relatively little variation within species and were in excellent agreement with the data in Table I. For example, the range of erucic acid content in *Crambe abyssinica* (6 samples, 3 locations) was 56 to 59%, in *Eruca sativa* (6 samples, 3 locations) 42 to 45%, and in *Raphanus sativus* (5 samples, 4 locations) 31 to 34%.

Seeds of selected Cruciferae are shown by this survey to contain oils which, on the basis of chromatographic analyses, appear to be suitable industrial sources of linolenic acid and eicosenoic acid in addition to the usual erucic acid.

Acknowledgments

The authors are grateful to C.A. Glass for infrared examination of the oils and to R.L. Cunningham for preparation of methyl esters.

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[Received June 7, 1961]

Dilatometric Properties of Some Glycerides of Confectionery Fats[1]

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Two triglycerides, 1-oleodistearin and 2-palmito-oleostearin, which are components of some confectionery fats, were synthesized and their melting behavior and dilatometric properties were determined. Expansivities and melting dilations of the various polymorphic forms were measured.

1-Oleodistearin was found to have two melting points, 30.3 and 42.1°C., while 2-palmito-oleostearin was found to have melting points at 24, 37, and 40.5°C. The rates of transformation of the thermodynamically unstable polymorphs at temperatures below their melting points were much more rapid than those for the corresponding 2-oleo isomers previously reported.

Mixtures of 2-oleopalmitostearin with 2-palmito-oleostearin and 1-oleodistearin with 2-oleodistearin were examined dilatometrically. In each of these mixtures the components apparently do not temper at the same rate to similar polymorphic forms and thus there is some degree of incompatibility even though in each of these mixtures the components are positional isomers. The properties of the intermediate melting mixtures are dependent on the method of tempering.

COCOA BUTTER-LIKE fats derived from domestic vegetable oils by processes involving interesterification and from animal fats by processes involving fractionation should contain sizable proportions of both 1-oleodistearin and 2-palmito-oleostearin. One of these glycerides is the positional isomer of 2-oleodistearin, and the other is a positional isomer of 2-oleopalmitostearin. The 2-oleo glycerides comprise about 80% of cocoa butter. Unfortunately in neither pair of isomers do the members exhibit the same polymorphic behavior (11). The behavior in mixtures also should be different. Despite the common occurrence of the two glycerides, 1-oleodistearin and 2-palmito-oleostearin, in fat products, there have been only a few investigations with the pure compounds, and relatively little is known about their properties.

The synthesis and purification of 1-oleodistearin has been reported several times (1, 5, 6, 11). Lutton (11) made an investigation of polymorphic behavior and found three forms which he called sub-alpha, alpha-3, and beta prime-3. The sub-alpha was claimed to exist in modifications *A* and *B*, and it was presumed that the sub-alpha transformed reversibly to the alpha. The alpha-3 and beta prime-3 melted at 30.4 and 43.5°C., respectively. Apparently no one has made a dilatometric examination of 1-oleodistearin or of well-defined mixtures containing it.

2-Palmito-oleostearin also has been prepared and examined by several investigators (4, 9, 11, 13). Lutton (11) again is the only one who has made a thorough examination of polymorphic behavior. The polymorphs which he found to exhibit melting and their associated melting points were alpha-2, 25.3°C.; sub-beta prime-3, 37°C.; and beta prime-3, 40.2°C. The alpha form was found to transform reversibly to sub-alpha. The beta prime-3 form was obtained from solvent only. Apparently no dilatometric data on 2-palmito-oleostearin have been published heretofore.

The purpose of the present investigation was to determine dilatometric and other properties of 1-oleodistearin and 2-palmito-oleostearin and of a simple mixture of each of these compounds with its positional isomer occurring in cocoa butter.

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[1] Presented at the 52nd Annual Meeting of the American Oil Chemists' Society, St. Louis, Missouri, May 1–3, 1961.

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Experimental

Synthesis of Glycerides. 2-Palmito-oleostearin was prepared by a modification of the procedure of Verkade *et al.* (14). The intermediate 1-stearoyl-2-palmitoyltritylglycerol was prepared as described in a previous publication (10). The latter compound was detritylated by hydrogenolysis in glacial acetic acid with 10% palladium-on-carbon as catalyst. After a reaction time of 5 hr. at 45–50°C. the catalyst was filtered off and washed several times with glacial acetic acid to remove adhering product. The filtrate and washings were combined and poured into about 10 times their volume of water at 10°C. The 1-stearo-2-palmitin which precipitated was filtered immediately and washed with cold absolute ethanol. The precipitate was dissolved in commercial hexane, washed with water until neutral, and dried over anhydrous sodium sulfate. After several crystallizations from petroleum ether, a mixture of ether and 95% ethanol, and finally commercial hexane, the 1-stearo-2-palmitin had a melting point of 69.4°C.

To prepare the 2-palmito-oleostearin, 0.0123 mole of oleoyl chloride in chloroform was added slowly to a chloroform solution containing 0.0081 mole of 1-stearo-2-palmitin and 0.085 mole of quinoline. After refluxing for 3 hr., the resulting mixture was allowed to stand overnight at room temperature. The mixture was dissolved in ethyl ether and washed successively with cold 0.5 *N* sulfuric acid, 5% potassium bicarbonate solution, and water. After drying over anhydrous sodium sulfate, the ether solution was filtered, and the ether was evaporated under reduced pressure. The 2-palmito-oleostearin was crystallized several times from a mixture of petroleum ether and acetone. The purified 2-palmito-oleostearin melted at 40.5°C.

The 1-oleodistearin was prepared essentially by the method of Daubert *et al.* (6). 1-Mono-olein in the amount of 0.0281 mole was dissolved in a solution containing 0.0852 mole of dry quinoline in 100 ml. of chloroform. To this solution 0.0617 mole of stearoyl chloride was added slowly with stirring. The solution was allowed to react for 3 days at 45°C. Then ethyl ether was added and the solution was washed successively with cold 0.5 *N* sulfuric acid, 5% potassium bicarbonate solution, and water. The solution was dried over anhydrous sodium sulfate and filtered, and the ether was removed by distillation under reduced pressure. The 1-oleodistearin was crystallized several times from a solution of ether and ethanol and from acetone to obtain the purified product melting at 42.1°C.

Analytical data for the two glycerides are recorded in Table I. The 2-oleodistearin and 2-oleopalmitostearin were the same compounds whose preparation and properties were described earlier (10).

Dilatometric Measurements. The dilatometric examinations were carried out in dilatometers of the gravimetric type with mercury as the confining liquid. The construction of these dilatometers, their method of use, and methods employed in correcting dilatometric readings for the expansion of mercury and glass and making various other calculations were in accordance with previously published descriptions (2, 3).

TABLE I

Analytical and Physical Data for 2-Palmito-oleostearin and 1-Oleodistearin

Property	2-Palmito-oleostearin	1-Oleodistearin
Capillary melting point, °C.		
Form I	40.5	42.1
Form II	37.0	30.3
Form III	24.0	
Density, g./ml. at 60°C.	0.8778	0.8771
Refractive index, n_D^{45}	1.45413	1.45489
Iodine value		
Found	28.6	27.3
Calculated	29.5	28.5

In general the procedure followed in examining the lower melting polymorphs was to melt the sample, solidify it in ice water, and then cool it to about −37°C. With most triglycerides this procedure will give the alpha form. After about 20 min. at this initial temperature and at each successive temperature, the mercury in the weighing flask of the dilatometer was weighed and the sample was heated to the next temperature. When polymorphic transition or melting was encountered, this period of 20 min. was not sufficient to reach equilibrium conditions. This nonattainment of equilibrium was indicated by the position of the mercury at the tip of the dilatometer after the flask containing the excess mercury was weighed and was ready to be reattached to the dilatometer. Formation of drops of mercury at the tip indicated melting was still in progress. A contraction of the mercury into the dilatometer away from the tip indicated a polymorphic transition was in progress. In either case additional time at that temperature was necessary to reach equilibrium.

Where rapid polymorphic transformations were encountered a "thrust-in" technique (10) was used to obtain dilatometric data on the unstable polymorphic forms. For example, 2-palmito-oleostearin was melted at temperatures above 60°C., solidified in ice water for 5 min., and then placed in the bath at 25°C. The mercury-containing flask attached to the dilatometer was loosened and lowered so that mercury could drop from the dilatometer into the flask as the sample expanded but could not be sucked backed. After about 1 min. the mercury stopped dropping into the flask and pulled up into the dilatometer stem away from the tip. This contraction continued for about 8 min. after which time volumetric equilibrium of the sample was reached and the end of the mercury column ceased to move. The expansion of the sample during the first minute measured the thermal expansion of the lowest melting polymorph before this expansion was counterbalanced by conversion to the intermediate melting polymorph. In this particular test the 2-palmito-oleostearin was converted completely at the end of 8 min. to the intermediate melting polymorph, which was much more stable at this temperature than was the lowest melting polymorph.

To obtain the highest melting polymorphs for dilatometric examination, the samples were partially melted and resolidified during the early stages of tempering; and then they were tempered by keeping them just below their melting points for a sufficient length of time.

Melting Points. The several melting points were determined by the capillary tube method. Determinations were made on samples obtained by fractional crystallization from solvents and on samples after melting, resolidification, and tempering. The samples were further examined over short temperature intervals by the "thrust-in" technique (7). The temperature and length of time required to transform a lower melting polymorph into one having a higher melting point were noted.

Results and Discussion

Melting Behavior. Three capillary melting points were found for 2-palmito-oleostearin. The lowest melting polymorph, Form III, was found after melting the sample in hot water and then solidifying it in ice water for about 10 sec. This polymorph melted slowly in a water bath at 24.0°C., requiring about 20 sec., but then quickly resolidified. When this sample was held at 24.0°C. for an additional 10 sec. after the start of resolidification, Form II was obtained, and it then had to be plunged into a bath at 37.0°C. to effect melting. If the sample was then held at 37.0°C., it became cloudy after about 1 min. and began to transform into the highest melting polymorph, Form I. After complete conversion into Form I, the sample melted at 40.5°C.

The melting points which were found are in fairly good agreement with those reported by Lutton (11). However, Lutton stated that the highest melting polymorph was obtained only by crystallization from solvent, whereas the present sample could be tempered readily to this form.

Only two melting points were found for 1-oleodistearin. When the sample was melted in hot water, chilled for 5 sec. in ice water, and then placed in a bath at 30.3°C., slow but complete melting occurred (Form II). When the sample was melted and chilled as above and then tempered for 40 min. at 25° C., complete melting at 30.3°C. no longer occurred, indicating a fairly quick partial conversion to the highest melting form. After complete conversion to Form I, melting occurred at 42.1°C.

1-Oleodistearin and 2-palmito-oleostearin were tempered to their highest melting polymorphic forms much more rapidly than were 2-oleodistearin and 2-oleopalmitostearin, which were examined earlier (10). This difference in relative rates was unexpected in view of the fact that the symmetrical, diacid triglycerides of the saturated fatty acids having an even number of carbon atoms temper more rapidly than do the corresponding unsymmetrical triglycerides (12).

Dilatometric Behavior. The volumetric expansion vs. temperature for the polymorphs of 2-palmito-oleostearin are shown graphically in Fig. 1. The most notable property of 2-palmito-oleostearin is its rapid rate of conversion to the highest melting form. Curve C was obtained on solidifying the sample in ice water, chilling to −37°C., and then raising the temperature gradually. The holding time at each test temperature was 20 min. Curve C exhibits a slight change in the rate of expansion at about −10°C. When the sample was held at 5°C. for 0.5 hr., a very slight conversion to the next higher melting polymorph occurred. At temperatures of 10 to 20°C. faster rates of conversion were found. At 25°C. conversion was complete within 0.5 hr. Because of this rapid conversion the dilatometric melting point of this form could not be determined.

Curve D was obtained by the "thrust-in" method in which individual points were determined independently of each other. The failure of Curves C and D to coincide could be due to slight tempering during the preparation for the run represented by Curve C. By the "thrust-in" method the dilatometric expansion curve could be extended to about 20°C. without too great an amount of conversion to the next higher polymorph.

The intermediate melting form, Curve B, was obtained by melting the sample, solidifying it in ice for 5 min., and then tempering for about 20 min. at 25°C. before chilling to −37°C. Near its melting point this polymorph converts rapidly to the highest melting form.

The highest melting or stable form, Curve A, was obtained by tempering the sample for several days at room temperature or for a lesser time at temperatures up to 37°C.

Dilatometric curves for the two polymorphs of 1-oleodistearin are given in Fig. 2. The low-melting form, which was obtained by solidifying the melted sample in ice water, exhibits a distinct change in expansivity just below 0°C., which tends to confirm Lutton's claim of the existence of two modifications of one polymorphic form of this compound (11). Above about 15°C. it was necessary to use the "thrust-in" method.

The high-melting form was obtained by slowly warming the low-melting form and then tempering just below 42°C. Using this procedure the conversion

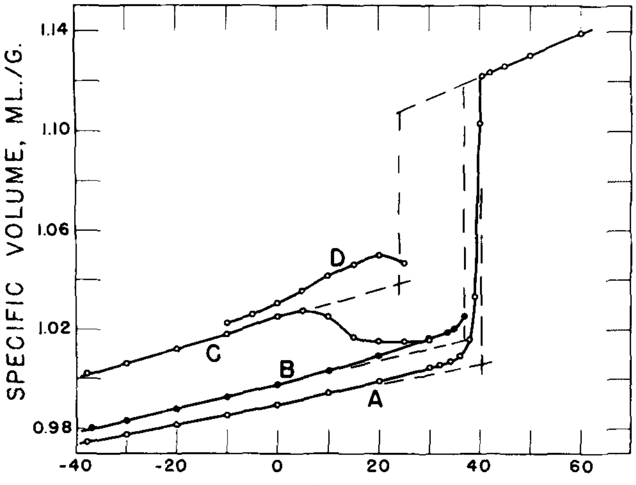

FIG. 1. Dilatometric curves for the polymorphs of 2-palmito-oleostearin: *A*, Form I; *B*, Form II; *C*, Form III; and *D*, Form III determined by "thrust-in" method.

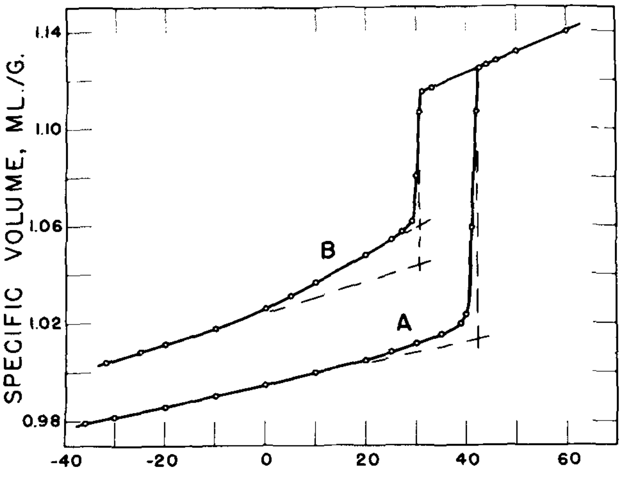

FIG. 2. Dilatometric curves for the polymorphs of 1-oleodistearin: *A*, Form I; and *B*, Form II.

was completed in 2–3 hr. The conversion could also be carried out by storing the quickly solidified sample overnight at about 25°C. Even at 16°C. about half converted to the highest melting form overnight.

Some evidence of another solid form less dense than the low-melting form mentioned above was obtained on very rapid solidification, but the evidence was not always reproducible. The possibility that 1-oleodistearin can be solidified in an amorphous form should not be ruled out. In an earlier dilatometric investigation it was found that 1,2-diaceto-3-olein could be readily cooled to a glass-like solid (8). The 1-oleodistearin did not behave like most triglycerides in that it was difficult to remove dissolved gas by the usual method of repeated melting and solidification during the process of filling the dilatometer. The melting and solidification had to be repeated a large number of times. The solid form had a very waxy appearance.

Dilatometric data were obtained on a 1:1 mixture of 2-palmito-oleostearin and 2-oleopalmitostearin. The low-melting form of the mixture, Curve C, Fig. 3, was obtained by melting the sample, solidifying it in ice for a few minutes, and then cooling to −30°C. Simple expansion was encountered up to about 10°C., but as the temperature was increased above this level the mixture converted to higher melting forms at increasing rates, and the "thrust-in" method was used to complete Curve C. At 22–25°C. conversion to higher melting forms was very rapid, and the solidification time had to be kept to a minimum (5 min.) to retard conversion.

When the sample was tempered for 1 hr. at 25°C. after being solidified in ice water, it exhibited relatively uniform melting over the range of about 18 to 32°C. With this sample no distinctive dilatometric curve was obtained; that is, the exact form of the curve was not reproducible.

Curve B of Fig. 3 was obtained by melting the sample, solidifying it in ice water, and then tempering overnight at 25°C. before starting the run. The shape of Curve B indicates a broad melting range with the midpoint occurring at about 27°C. The melting rate above 28°C. is more or less uniform to about 36°C. Somewhat independent melting of the individual components and polymorphic forms seems to occur.

When the sample was solidified as mentioned above and then tempered for several days at room temperature before the dilatometric examination was made, much of the sample apparently was converted to the highest melting polymorphs, but melting started about 5° lower than it did for a thoroughly tempered sample.

Curve A of Fig. 3 was obtained with a sample which was thoroughly tempered by holding it at 30°C. for 2.5 days. The greater portion of this tempered sample melts between 32 and 36°C., and the midpoint of melting is about 34°C.

This mixture of 2-palmito-oleostearin and 2-oleopalmitostearin possessed a melting range which was definitely longer than those of mixtures of 2-oleopalmitostearin and 2-oleodistearin examined earlier (10). The latter tended to behave like single compounds. With the present mixture the point of complete melting always was relatively high, apparently because the 2-palmito-oleostearin readily converts to its highest melting polymorph.

Dilatometric measurements were made on a mixture of 27.8% 2-oleodistearin and 72.2% 1-oleodistearin. This proportion was selected to facilitate comparison with a mixture of 2-oleopalmitostearin and 2-oleodistearin examined earlier (10). Solidification of the present mixture in ice water produced lower melting polymorphs which were fairly stable, Curve C of Fig. 4. However, at 30°C. conversion was rapid enough so that the "thrust-in" method had to be used to determine the melting portion of Curve C.

While quickly solidified samples of the mixture tended to undergo polymorphic transformation on being warmed to 30°C., a melt of the mixture cooled to 31.8°C. gave no indication of solidifying over a period of 16 hr. The tendency to remain in a supercooled condition was quite marked.

Limited tempering of the quickly solidified mixture yielded various types and proportions of intermediate forms. Curve B of Fig. 4 was obtained when the mixture was melted and heated to 50°C., chilled in ice water, and then tempered at 26°C. for 2.5 days. This curve shows a slight amount of conversion to the highest melting form because complete melting does not occur until the temperature reaches 42°C.

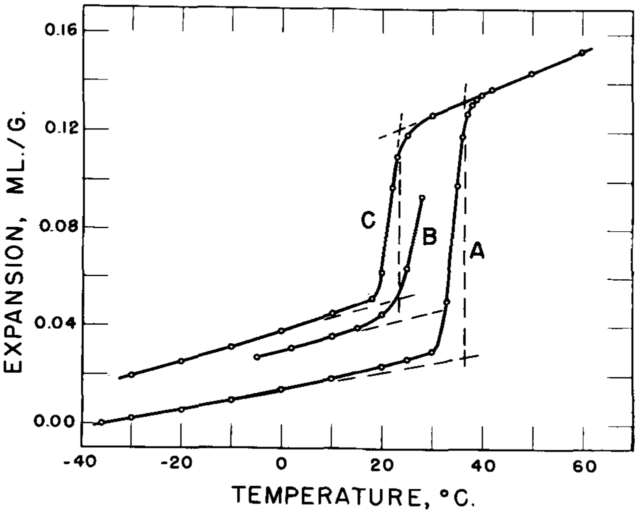

FIG. 3. Dilatometric curves for the composition 72.2% 1-oleodistearin and 27.8% 2-oleodistearin: *A*, both glycerides in Form I; *B*, both glycerides mostly in Form II, and *C*, both glycerides in Form III.

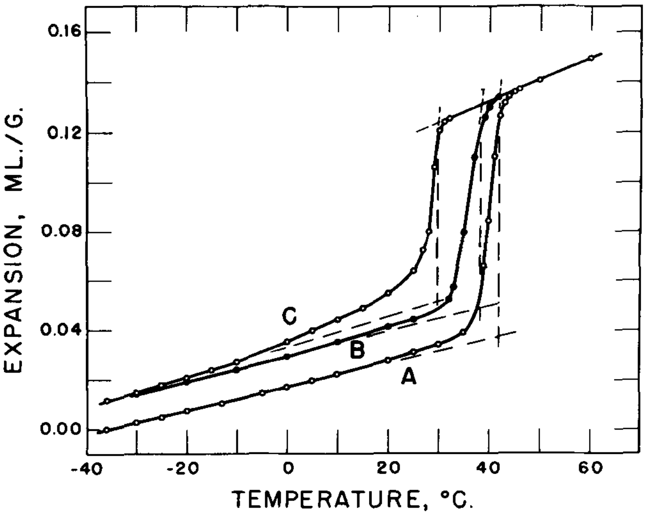

FIG. 4. Dilatometric curves for a mixture of equal parts of 2-oleopalmitostearin and 2-palmito-oleostearin; *A*, both glycerides in Form I; *B*, both glycerides mostly in Form II, and *C*, both glycerides in Form III.

TABLE II
Expansivity and Melting Dilation of the Products

Compound or mixture	Temperature interval, °C.	Polymorphic form	Coefficient of expansion, ml./g./°C.	Melting dilation, ml./g.	Melting point,[a] °C.
2-Palmito-oleostearin	−38 to 0	I	0.000403	0.1161	40.3 (40.5)
	−37 to 0	II	0.000485	0.1035	(37.0)
	−30 to −10	III	0.000581	0.0695	(24.0)
	50 to 60	Liquid	0.000881	....	......
1-Oleodistearin	−36 to 0	I	0.000441	0.1117	42.2 (42.1)
	−32 to −10	II	0.000614	....	......
	5 to 20	II	0.001116	0.0554	30.6 (30.3)
	45 to 60	Liquid	0.000859	....	......
2-Oleopalmitostearin and 2-palmito-oleostearin (1:1)	−36 to −10	I	0.000381	0.1050	36.5
	− 5 to 2	II	0.000543		
	−30 to −5	III	0.000586	0.0698	23.4
	42 to 60	Liquid	0.000858	....	......
2-Oleodistearin and 1-oleodistearin (27.8:72.2)	−36 to 0	I	0.000482	0.0966	42.0
	−30 to 0	II	0.000501	0.0822	38.3
	−30 to −15	III	0.000594	0.0722	29.8
	46 to 60	Liquid	0.000866	..	

[a] Melting points in parentheses are capillary melting points, others are dilatometric melting points.

When the mixture was solidified slowly from the melt by cooling in air at 25°C. and then held at this temperature for several days, melting occurred between the range of the intermediate forms, represented by Curve B, and the highest melting forms, represented by Curve A. Dilatometric curves obtained with slowly cooled samples exhibited slight breaks in the melting portions, indicating that individual melting of components tended to occur. Possibly under the conditions of slow cooling nonhomogeneous compositions were obtained.

The mixture in the highest melting forms, Curve A, was obtained by tempering for 4 days at 28°C. a sample containing seed crystals of the highest melting forms. The tempering of some seeded samples could be effected in less time by using a higher temperature. Only 2 hr. was required in one instance.

The coefficients of expansion in the solid and liquid states, the dilatometric or capillary melting points or both, and the volume changes accompanying melting were determined for the two glycerides and the two mixtures and are recorded in Table II.

The mixing of one triglyceride with another is generally recognized to retard the rate of any polymorphic transformations. In an earlier examination of 2-oleopalmitostearin and 2-oleodistearin, the major components of cocoa butter, this was found to be true (10). Furthermore, 2-oleopalmitostearin and 2-oleodistearin exhibited similar polymorphic behavior, and their mixtures tended to behave like a single compound.

In the present investigation 2-oleopalmitostearin and 2-oleodistearin were each mixed with a positional isomer, and the binary mixtures were examined. The admixed isomer in each case exhibited a much faster rate of polymorphic transformation and a different pattern of transformation. On the basis of the data obtained it must be concluded that the mixing of two glycerides which exhibit widely different rates of polymorphic transformation may actually serve to increase the rate of transformation of the slower one. Possibly the more easily transformed component can serve as "seed" for the other. The mixtures of the isomers which were examined behaved otherwise as would be expected of a triglyceride mixture.

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[Received June 21, 1961]

Further Studies on the Pancreatic Hydrolysis of Some Natural Fats

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A series of animal and vegetable fats has been subjected to hydrolysis with pancreatic lipase. From the results obtained, the triglyceride compositions of the original fats have been calculated by the method previously proposed by Coleman and Fulton.

These compositions show substantial agreement with those obtained by other methods. Similarities and differences between fats are shown to be reflections of similarities and differences in glyceride composition. The middle position of the triglycerides has been shown to be largely occupied by unsaturated acids in the case of shea, illipé, and cocoa butters; and by palmitic acid in the case of lard. From a study of the glyceride compositions of a series of lards, of differing fatty acid content, it is suggested that the glycer-