a) indicate hydroxy FA and polar compounds removed by alumina treatment. The dotted arrows indicate peaks partially reduced by alumina treatment. a = anteiso and i = iso.

iso-esters had higher m.p. than anteiso-esters in the chain-length range tested, and Table 2 shows that the m.p. of anteiso-FAME were depressed more compared with the corresponding normal-chain FAME than the iso-FAME (12). The shorter the chain length of branched esters, the larger was the relative m.p. depression. The addition of an IE group lowered the m.p. of all the esters below the m.p. of their corresponding ME. Probably branches on both ends of the chain are quite disruptive to chain alignment. Typically, the normal-chain FAME have 32–36°C lower m.p. than the corresponding FFA (12). This magnitude of difference also was observed when comparing the anteiso-FAME esters with branched FFA (1), but for iso-methyl esters this decrease in m.p. is ~10°C greater.

The m.p. of these branched esters were comparable with or

TABLE 1
FAME Composition[a] (GC %) of Liquid and Solid Phases of the Urea Countercurrent Distribution of Distillation Fraction 17[b]

	Liquid Phase									Solid Phase							
Tube #	13a	14i	15i	15a	15:0	16i	16:1	16:0	Tube #	13a	14i	15i	15a	15:0	16i	16:1	16:0
Start	0.4	0.7	0.7	12.5	7.6	65.0	3.9	7.1	Start	0.4	0.7	0.7	12.5	7.6	65.0	3.9	7.1
1	—	—	—	—	***40.8***	27.6	—	***29.3***	1	—	—	—	—	36.2	3.6	—	***58.1***
2	—	—	—	—	20.5	69.9	—	7.1	2	—	—	—	—	***40.6***	20.4	—	36.9
3	—	—	0.1	—	10.2	85.3	—	1.8	3	—	—	—	—	34.9	48.2	0.4	14.1
4	—	—	0.2	—	1.5	97.1	—	0.1	4	—	—	0.1	—	8.4	88.2	0.5	1.4
5	—	—	0.4	0.1	0.4	***98.3***	0.1	—	5	—	—	0.2	—	0.8	***98.2***	0.1	—
6	—	0.2	1.1	0.7	—	95.1	2.0	—	6	—	—	0.5	—	0.2	***98.2***	0.3	—
7	—	0.3	1.2	2.0	0.1	92.4	3.1	—	7	—	0.1	0.8	0.4	—	96.6	0.9	—
8	—	1.2	1.6	11.8	—	73.9	9.7	—	8	—	0.3	1.3	2.0	—	91.6	4.0	—
9	—	2.4	***2.1***	32.9	—	43.5	***16.2***	—	9	—	0.9	***1.9***	9.3	—	77.8	8.6	—
10	0.4	***3.1***	—	66.8	—	10.7	15.5	—	10	—	2.4	***1.9***	41.9	—	30.1	***20.1***	—
11	2.5	2.6	—	***84.4***	—	0.8	5.9	—	11	1.9	2.4	—	***82.1***	—	1.6	6.1	—
12	4.2	2.5	0.4	76.8	—	2.4	6.9	—	12	1.8	***2.8***	0.7	75.1	—	5.8	11.6	—
13	***7.5***	1.4	0.5	56.9	—	2.1	4.1	—	13	***19.6***	—	—	41.5	—	—	—	—

[a]GC %, percentage of FA compared with the total amount of FA, determined by comparing area under the gas chromatographic peak vs. area under all peaks.
[b]Numbers in boldface italic indicate the fraction(s) richest in each component.

lower than that of isopropyl soyate produced by Lee *et al*. (11). We also observed further depression of m.p. when the purity of ester was reduced.

The molar heats of fusion of branched-chain esters increase with chain length as shown in Table 2. Iso-esters have a higher ΔH than anteiso-esters, and the ΔH of ME was greater than IE.

Anteiso-C_{13}-ME, anteiso-C_{15}-ME, anteiso-C_{17}-ME, and iso-C_{14}-IE gave DSC melting curves with double peaks, indicating polymorphism. To investigate this phenomenon, we inserted a special step in the DSC method. The first heating and cooling steps remained unchanged, but when the sample was reheated, the temperature increase was stopped at the temperature representing the lowest temperature between the double peaks. After equilibration at this temperature for about 2 min, the sample was recooled to −80°C. On reheating to 80°C at 5°C/min, the modified temperature program gave a single DSC peak corresponding to the high-melting form but skewed to the low-melting side. Supposedly holding for 2 min at a temperature higher than the T_p of the low-temperature form allowed most of this form to change to the higher-melting polymorph.

The effect of different cooling rates on the crystal formation was also analyzed. Surprisingly, rapid cooling (40°C/min) tended to produce more crystals having a higher m.p., whereas slow cooling (2°C/min) encouraged a greater proportion of the lower m.p. crystal form.

The m.p. of anteiso-C_{17}-ME of 72% purity and 85% purity were measured to see how the minor components affected the melting curve. T_p of the high-melting peak shifted to lower temperatures by 5.9 and 2.4°C, respectively, compared with the sample of 95% purity. A single peak was observed in the two less pure samples instead of the double peaks of the purer sample.

NMR. The designations of carbon atoms in the chains are shown in Scheme 1. The chemical shifts of the carbon atoms on ^{13}C NMR spectra are given in Table 3. The results for carbons 1, 2, and 3 seemed significantly different for ME and IE

TABLE 2
The Melting Onset (T_{on}), Peak (T_p), and Completion (T_{com}) Temperatures, and Molar Heat of Fusion of Branched-Chain Methyl (ME) and Isopropyl Esters (IE) with Carbon Chain Length of 13 to 18 and the Reported Melting Points of Normal-Chain ME (12)

FFA carbon number	Branch and ester type	T_{on} (°C)	T_p (°C)	T_{com} (°C)	Heat of fusion (kJ/mol)	Purity (%)	Normal-chain ME m.p. (°C)
18	iso-ME	22.8	25.7	27.1	53.2	95	37.8
16	iso-ME	12.6	16.5	18.2	47.5	98	30.7
14	iso-ME	−1.5	2.7	4.8	38.9	95	19.1
17	ante-ME	0.3	3.8	5.2	43.9	98	29.7
15	ante-ME	−14.5	−10.9	−9.0	34.9	95	19.1
13	ante-ME	−34.5	−31.8	−30.5	27.8	94	5.8
18	iso-IE	3.2	6.3	7.5	43.4	96	—
16	iso-IE	−8.3	−4.8	−3.2	38.1	96	—
14	iso-IE	−21.0	−16.9	−15.3	30.8	94	—
17	ante-IE	−9.3	−6.3	−5.2	35.4	97	—
15	ante-IE	−24.3	−20.5	−18.1	30.5	94	—

TABLE 3
Averaged ^{13}C NMR Chemical Shifts (ppm) of Branched-Chain Esters[a]

	ME	IE	All esters	Ref. 13
C1	174.39	173.49	173.99	—
C2	34.15	34.60	34.35	34.10
C3	24.99	25.09	25.04	24.70
ω1 iso	—	—	22.73	22.68
ante	—	—	11.44	11.42
ω2 iso	—	—	27.59	27.98
ante	—	—	27.11	27.14
ω3 iso	—	—	39.10	39.08
ante	—	—	34.57	34.41
ω4 iso	—	—	27.92	27.45
ante	—	—	36.64	36.66
Methyl branch iso	—	—	22.73	22.68
ante	—	—	19.23	19.23
Ester methyl	51.48	—	—	—
Methylene envelope	—	—	29.2–30.0	28.8–30.0

[a]For abbreviations see Table 2.

and are listed separately. Most of the chemical shifts agree with Gunstone's result with very small variations (13).

In ^{1}H NMR spectra, the hydrogen atoms at the methyl ends of iso-compounds (i.e., the hydrogen atoms on the two ω-1 carbons) gave a doublet signal at 0.85–0.88 ppm, whereas those at the methyl ends of anteiso-compounds (i.e., the hydrogen atoms on the w-1 and methyl branch) gave an asymmetrical triplet signal at the same chemical shift. Normal-chain esters showed a symmetrical triplet at the same chemical shift. The other signal difference between iso- and anteiso-compounds was a septet for iso-esters at ~1.5 ppm, which was absent from the spectra of anteiso-esters. We believe this septet peak can be assigned to the hydrogen on the carbon at the ω-2 position of iso-branched esters. The intensity of this septet peak indicated that the peak was associated with one proton. A septet peak at a different chemical shift was also observed in the spectra of IE, which have an isopropyl group at the carboxyl end.

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