# Technical

# Determination of the Fully Saturated Triglyceride Composition of Fats[1]

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## ABSTRACT

A method is described for the quantitative determination of the fully saturated triglyceride composition of fats. The method involves a quantitative oxidation of the unsaturated triglycerides in the fat to a mixture of α ketol and dihydroxy compounds, using aqueous alkaline potassium permanganate and a phase transfer catalyst in a two phase reaction. The fully saturated triglycerides are fractionated from the oxidation products by column chromatography on silicic acid and their composition is quantified by gas liquid chromatography (GLC) using a flame ionization detector. Analyses of samples of lard, cocoa butter, olive oil, soybean oil, crude palm oil (ex Malaysia) and the stearin fraction from palm oil are given as examples of the method.

## INTRODUCTION

One method for the determination of fully saturated glycerides is to remove the unsaturated glycerides by oxidation with potassium permanganate in a two phase reaction. However, reaction between two substances located in different phases of a mixture is often inhibited because of the inability of reagents to come together. The stirred heterogeneous mixture of triglycerides in dichloromethane and an aqueous potassium permanganate solution is an example where little or no oxidation of the unsaturated triglycerides is reported even when vigorous stirring is used (1). Traditionally this problem has been solved by the use of an appropriate mutual solvent or a water miscible cosolvent such as an alcohol to obtain a homogeneous medium. However, if a hydroxylic solvent is selected reaction may still proceed slowly owing to extensive solvation of the anions.

An alternative solution to the heterogeneity problem is to employ a phase transfer catalyst (PTC). The catalyst transfer the water soluble reactant across the aqueous-organic interface into the organic phase where a homogeneous reaction can take place. Under normal conditions the PTC is not consumed but performs the transport function repeatedly. Organic soluble quaternary ammonium halides, e.g., $Q^+Cl^-$, have been found to be excellent agents for the transfer of anions from an aqueous phase to an organic phase (2). The water soluble permanganate anions are transferred efficiently into the organic phase by the PTC as the $Q^+MnO_4^-$ ion-pair (3) where it reacts with the ethylenic linkages in the unsaturated triglycerides. Migration of the cationic catalyst probably as the $OH^-$ or $Cl^-$ salt back to the aqueous phase completes the cycle (1). The process continues with the precipitation of manganese dioxide until either all of the permanganate or the unsaturated triglyceride has been consumed. The success of the catalytic effect depends to a large extent upon the high partition coefficient of the $Q^+MnO_4^-$ ion pair between the aqueous and organic phases compared with the corresponding value for the $Q^+Cl^-$ ion pair (4-6). As the reaction rate is directly dependant upon the partition coefficient for the ion pairs between the two phases, it is solvent-dependent. One of the most suitable organic solvents for triglyceride oxidation is dichloromethane (7,8).

The reactions were initially performed using neutral aqueous potassium permanganate as oxidant, but the oxidation products were diverse, consisting of cleavage and addition products at the ethylenic linkages. Isolation of the fully saturated triglycerides from the mixture of oxidation products was difficult and time consuming. The oxidation procedure offered no practical advantage as a method for the determination of the fully saturated triglyceride composition of a fat.

Using an aqueous alkaline medium, quantitative conversion of the unsaturated triglycerides into a mixture of α ketol and erythro dihydroxy compounds resulted. Fully saturated triglycerides were unaffected by the oxidation procedure and could be quantitatively recovered from the oxidation mixture by column fractionation using silicic acid. Triglycerides were quantified using high temperature GLC which is a rapid and quantitatively accurate method for separating triglyceride mixtures by molecular weight (9).

## EXPERIMENTAL PROCEDURES

### Materials

Trilaurin, trimyristin, tripalmitin, tristearin, triolein and methyl oleate (all of 99% purity) were purchased from Sigma Chemicals Ltd.

Dichloromethane and potassium permanganate were of Analar purity.

OV-17 stationary phase was purchased from Phase Separations Ltd.

DEGS-PS stationary phase was purchased from Supelco Inc.

Kieselgel 100 (35-70 mesh) was used for silicic acid column chromatography.

Tricaprylmethylammonium chloride ("Aliquat 336") was purchased from Fluka Chemicals Ltd. The alkyl groups are a mixture of $C_8$-$C_{12}$ straight chains with an average chain length of ten carbon atoms and a molecular weight of approximately 507.

### Samples

Olive oil, soybean oil and lard were commercial samples. Cocoa butter was supplied by J. Bibby Ltd. Crude palm oil

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(ex Malaysia) and Palm oil stearin fraction were supplied by Mr. B.K. Tan (Malaysian Agricultural Research and Development Institute).

### Thin Layer Chromatography (TLC)

Silicic acid preparative chromatography was used for the isolation of the oxidation products for identification by GLC and spectrophotometric methods. For this purpose, plates of silica gel G (20 cm. x 20 cm., 0.5 mm thick) were prepared by standard methods (10) and activated at 110 C for 1 hr before use. A mixture of petroleum ether (Bp 40-60 C), diethyl ether, formic acid (59:40:0.1 v/v/v) was used as the eluting solvent system. Bands were located by spraying the plate with a 0.2% alcoholic solution of 2,7-dichlorofluorescein and viewing under UV light. For identification and quantitation, the spots were located and the silica gel of each area immediately scraped off the plate, collected in separate glass tubes and covered with a solution of 5% methanol in diethyl ether. After filtration of the suspension and several washings with further portions of the solvent mixture, the eluted compounds were identified by GLC and spectrophotometric methods.

### Gas Liquid Chromatography (GLC) of Triglycerides

The gas chromatograph used was a Pye Unicam model 104 equipped with flame ionization detectors, automatic temperature programming and a 0.45 m x 2.5 mm ID glass column containing 2% OV-17 on Supelcoport 100/120 mesh. The column was programmed from 250-360 C at 4 C/min. with a nitrogen flow rate of 80 ml/min. The peaks were identified by cochromatography with known triglyceride reference compounds. Peak areas were measured by triangulation and triglyceride compositions were reported in mole percentage.

### GLC of Fatty Acids

Fatty acid compositions of the fat prior to oxidation and the triglycerides fractionated after oxidation were determined by GLC analysis of their methyl esters prepared by the Brockerhoff method (11). The methyl esters were analyzed on a 2.1 m x 2.5 mm ID glass column containing 10% DEGS-PS on Supelcoport 100/120 mesh. The column temperature was held constant at 175 C and samples were analyzed isothermally with a nitrogen flow rate of 80 ml/min. Typical chromatograms for cocoa butter are given in Fig. 1.

The crude oxidation mixture before fractionation on silicic acid contained a range of compounds of varying polarity. The methyl esters of the oxidized triglycerides were analyzed on a 1.83 m x 2.5 mm ID glass column containing 3% OV-17 on Supelcoport 100/120 programmed from 85-300 C at 6 C/min with a nitrogen flow rate of 80 ml/min. The fatty acids were identified on the basis of retention time and cochromatography with known standards as well as infra-red spectroscopy and mass spectrometry.

## OXIDATION WITH POTASSIUM PERMANGANATE

### Aqueous Alkaline Potassium Permanganate

The fat (0.1 g) and tricaprylmethylammonium chloride (0.25 g) dissolved in dichloromethane (20 ml) were placed in a 100 ml round bottomed flask. Sodium hydroxide (0.1 g) dissolved in distilled water (8 ml) was added to the flask and the two phase mixture was stirred vigorously at room temperature for 3 min. Potassium permanganate (0.4 g), sodium hydroxide (0.1 g) dissolved in distilled water (10 ml) were added to the flask and the mixture stirred vigorously for 2 hr. After reaction, the excess potassium permanganate and manganese dioxide produced during the oxidation were reduced by bubbling sulfur dioxide gas into the mixture until the pH of the aqueous phase was 2-3. The organic layer was separated and the residual aqueous layer washed with dichloromethane (3 x 5 ml). The combined organic extracts were washed with distilled water (2 x 8 ml), dried over anhydrous magnesium sulfate and the volume reduced almost to dryness.

This extract (crude oxidation mixture) was fractionated on a 10 cm x 1 cm ID glass column fitted with a sintered glass base and packed with silicic acid (2.5 g). The eluting

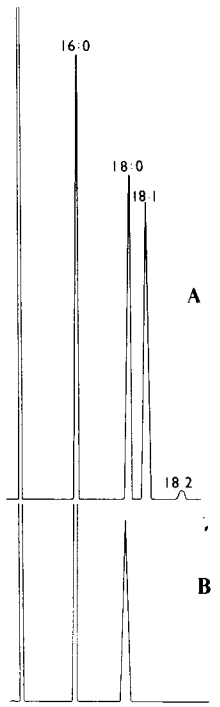


**FIG. 1. Fatty acid analysis of cocoa butter triglycerides: (a) methyl esters of fatty acids derived from cocoa butter before oxidation; (b) methyl esters of fatty acids derived from the neutral triglycerides fractionated from the crude oxidation mixture.**

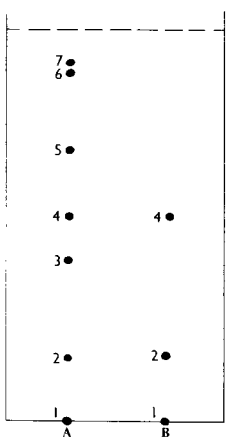


**FIG. 2. TLC of the oxidation products using methyl oleate as a model unsaturated acyl group: (A) Using a neutral aqueous medium; (B) using an alkaline aqueous medium. Conditions—preparative TLC plate coated with 0.5 mm thickness silica gel. Eluted with petroleum ether (Bpt. 40-60 C), diethyl ether, formic acid (59:40:0.1 v/v/v). Spots visualized by spraying the plate with a 0.2% alcoholic solution of 2,7 dichlorofluorescein or charred with chromic-sulfuric acid.**

TABLE I

Identification of Oxidation Products

| TLC SPOT (FIG. 2A) | Rf value | GLC retention time (min.)[a] | Identified as: |
|---|---|---|---|
| 1 | 0.0 | Complex thermal fragmentation pattern obtained. | Phase transfer catalyst. |
| 2 | 0.16 | 30.0 | Erythro 9,10 dihydroxy methyl stearate. |
| 3 | 0.41 | 14.0 | Mono methyl azelaic acid. |
| 4 | 0.52 | 28.0 | Mixture of 9,10- and 10,9-hydroxy keto methyl stearate. |
| 5 | 0.68 | 8.0 | Mono basic acidic fragments, mainly nonoic acid. |
| 6 | 0.88 | 26.8 | 9,10 diketo methyl stearate. |
| 7 | 0.90 | 22.4 | Methyl oleate. |

[a]Analyses carried out on a glass column 1.83 m x 2.5 mm I.D. containing 3% OV-17 on Supelcoport 100/120 mesh. Program: 85-300 C at 6 C/min. Nitrogen flow rate of 80 ml/min.

solvent was a mixture of petroleum ether (Bp 40-60 C) and diethyl ether (90:10 v/v). The fully saturated triglycerides were eluted in the first 25 ml, which was reduced in volume and the triglycerides quantified by GLC using OV-17 as the stationary phase.

### Aqueous Neutral Potassium Permanganate

The oxidation procedure was similar to that outlined above except that sodium hydroxide was omitted. A reaction time of 2½ hr was required for complete reaction. The oxidation products obtained from methyl oleate (model unsaturated acyl group) are given in Fig. 2 and Table I.

## RESULTS AND DISCUSSION

A commercially available quaternary ammonium salt "Tricaprylmethyl ammonium chloride" was a suitable transfer agent for the permanganate oxidation of unsaturated triglycerides using a two phase reaction.

The oxidation of unsaturated triglycerides using neutral aqueous potassium permanganate and a PTC was unsatisfactory as a quantitative procedure for the elimination of unsaturated triglycerides from a complex triglyceride mixture. Fatty acid analysis of the oxidized triglycerides, as their methyl esters (Table I), showed the presence of a complex mixture of products which analyzed as follows: 9,10- and 10,9-hydroxy keto methyl stearate (43%), 9,10-diketo methyl stearate (5%), 9,10-dihydroxy methyl stearate (trace). Cleavage products included monobasic and dibasic acids (as methyl esters) plus aldehydic compounds and comprised ca. 50% of the reaction products. Secondary decomposition products were also detected. Column

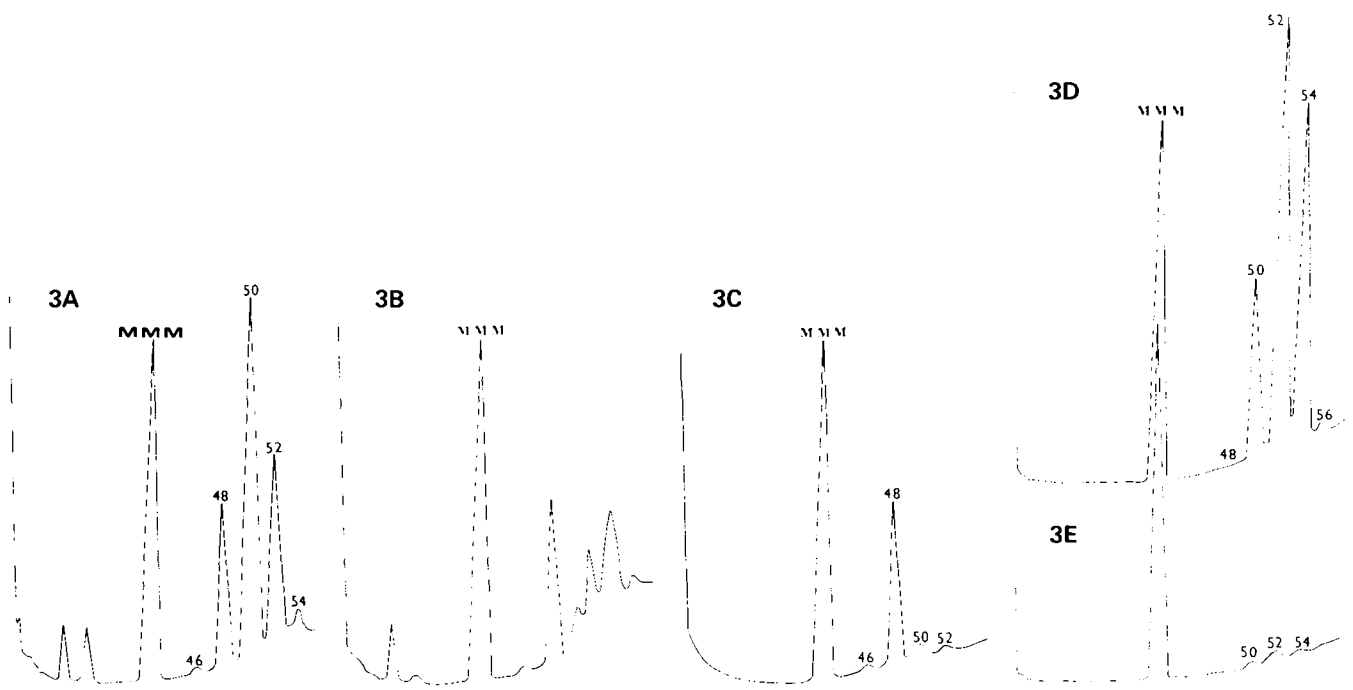


FIG. 3. Typical gas liquid chromatograms using the oxidation procedure described: (3A) total triglycerides in the stearin fraction of palm oil. Trimyristin (MMM) was added as an internal standard. Chromatographic conditions—Column 0.45 m x 2.5 mm I.D. glass column containing 2% OV-17 on Supelcoport 100/120 mesh. Program—250-360 C at 4 C/min. with a nitrogen flow rate of 80 ml/min. The injection port temperature and detector were maintained at 350 C; (3B) crude oxidation products chromatographed before fractionation on silica gel; (3C) fully saturated triglycerides fractionated from the crude oxidation mixture; (3D) total triglycerides in cocoa butter; (3E), fully saturated triglycerides in cocoa butter.

fractionation of the fully saturated triglycerides from the oxidation products was time consuming, as it required two or more column separations to remove the diketo and acidic products.

The alkaline oxidation procedure was found to be satisfactory for the quantitative oxidation of unsaturated triglycerides into more polar derivatives. Figure 1 shows the complete absence of unsaturated acyl groups in the methyl esters of the triglycerides eluted from the silicic acid column.

The optimum reaction conditions were determined using methyl oleate as a model unsaturated acyl group in a triglyceride and varying the mole ratios of potassium permanganate, sodium hydroxide and PTC. A mole ratio of 1 part methyl oleate, 5 parts potassium permanganate, 7 parts sodium hydroxide and 1 part PTC gave complete oxidation of the ethylenic linkages in a two hour reaction at room temperature. The reaction products were α-ketol and erythro 9,10-dihydroxy methyl oleate formed in approximately equimolar quantities. Lowering the temperature of the reaction to 0-5 C did not alter the reaction products but resulted in a decrease in the reaction rate. Increasing the ratio of sodium hydroxide from 7 parts to 55 parts only slightly increased the yield of 9,10-dihydroxy methyl stearate. Reducing the concentration of PTC reduced the rate of reaction. An excess of potassium permanganate was needed for complete reaction; this is particularly important when highly unsaturated fats are to be oxidized.

The phase transfer catalyzed oxidation with alkaline potassium permanganate occurs because of the ability of the quaternary ammonium salt to transfer both permanganate ion and hydroxide ion into the organic phase. However, the partition coefficients of the hydroxide and permanganate anions between the two phases greatly favors the formation of $Q^+MnO_4^-$ ion pairs. Hence complete formation of the erythro dihydroxy compound cannot be achieved using the present catalyst. Experimentally the reactions are very easy to perform, and the saturated triglycerides are readily isolated from the two phase reaction mixture.

Typical gas liquid chromatograms of samples before and after oxidation are shown in Figure 3. The crude oxidation mixture from the stearin fraction of palm oil (Figure 3B) chromatographs as a number of ill-defined peaks.

The fully saturated triglyceride compositions for a number of fats, determined by the PTC procedure, are given in Table II. Quantitative molar calibration factors for the GLC analyses were determined using a known composition mixture of trilaurin, trimyristin, tripalmitin and tristearin. Typical calibration factors are given in Table III and all peak areas were corrected accordingly.

The possible loss of saturated triglycerides by hydrolysis or interesterification during the oxidation procedure and subsequent column fractionation was checked by GLC. A known weight of trimyristin (0.09 g) was added to a fat sample prior to oxidation. Trimyristin was selected as the reference compound because none of the fats examined contained any $C_{42}$ triglycerides. Recovery of the trimyristin after oxidation and after fractionation was quantitative, showing that fully saturated triglycerides were stable to the oxidation conditions.

The fully saturated triglyceride compositions given in Table II compare favorably with literature results determined by silver nitrate TLC (12). The oxidation method is particularly useful for the analysis of fats of high monounsaturated triglyceride composition and for triglyceride samples of varying chain length and polarity when overlap on silver nitrate TLC is a problem.

Although 100 mg of sample was used for the analyses,

TABLE II

Triglyceride Composition (Mole %)

| No. of acyl carbon atoms | Cocoa butter | | Lard | | Crude palm oil | | Stearin fraction from palm oil | | Olive oil | | Soya bean oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total[a] | Fully saturated[b] | Total | Fully saturated | Total | Fully saturated | Total | Fully saturated | Total | Fully saturated | Total | Fully saturated |
| 46 | 0.0 | 0.0 | 0.3 | 0.1 | 0.2 | 0.2 | 0.6 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| 48 | 0.05 | 0.05 | 1.7 | 0.6 | 3.9 | 3.5 | 19.3 | 19.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| 50 | 17.95 | 0.55 | 16.3 | 1.9 | 42.3 | 0.5 | 54.6 | 2.4 | 4.0 | 0.0 | 1.3 | 0.0 |
| 52 | 45.1 | 0.9 | 63.8 | 3.0 | 43.6 | 0.0 | 24.8 | 1.0 | 32.2 | 0.0 | 24.4 | 0.0 |
| 54 | 36.4 | 0.6 | 15.2 | 0.2 | 10.0 | 0.0 | 0.7 | 0.0 | 63.4 | 0.0 | 74.3 | 0.0 |
| 56 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.0 | 0.0 | 0.0 |
| TOTAL | 100.0 | 2.1 | 100.0 | 5.8 | 100.0 | 4.2 | 100.0 | 23.3 | 100.0 | 0.0 | 100.0 | 0.0 |

[a]Total triglyceride composition determined experimentally by GLC using molar calibration factors.
[b]Fully saturated triglyceride composition determined experimentally by the oxidation procedure and quantified by GLC.

TABLE III

Molar Calibration Factors Calculated for Simple Monoacid Triglycerides on a 1.83 m x 2.5 mm I.D. Column Containing 2% OV-17 on Supelcoport 100/120 mesh.

| No. of acyl carbon atoms | Molar calibration factor |
|---|---|
| 36 | 1.00 |
| 42 | 0.88 |
| 48 | 0.85 |
| 54 | 0.93 |

lower sample weights can be used and the method may lend itself to the analysis of lipids from body fluids.

REFERENCES


1. Starks, C.M., J. Am. Chem. Soc. 93:195 (1971).
2. Dehmlow, E.V., Agnew Chem., Intern. Ed. Engl. 13:170 (1974).
3. Herriott, A.W., and D. Picker, Tetrahedron Lett. 1511 (1974).
4. Seeley, F.G., and D.J. Crouse, J. Chem. Eng. Data 11:424 (1966).
5. Cerai, E., Chromatogr. Rev. 6:154 (1964).
6. Scibona, G., J.F. Byrum, K. Kimura, and J.W. Irvine, Solvent Extr. Chem. Proc. Int. Conf. 398 (1965).
7. Foglia, T.A., P.A. Barr, and A.J. Malloy, JAOCS 54:858A (1977).
8. Weber, W.P., and J.P. Shephard, Tetrahedron Lett. 4907 (1972).
9. Litchfield, C., R.D. Harlow, and R. Reiser, JAOCS 42:849 (1965).
10. Mangold, H.K., in "Thin Layer Chromatography," Edited by E. Stahl, Academic Press Inc., New York, 1965, pp. 137-186.
11. Brockerhoff, H., Arch. Biochem. Biophys. 110:586 (1965).
12. Jurriens, G., and A.C.J. Kroesen, JAOCS 42:9 (1965).


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# Preparation of Some Fatty Glycolic Acid Derivatives and Screening for Antimicrobial Activity

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## ABSTRACT

Nine glycolic acid derivatives were prepared and screened as primary plasticizers for polyvinyl chloride (PVC) and for antimicrobial activity. Two types of compounds were represented: (a) those with a methyl or ethyl ester grouping at the carboxyl function of glycolic acid and a fatty acyl group at the hydroxyl function; and (b) those with three acyl groups attached at the hydroxyl functions of the amide, resulting from the reaction of glycolic acid and diethanolamine. None of the compounds was compatible with PVC at the 35% level of incorporation, and the compounds were not further evaluated as plasticizers. They were screened for antimicrobial activity against a gram-positive bacterium, *Staphylococcus aureus;* a gram-negative bacterium, *Escherichia coli;* a yeast, *Candida utilis;* and a mold, *Penicillium* sp. Six of the compounds inhibited two or more of the test organisms suggesting some of these materials merit further testing as biostatic agents.


## INTRODUCTION

During an investigation of potentially useful derivatives of vegetable oils, a number of new fatty derivatives of glycolic acid were prepared for evaluation as plasticizers. They failed as primary plasticizers for polyvinyl chloride (PVC) at the usual (35%) level of incorporation. When evaluated according to the procedure described (1), the compounds either did not mill with the PVC or showed exudation within a short time after being incorporated into the polymer. The compounds were not further investigated as plasticizers; however, they were screened for antimicrobial activity. Other research has shown that many fatty compounds have antimicrobial activity (2-9). This paper is a report of the preparation of the compounds and the screening results.

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## EXPERIMENTAL PROCEDURES

Glycolic acid, diethanolamine and the acid chlorides used were commercial products. Intermediates and final products were characterized by infrared (IR) and nuclear magnetic resonance (NMR) spectral analyses (10). Densities of the liquids were determined pycnometrically in a bath thermostatically controlled to ± 0.1 C. Melting points of the solids are uncorrected and were determined by immersing the bulb of a thermometer directly in the partially melted material while it was maintained in a water bath at a temperature slightly above the melting point. Refractive indices were determined at 30 C with a precision Bausch and Lomb refractometer, with the D sodium line. Isolated yields were 90% or more of the theoretical.

### Preparations

*Methyl and ethyl glycolate.* Ca. 500 g of glycolic acid (70% in water) was dried by azeotropic distillation of the water from a benzene solution with a Dean-Stark trap. The methyl and ethyl esters were each prepared in situ by refluxing with methyl or ethyl alcohol for 72 hr. The purified esters were obtained by fractional distillation.

*Carbomethoxymethyl palmitate.* Palmitoyl chloride (55 g, 0.2 mol) was added to a stirred solution of methyl glycolate (18 g, 0.2 mol) in pyridine (20 ml). The precipitated pyridine hydrochloride was filtered and washed with benzene; the resulting benzene solution was water-washed, dried over sodium sulfate and the solvent stripped off with a rotary evaporator, leaving a quantitative yield of product.

Carbomethoxymethyl oleate, carboethoxymethyl oleate, carboethoxymethyl hydrocinnamate, and *bis*(carbomethoxymethyl) adipate were prepared by the same procedure used for the palmitate, but the appropriate acid chloride and glycolate were substituted.