Technical

Synthesis and Purification of Polyunsaturated Triglycerides

R.O. ADLOF and **E.A. EMKEN**, Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture, 1815 North University Street, Peoria, IL 61604

ABSTRACT

Triglycerides (TG) were prepared by the sodium methoxide-catalyzed interesterification of triacetin and the appropriate methyl ester. Yields were optimized and side-reactions were investigated. Silver resin chromatography utilizing mixed solvent systems (acetonitrile/acetone) was used to separate the resulting TG/methyl ester mixtures. The isolated TG were analyzed by ultraviolet and infrared spectroscopy and were found to contain no conjugated or *trans* isomers. They also were analyzed by thin layer chromatography and gas chromatography and found to contain no diacyl-monoacetyl glyceride or other reaction components. Based on higher yield and lack of isomerized byproducts, this procedure was found to be superior in the synthesis of highly unsaturated TG.

INTRODUCTION

Multigram quantities of very pure deuterium-labelled triglycerides (TG) were required for our studies of the metabolism of fats in human subjects (1,2). Triglycerides composed of monounsaturated fatty acids have been effectively prepared by *p*-toluenesulfonic acid-catalyzed esterification (3). However, this method produced small quantities of isomerized byproducts when polyunsaturated fatty acids were utilized. The reaction of a fatty acid chloride with glycerol (4,5) does not produce isomerized byproducts, but overall yields are only 60-70% and recovery of the unreacted fatty acids is difficult. The sodium methoxide-catalyzed interesterification of triacetin and mono-, di- and triunsaturated methyl esters (6,7) was found to be an excellent procedure for the preparation of triglycerides. The major difficulties encountered were formation of small amounts of mono- and diglycerides and subsequent purification of the triglycerides. The final mixture (composed of the methyl ester, TG and a trace of diacyl-monoacetyl glyceride) was difficult to separate by chromatography on columns packed with silica gel or Florisil (8). Silver resin chromatography procedures (9-11) were developed to separate these mixtures efficiently. The columns used for most of the separations were packed with 100% Ag^+/Na^+ resin, and the elution solvent was varied (12) to separate the components. 100% Ag^+/Na^+ resin is XN1010 sulfonic acid resin (Na^+ form) in which 100% of the Na^+ ions have been replaced by Ag^+ ions. The investigation was also expanded to include the separation of fatty acids and triglyceride mixtures.

EXPERIMENTAL

Instrumentation

Ultraviolet (UV) spectra were obtained with a Cary 219 Spectrophotometer (Varian Associates, Palo Alto, CA). Infrared (IR) analyses were carried out with a Perkin-Elmer 621 grating infrared spectrophotometer. Liquid chromatography was accomplished using a single-piston pump (Metering Pumps Ltd., London) and the effluent was monitored by a differential refractometer (Waters Associates, Framingham, MA, R403).

Materials

Amberlyst XN1010 macroreticular sulfonic acid (H^+ or Na^+ form) resin (Rohm and Haas Co., Philadelphia, PA) was exchanged with silver ions as previously described (9). Methyl linoleate was separated from transesterified safflower oil (PVO International, San Francisco, CA) on a column containing 91% Ag^+/H^+ resin. Methyl linolenate was obtained from transesterified linseed oil (3.0-g samples, 2 ft × 1 in. column packed with 48% Ag^+/Na^+, 60/80 mesh, acetone elution). Other reagents used were triacetin (99+%, Aldrich Chemical Co., Milwaukee, WI) and sodium methoxide (powder, 98%, Harshaw Chemical Co., Cleveland, OH). All other solvents were analytical grade or better.

Methods

Silver resin chromatography was used to separate the methyl esters and the triglycerides from the reaction mixtures. All fractions were treated as follows: the solvent was removed by rotary evaporation, and the residue diluted with petroleum ether and washed twice with saturated saline solution. The petroleum ether layer was dried over sodium sulfate; the sodium sulfate was removed by vacuum filtration through a Buchner funnel. The petroleum ether was forced, by syringe, through a Silica Sep Pak (Waters Associates, Framingham, MA). The solvent was then removed by rotary evaporator. These procedures were used to remove traces of mesityloxide (11) or acetonitrile.

The purity of the prepared TG was determined by thin layer chromatography (TLC; Silica Gel-50F254, E. Merck, Darmstadt, Germany). The plates were developed with hexane/diethyl ether/acetic acid (80:20:1, v/v) and visualized with iodine vapors.

The triglycerides containing trienoic fatty acids were analyzed by UV (13) at 268 mμ to determine the amount of conjugation and by IR at 10.36 μ to determine the amount of *trans* isomer present (14). Some samples were further analyzed by gas chromatography on a Packard Model 428 Gas Chromatograph (GC). A 4 mm × 1 m glass column packed with 1% SE-30 was programmed from 180 to 320 C at 20 C/min using nitrogen gas as carrier and a flame ionization detector.

Syntheses and Separations

Triolein. Methyl oleate (4.50 g; 1.52×10^{-2} mol) and sodium methoxide (0.09 g) were combined in a 25-mL, 3-necked, round-bottomed flask equipped with N_2 inlet, needle septum and magnetic stirrer. Triacetin (1.00 g; 4.59×10^{-3} mol) was added dropwise by syringe over 1 hr. The temperature was maintained at 65 C by an oil bath and the mixture was stirred for one more hour. A vigorous stream of nitrogen was used to remove the methyl acetate. The mixture was then cooled, isooctane was added and the material chromatographed (90% isooctane, 10% diethyl ether) on a 30 × 250 mm glass column packed with 60 g silica gel (Baker Chemical Co., Phillipsburg, NJ). The eluant (500 mL) was evaporated by rotary evaporator to yield 4.45 g of pale yellow oil. Separation of triolein from excess methyl ester was accomplished with a 2.5 × 30 cm glass column packed with 100% Ag^+/Na^+ resin (200/270 mesh). The eluting solvent was acetone (see Fig. 1). Total triglyceride recovered was 3.03 g (75% yield). The recovered

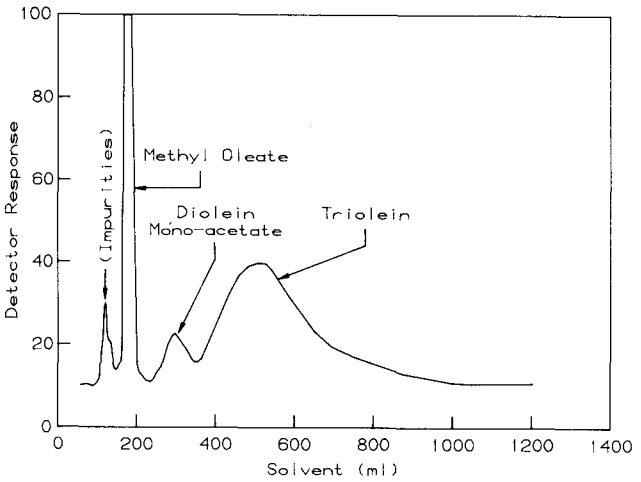

FIG. 1. Isolation of triolein on a 2.5 × 30 cm, 200/270 mesh, 100% Ag^+/Na^+ column. Flow rate: 7.5 mL acetone/min. Sample size: 1.30 g.

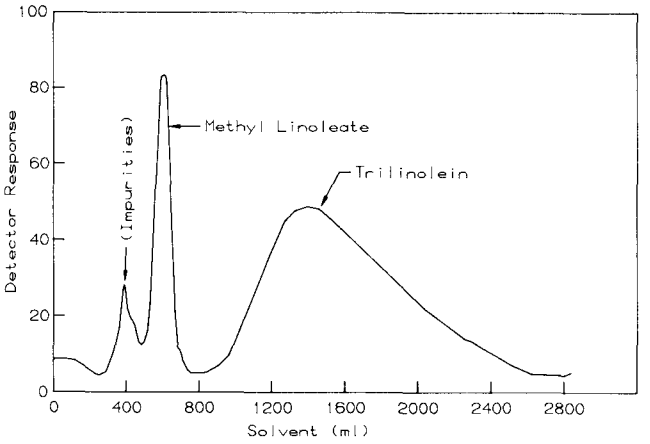

FIG. 2. Isolation of trilinolein on a 4 × 47 cm, 100/200 mesh, 100% Ag^+/Na^+ column. Flow rate: 7.8 mL of 8.5% acetonitrile in acetone/min. Sample size: 4.6 g.

methyl oleate (1.01 g) was recycled.

Trilinolein. Triacetin (2.00 g; 9.2 × 10^{-3} mol) and sodium methoxide (0.15 g) were combined in a flask equipped as in the previous example. Methyl linoleate (9.00 g; 3.06 × 10^{-2} mol; 10% excess) was added dropwise over 1 hr. The remainder of the work-up was the same, except that the residue was chromatographed through 90 g silica gel. The reaction mixture was separated with a 40 × 470 mm glass column packed with 100% Ag^+/Na^+ resin (100/200 mesh). The eluting solvent was 8.5% acetonitrile in acetone ([12]; see Fig. 2). The total sample recovered was 5.82 g trilinolein (72% yield) and 2.28 g methyl linoleate.

Trilinolenin. Methyl linolenate (4.5 g; 1.57 × 10^{-2} mol) and sodium methoxide (0.10 g) were combined as in the triolein preparation. Triacetin (1.0 g; 4.59 × 10^{-3} mol) was added dropwise over 45 min. Work-up and separation by silver resin chromatography gave 2.92 g trilinolenin (73% yield) and 0.92 g methyl linolenate. The same column was used as in the triolein separation above. The sample size was 1.05 g; the eluting solvent was 25% acetonitrile in acetone (see Fig. 3).

Separation of Oleate and Linoleate (Acid, Ester, TG)

A mixture of 38% methyl oleate, 38% oleic acid and 24%

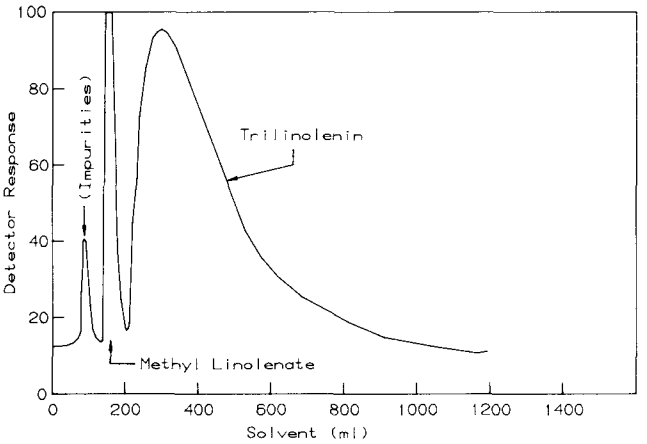

FIG. 3. Isolation of trilinolenin on a 2.5 × 30 cm, 200/270 mesh, 100% Ag^+/Na^+ column. Flow rate: 6.7 mL of 25% acetonitrile in acetone/min. Sample size: 1.05 g.

triolein (50 mg) was separated on a 71 × 0.6 cm glass column containing a 20 mL 100% Ag^+/H^+ resin (40/80 mesh). Acetone (0.3 mL/min) was used as solvent. The order of elution was ester (20.2 mL), acid (27.6 mL) and TG (48.2 mL).

A mixture of 33% methyl linoleate, 33% linoleic acid and 33% trilinolein (35 mg) was separated on a 75 × 0.6 mm glass column packed with ~22 mL of 39% Ag^+/Na^+ resin (40/80 mesh). Acetone (0.3 mL/min) was used as solvent. The order of elution was methyl linoleate (18.8 mL), trilinolein (27.8 mL) and linoleic acid (48.7 mL). A larger sample (303 mg) of the same mixture was separated on a 2.5 × 30 cm glass column packed with 100% Ag^+/Na^+ resin (200/270 mesh). Acetonitrile (8.5%) in acetone at 6.4 mL/min was used as solvent. The order of elution was methyl linoleate (175 mL), linoleic acid (350 mL) and trilinolein (765 mL).

RESULTS AND DISCUSSION

During the syntheses and purification of the triglycerides, several interesting points were noted. The order in which the ingredients were added did not affect the yields of TG. The interesterification procedure always yielded ~10% mono- and diglycerides, which suggests the presence of MeOH or H_2O. These byproducts occurred despite the use of 5-10% excess methyl ester and methanol free sodium methoxide. Redrying of the methyl esters and triacetin did not decrease the amounts of these byproducts. Reacetylation of the triacetin with acetyl chloride/pyridine (15) before use also did not change the diglyceride content of the reaction mixture. The diglycerides were readily removed from the reaction mixture by silica gel chromatography, but the diacyl-monoacetyl glyceride, methyl esters and TG were difficult to separate and required development of a silver ion chromatographic method for final purification.

Silver-saturated columns and mixed solvent systems are more versatile than partial argentation resin chromatography (PARC) for the purification of triglycerides. Utilizing mixed solvents, a single silver-saturated XN1010 macroreticular sulfonic acid resin column can be used to separate a wide range of mono-, di- and trienoic fats. Similar separations utilizing PARC technology would require the preparation and storage of 4-6 columns containing 20-100% Ag^+/Na^+ resin. Acetone was used due to its low toxicity and its ability to solubilize triglycerides.

The elution order of linoleic acid and trilinolein reversed

when a different solvent composition/column type was used [see previous section]. Although this phenomenon has been observed in other types of liquid chromatography, we believe this to be the first time such an occurrence has been observed in the separation of lipids by silver resin chromatography. Thus, care should be exercised in the identification of components when the solvent composition or column types have been changed.

Analyses of the triglyceride fractions by UV and IR indicated the presence of <0.1% conjugation and *trans* isomers. Analysis by TLC and GC did not indicate the presence of diacyl-monoacetyl glycerides or other byproducts or intermediates. The purity of the eluted TG was >99%. Trilinolenin prepared by the *p*-toluenesulfonic acid-catalyzed esterification of linolenic acid by glycerol was found to contain 0.5-1.0% conjugated and/or *trans* isomers. In situations where high-purity triglycerides are required, our procedure can be used to prepare large-scale quantities of these important compounds.

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Chemical Composition and Characteristics of *Moringa peregrina* Seeds and Seeds Oil

M.A. SOMALI, M.A. BAJNEID and **S.S. AL-FHAIMANI**, Quality Control Laboratory, Ministry of Commerce, Halat Ammar (Tabuk), Saudi Arabia

ABSTRACT

The *Moringa peregrina* kernel contains 1.8% moisture, 54.3% oil, 22.1% protein, 3.6% fiber, 15.3% carbohydrate and 2.5% ash. The composition and characteristics of the extracted oil were determined. Gas liquid chromatography of methyl esters of the fatty acids shows the presence of 14.7% saturated fatty acids and 84.7% unsaturated fatty acids. The fatty acid composition is as follows: palmitic 9.3%, palmitoleic 2.4%, stearic 3.5%, oleic 78.0%, linoleic 0.6%, linolenic 1.6%, arachidic 1.8% and behenic 2.6%.

INTRODUCTION

The *Moringa* family consists of ca. 10 Xerophytic species distributed from tropical Africa to the East Indies (1). Four main species exist: *Moringa aptera, Moringa concanensis, Moringa olifera* and *Moringa pterygosperma.* All the species except *M.peregrina* species grow wild and are rapidly growing trees of 25-30 ft high which bear long seed pods, each pod containing ca. 20 seeds. The chemical compositions of the oils of *M.aptera, M.concanensis, M.olifera* and *M.pterygosperma* have been reported.

M.aptera contains 53% oil (2), and in separate studies *M.concanensis* was shown to contain 31.4% oil (1) and 33% oil (3), *M.olifera* contained 21% oil (4) and 27% oil (3) and *M.pterygosperma* contained 34.4% oil (1).

Moringa peregrina, locally called "Yassar", contains seeds which have long been used as a source of oil. The oil is extracted by boiling seeds with water and collecting the oil from the surface of the water. The oil extracted is called "Al-Yassar". At present, the oil is not popular. The purpose of this work was to study the chemical composition of the seed, the physicochemical constants and fatty acid composition of the oil *M.peregrina* which have not been previously reported.

EXPERIMENTAL METHODS

Sampling

The *Moringa peregrina* seeds of wild cultivated trees were collected from the Chewag region of northwest Saudi Arabia. A total of five samples were used.

Methods of Analysis

The specific gravity, water content, nitrogen content, fat and ash of the kernel were estimated by usual standard methods recommended by AOAC (5). The percentage of protein was calculated by multiplying total nitrogen by a factor of 6.25.

Extraction and Analysis of Oil

Moringa oil was extracted (soxhlet) from the seed with petroleum ether (40-60 C) and analyzed immediately for iodine value, saponification number, refractive index, unsaponifiable matter, acid value and peroxide value by AOCS (6).

GLC Analysis

Methyl esters of extracted oil were prepared according to AOCS Method Ce 2-66 using 14% boron trifluoride solu-