Titration of Cyclopropene Esters With Hydrogen Bromide[1]

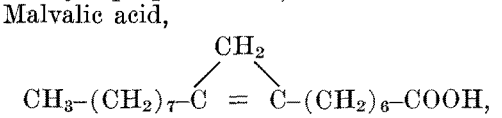
R. O. FEUGE, ZIGRIDA ZARINS, JOHN L. WHITE and RAIFORD L. HOLMES,
Southern Regional Research Laboratory,[2] New Orleans, Louisiana 70119

Abstract

Esters of the naturally occurring cyclopropene acids have heretofore been determined by titration with hydrogen bromide in glacial acetic acid. However, highly purified cyclopropenes had an apparent purity of only 83–86% by this method. The catalyzed addition of acetic acid during the titration has been shown to occur. Substituting toluene for the acetic acid not only gives the correct cyclopropene content, but also sharpens the end point of the titration. The new titration is performed at 70–75 C and 1,3-diphenylguanidine, which is soluble in toluene, should be used as a primary standard. The indicator solution is 0.03% crystal violet in butyric acid. Mono- and diglycerides and oxidized fatty compounds must be removed before titration. Oxirane oxygen can be determined by the new procedure, probably with an accuracy greater than that possible with hydrogen bromide in glacial acetic acid.

Introduction

The seed oils of many species of the order Malvales, including *Gossypium hirsutum* or Upland cotton, contain the cyclopropene acids, malvalic and sterculic (1). Malvalic acid,



occurs in cottonseed oil to a greater extent than does the C_{19} homolog sterculic (2), but their total amount is usually below 1%. Because a number of investigators have shown these cyclopropene acids to have unusual physiological activity (3), interest in methods of analysis is obvious.

The simplest and most convenient of these analytical methods consists of a titration with hydrogen bromide. To bring about reasonably rapid addition of hydrogen bromide to the cyclopropene moiety, anhydrous conditions and the proper organic solvent must be employed. In the methods described heretofore (4–8), Durbetaki reagent (acetic acid-hydrogen bromide) was employed. The sample was dissolved in three parts glacial acetic acid and one part benzene and titrated at about 55 C with 0.1 N hydrogen bromide in glacial acetic acid with crystal violet as indicator. Most of the research on and with these methods was conducted with impure esters of the cyclopropene acids, so there was little opportunity to verify the supposition that 1 mole of hydrogen bromide added to 1 mole of the cyclopropene moiety. However, in at least two instances the finding of cyclopropene concentrations of about 97% by such titrations has been reported (9,10). The cyclopropene contents determined by these methods were always reproducible and were not affected by ordinary variations in titration time, indicating that the acetic acid did not by itself react with the cyclopropene moiety under the titration conditions. However, in our laboratory the most highly purified samples of methyl malvalate and methyl sterculate derived from such diverse sources as cottonseed oil and the seed oils of *Sterculia foetida* and *Hibiscus syriacus* invariably analyzed 83% to 86% cyclopropenes by these same methods. Other criteria of purity indicated the values should have approached 100%.

The titration of the cyclopropenes with hydrogen bromide was examined with the objectives of identifying the problem with the heretofore published procedures and devising an improved procedure.

Experimental Procedure

Materials and Reagents

Two samples of methyl sterculate derived from different samples of *Sterculia foetida* oil were employed. In each case the oil was extracted from dehulled and crushed seeds with hexane at room temperature. The oils were refined with alkali and subjected to a methanolysis catalyzed by sodium methoxide. The methyl esters were fractionated in one case by repeated urea clathrations and in the other by crystallizations from methanol (11). The best methyl sterculate fraction from each procedure was dissolved in petroleum ether and passed through activated alumina (11).

The methyl sterculate obtained by repeated urea clathration contained impurities amounting to only a few tenths of 1%, at most. Analysis by gas liquid chromatography (GLC) and thin layer chromatography (TLC) of both the methyl sterculate and its methyl mercaptan addition product (9) revealed no impurities. The melting point of this methyl sterculate was −11.3 C. Hydrogenation of the sterculate yielded a product which on GLC analysis was found to consist of approximately 98% methyl dihydrosterculate and about 2% methyl esters of C_{19}, branched-chain fatty acids.

The methyl sterculate prepared by fractional crystallization, which will hereafter be referred to as methyl sterculate concentrate, was subjected to the examinations described above. Its purity was estimated to be 93 ± 1%, the impurities being almost entirely methyl oleate and methyl linoleate.

The cottonseed oil was a freshly refined and bleached product obtained from a commercial processor. It was further purified by passing a petroleum ether solution of the oil through a column of activated alumina (one part oil and four parts alumina). Methyl esters were prepared from a portion of the purified oil by a methanolysis catalyzed by sodium methoxide, and these esters also were treated with alumina.

Corn oil (refined, bleached and deodorized) was obtained from a commercial supplier. It was further purified and methyl esters were prepared as in the case of the cottonseed oil. A portion of the corn oil was held in an open dish at 60 C until a peroxide value of 31 milliequivalents/kg of oil was reached. A portion of the oxidized oil was bleached at 120 C with 6% of a neutral, activated clay.

The 1-monostearin and 1,3-distearin employed to established the effect of mono- and diglycerides in oils being analyzed were highly purified compounds (>99%) available from previous research. The

[1] Presented at the 41st Fall Meeting of the American Oil Chemists' Society, Chicago, Ill., October 15–18, 1967.
[2] South. Utiliz. Res. and Dev. Div., ARS, USDA.

methyl 9,10-epoxystearate, prepared by treating highly purified methyl oleate with *m*-chloroperbenzoic acid, contained 94.5% methyl epoxystearate, calculated from the content of oxirane oxygen determined by the AOCS method.

All of the solvents, fatty acids and other compounds were of analytical grade.

Equipment. Several titration assemblies were devised and tested. The one finally adopted used a 10 ml, automatic burette with a Teflon plug (Kimax No. 17138-F). The burette was modified by fitting it with a Luer tip to accept Teflon needles. A tube of Drierite protected the contents of the burette from moisture. A Teflon needle (No. 22 or 24) about 30 cm long was attached to the Luer tip and the lower end of the needle was passed through a neoprene stopper so that the tip of the needle just touched the bottom of the 50 ml Erlenmeyer used to hold the sample being titrated. The neoprene stopper was fitted with a second Teflon needle which did not extend into the solution being titrated. The second needle served as a breather and was also used to add the indicator solution midway in the titration.

To control the temperature of the titration, the sample-containing Erlenmeyer was placed in a crystallizing dish partly filled with paraffin oil. This assembly was placed on a magnetic stirrer—hot plate combination. A Teflon-coated stirring bar was used in the Erlenmeyer flask.

Hydrogen Bromide Catalyzed Addition of Acetic Acid. To demonstrate that hydrogen bromide catalyzes the addition of acetic acid to the cyclopropene moiety in the heretofore used methods (6–8), a series of titrations was conducted at 55 C in which 0.1 N hydrogen bromide in benzene was substituted for 0.1 N hydrogen bromide in glacial acetic acid and varying ratios of acetic acid to benzene instead of the usual 3:1 ratio were used in the solvent (12).

The reaction products of two of these titrations were recovered by evaporating the solvents at a low temperature and then analyzed by TLC (silicic acid plate; solvent, hexane-diethyl ether). Also analyzed by TLC were the original methyl sterculate, the sterculate after refluxing with glacial acetic acid for 4 hr, and the reaction product from a titration conducted in the heretofore normal manner. It was established that titrations in acetic acid-benzene solutions produced compounds in addition to those found on titrating in benzene only and that the additional compounds behaved on TLC analysis like the compounds produced on refluxing methyl sterculate with glacial acetic acid.

The same reaction products and others were examined by reversed-phase TLC and the same conclusions were reached. Infrared spectra of the reaction products and thin layer chromatographs of these products and of fatty acids confirmed that the reaction products contained no free carboxyl groups. Other tests established that no unreacted methyl sterculate remained after the refluxing with glacial acetic acid and that this reaction product contained acetyl groups. The bromine content of the product from the titration in benzene only was higher than that for the product from the titration in glacial acetic acid.

Selection of Solvent. In searching for a substitute for acetic acid in the cyclopropene titration, preliminary tests were made with a number of solvents. Water-containing solutions, alcohols and esters of low molecular weight were entirely unsatisfactory. Titrations in propionic acid proved to be relatively slow and the end points were poor. Solvents such as bromobenzene and trichlorotrifluoroethane dissolved relatively little hydrogen bromide.

The aromatic hydrocarbons, benzene, toluene and xylene, proved to be the best solvents generally available. They exhibit some of the properties of Lewis bases and form loose addition compounds with hydrogen bromide. Toluene was considered superior to benzene because it has a lower vapor pressure. By passing gaseous hydrogen bromide through toluene at atmospheric pressure and room temperature (25.8 C) solutions up to 0.84 N were prepared.

Analytical grade toluene as received generally contains from 0.01% to 0.03% of water. Passing hydrogen bromide into such toluene produces an immediate haze and after standing for several days small droplets of aqueous solution separate. Therefore, the toluene should first be dried, which can be accomplished by passing dry nitrogen through the toluene for a short time. Solutions of hydrogen bromide in dry toluene, unlike solutions in acetic acid, are quite stable; the normality changes little with time and there is little change in color.

Substitution of toluene for glacial acetic acid has another advantage; the color change on titrating to the crystal violet end point is much more rapid. When adding 0.1 N hydrogen bromide to 20 ml of glacial acetic acid containing the crystal violet indicator, several drops are required to change the color from purple to yellow. With toluene the change occurs with a fraction of one drop or about 0.01 ml.

Titrant. The suitability of titrants other than hydrogen bromide was considered briefly. Hydrogen chloride, as anticipated, was less soluble in toluene than was hydrogen bromide, and its reactivity with the cyclopropene moiety was much lower. Hydrogen iodide, on the other hand, was more reactive than was hydrogen bromide, but hydrogen iodide decomposes easily to yield free iodine.

Indicator Solution. While toluene dissolves a sufficient amount of the indicator crystal violet, the solution is not deeply colored, and on the addition of hydrogen bromide the acid form of crystal violet becomes quite insoluble and precipitates. A number of other indicators were tested and also found to be unsatisfactory. Subsequently, the performance of crystal violet was improved by dissolving it in a fatty acid (acetic, butyric, caproic or caprylic) and adding a small amount of this solution midway in a titration. Each solution colored toluene a deep purple, the basic color of crystal violet. On adding hydrogen bromide the yellow or acid form of crystal violet was produced in each case, but with the acetic acid turbid solutions frequently formed. Butyric acid was considered to be the best solvent for the crystal violet.

In a titration employing 20 ml toluene as solvent, the addition of 0.1 ml of butyric acid containing 0.03% crystal violet was sufficient when added after the midpoint of the titration had been reached. However, adding several 0.1 ml portions during the titration and one just before the end point did not appear to adversely affect the titration and made determination of the end point easier.

To establish that the addition of a small amount of butyric acid during a titration has no significant effect on the values obtained, a series of titrations was conducted at 70–75 C in solutions of toluene-butyric acid and, for comparison, in toluene-acetic acid (see Table I).

Primary Standard. 1,3-Diphenylguanidine, one of the standards which has been used for acid-base

TABLE I

Titration of Methyl Sterculate Concentrate in Toluene-Acetic Acid and Toluene-Butyric Acid Solutions[a]

Fatty acid in solvent, %	Sterculate found,[b] %	
	Acetic acid solution	Butyric acid solution
0	100.0	100.0
5	97.0	99.9
30	92.3	97.0
100	85.2	93.7

[a] Titrated at 70–75 C with 0.1 N HBr in toluene.
[b] Calculated on basis of total amount in solution. Concentrate contained 93.3% methyl sterculate.

titrations, was found to be the best compound for standardizing hydrogen bromide in toluene. Diphenylguanidine is soluble in toluene and can be used under the same conditions employed to titrate the cyclopropenes. While the resulting reaction product is insoluble in toluene, its formation does not appear to interfere with the standardization.

Interfering Substances. Both 1-monostearin and 1,3-distearin in toluene solution can be titrated with 0.1 N hydrogen bromide in toluene at 70–75 C. However, the reaction rate is quite slow. The addition of two or three drops of titrant will change the color of the crystal violet from purple to yellow. Then in the course of 5 to 10 sec the color will return to purple. Obviously, in titrating for cyclopropenes the samples have to be free of mono- and diglycerides.

Corn oil oxidized to a peroxide value of 31 behaved much like the mono- and diglycerides. Even after the oil was bleached with 6% neutral activated clay at 120 C some compounds which reacted with hydrogen bromide were still present.

Titration of Oxirane Oxygen. As anticipated, methyl 9,10-epoxystearate dissolved in toluene could be readily titrated. However, for the reaction to proceed at a good rate the temperature had to be raised to 40 C. The precision obtained was good but the percentage of methyl epoxystearate found was slightly higher than that calculated from titration data for oxirane oxygen determined according to the AOCS method. The values were 95.6 and 94.5, respectively. This suggests the possibility that hydrogen bromide also catalyzes the addition of acetic acid to epoxides.

Titration Temperature. The use of toluene (bp 110 6 C) as a solvent for the sample and the use of a Teflon needle to introduce the titrant below the surface of the solution being titrated permits an increase in temperature well above the 55 C previously employed and eliminates the necessity for close temperature control. At 55 C the reaction between hydrogen bromide and the cyclopropenes was quite slow. At 70–75 C, the preferred temperature, the reaction rate was satisfactory. Titrations were conducted at temperatures up to 90 C without noticing adverse effects.

Type and Size of Sample. The cyclopropene content of mixed triglycerides or of a natural oil could be determined when the product contained no oxidized components, monoglycerides or diglycerides. The refined and bleached cottonseed oil, which was of good quality and which had been freed of any minor amounts of these impurities by treatment with alumina, consistently analyzed 0.76% cyclopropenes, calculated as methyl malvalate, when 5 to 12 g was dissolved in 20 ml of toluene and titrated with 0.1 N hydrogen bromide. Methyl esters prepared from the cottonseed oil also titrated 0.76% cyclopropenes.

Under the same conditions 5 g of purified corn oil required only one drop (0.01 ml) of 0.1 N hydrogen bromide to titrate to the crystal violet end point, and 10 g of oil required less than two drops. However, as the amount of corn oil increased above about 12 g/20 ml of toluene, the end point became less sharp.

TABLE II

Analysis of Mixtures of Methyl Sterculate and Methyl Esters of Corn Oil Fatty Acids

Approx. wt. of Sample, g	Methyl sterculate	
	Present	Found[a]
0.15	100.0	99.9
0.34	72.1	72.2
0.50	47.9	47.9
1.0	23.83	23.87
2.5	9.57	9.60
4.9	4.87	4.84
13.0	1.99	1.93
5.0	0.00	0.00

[a] Average of two analyses.

Four successive titrations of the highly purified methyl sterculate indicated purities of 100.0%, 99.8%, 100.1% and 99.9%. For these titrations the sample weights range from 0.1240 to 0.1855 g.

Solutions of the highly purified methyl sterculate and the purified methyl esters derived from corn oil were prepared and titrated. The results are recorded in Table II.

As is evident from the table, the sample weights varied about 38-fold. The amount of 0.1 N hydrogen bromide (not shown in the Table) ranged between 7.63 and 8.30 ml for the mixtures.

Method

Reagents. Dry toluene; 1,3-diphenylguanidine; anhydrous hydrogen bromide; crystal violet indicator; and butyric acid.

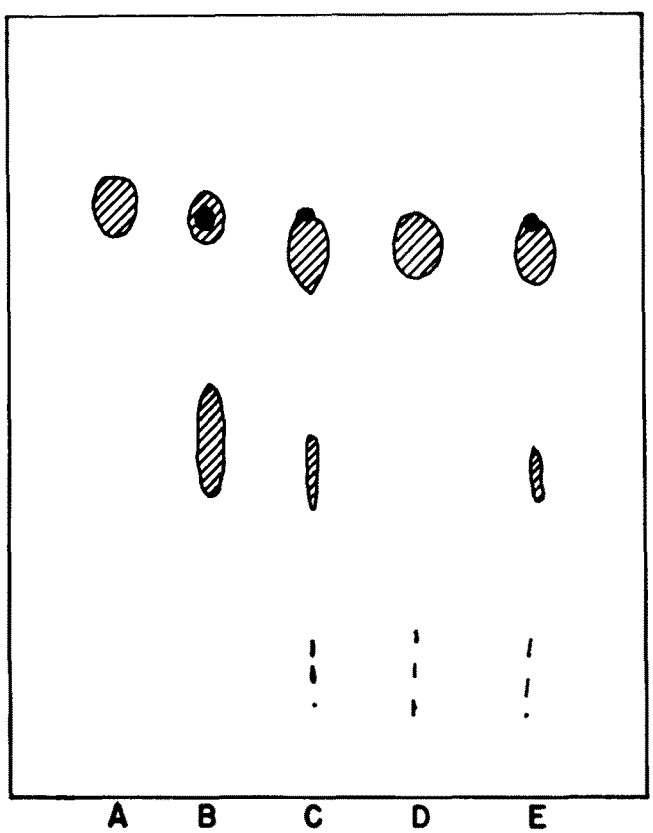


FIG. 1. Thin layer chromatograms of (A) pure methyl sterculate, (B) methyl sterculate refluxed with glacial acetic acid, (C) methyl sterculate dissolved in glacial acetic acid and titrated with hydrogen bromide dissolved in benzene, (D) methyl sterculate titrated with hydrogen bromide in benzene solution (no acetic acid present), and (E) methyl sterculate dissolved in acetic acid-benzene solution and titrated with hydrogen bromide in acetic acid (6).

Solutions. Prepare a solution of 0.1 N hydrogen bromide by bubbling anhydrous hydrogen bromide through analytical grade toluene which has been stripped with dry, purified nitrogen to remove practically all of the 0.01% to 0.03% moisture normally found in toluene; prepare a solution of 0.03% crystal violet in butyric acid.

Apparatus. A Machlet automatic buret or a Kimax No. 17138-F buret (10 ml) equipped with a Teflon plug, a drying tube and a Luer tip. Teflon needles, No. 22 or 24; a hotplate-magnetic stirrer combination and Teflon coated stirring bars; thermometer; crystallizing dish partially filled with liquid paraffin for use as a heating bath; and glass-stoppered, 50 ml Erlenmeyer flasks.

Procedure. Standardize the solution of hydrogen bromide in toluene by titrating a weighed amount of 1,3-diphenylguanidine dissolved in toluene. Follow the procedure used for the cyclopropene samples as described below.

Weigh out samples of the cyclopropene-containing methyl esters of such amounts that a titration with 0.1 N hydrogen bromide requires from 1 to 10 ml of solution. Do not use more than 10 g of mixed methyl esters per sample. Transfer the weighed samples to the 50 ml Erlenmeyer flasks, add 20 ml of dry toluene to each and add a magnetic stirring bar to each flask. As each sample is titrated, remove the flask stopper from the Erlenmeyer flask and substitute a neoprene stopper through which two Teflon needles have been inserted. One needle, which must extend below the surface of the solution in the Erlenmeyer flask, is used to deliver the 0.1 N hydrogen bromide solution. The other needle, which must not extend below the surface of the solution, serves as a breather and is also used to add the indicator solution midway in the titration.

Place in the liquid paraffin bath the sample to be titrated, turn on the magnetic stirrer, and heat the system to 70–75 C. Start the titration. When the titration is about half way complete, add 0.1 ml of butyric acid containing 0.03% crystal violet indicator. Titrate to the first color change.

Note. For these titrations methyl esters are normally employed, and they must be free of epoxides, mono- and diglycerides, hydroperoxides, and other oxidized materials. These impurities can usually be removed by passing the mixed methyl esters in a petroleum ether solution through an alumina column (one part ester to four parts alumina). Removal of some of these impurities from triglycerides by an alumina treatment is difficult.

ACKNOWLEDGMENT

This work was supported in part by a grant from the Foundation for Cotton Research and Education.

REFERENCES

1. Carter, F. L., and V. L. Frampton, Chem. Rev. *64*, 497–525 (1964).
2. Shenstone, F. S., and J. R. Vickery, Nature *190*, 168–169 (1961).
3. Phelps, R. A., F. S. Shenstone, A. R. Kemmerer and R. J. Evans, Poultry Science *44*, 358–394 (1965).
4. Smith, C. R., Jr., M. C. Burnett, T. L. Wilson, R. L. Lohmar and I. A. Wolff, JAOCS *37*, 320–323 (1960).
5. Wilson, T. L., C. R. Smith, Jr. and K. L. Mikolajczak, Ibid. *38*, 696–699 (1961).
6. Harris, J. A., F. C. Magne and E. L. Skau, Ibid. *40*, 718–720 (1963).
7. Harris, J. A., F. C. Magne and E. L. Skau, Ibid. *41*, 309–311 (1964).
8. Magne, F. C., J. A. Harris, R. A. Pittman and E. L. Skau, Ibid. *43*, 519–524 (1966).
9. Raju, P. K., and Raymond Reiser, Lipids *1*, 10–15 (1966).
10. Hammonds, T. W., and G. G. Shone, Analyst *91*, 455–458 (1966).
11. Nordby, H. E., B. W. Heywang, H. W. Kircher and A. R. Kemmerer, JAOCS *39*, 183–185 (1962).
12. Feuge, R. O., Zigrida Zarins, J. L. White and R. L. Holmes, Ibid. *44*, 548 (1967).

[Received July 8, 1968]