Determination of Cyclic Fatty Acids by Gas-Liquid Chromatography[1]

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Abstract

A method is described to determine cyclic fatty acids in cyclic monomers by gas-liquid chromatography (GLC), which separates saturated straight-chain esters from cyclic esters. The content of cyclic esters can be determined by integration of the area on the chromatograph under the cyclic peaks. GLC was applied to cyclic monomers made from linseed oil and from linolenic acid. Samples of both hydrogenated and nonhydrogenated cyclic monomers were analyzed; however, more reliable results were obtained on the hydrogenated samples. The results show a standard deviation of 0.25 for linseed oil and 0.36 for linolenic acid.

Accuracy of the analysis was established by comparing data with that obtained by crystallization. The GLC method is approximately three times faster.

Introduction

PREPARATION of cyclic fatty acids by heating linseed oil in ethylene glycol with excess sodium hydroxide has been described (2,4,9). The time of heating required for maximum cyclization depends on temperature, amount of catalyst, and solvent-to-oil ratio. The fatty acids recovered by neutralizing with sulfuric acid and extracting with hexane are esterified with methanol and distilled under vacuum to give monomeric and polymeric ester fractions. The monomeric fraction contains cyclic fatty acid esters, which may be useful in making plasticizers or coatings, as well as other products, whose uses are presently being investigated (1,8).

Previously, after the monomer was hydrogenated, cyclic fatty acid content was determined by crystallization from acetone (3). In the new method, gas-liquid chromatography (GLC), employed in a variety of ways for the analysis of methyl esters of fatty acids (5–7), was applied to the determination of the cyclic fatty acid content of the monomer. The analyses by GLC are compared with those by crystallization. An experienced operator can analyze a sample of monomeric acids for cyclic acid content in approximately 30 min by solvent crystallization. The same operator can analyze the same sample using GLC in 10 min with improved accuracy.

Development of the GLC method was approached in two ways: (a) Samples of hydrogenated cyclic monomers were analyzed by GLC, which produced a chromatogram with well-separated peaks representing the saturated straight-chain fatty acid esters and the saturated cyclic fatty acid esters. The cyclic content of the monomer was then determined by integration of the area under the peaks. (b) A chromatogram was also prepared from samples of unsaturated cyclic monomers, and the peaks were again integrated to obtain the percentage of cyclic fatty acids in the monomer.

Apparatus and Procedures

The chromatographic instrument used for developing the method to determine cyclic fatty acids was a Beckman GC-2A. Helium was used as the carrier gas in preference to hydrogen because of the safety factor. The apparatus was modified by replacing the helium capillary with a shortened tube to allow increased helium flow and boost resolution. The sample of hydrogenated monomeric esters, if solid, was liquified by heating just before injection.

A liquified sample of cyclic monomer methyl esters (0.1 μl) was measured in a 10-μl Hamilton microsyringe and injected into the preheater where the sample was vaporized. Best results were obtained using a microsyringe with a 3-in. needle, which injected the sample very near the end of the column. The sample passed into a 6-ft, ¼-in. stainless-steel column containing 10% diethylene glycol succinate (DEGS) adsorbed on 120/140 mesh acid-washed Celite.

Column packing was prepared by dissolving 2 g of DEGS in 100 ml of chloroform. This mixture was thoroughly blended with 20 g of 120/140 mesh acid-wash Celite, and the chloroform was evaporated on a steam bath. The packing was placed in a vacuum oven for 2 hr at 80C. The column, packed by vacuum and continuous tamping, was tempered in the chromatograph for 24 hr before use or until base line stability was attained. Column temperature was 220C, and the helium flow rate was 60 to 75 ml/min. The thermal detector cell with an applied current of 300 ma indicated a difference in the conductivity of the helium stream on a Brown recorder, equipped with a Disc integrator.

A sample containing principally methyl stearate was run before the unknown cyclic monomer. The retention time of the methyl stearate was calculated and used to locate the methyl stearate peak in the sample of cyclic monomer. Unsaturated cyclic monomer contains C_{16} and C_{18} linear esters plus the cyclic esters. Hydrogenation converts all the linear unsaturated C_{18} esters to methyl stearate. A vertical line is drawn after the methyl stearate down through the integrator lines (Fig. 1). Fatty esters eluted after the methyl stearate are considered to be cyclic fatty acid esters. Calculation for cyclic content is based on the integrated area under the cyclic peaks in relation to the total area.

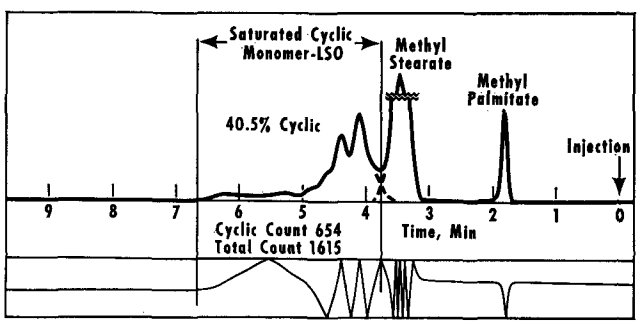

FIG. 1. A typical gas-liquid chromatogram of hydrogenated methyl esters from a cyclic monomer.

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Results and Discussion

Column packing is the most important single factor in GLC analysis and is the most difficult variable. During early work a 6-ft, ¼-in. stainless-steel column packed with a 40/60 mesh solid support and 25% liquid phase was used. Decreasing the size of the solid support and decreasing the percentage of liquid phase greatly increased the resolution. The first substrate used, Craig polyester succinate, gave poor resolution. Resoflex 446 was tried, but at the operating temperature it was continuously eluted from the chromatograph, and this elution caused poor base line stability.

The helium flow rate that produced the best results was between 60 and 75 ml/min. Below this range the chromatogram was too spread out and yielded less resolution. Column temperature proved highly significant. Below 220C elution time greatly increased and resolution fell off. Much above 220C the DEGS gradually eluted from the column. Increasing the column length from 6–12 ft produced very little increase in resolution, with a great loss in flow rate. The diameter of the column was changed to ⅛ in. O.D. again with very little effect.

Another important variable is the current flow through the thermal detector cell. The current used, 300 ma, is the recommended maximum for column temperature of 220C. Less current than 300 ma gave poorer resolution. With a packing containing a relatively small amount of substrate it was important not to overload the column by injecting a large sample. The most suitable sample size was from 0.1–0.3 μl. Peak height was controlled by an attenuator. The chart speed on our recorder was ½ in./min.

Previously the only method of determining the cyclic acid content of a cyclic monomer was by low-temperature crystallization. A hydrogenated sample of the methyl esters or acids of a cyclic monomer was dissolved in acetone, the temperature reduced to −50C, and the solution filtered. The solid fraction was recrystallized in acetone to remove any remaining cyclic fatty esters. After removal of acetone the liquid sample was weighed. Each analysis required about 30 min.

Determination of cyclic acids by GLC was first explored by analyzing the same hydrogenated samples that had been crystallized. The results from GLC on cyclic acid samples prepared from linseed oil (Table I) were constantly 2–3% lower than those from crystallization. The difference was later shown to be caused by some of the palmitic acid that remained in the cyclic acids obtained by solvent crystallization (Fig. 2). The difference did not appear as great on the cyclic acid prepared from linolenic acid (Table I) because the original linolenic was low in palmitic. The crystallization data indicate that an experienced technician must operate the crystallizer in order to obtain valid results. Several of the cyclic acid reactions, where several samples were taken at intervals during the reaction, do not show a smooth production curve by crystallization, whereas the curve by GLC is excellent. The entire determination of cyclic acids by GLC requires only 10 min, and the accuracy is within a standard deviation of 0.25 for linseed oil and 0.36 for linolenic acid. Samples that did not reach the base line after the analysis were re-run to prevent inflating the amount of cyclic acid.

TABLE I

Comparative Data from a Series of Runs to Determine the Amount of Cyclic Fatty Acids in Linseed Oil and Linolenic Acid

Percentage Cyclic Acid Methyl Esters					
By crystallization		By GLC			
		On saturated monomer		On unsaturated monomer	
Linseed oil	Linolenic acid	Linseed oil	Linolenic acid	Linseed oil	Linolenic acid
44.7	86.2	41.7	85.8	46.5	87.5
45.5	81.2	43.6	85.6	46.8	82.5
45.9	92.5	43.4	91.6	46.3	92.7
46.7	94.2	44.3	93.3	45.1	91.2
46.6	82.5	44.1	80.9	43.3	83.6
48.9	84.7	45.5	83.0	48.2	88.2
46.9	85.4	45.9	84.1	49.1	88.8
47.9	87.5	47.3	85.8	48.3	87.9
58.4	45.5	50.3	44.2	54.4	67.5
52.6	82.0	50.5	81.9	53.8	89.4
54.0	90.2	50.7	89.0	53.4	90.6
59.0	91.8	50.7	91.4	51.6	92.9
54.3	71.6	46.8	68.8	52.6	68.8
51.6	85.1	47.7	84.8	53.6	81.7
53.5	91.9	47.5	91.5	53.5	91.3
66.1	95.0	49.4	94.7	52.8	92.1

Further work was carried out in an attempt to eliminate the hydrogenation step by performing the GLC determination on samples of unsaturated cyclic monomer prepared from linseed oil and from linolenic acid. It was observed that most of the unsaturated cyclic monomer was eluted after the unsaturated straight-chain esters but that a small amount of unsaturated cyclic esters was eluted with the straight chain esters and accounted for the difference between the hydrogenated and unhydrogenated samples. Unsaturated straight-chain esters in the unsaturated cyclic monomer were identified by adding standards of each of the individual straight-chain esters including conjugated esters (Fig. 3). Calculation of the cyclic content of the unsaturated monomer was made by measuring the area under the peaks eluted after the conjugated linolenic acid esters. Cyclic content of a sample of unsaturated cyclic monomer was thus compared with a saturated cyclic monomer from the same series. The results showed poor agreement. The actual line of demarcation between the unsaturated cyclic and the straight-chain esters was then determined by comparison with the analysis of the saturated cyclic sam-

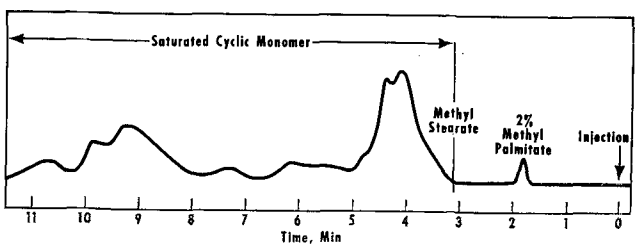

FIG. 2. A typical gas-liquid chromatogram of a cyclic monomer prepared by crystallization from linseed oil.

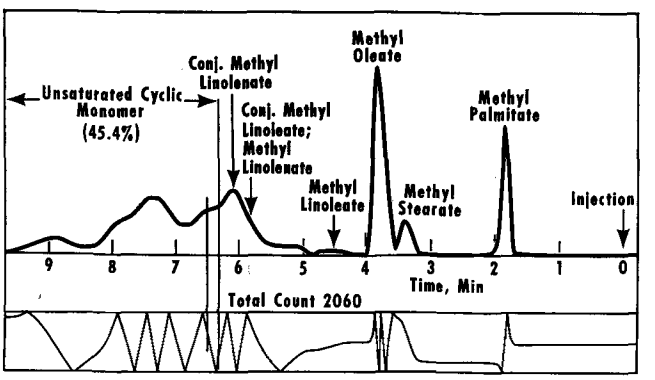

FIG. 3. Gas-liquid chromatogram of unhydrogenated methyl esters from cyclic monomer.

TABLE II

Standard Deviation of Cyclic Monomers from Linseed Oil and Linolenic Acid as Determined by GLC

Number of tests on different days	Linseed oil		Linolenic acid	
	Saturated	Unsaturated	Saturated	Unsaturated
1	52.5	52.6	90.4	88.5
2	53.0	53.6	89.9	89.3
3	52.7	51.9	89.7	88.9
Standard deviation	0.25	0.85	0.36	0.40

ple. The true separation point was determined by elution time. Unsaturated samples of the same series could then be determined for cyclic content. Data given in Table II agree very well; however, this agreement was frequently inconsistent.

This method of determining cyclic content on unsaturates was considered generally inferior to the method using saturated esters because a breakoff point between unsaturated cyclic and unsaturated straight-chain esters could not be located directly.

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Analysis of Soaps with Hydrogen Bromide in Glacial Acetic Acid

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Abstract

Alkali metal carboxylic acid salts of long-chain fatty acids and of α-sulfo fatty acids are determined by titration with hydrogen bromide in glacial acetic acid medium. Crystal violet is used as indicator.

Introduction

THE ANALYSIS of soaps of epoxidized fatty acids by the Durbetaki (1) method led to the discovery that the titrant, a solution of hydrobromic acid in acetic acid, reacted not only with the oxirane function, but with the carboxylic acid salt as well. A survey of the literature disclosed no study of the quantitative determination of soaps by this method, although the reaction of soaps with hydrogen bromide was known (2). An investigation to discover whether the Durbetaki method was applicable to the quantitative determination of soap therefore seemed desirable, especially if the procedure could be carried out in the presence of salts of strong acids.

As early as 1927, Hall and Conant (3,4) experimented with the titration of bases in glacial acetic acid. These authors used either potentiometric methods or visual indicators to obtain sharp endpoints in this medium.

Of the various indicators used in non-aqueous titrations, crystal violet is fairly popular. There have been reports of some difficulties with this indicator due to the several color changes it undergoes (5) when various types of compounds are titrated. However, potentiometric titration may be used to select the crystal violet color change which coincides with the equivalence point of the particular type of compound titrated. The accuracy and precision of the results is directly related to the length of experience of the analyst (6). For the determination of soaps, Palit (7) employed a double indicator method using phenolphthalein or cresol red in combination with methyl orange or methyl red. The analysis was carried out in a solvent mixture of a glycol with either a higher alcohol or chlorinated hydrocarbon. Pure sodium oleate and commercially available soaps, dissolved in a mixture of either ethylene or propylene glycol and isopropyl alcohol, were titrated with either hydrochloric acid or perchloric acid in the same medium. Potentiometric titrations were employed to substantiate the results.

Markunas and Riddick (8) determined carboxylic acid salts and other weak bases with potentiometric and visual indicator titrations using glacial acetic acid as solvent and perchloric acid in acetic acid as the titrant. The authors did not, however, analyze any long chain fatty acid salts.

Fritz and Fulda (9) titrated tertiary amines and lithium nitrate potentiometrically in glacial acetic acid, using perchloric acid as titrant. Acetic anhydride was added to remove traces of water. Again, soaps were not analyzed.

Pellerin (10) stated that salts of acetates, propionates, citrates, tartrates, benzoates, salicylates, etc. can be titrated with perchloric acid in acetic acid.

Reagents and Apparatus

Acetic acid, glacial, analytical reagent grade.

Hydrogen bromide in acetic acid, 0.1N, anhydrous.

Crystal violet indicator solution. Prepare a saturated solution (approximately 1%) of crystal violet (Eastman) in glacial acetic acid.

Sodium carbonate, primary standard grade, dried for 3 hr at 240C, or potassium acid phthalate, primary standard grade, dried to constant weight at 125C.

Automatic buret, 50 ml, reservoir type, equipped with drying tubes to protect content from atmospheric moisture.

Procedure

Weigh accurately a sample of 0.4–0.6 g into a 250 ml Erlenmeyer flask fitted with a rubber stopper. Add 50 ml glacial acetic acid and dissolve the sample, with

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