Fractionation of Triglyceride Mixtures by Preparative Gas Chromatography

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ABSTRACT

A semiautomatic system is described for gas-chromatographic separation and recovery of triglycerides of uniform molecular weight in milligram quantities. It employs an Aerograph Autoprep 700 (Wilkens Instrument and Research, Inc.) equipped with a stream splitter and a hydrogen flame ionization detector. The column is an aluminum or stainless steel tube (¼ in. O.D. x 2 ft) and contains silanized Chromosorb W (60-80 mesh) coated with 5% (w/w) JXR or SE-30. Five to ten milligrams of mixed triglyceride are injected at a time and the temperature is programmed exponentially from 150 to 350°C. With split ratios of 1:5 to 1:10 collections of 20 to 50 mg of each peak can be made with some 10 to 20 injections.

INTRODUCTION

THE SEPARATION of natural triglycerides by gas-liquid chromatography (GLC) results in a segregation of the triglycerides on the basis of their molecular weight (1,2). Although each fraction contains only glycerides of the same carbon number these triglycerides usually contain an assortment of fatty acids esterified to glycerol. In order to identify the glycerides it is necessary to isolate the individual peaks and to determine their fatty acid compositions. The present report describes the separation and recovery of some synthetic and natural triglyceride mixtures in quantities large enough for rechromatography in the GLC apparatus and on thin-layer plates before and after enzymic and chemical transformations.

MATERIALS AND METHODS

Glycerides

The butter oil, its molecular distillates (3) and the coconut oil (4) used in this study have been described previously. The medium chain length (MCT) and long chain length (LCT) triglycerides were obtained from E. F. Drew and Company, Boonton, N.J. These triglycerides had been prepared by transesterification of fatty acid methyl esters of selected chain length with glycerol. The corn oil was Mazola and was purchased from a local market. Simple synthetic triglycerides of even carbon number and ranging from tricaprin to tristearin and triolein were obtained from the Applied Science Laboratories, Inc., State College, Pa.

Gas-Liquid Chromatography

An Aerograph Autoprep 700 (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) was modified by introducing a stream splitter and a hydrogen flame ionization detector. The flame detector was mounted on top of the thermal conductivity (TC) detector and was connected to the column through a post-column effluent splitter. The splitter (Fig. 1) consisted of two stainless steel tubes (¼ in. and 1/16 in. O.D.) silver-soldered into the male Swagelok fitting (B) accepting the outlet end of the ¼ in. O.D. chromatography column (A). During chromatography the column effluent entered the splitter and was divided between the 1/16 in. bypass tube to the flame detector (C) and the ¼ in. tube leading through...
the thermal conductivity detector (E) to the collector (H). With a 1/16 in. O.D. outlet hole (D) in the Swagelock fitting accepting the 1/8 in. tube at the entrance to the TC detector, the approximate split ratio was 1:1 under the selected working conditions. By placing a 21 gauge needle (F) into the bypass tube at B the gas flow to the flame detector was restricted and the split ratio became 1:4.5 in favor of the collector. A further restriction resulting in a split ratio of about 1:10 was obtained by inserting a 0.007 in. diameter wire (G) into the needle. (A standard kit is now available from Wilkens Instrument and Research Inc., which converts the A-90-P or A-700 instruments to flame units).

The splitter needle actually sticks into the packing material in the column. In order to prevent the solid support from plugging the splitter needle, the column exit (AA) is prepared for entrance of the splitter by a preliminary penetration of the solid support by a 20-gauge needle.

The restrictor wire (G), about 2 in. long, is completely inserted in the needle. For optimum sensitivity the flow to the flame tip was kept at about 25-30 ml/min requiring an overall flow of about 110—120 ml/min through column and about 90—100 ml/min to the collector (1:4.5 ratio). For a 1:10 split ratio, the flow through the column was set at about 300 ml/min. During the run, the detector oven housing, the splitter and the flame ionization detector were maintained at 325°C. A special elbow heater (Wilkens Instrument and Research, Inc. accessory) was used to obtain the column temperature to reach the upper limit and adjusting the cooling cycle timer to the number of minutes necessary to cool the column down to the starting temperature or slightly below it; 4) setting the precollection timer to ignore the solvent and any low boiling components emerging prior to the triglycerides; 5) injecting the sample into the flash evaporator or directly onto the column using a Hamilton syringe with either a 2 or a 6 in. needle, respectively, and recording the time and temperature of injection; 6) recording the temperature rise at regular time intervals and collecting peaks automatically by setting the peak signal activator switch on the recorder to the desired level. (As each peak appears on the recorder, the signal switch is tripped and the collector table is automatically advanced); 7) if necessary, using a postrjection timer to postpone the opening of the oven lid and the start of the cooling cycle until the remaining high molecular weight peaks have been vented or collected together in a separate collection bottle.

**Thin-Layer Chromatography**

The thin-layer chromatographic (TLC) examinations of the triglycerides before and after GLC and enzymatic or chemical transformations were carried out using 20 x 20 cm plates coated with a 250 µ thick layer of Silica Gel G (Merek and Co.) (5). The prepared plates were reactivated after washing with methanol. The lipids were applied in spots containing about 0.1 to 0.5 mg of material per spot. The plates were developed with a mixture of petroleum ether and ethyl ether (120:80, v/v) to which 3 ml of formic acid was added. The developed plates were sprayed with a 0.2% solution of 2,7-dichlorofluorescein in ethanol. The lipid components were located by examining the plates under ultraviolet light. The system was capable of separating (in order