Potentiometric Titration of Cyclopropene Esters

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ABSTRACT

The quantitative determination of cyclopropene acids occurring as esters in seed oils can be made by a direct titration with hydrogen bromide in toluene, but the color indicator used heretofore to determine the end point presented problems. A potentiometric titration that avoids these problems was devised. A silver-silver chloride indicator electrode and a calomel reference electrode connected to the titration cell by a salt bridge were employed. The precision of the cyclopropene analysis of purified samples seems to depend primarily on the accuracy to which the amount of titrant can be determined. At the 1% level, reproductible values to about ±0.01% are obtained.

INTRODUCTION

The cyclopropene acids malvalic and sterculic occur as esters in the seed oils of many species of the order Malvales, including *Gossypium hirsutum*, or Upland cotton. Various chemical and physical methods and combinations of these have been suggested for the quantitative determination of cyclopropenes in lipids. None of the reported methods is entirely satisfactory. A titration with hydrogen bromide in an anhydrous medium would be preferred in some cases if certain problems could be overcome. Some earlier analytical procedures with hydrogen bromide specified a titration in an anhydrous solution containing acetic acid (1-3). But under the conditions employed, the acetic acid reacted with the cyclopropene moiety (4). Subsequently, the titration in acetic acid solution was modified by applying a correction factor of about 15% (5).

Cyclopropenes have been titrated with hydrogen bromide in toluene solution to the color end point of crystal violet (6). However, crystal violet has a low solubility in toluene, and the protonated form is practically insoluble. Thus, determination of the end point requires several additions of an indicator solution plus experience and skill. Rosie and Shone (7) modified the titration by adding to the sample an excess of hydrogen bromide in benzene, allowing the reaction to proceed for 30 to 40 min at room temperature, adding water, and back titrating with a sodium hydroxide solution. Apparently, a direct, potentiometric titration to eliminate the need for a color indicator has never been investigated.

EXPERIMENTAL PROCEDURES

Equipment

A Beckman Research pH Meter, Model 101900 (Beckman Instruments, Inc., Palo Alto, CA) was used in the potentiometric mode for all titrations. Data were recorded directly from the millivolt scale, which was graduated in 0.2-mv increments over a range of ±50 to ±1460 mv. A silver-silver chloride indicator electrode (Ag/AgCl Internal, No. 476024, Corning Glass Works, Science Products Division, Corning, NY) recommended for halide ions was used with a standard, calomel reference electrode. The latter was placed well outside the titration cell and connected to it by a salt bridge of a saturated solution of potassium chloride in an adapter.

[1]One of the facilities of the Southern Region, Science and Education Administration, U.S. Department of Agriculture.

The adapter consisted of the outer part of a 14/20 standard-taper, ground-glass joint fused to glass tubing, 260 mm long × 4 mm od. An asbestos fiber junction was fused to the lower end of the adapter to permit contact between the saturated electrolyte and the anhydrous solution in the titration cell. Between titrations, the adapter was flushed with deionized water and refilled with fresh electrolyte.

The titration cell, Figure 1, was constructed from glass tubing and was water jacketed, measured 45 mm in inside diameter by 85 mm in depth. Water from a controlled temperature bath was circulated through the jacket.

A Teflon-coated magnetic stirring bar was used to mix the contents of the cell. The titration cell was closed with a neoprene stopper through which were inserted the indicator electrode; the adapter tube from the reference elec-

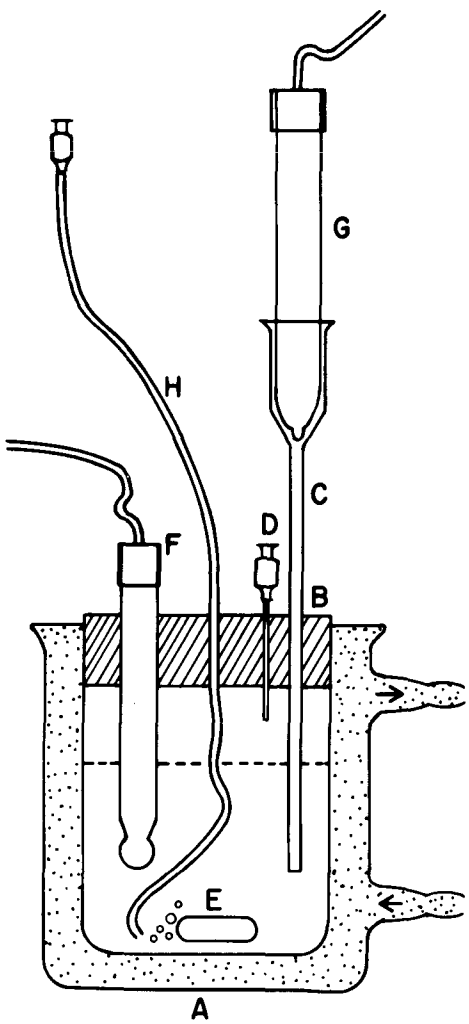

FIG. 1. Titration cell and connecting equipment. (A) Water-jacketed glass cell; (B) neoprene stopper; (C) salt bridge for reference electrode; (D) breather needle; (E) Teflon-coated magnetic stirring bar; (F) indicator electrode; (G) reference electrode; (H) No. 22 Teflon needle connected to 10-ml automatic burette.

trode; a No. 22 Teflon needle, which permitted introduction of the titrant below the surface of the solution in the cell; and a No. 22 stainless-steel needle, which served as a breather.

The titrant was measured into the titration cell with a 10-ml automatic burette equipped with a Teflon plug (Kimax No. 17138-f).

Reagents and Materials

Reagent grade toluene that contained about 0.03% water was freed of water by nitrogen stripping under vacuum at room temperature. Solutions of 0.1 N HBr in toluene were prepared by passing gaseous HBr through the toluene at atmospheric pressure and room temperature.

The cottonseed oil samples, obtained from a commercial source, consisted of a refined oil and a refined and bleached oil, both made from the same crude. The peanut oil was a refined-only product made commercially (Planters Peanuts, Suffolk, VA). Refined and bleached *Sterculia foetida* oil was prepared in the laboratory from crude oil extracted from ground seed with hexane.

Titrations were run either on the oils or on methyl esters prepared from the oils by a sodium methoxide catalyzed methanolysis. Usually both the oils and methyl esters were further purified just before titration by passage of a hexane solution of the oil or methyl ester (1:1 by weight) through a column of activated alumina (Alumina F-20, Aluminum Corporation of America, 80-200 mesh, 4 parts alumina to 1 part of oil or methyl ester, w/w) (2).

Procedure

In a typical run, 2.5 to 10 g of oil or methyl esters derived from the oil was weighed accurately into the titration cell, and 30 ml of dry toluene and a Teflon-coated magnetic stirring bar were added. The neoprene stopper holding the indicator electrode, the lower part of the salt bridge, the Teflon needle and the breather needle was positioned on the titration cell. Water preheated to 75 C was circulated through the outer jacket of the titration cell.

The titrant, 0.1 N HBr, was added usually in 0.1-ml increments for a first run on an unknown sample. After each increment, millivolt readings were allowed to reach a constant value and then recorded. When sizable changes in the recorded readings were noted for successive increments, smaller increments of titrant were added to obtain a sharper end point. Subsequent runs on the same sample were performed more quickly because larger increments of titrant could be added before the break in the potentiometric curve was reached.

Potentiometric readings were plotted against titrant added. The end point was determined graphically from the intersection of the S-shaped section of the potentiometric curve with a line drawn parallel to and equidistant from the two horizontal segments adjacent to the S-shaped section.

Standardization of the titrant was performed at room temperature. Approximately 0.15 g of potassium acid phthalate was dissolved in 14 ml of glacial acetic acid and 5 ml of toluene, and the solution was titrated potentiometrically.

RESULTS AND DISCUSSION

Triglyceride oils frequently contain such compounds as monoglycerides, diglycerides and oxidation products that also react under the conditions employed (4). In the potentiometric titrations, these interfering compounds reacted at about the same rate as the cyclopropenes, and the titration of these interfering compounds could not be separated from that of the cyclopropenes. Unless the oil

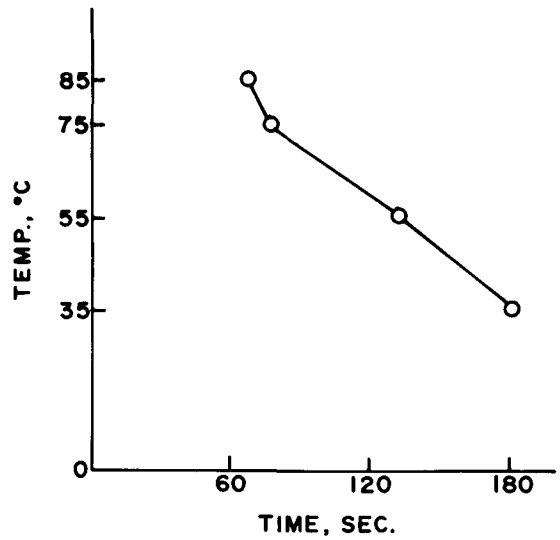

FIG. 2. Time required at different temperatures for potentiometric reading to reach a steady value.

to be analyzed is of good quality and known to be free of the interfering impurities, conversion of the glyceride fatty acids to methyl esters and further purification of the methyl esters by passage through activated alumina are necessary. The alumina treatment suggested heretofore (2) consisted of passing a hexane solution of oil or methyl esters through a column of alumina (1 part fatty product to 4 parts alumina) but did not specify the activity of the alumina, which apparently was used as received and had not been freshly activated. In the current investigation, activated alumina was used as received. Removal of interfering substances was monitored by thin layer chromatography.

The most appropriate titration temperature was established in a series of runs with a commercially refined and bleached cottonseed oil of good quality. In each run, about half (1.25 ml) of the approximately 0.1 N HBr required for titration to the end point was added, and the time required for the potentiometric reading to reach a steady value was recorded (Fig. 2). As the temperature increased, the time required to reach a steady potentiometric reading decreased. A temperature of 75 C was selected for subsequent titrations and represents a compromise between the increased vaporization of toluene at the higher temperatures and the increased time at the lower temperatures. The difference in the required time at 75 C and 85 C was only 10 sec.

Possible vaporization of hydrogen bromide at the selected temperature of 75 C was not a factor. At this temperature, HBr reacts with the cyclopropenes in the earlier stages of titration as rapidly as the HBr solution can be added (4). Close to the end point, when small increments of titrant are added, the HBr is consumed within seconds. The attainment of a steady potentiometric reading depended primarily on the characteristics of the electrodes and salt bridge and not on the rate of reaction between cyclopropenes and hydrogen bromide.

The standardization of the HBr solution at room temperature by a potentiometric titration of potassium acid phthalate dissolved in glacial acetic acid and toluene presented no problems. The potential during the first stage of a titration remained at a practically constant value and changed abruptly at the end point. Additions of titrant beyond the end point again resulted in practically no change in potential. The normality determined by potentiometric titration always agreed within the limits of

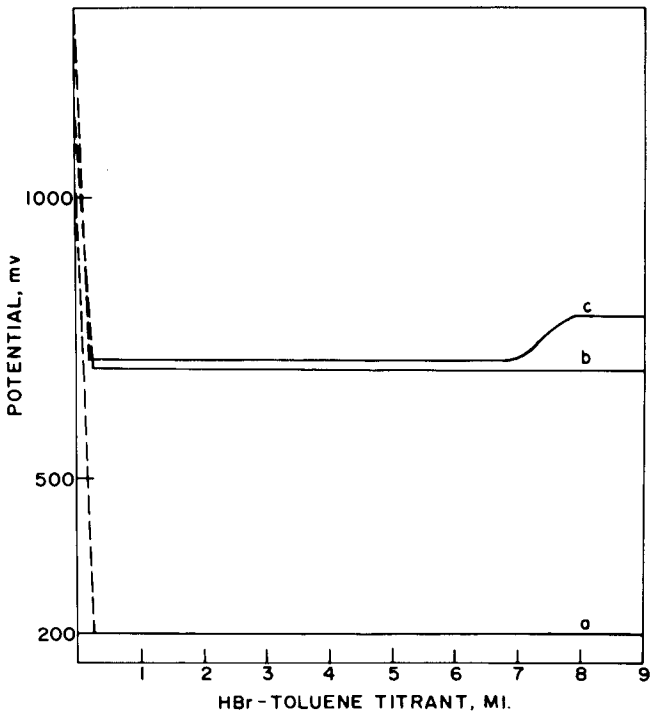

FIG. 3. Potentiometric titration curves: (a) 30 ml of toluene, (b) 5 g of methyl esters of peanut oil fatty acids dissolved in 30 ml of toluene, and (c) 0.3 g of *Sterculia foetida* oil and 5 g of methyl esters of peanut oil fatty acids dissolved in 30 ml of toluene. Titration with approximately 0.08 N hydrogen bromide in toluene.

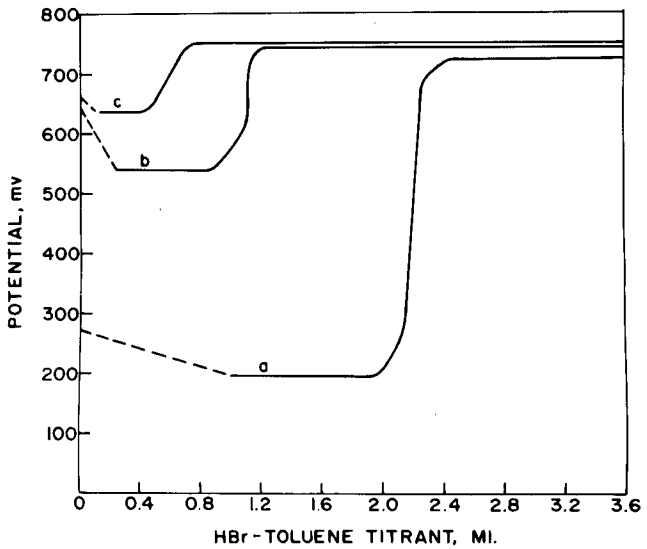

FIG. 4. Potentiometric titration curves of methyl esters in 30 ml of toluene: (a) approximately 10 g of methyl esters of cottonseed oil fatty acids, (b) approximately 5 g of methyl esters of cottonseed oil fatty acids plus 5 g of methyl esters of peanut oil fatty acids, and (c) approximately 2.5 g of methyl esters of cottonseed oil fatty acids plus approximately 7.5 g of methyl esters of peanut oil fatty acids.

experimental error with that determined by titration to the blue-green end point of crystal violet.

The potentiometric curve obtained on titrating 30 ml of toluene with hydrogen bromide solution is shown as curve *a*, Figure 3. The initial drop in potential as the titrant was added was typical of toluene and solutions of toluene and oil or methyl esters. In pure toluene, before the addition of hydrogen bromide solution, the potentiometric reading varied, apparently depending on minute, transient variations in the essentially nonconducting system. After the first increments of titrant, changes occurred in conductivity, characteristics of the electrodes and possibly other characteristics of the system. After the initial change, additional increments of titrant did not change the potential. The dashed portion of curve *a*, Figure 2, connects the original potential reading with that obtained after the first indicated amount of titrant had been added. This change in potential at the start of the titration did not consume any measurable amount of titrant. Variations in the amount of drop did not affect the amount of cyclopropenes found by titration.

The potentiometric curve for the titration of the cyclopropene-free methyl esters prepared from peanut oil (curve *b*, Fig. 3) does not show the absolute potentials recorded for pure toluene. Duplicate samples usually did not show the same absolute potentials. Curve *c*, Figure 3, represents the titration of about 0.3 g of purified *Sterculia foetida* oil. According to this titration it contained 56.67% cyclopropenes, calculated as sterculic acid. Analysis by essentially the same method, except that crystal violet was used as indicator (4), showed the same cyclopropene content within experimental error.

Methyl esters derived from a refined cottonseed oil of good quality but not further purified by passage through alumina were analyzed for cyclopropenes by potentiometric titration and by titration under the same conditions,

TABLE I

Cyclopropene Content of Methyl Esters

Amount of methyl esters, g		Cyclopropene content,[a] %	
Cottonseed oil	Peanut oil	Found	Present
9.9301	None	0.573	
4.9600	4.9495	0.277	0.286
2.4974	7.4331	0.143	0.144
None	5.0808	0.000	0.000

[a]Calculated as malvalic acid.

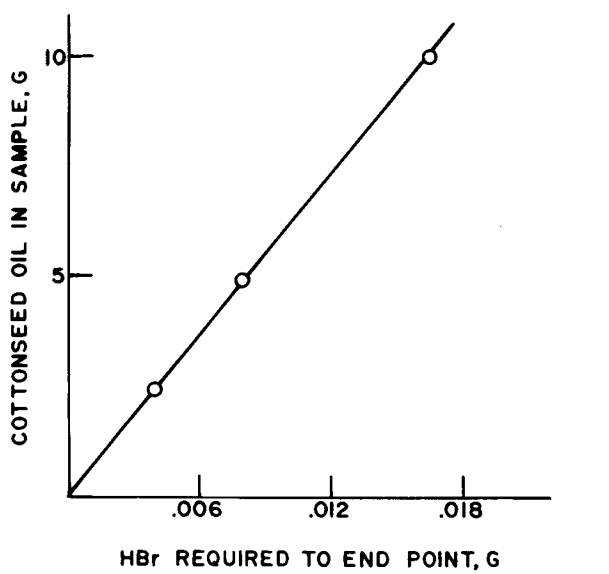

FIG. 5. Correlation between amount of methyl esters derived from cottonseed oil and amount of hydrogen bromide solution required for titration.

except that the color indicator crystal violet was employed to determine the end point (4). The percentage of cyclopropenes, calculated as malvalic acid, found in triplicate determinations by each method, were as follows:

Potentiometric titration	Titration to crystal violet end point
0.686%	0.688%
0.696%	0.692%
0.682%	0.684%
Average, 0.691%	Average, 0.688%

Samples of methyl esters derived from refined cottonseed oil and from refined peanut oil were further purified by passage through alumina. The two methyl esters and two mixtures of the methyl esters were analyzed for cyclopropene content. Three of the titration curves obtained are shown in Figure 4, and the amounts of cyclopropenes are recorded in Table I. The titrant required was directly proportional to the amount of methyl ester from the cottonseed oil (Fig. 5).

ACKNOWLEDGMENTS

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Alteration of Long Chain Fatty Acids of Herring Oil during Hydrogenation on Nickel Catalyst[1]

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ABSTRACT

During hydrogenation of a refined herring *(Clupea harengus)* oil iodine value (IV) 119, on a commercial nickel catalyst, samples were collected at IV 108, 101, 88 and 79. In the early stages of the process, IV 119 to IV 101, the positional and geometrical isomerization of the long chain monoenoic fatty acids (20:1 and 22:1) was hindered by the stronger absorption on the catalyst surface of the polyenes with 4, 5 and 6 double bonds. Consequently at IV 101, 70% of these polyenes had been converted to dienoic and trienoic fatty acids, but only 3-4% *trans* 20:1 and 22:1 accumulated. As the hydrogenation proceeded, IV 101 to IV 79, the original *cis* 20:1 and 22:1 isomers (mainly Δ11 with some Δ9 and Δ13) decreased and new positional and geometrical isomers (both *cis* and *trans* in positions Δ6 to Δ15) were formed. The major *trans* isomers were Δ11 accompanied by important proportions of Δ10 and Δ12. At IV 79, more *trans* 20:1 (ca. 36%) than *trans* 22:1 (ca. 29%) was detected. Monoethylenic fatty acids newly formed from polyethylenic fatty acids made only minor contributions to the total 20:1 and 22:1 at these levels of hydrogenation, but a "memory effect" which skews the proportions of minor *cis* and *trans* isomers can be attributed to the proportions of minor *cis* 22:1 isomers (Δ9, Δ13 and Δ15) orginally present.

INTRODUCTION

Vegetable oils dominate world commerce (1) and their hydrogenation has received much attention (2-8). Much less data is available on the subject of fish oil hydrogenations (9-13). The main difference between unprocessed vegetable and marine oils serving as raw materials is the higher degree of unsaturation in some of the fatty acids of the latter. In the marine oils a further difference lies in the position of the ethylenic unsaturation in the eicosenoic (20:1) and docosenoic (22:1) acids relative to the position of bonds in the corresponding polyethylenic fatty acids. The polyethylenic acids of vegetable oils, mainly 18:2 with some 18:3, have bonds in Δ9, Δ12 and Δ15 positions, the former corresponding to the position of the principal octadecenoic acid isomer (18:1Δ9). Similarly in marine oils, the polyethylenic acid 20:5 has bonds in the Δ5, Δ8, Δ11, Δ14 and Δ17 positions, the Δ11 corresponding to the dominant eicosenoic isomer (20:1Δ11). However, in the important 22:6 acid, the positions of the ethylenic unsaturation are Δ4, Δ7, Δ10, Δ13, Δ16 and Δ19, none of which correspond to an ethylenic bond position in the dominant docosenoic isomer (22:1Δ11). Comparisons among products relating to this positional difference could be informative as to the mechanisms of hydrogenation.

Discrepancies exist among the publications of Ackman et al. (9); Conacher et al. (10) and Lund and Hølmer (12) as to the positional distributions of ethylenic bonds in the *cis* and *trans* 20:1 and 22:1 fatty acids of partially hydrogenated marine oils. Accordingly, we have reinvestigated the formation and behavior of these long chain monoethylenic fatty acids during the hydrogenation process. The high content of 20:1 and 22:1 in Canadian herring oil (14), hydrogenated according to commercial practice (practically no change in saturates and monoenes content), facilitates comparison of the original monoethylenic fatty acids with those produced by isomerization only, and with those produced from reduction of the more highly unsaturated fatty acids.

MATERIALS AND METHODS

Samples of herring oil were collected during a pilot scale hydrogenation executed according to Canadian commercial practice with nickel catalyst (0.2%), at 190-225 C, and

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