T. P. HILDITCH SYMPOSIUM ON ANALYSIS OF NATURAL FAT TRIGLYCERIDES. III

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CARTER LITCHFIELD, Chairman

Synthesis and Analysis of Optically Active Triglycerides

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Abstract

Physical-chemical methods for steric analysis of asymmetrical triglycerides and 1,3-diglycerides have been investigated.

Aliphatic triglycerides exhibit optical rotation at the D line and in the ultraviolet to 313 mμ when they contain greatly different fatty acid substituents, such as isobutyric, sorbic or others contrasted with palmitic and similars. However, optical rotation is not demonstrable with triglyceride antipodes which have only palmitic, oleic and similar component acids. Since their optical rotation is not apparent, they are called cryptoactive glycerides.

Presence or absence of the piezoelectric effect permits diagnosis of antipode or racemate with crystalline tri- and diglycerides.

Mixed melting point diagrams of one antipode and/or of the racemate with the triglyceride of unknown configuration can be used to identify the latter. X-ray diffraction patterns of antipode and racemic glycerides are different, and configuration assignment is possible by making comparisons.

The optical rotation, $\alpha_D = +0.19°$, of dolphin oil was found to be due to S(+)-methylethylacetic acid which occurs as a component acid in the triglycerides. The optical activity of *euphorbiaceae* triglycerides is discussed and some pertinent model compounds have been prepared.

2-Oleo-palmitostearin from fresh cocoa beans was found to be the racemic compound.

Application of the Cahn-Ingold-Prelog R,S nomenclature to glycerides is outlined. Numerous glycerides have been synthesized in stereospecific form and their physical data are given. Some comments are made on stereospecific analysis of triglycerides without separating them.

The Problem and Its History

DISTRIBUTION OF FATTY ACIDS in biogenous glyceride molecules has been and still is a topic of extensive research. Up to now there seems to be divergency of opinion among investigators about the principles of distribution and the modifications of such principles which nature might follow in biosynthesis of glycerides (1–3). There is, however, a problem concerning triglycerides which is equally fascinating but can be posed and answered independently of the question of distribution. Regardless of even, random, partial random, restricted random or positionally specified random distribution in a given biogenous mixture of triglycerides, in all cases it is uncertain whether the individual glycerides represent one antipode or the racemate. The problem concerns all triglycerides which have different acids in positions 1 and 3. Glyceride molecules of this sort have an asymmetric carbon atom in position 2 of the glycerol, whatever the substituent in position 2 may be (see Fig. 1).

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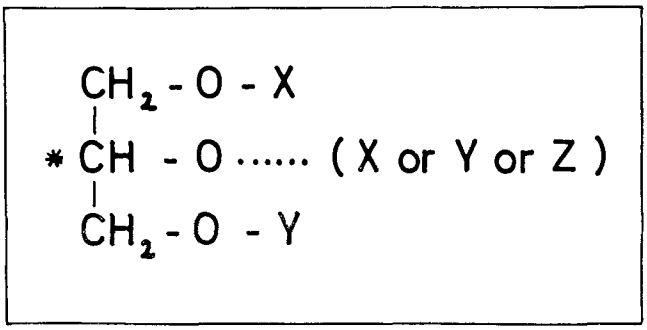


FIG. 1. The asymmetric carbon atom, C*, in position 2 of tri- and diglycerides.

The problem "antipode or racemate" has been solved long ago for other natural compounds like carbohydrates or amino acids. The same problem of natural triglycerides has not been dealt with until rather recently and one may state that it has not even been discussed adequately when compared with the question of fatty acid distribution. Although the problem of steric configuration of glycerides had been recognized, any experimental attack met with enormous difficulties. First of all, it was, and still is, very difficult to isolate a single triglyceride from biogenous mixtures for its further investigation; secondly, methods for syntheses of triglyceride antipodes are only of recent date, but such synthetic compounds are necessary for systematic study. Finally, the great majority of triglycerides, although not all of them, have the unpleasant property that their antipodes are not "optically active." The chemist is deprived of his primary tool, the polarimeter, for distinguishing antipode and racemate.

The credit for decisive improvement in such a discouraging situation goes to Fischer and Baer (4). Their efficient method for synthesis of triglyceride antipodes provided a basis for further experimental progress in the problem of antipode or racemate. The Fischer-Baer synthesis starts out from the readily available D-mannitol, and leads to an asymmetric monoglyceride.

Figure 2 illustrates the course of the syntheses. Intermediates are diisopropylidene mannitol, isopropylidene glycerol aldehyde and D-isopropylidene glycerol. Acylation of the hydroxyl group in the latter and hydrolysis of the isopropylidene group yields an optically active monoglyceride which Fischer and Baer defined as L-monoglyceride and which, according to present nomenclature, belongs to the R-series. Further simultaneous or successive acylation in positions 3 and 2 opens the way to any triglyceride antipode. Examples for alternative substitutions in positions 2 and 3 will be shown later.

Fischer and Baer reported the synthesis of four aliphatic triglycerides (see Table I) according to this scheme (4). Although none of the compounds showed any measurable optical rotation, the authors were convinced that these triglycerides represent antipodes rather than racemates. Triglycerides containing benzoyl or nitrobenzoyl substituents were obtained by the same method and they exhibit appreciable optical activity. However, direct proof of the identity of the aliphatic triglycerides as antipodes independent from the course of synthesis was not available at that time. The present report provides methods for such proof and it is indisputable that the Fischer and Baer synthesis leads to triglyceride antipodes.

TABLE I

Synthetic Triglycerides Prepared from Optically Active α-Monoglycerides (Fischer and Baer, 1941)

Triglyceride	Melting point, °C	$[\alpha]_D$
S-1-Aceto-distearin	56.5–57	0.0° (chloroform)
S-1-Lauro-distearin	48.5	0.0° (pyridine)
R-1-Stearo-dipalmitin	62.5	0.0° (chloroform or pyridine)
R-1-Palmito-dilaurin	44	0.0° (pyridine)

TABLE II

Optically Active Triglycerides

Glyceride	$[\alpha]_D$	Solvent
R-1-Pivalyl-divalerin	+ 1.8°	Methanol
R-1-Lauro-dibutyrin	+ 1.1°	Benzene
R-1-Myristo-dibutyrin	+ 0.85°	Benzene
S-1-Myristo-diisovalerin	− 0.75°	Methanol
S-1-Isovalero-dimyristin	− 0.06° (436 mμ)	Benzene
S-1-Sorbino-dipalmitin	− 5.5°	Benzene
S-1-Palmito-disorbin	− 6.85°	Benzene
R-1-Lauro-dipalmitin	+ 0.09°	Benzene

Fischer and Baer summarized their experiences as follows: "The lack of observable rotation in our synthetic unsymmetrical triglycerides with at least two different fatty acids leads to the suspicion that the *natural* triglycerides of the same type may show no rotation in sodium light." And they conclude: "Therefore, the natural triglycerides, though they do not show a rotation, are not necessarily racemic, but might easily occur in either of the two enantimorphic forms" (5).

This re-states the stereochemical problem but further research in this direction became stagnant again for a considerable time. The conservative and cautious wording "are not necessarily racemic, but might easily occur . . ." indicates that no way was seen to confirm their results, and, therewith, no way was seen for further progress in studying the configuration of natural triglycerides.

When we began our investigations in 1957 (6), we divided the problem into the following questions:

1) Can one demonstrate optical activity with aliphatic triglycerides of types occurring in nature when they are synthetic and supposedly antipodes?
2) Can other means distinguish antipodes and racemates of synthetic triglycerides when polarimetry fails, and can they be used to determine the absolute configuration of such optically inactive "active" glycerides?

Our experiments answered the questions sufficiently for several practical approaches. Triglycerides from dolphin oil containing isovaleric acid were then investigated. Furthermore, the configuration of 2-oleopalmitostearin from cocoa butter has been determined (6).

Further discussion will concern another procedure

TABLE III

Piezoelectric Investigation of Glycerides

	Form	Piezo effect
Triglyceride		
1-Lauro-2,3-dipalmitin	Antipode	Yes
	Racemic	No
1-Stearo-2,3-dipalmitin	Antipode	Yes
	Racemic	No
1-Palmito-2-oleo-3-stearin	Antipode	Yes
	Racemic	No
Diglyceride		
1-Isovalero-3-myristin	Antipode	Yes
	Racemic	No
1-Stearo-3-olein	Antipode	Yes
	Racemic	No

TABLE IV

Glycerides Having Different X-ray Diffraction Patterns for Antipodes and Racemates

Triglycerides	Diglycerides	Monoglycerides
1-Lauro-dipalmitin	1-Butyro-3-myristin	Monopalmitin
1-Stearo-dipalmitin	1-Lauro-3-palmitin	Monostearin
2-Oleo-palmitostearin	1-Stearo-3-palmitin	
	1-Oleo-3-stearin	

which has been published recently for stereospecific analysis of triglycerides (7). It is based on positional analysis of fatty acids. Inherently, there are limitations to stereo-chemical conclusions when such a procedure is applied to the sum of biogenous triglycerides rather than individual triglycerides.

Alipathic Triglycerides with Detectable Optical Activity

Most aliphatic triglycerides previously tested for optical activity had very similar substituents in positions 1 and 3. Their molecular weight was rather high and this may explain the lack of demonstrable optical activity. Our first step was to check this hypothesis and to evaluate the structural features which render polarimetry useless. Several aliphatic triglycerides of relatively low molecular weight were prepared with distinctly different substituents in positions 1 and 3. Eight examples are listed in Table II where optical activity is well demonstrable. For clarity, only the rotation at the D line is given. Rotation in the UV region has also been measured and this was particularly important with myristodibutyrin, isovalerodimyristin and laurodipalmitin where the rotation values are very small.

The rotary dispersion of lauro-dipalmitin is shown in Figure 3, upper part. The lower part of Figure 3 shows the rotatory dispersion of the precursor, R-1-monopalmitin. The scale of optical rotation is in the upper part about 25 times larger than in the lower part. The rotation of the triglyceride is much lower than that of the monoglyceride and it is noteworthy that the directions of rotations are opposite. The results were further ascertained for myristo-dibutyrin and lauro-dipalmitin by synthesis and measurement of both antipodes (see Experimental Section, Table VIII).

The syntheses for R- and S-lauro-dipalmitin are outlined in Figure 4. Starting material for both antipodes was D-(+)-isopropylidene glycerol which had been prepared according to Fischer and Baer from D-mannitol. The absolute configuration of D-(+)-isopropylidene glycerol is known to be S. Therefore, the absolute configuration of the resulting optically active triglycerides is also known.

TABLE V

Melting Points of Crypto-active and Racemic Glycerides

	mp °C	
	Found	Lit.
Triglyceride		
S-1-Lauro-dipalmitin	53.6–53.9	
D,L-1-Lauro-dipalmitin	55.0–55.8	54 [a]
S-1-Stearo-dipalmitin	63.2–64.0	
D,L-1-Stearo-dipalmitin	62.0–62.9	62.5 [a]
S-1-Palmito-distearin	65.5–67.5	
D,L-1-Palmito-distearin	65 –66	65 [a]
S-2-Oleo-palmitostearin	33.9–34.5	
D,L-2-Oleo-palmitostearin		37.5 –38 [b]
Diglyceride		
R-1-Lauro-3-palmitin	65.0–65.5	
D,L-1-Lauro-3-palmitin	59.0–60.3	
S-1-Palmito-3-stearin	75.0–76.0	
D,L-1-Palmito-3-stearin	59.0–60.3	

[a] Ref. (20).
[b] Ref. (33).

The optical rotation of the eight structurally different asymmetric triglycerides (see Table II) follows a simple rule. The R-forms are dextrorotatory and the S-forms are levorotatory. It must be emphasized that the correlation of (+) with R and (−) with S has been derived only from eight examples and may not be universally valid. Asymmetric 1,3-diglycerides do not exhibit such regularity. S-1-Butyro-3-myristin is levorotatory, S-1-isovalero-3-myristin and S-1-sorbino-3-palmitin are dextrorotatory (see Experimental Section, Table VII).

In the experiments with asymmetrical triglycerides, the following observations are of importance for future investigations. In the beginning of this work, we were very anxious to carry each synthesis through the several steps without delay, and each product was investigated immediately after its preparation. This was under the impression of Fischer and Baer's report that their optically active monoglycerides had lost more than half of their activity after one year of storage (5). However, our preparations of active mono-, 1,3-di- and triglycerides did not show any detectable racemization after storage for 6 to 7 years. Moreover, optically active triglycerides apparently are rather stabile at higher temperature. For example, 1-laurodibutyrin was distilled in high vacuum at 170C without loss of activity. These statements refer to the pure products and the situation is certainly quite different in presence of trace amounts of alkali or other potential contaminants. Similarly, some chromatographic materials may be suspect in causing changes, in particular, certain preparations of aluminum oxide, as long as they have not been tested with stereochemically defined model compounds.

The unexpected stability of triglycerides does not pertain to autoxidation and polymerization. For example, 1-palmito-disorbin is much less stabile.

TABLE VI

Rotary Dispersion of 1-Monoglycerides

		[α] at mμ, (°)							[α]D in Pyridine	
	% in Solvent	578	545	436	405/8	366	334	313	Found	Lit.[a]
R-1-Monobutyrin	10 pyridine	−8.26	−9.39	−15.87	−18.94	−24.53	−29.97		−8.1	−8.3
R-1-Monopivalin	none [b]	+2.2	+2.8	+ 4.6	+ 5.2	+ 6.2	+ 7.2	+ 7.8		
	10 pyridine	−2.32	−3.2	− 5.2	− 6.2	− 8.0			−2.20	
	10 benzene [b]	+3.8	+4.3	+ 6.7	+ 7.7	+ 9.2	+10.6	+11.6		
	10 methanol	−5.2	−6.0	−10.4	−12.4	−19.7	−19.7			
	10 dioxane		−0.1	− 0.5	− 0.8	− 1.6	− 2.5	− 3.4		
R-1-Monolaurin	10 pyridine	−5.2	−5.54	− 9.46	−11.4	−14.7	−18.9	−22.4	−5.0	−4.9
	5 benzene	−1.10	−0.35	− 0.65	− 0.73	− 1.73	− 2.65	− 4.7		
	10 methanol	−5.85	−6.58	−11.15	−13.7	−17.7	−22.5	−26.9		
R-1-Monomyristin	10 pyridine	−4.48	−5.38	− 9.00	−10.62	−14.42	−18.42		−4.4	−4.6
	1.2 benzene	−1.2	−1.6	− 4.0	− 4.8	− 6.3	−11.2	−12.7		
	10 methanol	−5.85	−6.58	−11.15	−13.7	−17.7	−22.5	−26.9		
R-1-Monopalmitin	10 pyridine	−4.40	−4.83	− 8.20	− 9.85	−12.8	−15.8		−4.3	−4.37

[a] Ref. (27).
[b] The sign of rotation of R-1-monopivalin in substance and in benzene is opposite to that in other solvents. See also the abnormal rotary dispersion of R-1-pivalyldivalerin in benzene, Table VIII.

TABLE VII

Rotatory Dispersion of 1,3-Diglycerides

	% in Solvent	[α] at mμ, (°) 578	545	436	405/8	366	334	313
S-1-Butyro-3-myristin	30 methanol	−0.42	−0.47	−0.80	−0.93	−1.23	−1.50	−1.69
	30 pyridine	−1.12	−0.17	−0.36	−0.45	−0.62	−0.84	−1.05
S-1-Isovalero-3-myristin	30 methanol	+0.07	+0.07	+0.11	+0.15	+0.20	+0.20	+0.37
S-1-Sorbino-3-palmitin	2.5 methanol	+1.84	+1.95	+2.93	+2.93	+5.08	+7.82	
	10 pyridine			+0.7	+1.5	+2.5		

Nomenclature

A brief discussion of the Cahn-Ingold-Prelog nomenclature for designating the absolute configuration of antipodes as R or S may be helpful at this point (8–10). Knowledge of the absolute configuration of the molecule is prerequisite for use of this nomenclature. Steric representation is possible then, for example, in a Fischer projection formula.

The left side of Figure 5 shows a triglyceride with acyls ac, ac′ and ac″. Equivalent, but easier to visualize, is the representation of the molecule as shown in the center of Figure 5: the asymmetric carbon atom is in the plane of the paper, the broken lines point away from the observer (below the plane), and the wedges point towards the observer (above the plane). The next step leads to the representation on the right of Figure 5. For the observer's view the substituent of lowest priority (see below) on the asymmetric carbon atom is moved vertically behind the asymmetric carbon atom. This brings the three other substituents above the paper plane without changing the relative position of the asymmetric carbon atom. A picture results which may be compared with the view into a goblet or a protruding three-spoke steering wheel as seen by the driver. The connecting atoms of the three substituents −Oac, $-CH_2Oac'$ and $-CH_2Oac''$ are in the plane of the elevated rim. The decision of R or S form can now be made according to the priority rule or rule of sequences. The compound is "R" (*rectus*) when the sequence of descending priority is clockwise; it is "S" (*sinister*) when descending priority is counterclockwise.

The priority or "relative weight" of substituents on the asymmetric carbon is defined by the sequence rule which is a code set by convention. The atom having the highest atomic number in the periodic system has highest rank. Among the substituents of carbon 2 of a glyceride, the oxygen atom has highest rank while

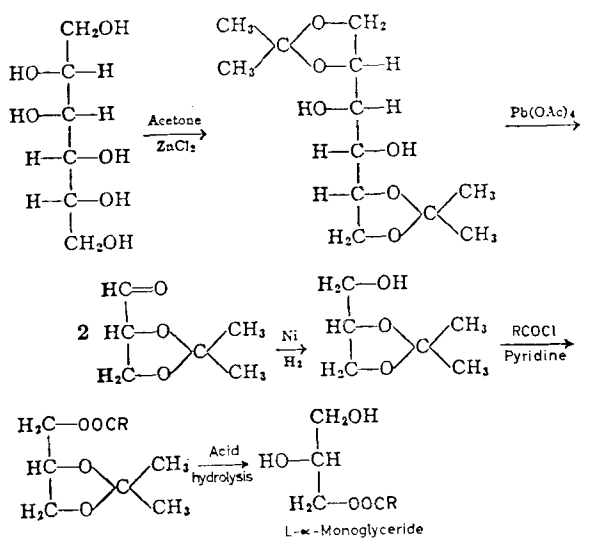


FIG. 2. The synthesis of L-monoglycerides according to H. O. L. Fischer and E. Baer.

the hydrogen atom has lowest rank. Therefore, the sequence begins always with −Oac and ends with −H. It remains to compare $-CH_2Oac'$ and $-CH_2Oac''$. The atoms next to the center of asymmetry are −C−O in both substituents so that the decision of second and third rank rests with ac′ and ac″. Proceeding further in their comparison, C atom by C atom, the longer one of saturated straight-chain acyls has higher priority than the shorter one. When entering, for example, n-myristyl as ac′ and n-valeryl as ac″ into the properly positioned molecule at the right of Figure 5, the priority sequence is −Oac above $-CH_2Oac'$ above $-CH_2Oac''$. The sequence is clockwise and the compound is the R-form. For ac′ and ac″ being saturated straight-chain acids, the higher molecular weight determines relative priority but this does not hold true when ac′ and ac″ in positions 1 and 3 are branched or unsaturated. One has to compare the carbon atoms step by step until one arrives at a branch or double bond in one of the acids. These features decide for priority; e.g., isovaleryl ranks above n-myristoyl, or 2-hexenoyl ranks above stearoyl. Double branching has priority over single branching; e.g., pivalyl ranks above 2-methylbutyryl. A double bond in C(n) has priority above single branching in C(n) but ranks behind double branching in C(n); e.g., pivalyl above 2-hexenoyl above 2-methylbutyryl.

Dolphin Oil

It has been shown above that optical activity is demonstrable for triglycerides which a relatively low molecular weight and distinctly different fatty acids. The findings were to be applied to natural triglycerides which fulfill these requirements. We chose dolphin oil since it is known to contain a considerable amount of isovaleric, and among others, also myristic acid (11). Early reports actually state that oil from this source is optically active, $\alpha_D = +0.3°$ (12) and +0.5° (13). However, none of the authors elaborated on this point. We confirmed the rather weak optical activity, $\alpha_D = +0.19$ in oil from *Delphinus delphis ponticus*.

Distillation of the oil in high vacuum at 190–250° involved heat exposure of less than 15 seconds and enriched the optical activity in the more volatile portion of the triglycerides, as was to be expected. The distillate amounted to about half of the material and had $\alpha_D = +0.36°$, while the residue was optically inactive. However, when these low-boiling triglycerides were saponified, the mixture of acids turned out to be optically active.

Distillation of the acids enriched S(+)-methylethylacetic acid in the more volatile portion. It was identified as its methyl ester by gas-liquid chromatography and quantified. The ratio methylethylacetic acid: isovaleric acid was 1:10. (+)-1-[S-methylethylacetyl] -R,S-dimyristin and (+)-1,2-di[S-methylethylacetyl] -R,S-myritsin were synthesized using optically active methylethylacetic acid. The optical rotation of these triglycerides is $\alpha_D = +3.45°$ and +10.16°, respectively,

and an estimate shows that the optical rotation of dolphin oil glycerides can be accounted for by the amount of (+)-methylethylacetic acid present. This does not rule out that R or S glyceride antipodes may exist in dolphin oil instead of, or together with, racemates of glycerides. It would have been wrong, however, to correlate the measured optical activity with the glycerol moiety.

An asymmetric acyl substituent is a rather conventional reason for optical activity of a compound, but the occurrence of S(+)-methylethylacetic acid in triglycerides is unusual. We are not aware that this acid has been reported as a component of biogenous glycerides although it has been shown to be a potential precursor of long-chain "anteiso" acids (14).

Euphorbiaceae Oils

Seed oils of the Chinese tallow tree, *Sapium sebiferum,* and of *Sebastiana lingustrina* are another example which cautions against concluding glyceride structure from optical rotation. Holman et al. had reported the optical activity of these oils (15) and more recently Maier and Holman (16) assigned the optical activity to carbon 2 of the glycerol. They suggested that the surprisingly high rotation, $\alpha_D^{20} = -21.5°$ and $-17.5°$, respectively, are due to the presence of a 2,4-conjugated dienoic acid which acts as chromophor. In context with such reasoning we synthesized asymmetrical triglycerides containing sorbic acid which has this conjugated structure (17a). Their optical rotation is unusually high when compared with that of our less exotic triglycerides. The result seemed to support the suggested concept although the sorbic acid triglycerides had only one-third or less of the rotation found in euphorbiaceae oils. More recent findings led Holman to the conclusion "that the optical activity in the euphorbiaceae glycerides is probably due to the asymmetric allene function which is present in one of the acids rather than due to an asymmetrical glycerol carbon" (17b).

It must be concluded that biogenous triglycerides with demonstrable optical rotation due to asymmetry in the glycerol have not been found yet.

Crypto-active Triglycerides

Some terminology should be clarified before reporting on long-chain triglycerides. It is quite usual to distinguish D- (or R-) and L- (or S-) lactic acids from racemic lactic acid by the term "optically active" or "active" lactic acid when specific reference to one distinct antipode is not necessary. The term "active" signifies one of the antipodes. However, to equate optical activity and antipode would be wrong when optical rotation of antipodes is only postulated by theory but cannot be demonstrated. The majority of the long-chain acid triglycerides which we prepared, e.g., 2-oleo-palmitostearin, failed to show optical rotation even in the ultraviolet. In such case, it is incorrect to contrast R or S antipode and the racemate as active and inactive compounds. Therefore, we suggest the less ambiguous term "crypto-active" when activity is expected but hidden as in the present situation.

The Piezoelectric Effect

In regard to polarimetry, the problem of distinguishing triglyceride antipodes and racemate is still where Fischer and Baer had left off; it could not be solved by modern UV polarimetry.

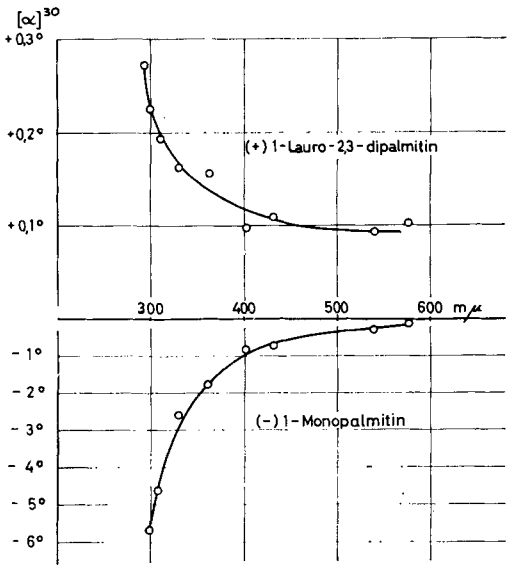


FIG. 3. Rotatory dispersion of (+)-1-lauro-dipalmitin and its precursor (−)-1-monopalmitin.

It has been known for a long time that the surface of crystals, the lattice of which has no center of symmetry, can become electrically charged when mechanical pressure acts upon them in direction of a structural polarity (18). As a consequence, crystals which consist of asymmetric molecules in one antipode form should exhibit such piezoelectric properties. It is to be expected that crystals of triglyceride antipodes are piezoelectric. A piezo-electrometer designed by Bergmann (19) (see Figs. 6 and 7) permits demonstration of the piezoelectric effect.

The apparatus is used with a single crystal or, as in the case of glycerides, with some crumbs of the powdery crystals. They are placed between two metal plates which are vibrating with the frequency of the alternate current to apply and release pressure on the sample. The plates serve at the same time as electrodes and are connected with a volt meter to indicate the piezo charge of the crystals.

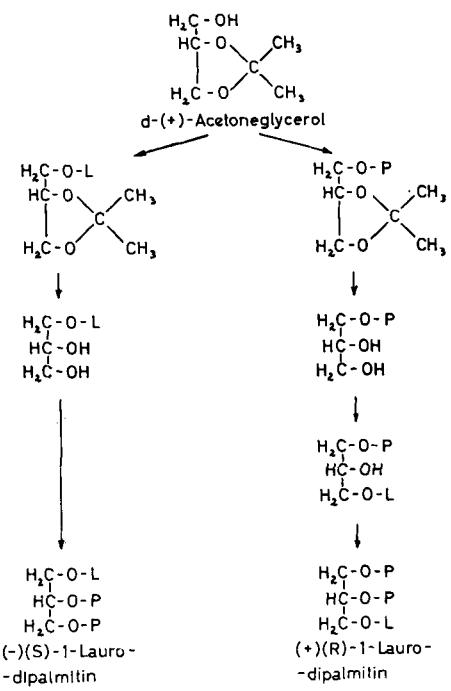


FIG. 4. Synthesis of the antipodes of 1-lauro-dipalmitin.

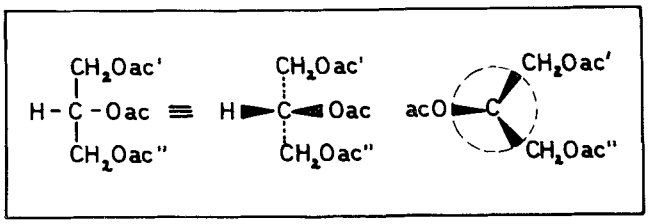


FIG. 5. Antipode of a triglyceride. Illustration of the Cahn-Ingold-Prelog nomenclature, developed from E. Fischer projection formula.

When this technique was applied to synthetic active and crypto-active glycerides, it was found that they had piezoelectric properties while the same compounds as racemates were not piezoelectric. Triglycerides, 1,3-diglycerides and monoglycerides were investigated. The magnitude of response depends on the substance. S-monomyristin and S-monopalmitin were the only glycerides where the effect was too small to distinguish between racemate and antipode with certainty. These exceptions are irrelevant since asymmetrical monoglycerides are optically active and can be measured with the polarimeter. Results with triglycerides and diglycerides are listed in Table III.

Melting Point Diagrams

Comparison of melting points is, besides polarimetry, a traditional method for distinguishing racemate and antipodes but in practice it is bound to fail with triglycerides. The differences of melting points of crypto-active glycerides and their racemates are mostly too small to be a deciding criterion. This is aggravated by the difficulty in obtaining from a biological mixture an individual glyceride of a melting point sufficiently sharp for convincing conclusions. The situation may be exemplified with R,S-stearodipalmitin, mp 62.9C, and crypto-active S-stearodipalmitin, mp 64.0C. Both of these compounds had been synthesized and measured in our laboratory. Fischer and Baer reported mp 62.5C for a stearodipalmitin which, according to the synthetic method, was the R antipode, whereas the same melting point has been reported also for R,S-stearodipalmitin (20). A more reliable procedure is the determination of mixed melting points so that a melting point diagram is obtained of mixtures consisting of an unknown and a known test substance in different proportions.

It is noteworthy that such a procedure had been already suggested 60 years ago "for diagnosis of an 'inactivum'" (21). At that time inactivum meant racemate or conglomerate since the problem of a crypto-active inactivum was not known.

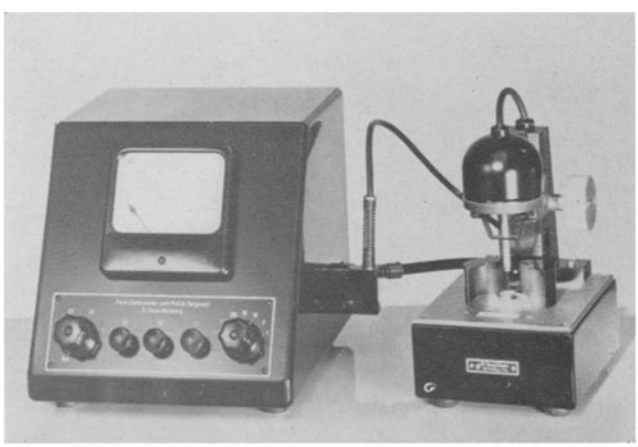


FIG. 6. Piezo-electrometer according to L. Bergman, with volt meter.

We investigated the phase diagrams for racemic and crypto-active glycerides with several compounds and, in most cases, the characteristic eutecticum of racemate and crypto-active compound was found. Figure 8 gives two examples for such data. Curves have not been drawn in these diagrams to enable more critical evaluation of the method. The eutectica are easily recognized.

Identifying the steric structure of a triglyceride of known chemical structure in this way requires synthesis of one of the antipodes as test substance and determination of the phase diagram in mixture with the unknown. Both substances, known and unknown, must be available in high purity.

Five possibilities can be foreseen:

1) The substances are identical when the melting point curve of the mixtures is horizontal.
2) The unknown triglyceride is enantiomeric to the test antipode when their melting points are identical and when the melting point curve of their mixtures has one or two eutectica.
3) The unknown is a racemate which crystallizes as such when melting points of unknown and test antipode are not identical, and the melting point curve has a minimum between 100% known and 100% unknown.
4) The unknown is a racemate which crystallizes as conglomerate when it has a melting point lower than any of its mixtures with the test antipode.
5) The method would fail when the antipodes of the glyceride form a continuous series of mixed crystals having identical or very similar melting points.

X-Ray Diffraction

Roentgenographic comparison of racemic and crypto-active compounds was found to be a simpler method for their distinction. X-ray diffraction differentiates glyceride antipode and racemate by their crystal structures and this was found valid for tri-, 1,3-di-, and 1-monoglycerides so far without any exception. Table IV lists the glycerides which have been investigated. Diffraction patterns are exemplified in Figure 9 by those obtained from 1-lauro-2,3-dipalmitin compounds.

Patterns 1 and 2 of Figure 9 represent the antipodes and are, of course, identical. The pattern of the racemate in line 3 is greatly different from 1 and 2. Pattern 4 confirms that the substances of patterns 1 and 2 are antipodes and that substance 3 is their racemate. The material for pattern 4 had been obtained by evaporating the solution of an equal mixture of compounds 1 and 2. The diffraction of the mixture is identical with that of the racemate, pattern 3.

The configuration of a given triglyceride can be determined on this basis provided its chemical structure is known. For example, with a 1-stearo-dipalmitin being given, one has to synthesize the racemate, R,S-1-stearo-dipalmitin and one of the antipodes, R- or S-1-stearo-dipalmitin. The x-ray diffraction patterns of the three compounds must then be compared. The problem is solved when the unkown and the racemate render identical patterns. However, the pattern of the unknown may be identical with that of the synthetic antipode. Then the question arises whether the unknown is R or S since normal x-ray patterns of enantiomers are identical. Another roentgenogram, of

the unknown sample mixed with the synthetic antipode, 1:1, decides for one of the alternatives. The unknown is the enantiomer of the synthetic antipode when the pattern of the racemate appears from the mixture; it has the same configuration as the synthetic antipode when the pattern of the mixture remains that of the individual compound.

The method would fail when a R,S-glyceride crystallizes as a conglomerate of the enantiomorphous crystals of the antipodes. The diffraction of such a conglomerate is not distinct from that of the individual antipodes. In such cases, one would have to resort to determination of melting point and possibly mixed melting point to find the correct steric assignment for the unknown glyceride. The melting point of a conglomerate is always lower than that of one antipode. The mixed melting point of the two must be determined when melting points of unknown and synthetic antipode are equal. Depression of the mixed melting point indicates that the unknown is the enantiomorph of the synthetic product; unchanged mixed melting point shows that they are of identical configuration.

2-Oleo-palmitostearin from Cocoa Beans

The above described investigations opened the way to determine the steric structure of biogenous triglycerides. The procedures were applied to 1-palmito-2-oleo-3-stearate. This triglyceride is a major component of fat from cocoa beans and it can be obtained in acceptable purity by fractional crystallization. The glyceride was isolated from cocoa butter, from fermented beans which had not been roasted and, finally, from viable seeds which had been harvested a few days before work-up. Any falsification of results by industrial processing of cocoa beans would have become apparent by comparison of the products.

Isolation of the triglyceride was carried out under conditions which with certainty do not cause racemization; extraction with diethyl ether and crystallization from acetone were applied. The antipodes and the racemic mixture were synthesized. Figure 10 shows in the upper line the x-ray diffraction pattern of one synthetic antipode, in the center line that of the racemate and in the lower line that of the glyceride which had been obtained from the biological mixture.

2-Oleo-palmitostearin from cocoa beans is the racemate since its diffraction is identical with that of the synthetic racemate and grossly different from that of the antipode. Isolation from cocoa butter, fermented beans or the fresh seeds did not influence the result. For the first time, the configuration of a biogenous triglyceride has been identified. However, it is not permissible to draw the general conclusion that all natural triglycerides are racemates, and it may well be that both racemic and single-antipode glycerides will be found in future investigations.

Outlook

Optical activity, piezoelectricity, mixed melting point diagrams and x-ray diffraction are applicable to stereochemical analysis of glycerides. Except for the first method, crystalline materials are required. It is conceivable that crystalline compounds can be derived from liquid unsaturated glycerides without affecting their configuration to make the other methods applicable. The simplest case of this approach would be hydrogenation of a glyceride containing two or three acids of different chain lengths.

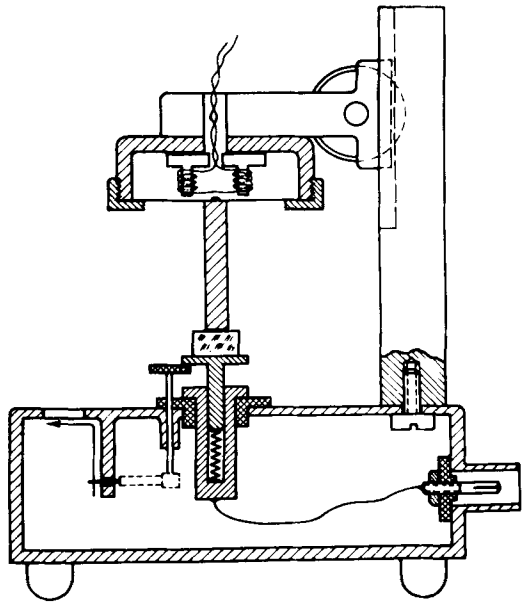


FIG. 7. Diagram of the piezo-electrometer of Fig. 6.

The stereo-analytical procedures apply to individual triglycerides. Enzymatic methods for determining the chemical structure of triglycerides are available and may be supplemented by mass spectrometry (22); the necessary test compounds of defined configuration are well accessible; great progress has been made in fractionating glycerides of biological origin. When the configurations of triglycerides withstand the new fractionation methods a broader investigation of the stereo-chemistry of biogenous triglycerides by physical-chemical means appears feasible.

L-α-glycerophosphoric acid is a precursor in the biosynthesis of lipids. The most direct biological route to triglycerides is outlined in Figure 11 (23). L-α-glycerophosphoric acid is acylated by two fatty acids in form of their CoA esters to yield phosphatidic acid; after dephosphorylation of the latter, the third acyl group is introduced. The precursor is optically active but the problem of configuration arises only when different fatty acids are introduced in positions 1 and 3. A pure triglyceride antipode should result when biosynthesis is selective and distinct in regard to the fatty acids which enter these positions. Nonselective (random) use of fatty acids to esterify posi-

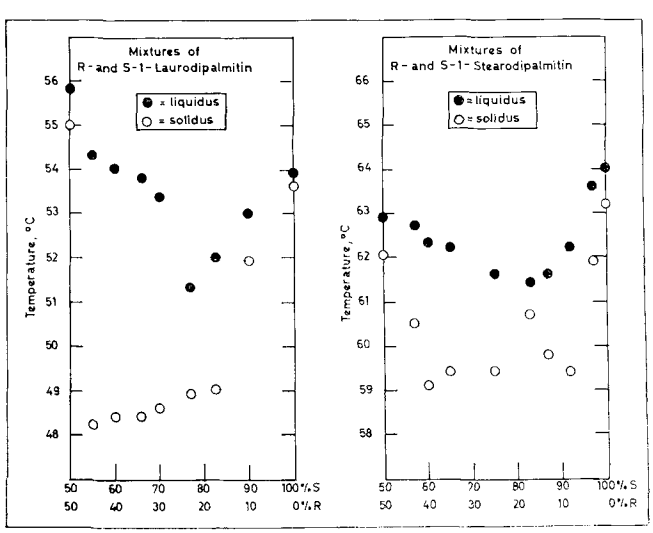


FIG. 8. Melting point diagrams of antipode + racemate.

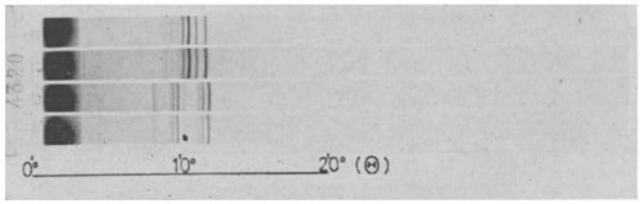


FIG. 9. X-ray diffraction patterns of 1-lauro-dipalmitin: 1 and 2, antipodes; 3, racemate by synthesis; 4, racemate by mixing antipodes.

tions 1 and 3 would yield racemic triglycerides. Selective acylation in one and nonselective acylation in the other position would give the mixture of an antipode with the racemate. The nature of the acid in position 2 does not play any role in these principles; it may be equal to one of the former acids or different from them.

The partial enzymatic hydrolysis of triglycerides by hog pancreatic lipase in positions 1 or 3 is essentially nonselective with similar fatty acid substituents. However, some differentiation has been reported when the acyl substituents differ greatly (24). One may expect enzymatic synthesis more likely to be selective with markedly different acids competing for positions 1 or 3 than with very similar acids.

Another factor must be born in mind when speculating about steric specificity of biogenous triglycerides. The enzymatic acylation may lead to an antipode when it is selective towards particular acids and positions. However, the configuration of the product of straight-forward synthesis, as in Figure 11, can be altered by exchange of fatty acids in alternative or reversible reactions (25) under a competitive situation different from that of the primary synthesis.

Stereospecific Analysis of Triglycerides

According to Brockerhoff (1965)

The procedures for configurational analysis of triglycerides discussed in the foregoing require individual triglycerides. Brockerhoff reported recently an ingenious method for stereospecific analysis of triglycerides (7). However, this method, like the others, requires investigation of individual triglycerides to arrive at conclusions in regard to configuration.

Brockerhoff defines positions 1 and 3 of the glycerol moiety according to Fischer's projection formulas as shown in Figure 12, first line, with the esterified hydroxyl group in position 2 always to the left. The sequence of enzymatic and chemical reactions outlined in Figure 12 affords separate analysis of the fatty acids in the specified positions 1 and 3.

The first step involves pancreatic lipolysis in positions 1 or 3 so that the mixture represented in the second line is obtained. The mixture of diglycerides is purified from other components by chromatography. It is reacted with phenyl dichlorophosphate to form the corresponding L- and D-phosphatidyl phenols, as shown in the third line. Phospholipase A (from snake venom, *Crotalus atrox*) is stereo-specific for the L-phosphatidic compound and hydrolyzes only the ester linkage in position 2 while D-phosphatidyl phenol is not attacked. The components of the last line in Figure 12 are again separated chromatographically. Fatty acid analyses of intermediary and final products permit positional identification.

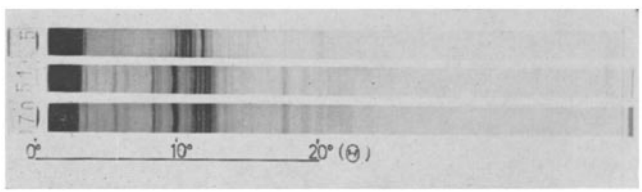


FIG. 10. X-ray diffraction pattern of 2-oleo-palmitostearin: upper, antipode; center, racemate by synthesis; lower, isolated from cocoa beans.

The method was tested with two complex mixtures of glycerides where the positional distribution of fatty acids was known from the course of their synthesis or partial synthesis. The positional analyses were in good agreement with the theoretically expected values. Corn oil triglycerides were investigated then with the result, "the distribution of the major fatty acids between positions 1 and 3 is almost random." Brockerhoff offers a rigorous critical evaluation of the analyses but does not further elaborate on stereospecific aspects. With this attractive method for analyzing separately the fatty acids of the different positions, it appears warranted to caution against drawing stereospecific conclusions on the basis of summarizing analyses of triglyceride mixtures.

The fatty acids in positions 1 and 3 of a glyceride molecule bring about, or are the criterion for, differences in configuration. Selective analysis of acids in these positions using a mixture of triglycerides permits stereochemical conclusions under the specific condition that one certain fatty acid, e.g., palmitic, is found only in one position, either 1 or 3. This is illustrated by the hypothetical example in Figure 13 where the analysis of a binary mixture shows the molar ratios in position 1, P:S = 1:1; position 2, 100% O; position 3, 100% S. Palmitic acid occurs only in position 1. Therefore, all glycerides containing palmitic acid are one type of antipode.

On the other hand, a certain fatty acid may be found equally distributed in positions 1 and 3, or preferentially in one of them. The conclusion that this acid concerns racemates or partial racemates is not permissible. This is illustrated by the hypothetical examples of Figure 14. Both, mixture I and mixture II, yield the same analysis: position 1, S:P = 1:1; position 2, O:P = 1:1; position 3, S:P = 1:1. Moreover, an equimolar mixture of all six compounds listed in Figure 14 would also yield the above positional analyses. The six-component mixture, however, would consist of ⅓ racemic 2-oleo-palmitostearin + ⅙ R-2-oleo-palmitostearin; ⅓ racemic 1-stearodipalmitin + ⅙ S-stearodipalmitin. Obviously, conclusions as to racemates, antipodes or mixtures thereof are not possible.

Brockerhoff is aware of these limitations and did not enter into the stereospecific aspects. The discussion here is offered to prevent possible pitfalls where the enzymatic procedure may instigate summarizing stereochemical deductions when separation of biological glyceride mixtures is not yet achieved.

Experimental

Syntheses

The active and crypto-active glycerides were synthesized according to the methods described by Fischer and Baer (4,5,26,27). In cases, lithium-aluminum hydride was used instead of Raney-nickel/H_2 for the reduction of D-isopropylidene glyceryl aldehyde to D-isopropylidene glycerol. Synthesis of D,L-glycerides followed the same scheme starting from D,L-isopropylidene glycerol.

Purification

Quality of products was always checked by thin-layer chromatography. Usually the substances were purified by recrystallization up to 10 times, preferentially from acetone or hexane. Di- and triglycerides were purified by chromatography in chloroform on columns of silicic acid to remove small amounts of cross-contamination or of monoglycerides and fatty acids. Isopropylidene glycerides and, in some cases, monoglycerides were fractionated without decomposition by vacuum distillation through a small packed column. R-(+)-Pivalyl-divalerin was distilled at 156C/1 mm. R-(+)-Laurodibutyrin was subjected to a short-path distillation in an apparatus according to Utzinger (28) at 1 μ. Thermocouples were placed in the circulating heater liquid at the beginning and the end of the evaporation trough. They indicated a temperature gradient from 160C to 175C and the material was exposed to heating for not more than 15 seconds while flowing through the trough. The exposure is shorter for the distilling portion of a mixture.

Measurements

Melting points were taken on a microscope hot stage according to Kofler (29). Sintering and melting temperatures of binary mixtures were determined in melting point tubes (30) using an apparatus as described by Tottoli (31). The capillaries had I.D. 0.8 mm and were filled with substance to a height of 4–5 mm. A steel wire, diameter 0.2 mm, served for stirring. The temperature gradient was about 0.2C/min during the measurements.

Twenty milligrams of mixtures of accurately known composition were dissolved in 0.2 ml of warm acetone. The solvent was evaporated at room temperature and finally in a vacuum dessicator. The crystalline residue was thoroughly mixed by *crunching and stirring with* a micro spatula. The more usual procedure of homogenization by melting and solidifying a mixture is not applicable since glycerides often crystallize from the melt in lower melting modifications. Melting point data for diglycerides and triglycerides are listed in Table V; data for phase diagrams are found in Figure 8.

The *piezoelectric effect* was investigated in a piezoelectrometer according to Bergmann (19). Powdery materials had to be investigated since single crystals of glycerides suitable for these measurements could not be obtained. Practice showed that it is best to use very little material, up to 5 crumbs, or < 2 mg, between the plates. The powder was placed on a small piece of a microscope cover glass, thickness 0.2 mm. With samples melting below 45C, it was advantageous to carry out the measurements in a cold room.

The metal plunger of the commercial apparatus has a diameter of 5 mm. It was replaced for our measurements by a plunger of 1 mm diameter. About 10 to 20 measurements were made and the position of the material had to be changed each time so that new portions of the sample were exposed to the plunger. The latter was cleaned for each test.

The piezoelectric effect may not become apparent in some of the tests since the crystals are placed without orientation and only a small portion of them may be in the optimal position having the polar axis in the direction of pressure. Moreover, the effect is weakened or cancelled when several crystals are pressed at the same time while their polar axes are in opposite directions. Therefore, numerous measurements are necessary with a powder to demonstrate unambiguously the presence or absence of a piezoelectric effect. Single measurements are not reproducible.

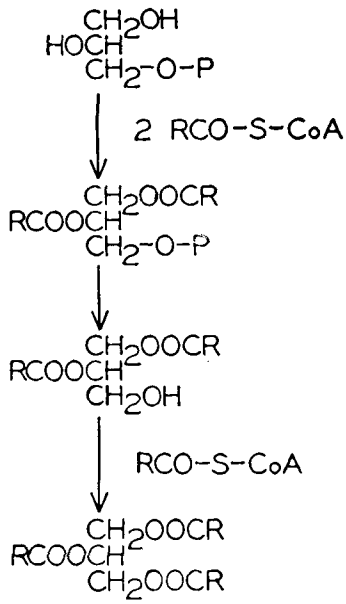


FIG. 11. Pathway of biological conversion of glycerol to triglyceride (Weiss, Kennedy and Kiyasu (22)).

Piezoelectric investigations so far were exclusively the realm of physicists and, to our knowledge, experiences have not been reported yet from organic chemistry laboratories.

Rotary dispersion was measured in a spectropolarimeter (O. C. Rudolph and Sons, model No. 70, serial No. 512), combined with a Zeiss mirror monochromator of earlier date. Light source was a mercury high-pressure lamp (Hanauer Quarzlampen Gesellschaft, S 81, 80 watt) operated with stabilized direct current. Rotatory dispersion values of mono-, di- and triglycerides are compiled in Tables VI–VIII.

X-ray diffraction pictures were taken in a Guinier-de Wolff camera, R = 114.7 mm, using monochromatic CuK_α radiation (1.54Å). The dimensions were such

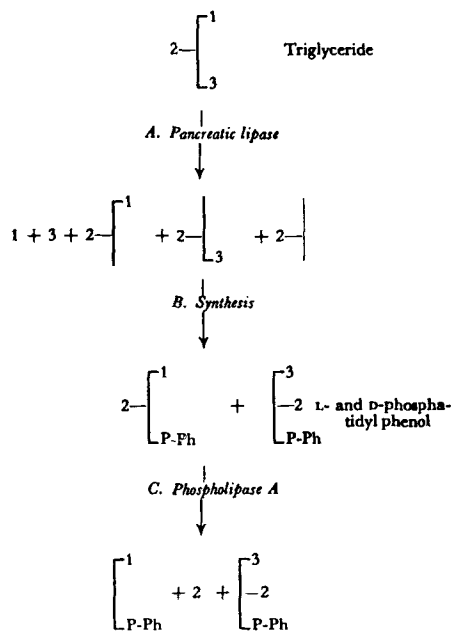


FIG. 12. Stereospecific analysis of triglycerides, according to Brockerhoff (7).

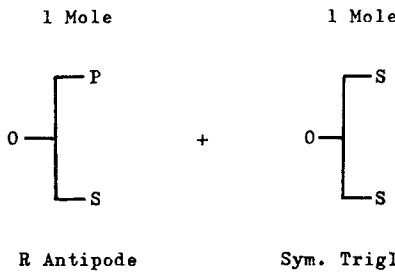
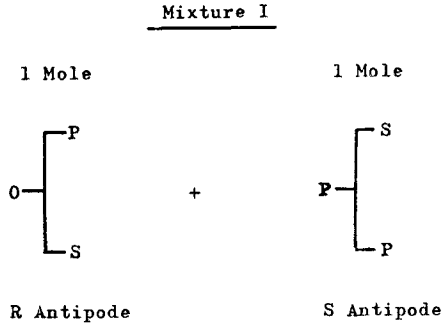


FIG. 13. Mixture of antipode and symmetrical triglyceride: P, palmitic; O, oleic; S, stearic acid. When one acid (P) is found only in one position, it pertains to glycerides occurring only as the antipode.



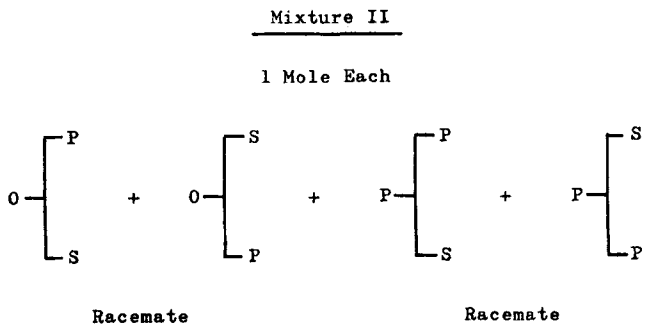


FIG. 14. Mixtures of 2 antipode triglycerides and of 2 racemic triglycerides yield the same positional analyses of fatty acids, as does the mixture of all 6 components. P, palmitic; O, oleic; S, stearic acid.

that 4 mm on the film corresponded to 1°(⊛). The relative intensities were visually estimated on uncalibrated films without further correction. The data are reported in Table IX (H.-U. Lenné).

Dolphin Oil, S(+)-Methylethylacetic Acid

Body oil of *delphinus delphis ponticus* was obtained from Turkey. The pertinent data were: saponification value, 214; free fatty acids, 2.68%; unsaponifiables, 0.75%; iodine value (Hanus), 128; refractive index, $n_D^{20} = 1.4712$; specific gravity, $d_4^{20} = 0.93$; optical rotation, $\alpha_D = +0.19°$. About 28 gm of the material was subjected to short-path distillation (28) at 0.2 μ. The temperature along the distillation trough was between 197 and 255C. Four fractions were obtained which are characterized in Table X. It is seen that the optical activity of the oil is in the low-boiling portions.

Total oil was saponified with 0.5N alcoholic KOH. The greater portion of the alcohol was evaporated, the residue was acidified and the acids were recovered by extraction with ether. Acids from 19.75 gm oil were subjected to steam distillation until a total of 1.2 liter water had distilled. The value of 7 mMol acid in 100 ml distillate at the beginning had then receded to 0.5 mMol acid which indicated that the greater portion of the C_5-acids had distilled. The acids were recovered in ether and the solvent was removed by distillation through a short packed column. The residue was 3.1 gm acids, acid value, 532 (pentanoic acid, 549); $n_D^{20} = 1.4020$; $\alpha_D = +1.61°$. After esterification with CH_2N_2, the esters were analyzed by gas-liquid chromatography.

The GLC apparatus and the flame ionization detector were constructed in these laboratories. A steel capillary, length 50 meters, was used with Lubrol NO (polyethyleneglycol ether of ICI) as stationary phase at 55C, and a N_2 flow of 2 ml/min. The retention value of the isomeric C_5-esters was 1.09. Quantification by peak areas of the recording showed isovalerate to be 70.2–74.1%, methylethylacetate 7.4–7.8%; two further esters were tentatively identified as isobutyric, 2.5% and n-butyric ester, 0.5%; solvent, 15.5–19.1% (R. Kaiser).

Corrected for the presence of solvent, the average

TABLE VIII

Rotatory Dispersion of Optically Active Triglycerides

		[α] at mμ, (°)								
	% in Solvent	578	545	436	405/8	366	334	313	302	297
R-1-Pivalyl-divalerin	none	+1.35	+1.60	+ 2.70	+ 3.20	+ 4.12	+4.9	+5.6	+6.0	
	10 pyridine	+0.2	+0.22	+ 0.42	+ 0.48	+ 0.55				
	10 benzene[a]	+0.30	+0.25	+ 0.15	+ 0.05	− 0.05	−0.20	−0.85		
	10 methanol	+1.90	+2.20	+ 3.20	+ 3.70	+ 4.70	+6.00	+7.00	+7.65	+8.00
	10 dioxane	+1.04	+1.08	+ 2.00	+ 2.56	+ 3.48				
	10 acetone	+1.5	+1.65	+ 2.82	+ 3.40	+ 4.42				
	10 ethyl acetate	+1.22	+1.42	+ 2.40	+ 2.85	+ 3.80				
	10 cyclo-hexane	+1.80	+2.06	+ 3.46	+ 4.20	+ 5.60	+6.8	+8.0	+8.8	
R-1-Lauro-dibutyrin	none	+0.05	+0.05	+ 1.11	+ 0.17					
	10 benzene	+1.10	+1.40		+ 2.20	+ 2.65				
	10 methanol	+0.02	+0.10	+ 1.10	+ 1.12	+ 0.14				
R-1-Myristo-dibutyrin	none	+0.09	+0.10	+ 0.16	+ 0.18					
	30 benzene	+0.88	+0.94	+ 1.40	+ 1.66	+ 2.20				
	34 methanol	+0.13	+0.15	+ 0.18	+ 0.32					
S-1-Myristo-dibutyrin	none	−0.09	−0.13	− 0.17						
	30 pyridine	−0.99	−1.12	− 1.96	− 2.36	− 3.08				
	30 benzene	−0.79	−0.91	− 1.62	− 1.91	− 2.57	−2.74			
	31 methanol	−0.13	−0.17	− 0.20	− 0.24	− 0.29				
S-1-Myristo-diisovalerin	none	−0.55	−0.64	− 1.25	− 1.39	− 1.72				
	34 pyridine	−0.33	−0.42	− 0.67	− 0.90	− 1.16				
	33 benzene	−0.41	−0.48	− 0.92	− 1.11	− 1.53	−1.95			
	33 methanol	−0.79	−0.86	− 1.51	− 1.88	− 2.50	−3.12			
S-1-Palmito-disorbin	7.3 benzene	−6.85	−8.00							
S-1-Isovalero-dimyristin	30 benzene			− 0.06	− 0.12	− 0.25	−0.43	−0.43		
S-1-Sorbino-dipalmitin	16.1 benzene	−5.57	−9.3	−17.9	−21.1	−30.95				
R-1-Lauro-dipalmitin	32 benzene	+0.10	+0.10	+ 0.11	+ 0.10	+ 0.16	+0.16	+0.20	+0.23	
S-1-Lauro-dipalmitin	21 benzene	−0.06	−0.07	− 0.08	− 0.11	− 0.18				

[a] The rotatory dispersion of R-1-pivalyl-divalerin in benzene is surprising. See also the anomalous sign of rotation of R-1-monopivalin in benzene, Table VI.

TABLE IX

X-Ray Diffraction Data of Glycerides

1-Stearo-3-palmitin				1-Oleo-3-stearin				1-Lauro-dipalmitin				1-Stearo-dipalmitin				2-Oleopalmitostearin					
Active (S)		Racemic		Active (S)		Racemic		Active (R)		Racemic		Active (S)		Racemic		Active (R)		Racemic		From Cocoa butter	
d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1	d(Å)	J/J1
19.0	cut off	13.3	cut off	13.8	cut off	13.7	cut off	19.0	cut off	16.4	cut off	14.3	cut off	15.2	cut off	13.9	cut off	13.7	cut off	15.0	cut off
16.7	10							17.0	2							13.0	20	12.4	15	12.6	15
10.11	1					13.0	2	16.0	2							10.89	2	10.44	2	10.52	2
9.11	1	9.46	1	9.50	3			12.9	20	13.2	6			13.7	10	9.46	1	9.11	1	9.07	1
8.34	1	7.85	2	7.93	5			11.49	1							8.18	5	8.08	6		
6.09	2			6.82	1			11.09	1	10.10	2					7.54	5	7.86	8	7.97	8
5.67	1			5.96	5					10.04	3					7.15	2	7.23	1		
5.25	2							9.84	5	9.36	3					6.89	5	6.98	2	7.04	2
5.07	1							8.48	5							6.56	1	6.82	1	6.86	1
4.86	1			4.68	40			8.02	1							5.95	3	6.50	1	6.44	1
4.58[a]	100[a]	4.61	100	4.61	50	4.65	100	7.37	2	7.15	1					5.54	5	6.04	1		
4.51[a]	60[a]	4.50	60	4.52	80	4.51	30							6.97	1	5.41	3	5.67	1		
		4.44	6	4.40	40	4.44	30			6.70	1	6.60	1			5.02	5	5.35	20	5.34	20
4.36	5	4.38	1					6.37	5	6.27	1	6.15	1			4.84	7	5.15	6	5.15	6
4.30	1			4.31	5	4.33	5	5.67	3							4.67	10	5.03	6	5.04	6
4.16	2	4.12	3	4.15	5	4.22	10	5.48	7							4.50	30	4.87	1		
4.09	5	3.99	1	4.08	5	4.14	7	5.38	2	5.36	70	5.40	3	5.33	10	4.44	10	4.70	2	4.72	2
3.81	100	3.83	30	4.00	5	4.03	2	5.19	2	5.24	10			5.22	4	4.36	20	4.53	100	4.55	100
		3.73	15	3.91	1	3.93	7			5.16	2					4.30	30	4.45	2	4.46	2
3.68	80	3.70	6	3.84	1	3.82	8	5.04	3	5.05	15			5.06	3	4.19	25	4.39	1		
		3.65	60	3.78	100	3.76	1	4.90	10	4.93	10	4.90	2			4.08	100	4.20	3	4.23	3
		3.60	12	3.74	50	3.70	2			4.85	2			4.83	1	3.97	20	4.15	5	4.15	5
3.53	1	3.54	1	3.66	80	3.65	2	4.72	7	4.71	70					3.91	2	4.04	10	4.04	10
		3.49	2	3.58	20	3.59	2	4.63	2	4.62	90					3.79	80	3.93	40	3.93	40
3.43	2			3.48	5	3.48	15	4.53	4					4.57	100	3.74	8	3.81	25	3.82	25
		3.39	1	3.44	1			4.48	5	4.47	100	4.45	3	4.48	10	3.70	8	3.70	30	3.71	30
3.32	1			3.38	5	3.39	4	4.35	60			4.31	30	4.35	1	3.62	8	3.61	25	3.62	35
3.23	1			3.22	2	3.28	2			4.29	50	4.20[a]	100[a]	4.25	1	3.59	8				
				3.17	2			4.20	100			4.18[a]	100[a]	4.15	8	3.52	1	3.51	8	3.52	8
		3.13	1	3.14	2	3.15	1			4.09	2	4.06	40			3.43	5	3.45	2	3.46	2
				3.01	1			4.01	50	3.95	10	4.02	30	3.98	10	3.37	1	3.31	4	3.32	4
				2.90	1			3.93	2	3.91	70	3.98	20	3.87	2	3.27	8				
				2.84	1					3.83	100	3.90	5	3.83	30	3.20	2	3.21	2	3.22	2
				2.80	1	2.73	1	3.79	80					3.80	1	3.12	2	3.11	1		
				2.68	1	2.70	1	3.73	2	3.71[a]	100[a]	3.74	70	3.74	1	3.07	1	3.05	1	3.06	1
				2.66	1	2.66	2			3.67[a]	100[a]	3.70	30	3.68	40	2.99	5	3.01	1		
				2.62	1	2.61	2	3.60	2			3.62	5	3.63	2	2.95	2				
				2.59	2	2.57	1							3.57	2	2.89	1	2.89	1	2.93	1
				2.55	2			3.52	2	3.48	2	3.52	1	3.54	2	2.85	1				
2.50	10	2.50	8	2.50	5			3.46	3	3.43	4			3.47	1	2.79	1	2.80	1	2.81	1
2.48	6	2.48	1	2.45	3	2.45	1	3.38	4	3.36	2	3.42	2	3.42	1	2.76	1				
				2.42	7					3.32	1	3.35	1	3.35	2	2.70	1	2.71	1	2.72	1
		2.40	2	2.40	1			3.26	2	3.24	2			3.29	1	2.66	1				
2.38	10			2.37	1			3.15	7	3.19	1			3.17	1	2.61	1	2.63	1	2.64	1
		2.35	1	2.34	2	2.35	4	3.10	2	3.11	10					2.58	1	2.58	1	2.59	1
2.31	1			2.31	2	2.32	4	3.06	4	3.08	1	3.10	1	3.07	1	2.55	6	2.55	10	2.55	10
2.28	8	2.26	1	2.27	3			3.00	2	3.03	3					2.51	3	2.51	2	2.52	2
		2.23	2	2.22	5	2.21	1			2.98	1			2.95	2	2.47	2				
2.18	1			2.18	1			2.92	1					2.91	1	2.42	7			2.45	1
2.16	2			2.16	4			2.84	3			2.82	1	2.85	1	2.38	2				
		2.13	4	2.14	6									2.82	1	2.36	1	2.35	2	2.36	2
2.11	3	2.09	1	2.11	4			2.77	2	2.76	10					2.33	1				
2.05	25	2.06	2	2.06	4			2.72	2	2.70	1					2.30	2				
				2.05	2			2.68	1							2.25	9	2.26	1	2.27	2
		2.01	5	2.03	4			2.65	1		1	2.64	1			2.23	10	2.23	2	2.24	2
		1.97	1	1.99	5			2.58	4			2.59	1	2.56	15	2.19	3	2.17	2	2.17	2
				1.92	1			2.53	3							2.15	1				
1.90	10	1.90	1	1.89	3			2.51	4	2.52	35	2.51	1	2.51	1	2.14	7	2.14	1		
				1.85	7			2.46	1	2.47	1	2.47	5			2.11	1				
1.83	3			1.83	1					2.39	12					2.10	2	2.10	2	2.10	2
1.80	1	1.81	2					2.35	2	2.37	10			2.36	8	2.05	7	2.06	2		
										2.33	2					1.98	3	2.04	2	2.04	2
								2.28	4	2.27	2	2.26	5	2.28	2	1.94	3	1.97	1	1.95	2
								2.25	7	2.23	2	2.23	5			1.92	3	1.93	2	1.93	2
								2.22	4	2.19	3			2.19	2	1.90	6	1.92	2		
								2.16	1		2			2.13	2						
								2.11	2	2.12	1										
								2.09	1	2.10	12			2.06	3						
								2.05	1	2.06	3										
								2.00	1	2.04	1			1.95	2						
										1.96	10			1.91	1						
										1.92	1										
								1.89	5	1.90	1			1.88	1						
										1.88	12			1.83	1						
										1.84											

Addtional weak lines

Additional weak lines

Additional weak lines

[a] Strong overlap.

TABLE X
Short Path Distillation of Dolphin Oil
(0.2 μ: Temperature Along Trough 197-255C)

Fraction	Gram	n_D^{20}	$[\alpha]_D^{20}$
1	2.5	1.4588	+0.36°
2	5.6	1.4661	+0.34°
3	5.2	1.4675	+0.36°
Residue	13.7	1.4785	0°
Original oil		1.4712	+0.19°

values are 88.3% isovaleric, 9.0% methylethylacetic acid and 2.75% butyric acid. A synthetic mixture in these proportions was prepared using S(+)-methylethylacetic acid, $\alpha_D^{21} = +18.5°$. The mixture had the rotation $\alpha_D = +1.64°$. This value is very close to $\alpha_D = +1.61°$ which had been measured with the mixture of steam volatile acids before esterification. One can conclude that the methylethylacetic acid of dolphin oil is the pure S antipode or (+) form.

2-Oleo-palmitostearin from Cocoa Beans

Oleo-palmitostearin is the prominent triglyceride of cocoa butter and investigations by Lutton (32), Chapman, Crossley and Davies (33) ascertained that the oleic acid is in position 2 of the glyceride. 2-Oleo-palmitostearin represents about 41% of cocoa butter triglycerides and is accompanied by about 22% oleo-distearin and 12% oleo-dipalmitin (34). Approximately one-fourth of the glycerides are unaccounted for. According to Woidich, Gnauer, Riedl and Galinovsky (35), they are likely to contain linoleic acid. Furthermore, the presence of a small portion of trisaturated glyceride is expected.

The seeds (*Theobroma Cacao L,* Amazonas variety) from the western district of Nigeria were taken from the matured fruit and any remaining fruit flesh was removed by hand. After wiping with a towel, the seeds were mashed in a Multimix homogenizer. Extraction of 108 gm of homogenized material with ether for 24 hr in a Soxhlet apparatus yielded 42 gm of a slightly cloudy yellow oil. Virtually all of this was recovered as a yellowish semisolid by crystallization from 425 ml of acetone at 0C for three days. Crystallization from 425 ml acetone was repeated for 24 hr thermostatically controlled at 22C and yielded 6.96 gm solids. The mother liquor, after 24 hr at 15C, precipitated 22.8 gm. This fraction was dissolved in 228 ml of acetone and kept for 24 hr at 22C, but no precipitate formed under these conditions. The temperature was lowered to 20C and 8.56 gm precipitate was collected after 24 hr. This preparation had mp 36.5–38.3C which is rather close to that of D,L-2-oleo-palmitostearin, mp 37.5–38C (33). Further recrystallizations did not improve the melting point. Numerous other experiments under different crystallization conditions did not yield a better quality.

Hydrogenation with PtO_2 in isooctane + acetic acid, 2:1, yielded a calculated iodine value of 31 (theoretical for 2-oleo-palmitostearin, 29.4). The refractive index was $n_D^{40} = 1.4562$, while synthetic 2-oleo-palmitostearin had $n_D^{40} = 1.4561$.

Reversed phase thin-layer chromatography of the triglyceride was carried out as follows: Kieselgur-tetradecane with acetone + acetonitrile, 8:2, as mobile phase (36); solvent equilibrated with tetradecane to 80% saturation; horizontal plate with irrigation procedure (37); sample, 4γ of triglyceride; developing time, 2 hr; indicator, α-cyclodextrin/I_2 (38). Besides the spot of oleopalmitostearin, R_f 0.48, a minor spot, R_f 0.41 was detected and in some chromatograms another spot, R_f 0.55 was indicated. Chromatography of the authentic compounds made likely that the contaminants are 2-oleo-distearin and 2-oleo-dipalmitin, the latter in negligible amount. Minute traces of trisaturated glycerides were also detected. They remain close to the starting point under these conditions.

GLC of the methyl esters showed that palmitic, stearic and oleic acids represent > 99% of the total acids in the triglyceride and that their molar amounts are 29.4%, 38.4% and 32.1%. The limit of error is ± 0.6% according to GLC of a model mixture having similar composition. When assuming all oleic acid in position 2 and disregarding the possible presence of 2-oleo-dipalmitin in trace amounts, one can estimate the purity of 2-oleo-palmitostearin as 88.2% with a contamination of 8.1% 2-oleo-distearin and 3.6% tristearin. GLC of oversized samples showed the presence of arachidic acid and of much less heptadecanoic acid, both in negligible amounts.

The results of the structural investigation are based on comparative x-ray diffraction of 2-oleo-palmitostearin from cocoa beans, synthetic D,L-2-oleo-palmitostearin and synthetic crypto-active 2-oleo-palmitostearin. A contamination of 2-oleo-distearin and possibly 2-oleo-dipalmitin in the natural product is not serious since their x-ray diffractions are very similar to that of D,L-2-oleo-palmitostearin. They do not interfere when comparing the preparation from cocoa with racemic and crypto-active forms.

Fermented cocoa beans (*Theobroma Cacao L.,* Forastero variety, "Good fermented Ghana," obtained through Fa. Schokinag, Mannheim) from the Gold Coast had been fermented there but had not been roasted. Cocoa butter was a product from Merck, Darmstadt. Isolation of 2-oleo-palmitostearin proceeded as described above. The samples were identical with that from fresh cocoa beans within limits of analytical error. Most important, the x-ray patterns of these preparations were identical.

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Glyceride Studies. Part III. The Component Glycerides of Five Seed Oils Containing Linolenic Acid

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Abstract

The component glycerides of linseed, wild rose, candlenut, soya and stillingia oils have been estimated by chromatography on thin layers of silica impregnated with silver nitrate. The separated glycerides are identified, qualitatively and quantitatively, by gas-liquid chromatography (GLC) of their methyl esters in presence of added methyl heptadecanoate as an internal standard. The results agree with those obtained by lipolysis or calculated directly from the component acids on the basis of the theory of positional distribution.

Introduction

THE COMPONENT GLYCERIDE analysis of drying oils containing linolenic acid has been undertaken by the classical crystallisation procedures of Hilditch and his colleagues (1-4), by the rather tedious countercurrent distribution technique of Dutton and Scholfield et al. (5,6) and by Youngs' oxidation procedure (7–9). This last method gives useful information about the distribution of saturated acyl groups but is less informative about the distribution of those unsaturated acyl groups which are oxidised to azelaic acid derivatives and thus become indistinguishable from one another. We find that separation on thin layers of silica impregnated with silver nitrate (10–13) provides a satisfactory basis for the quantitative determination of component glycerides and we have applied this to five seed oils: linseed, wild rose, candlenut, soya, and stillingia. Some workers have weighed the glycerides obtained (14,15), others have estimated the glycerol (12), or used densitometry (10) or the chromotropic acid colour reaction (13); we have added methyl heptadecanoate to the separated glycerides as an internal standard.

Experimental Procedure

Isolation of Neutral Glycerides

Seeds were obtained from J. Bibby and Sons (linseed and soya) and from the Tropical Products Institute [candlenut ex Uganda and stillingia (Sapium sebiferum) ex Bombay]. The wild rose seeds were obtained from hips collected locally. The outer shell was removed from the stillingia seeds and oil obtained from the kernel only. Crushed seeds were thoroughly extracted with boiling petrol ether (bp 40–60C) and neutral triglycerides were isolated by eluting the oil (1 gm) from a column of silica (30 gm) with benzene (200 ml) (16).

Separation of Glycerides by Thin-Layer Chromatography

The triglycerides (20–40 mg) are applied, in ether solution, in a band about 4 cm from one of the short edges of a glass plate (20 × 40 cm) layered (0.3–0.4 mm) with silica gel containing 15% of silver nitrate (10,11). These plates are developed by horizontal elution (17) for 2–3 hr using ether to separate the more unsaturated triglycerides and benzene containing 10% of ether to separate the less unsaturated triglycerides. Thereafter the plates are dried at room temperature in a current of nitrogen to remove all solvent and sprayed with a methanolic solution (0.2%) of 2′,7′-dichlorofluorescein. Six to ten bands appear and these are scraped from the plate with a sharp razor blade; the glycerides are thoroughly extracted with a mixture of methanol, ether, and water (5:5:1).

A known amount of methyl heptadecanoate in the form of a dilute methanolic solution is added to each glyceride extract which is then poured into water to remove silver nitrate and extracted three times with n-hexane. The triglycerides are converted to methyl esters by transesterification with methanol containing sodium methoxide (18). The esters are examined quantitatively by gas liquid chromatography (GLC) using a Perkin Elmer Fractometer fitted with a flame ionisation detector and 1 meter or 2 meter columns containing poly(ethylene glycol succinate) as stationary phase. The ratio of the area of the C_{17} ester peak to the total area of all other peaks is a measure of the amount of glyceride in each fraction.

Lipolysis

Our lipolysis procedure is based on that of Coleman (20), but the partial glycerides are separated on a thin layer (0.3-0.4 mm) of silica by developing with chloroform-acetone-ammonia (80:20:1, S.G. 0.88). The separated components are detected with 2′,7′-dichlorofluorescein and the monoglycerides (R_f about 0.3) are extracted three times with ether and then converted into methyl esters by transesterification (18).

Discussion and Results

Oils containing saturated acids (considered as a single group) along with oleic, linoleic, and linolenic acids may have twenty different glycerides, even when possibilities of isomerism are ignored, and we find it necessary to examine these oils on two chromatoplates. Development with ether separates the more unsaturated glycerides but not the less unsaturated ones which crowd together near the solvent front; development with a mixture of benzene and ether separates