Methods for the Determination of Cyclopropenoid Fatty Acids: VIII. The HBr Titration Method Applied to Small Samples

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Abstract

A method is described for the determination of cyclopropenoid fatty acids in small samples of refined and crude glyceride oils and methyl esters. Approximately 0.5 g of cottonseed oil is titrated with HBr-HOAc by a stepwise procedure at 3 C and 55 C after removal of interfering substances by adsorption on activated alumina. Side reactions occurring to the extent of 15% during the titration were uniform and highly reproducible, permitting application of a correction factor. A procedure is given for preparing methyl esters on a small scale to determine the cyclopropenoid content of highly oxidized oils.

Introduction

A previous publication (1) described the determination of the cyclopropenoid fatty acids in refined and crude cottonseed oils by a stepwise titration with HBr in acetic acid at 3 C and at 55 C. Traces of interfering substances were removed by adsorption on activated alumina. Higher concentrations of interfering substances as found in very rancid or highly oxidized oils could not be removed on alumina, but methanolysis of the oil followed by adsorption on alumina was found to be effective. Magne et al., pointed out that removal of high levels of interferences introduced error in the subsequent analysis and suggested a dilution technique (2) to correct the bias in lieu of a difficult materials balance separation. Twenty-five gram samples were taken for treatment on alumina and subsequent analysis. A method requiring less sample was desired for experiments in which oil yield was severely limited. By suitable modifications of the procedure of Harris et al. (1), satisfactory determination of the cyclopropenoid content can be done on 1.5–2 g of cottonseed oil or a drop of concentrate.

Experimental Procedures

Titrations were done in 10 ml Erlenmeyer flasks with heavy rim. An occasional erratic blank or standardization indicated contamination. A set of flasks was selected and used repeatedly without contacting tap water. After cleaning with solvents or distilled water the final rinse should be acetic acid.

Two sizes of columns for chromatographic adsorption were used. For 1.5–2 g samples the column measures 7 mm o.d. × 6 cm tip, 12 mm o.d. × 17 cm body and 15 mm o.d. × 8 cm top. For smaller samples the column measures 6 mm o.d. × 6 cm tip, 10 mm o.d. × 15 cm body and 15 mm o.d. × 8 cm top.

A 5 ml reservoir buret (Kimble No. 17138F) graduated in 0.01 ml permits estimation of volume delivered to .001 ml. A Teflon plug is essential since acetic acid rapidly removes grease from a glass plug. Since the titrations are done in a closed system, the HBr-acetic acid reagent must be added in whole drop increments. A drop is approximately 0.012 ml. With 0.01N HBr-HOAc one drop produces a recognizable color change in the titration of sodium carbonate, but with an oil the change is less distinguishable. A concentration of 0.015 to 0.02 N was chosen for use. A precision of approximately 0.01% cyclopropene acid was desired. Calculation showed that 0.5 g of cottonseed oil would be the minimum that could give such precision.

Additives to the Durbetaki reagent recommended for the oxirane determination such as hydroquinone (3) and quaternary ammonium halides (4,5) did not improve the cyclopropenoid titration.

Reagents

Activated alumina grade F-20, 80–200 mesh, Aluminum Company of America (6).

Standard 0.02 N hydrogen bromide in glacial acetic acid (Durbetaki reagent). The concentrate (Eastman No. 1161) is diluted to approximately 0.02 N with acetic acid. The reagent is standardized daily against approximately 4 mg samples of primary standard sodium carbonate weighed on a microbalance and dissolved in 0.7 ml benzene and 1.5 ml acetic acid. In the absence of a microbalance a suitable aliquot (1–2 ml) of a standard solution of sodium carbonate in acetic acid can be used.

Crystal violet indicator, 0.04% in acetic acid.

As solvent for the oil, 1.5 ml acetic acid and 0.5 ml of benzene was tried, but an occasional sample froze in the 3 C bath. Increasing the benzene to 0.7 ml removed this difficulty.

Alumina Treatment

As previously shown (1), refined cottonseed oils (alkali refined, washed, dried) and commercial salad oils (refined, bleached, deodorized) require a single column treatment using petroleum ether (bp 30–60 C) as solvent to remove interferences. Crude oils and esters require a dual column treatment. The sample is first passed through an alumina column using a diethyl ether-methanol (39:1) solvent as in the official AOCS method for neutral oil (6) followed by a petroleum ether column treatment. An amount of alumina four times the weight of sample (1.5 g of refined oil or salad oil, 2 g of crude oil or ester) is poured slowly into a chromatographic tube containing sufficient solvent to cover the adsorbent. Occasional swirling of the tube helps to wet the alumina and prevent blockage. The sample dissolved in 2–4 ml of solvent is poured on the column when the solvent has drained within 0.5 cm of the top of the packing. Two or three washings with additional solvent serve to transfer the sample essentially quantitatively to the column. It is allowed to percolate by gravity and is eluted with 25–30 ml of solvent added from an automatic siphoning separatory funnel, never allowing the top of the packing to be exposed to the atmosphere. The eluate is received in a 50 or 100 ml round bottom flask. BHA or hydroquinone (0.01% of sample weight) dissolved in ca 1 ml ether-hexane added to the receiver prevents reoxidation of the sample. The solvent is stripped from the sample on a rotary evaporator under reduced pressure at 55 C ± 5. Nitrogen is

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used to break the vacuum and provide an oxygen free environment.

Titration

Duplicate samples from the adsorption column are accurately weighed into 10 ml Erlenmeyer flasks, followed by 0.7 ml of benzene, 1.5 ml acetic acid, 4 drops of indicator and a micro stirring bar (Arthur H. Thomas No. 9235). Sample size may range from 0.5 g for cottonseed oil to 0.02 g for pure material. The flask is attached to the buret with a rubber stopper, incorporating a hypodermic needle or other means of pressure relief. After 1 min in an ice bath with magnetic stirring, the sample is titrated at 3 C to a bluegreen end point of at least 30 sec duration. The ice bath is replaced with a water bath maintained at 55 C by means of a thermostatically controlled hot plate equipped for magnetic stirring. Reagent is added dropwise until the same blue-green end point is reached. The two titrations are recorded separately. Solvent blank corrections are applied. The net titration at 3 C should not exceed one drop; more than one drop indicates interferences still present. In such cases an additional alumina treatment or a higher ratio (e.g., 5:1) is necessary. The titration at 55 C is a measure of the cyclopropenoid content.

$$\% \text{ Malvalic Acid} = .2804\ (Nv/w)1.175.100$$

Where v is the number of ml of HBr-HOAc solution of normality N required for w g of sample. Other cyclopropenoid compounds are similarly calculated, using the appropriate milliequivalent weight in place of .2804.

Methanolysis

The interferences in rancid and highly oxidized oils are not easily removed, even by several successive alumina treatments. Time and sample will be conserved by converting the oil to methyl esters and analyzing the esters. The validity of this approach has previously been established (1). Sodium methoxide is prepared by adding a weighed piece of clean sodium metal (30–40 mg) to 8 ml of absolute methanol in a 25 ml stoppered Erlenmeyer flask. When reaction is complete more methanol is added to give a concentration of 3 mg of sodium per ml in the final solution. The sample of approximately 2.5 g is weighed into a 25 ml Erlenmeyer flask with 3 ml of methanol and a stirring bar. Sodium methoxide is added a drop or two at a time with stirring until the mixture becomes alkaline to m-cresol purple indicator paper. More than 8 or 10 drops indicates a high free fatty acid content; in this case the neutral oil should be prepared as previously described for the alumina treatment of a crude oil (6). An additional 2.5 ml of sodium methoxide is added and the mixture refluxed for 1 hr with stirring. The flask is removed and cooled to room temperature. The solution is transferred to a separatory funnel (60 ml French style, Kimble No. 29044F is most effective) with several washes of petroleum ether totaling 15–20 ml. The esters are washed with a large excess of water containing 2 drops of acetic acid. The aqueous layer is discarded and the esters washed again with water containing 1 drop of acetic acid. At least three more portions of distilled water complete the washing. The solution is transferred to a 125 ml Erlenmeyer and dried with anhydrous sodium sulfate (2–5 g) with stirring at least 30 min. Finally the solution is filtered into a 100 ml round bottom flask and stripped on a rotary evaporator at 55 C ± 5. Generous washing with petroleum ether at each transfer or decantation will keep losses at a minimum; a large excess of petroleum ether is desirable, especially during the water washing. Yield of esters will be at least 2 g.

TABLE I

Comparative Analyses for Cyclopropenoid Content of Cottonseed Oils and Esters by Established Macroprocedure and This Procedure

Sample	Description	Macroprocedure, %	This Procedure, %
Malvalic acid[a]			
1	Corn oil	0.00, 0.00	0.00, 0.00
2[b]	Cottonseed salad oil	0.31, 0.32, 0.31	0.31, 0.31, 0.31
3	Cottonseed salad oil	0.33, 0.33	0.33, 0.32
4	Cottonseed salad oil	0.05, 0.06, 0.06 0.06	0.05, 0.05
5	Cottonseed salad oil	0.06, 0.06	0.05, 0.04
6	Cottonseed salad oil	0.29, 0.30, 0.30	0.34, 0.32
7	Refined cottonseed oil	0.62, 0.61	0.64, 0.61
8	Refined cottonseed oil	0.69, 0.68	0.65, 0.65
9	Refined cottonseed oil	0.64, 0.65	0.62, 0.62
10	Refined cottonseed oil	0.64, 0.64	0.62, 0.62
11	Refined cottonseed oil	0.62, 0.61	0.62, 0.60
12	Refined cottonseed oil	0.62, 0.62	0.57, 0.58
13	Crude cottonseed oil, hydraulic	0.60, 0.60	0.60, 0.61
14[b]	Crude cottonseed oil, screw pressed	0.66, 0.65	0.67, 0.68
15[b]	Crude cottonseed oil, screw pressed	0.55, 0.56	0.58, 0.58
16	Crude cottonseed oil, prepress-solvent	0.60, 0.61	0.57, 0.58
17[b]	Crude cottonseed oil, solvent		
Methyl Malvalate[a]			
18	Methyl esters of sample 2	0.31, 0.30	0.29, 0.27
19	Methyl esters of sample 14	0.62, 0.62	0.60, 0.59
20	Methyl esters of sample 15	0.55, 0.54	0.52, 0.51
21	Methyl esters of sample 17	0.58, 0.58	0.61, 0.62
22	Concentrate of cottonseed oil methyl esters	5.44, 5.46	5.45, 5.47

[a] Original data, before applying proposed factor.
[b] Methyl esters also prepared and analyzed.
[c] Necessary to prepare esters, interferences could not be removed.

Results

The effectiveness of chromatographic adsorption for removing interferences without affecting the cyclopropenoid content of cottonseed oils was demonstrated by previous workers (1) except for very high levels as previously noted (2). That the scaled down procedure reported here is equally effective is shown by the comparative data in Table I where salad oils,

TABLE II

Hydrobromination of Pure Cyclopropenoids

Sample	Description	Hydrobromination %	Cyclopropene factor
1	Methyl sterculate	85.25	1.173
		84.90	1.178
		84.57	1.182
		85.12	1.175
		84.73	1.180
		85.08	1.175
		84.50	1.183
2	Methyl sterculate	84.78	1.180
		85.37	1.171
		85.37	1.171
		84.93	1.177
		85.05	1.176
3	Methyl sterculate	85.20	1.174
		85.18	1.174
		85.45	1.170
		85.28	1.173
		85.58	1.168
3a[a]	No. 3 diluted to 57.65%	48.94	1.178
3b[a]	No. 3 diluted to 26.73%	22.71	1.177
3c[a]	No. 3 diluted to 12.28%	10.44	1.175
4	Methyl malvalate	84.35	1.186
		85.24	1.173
		85.22	1.173

[a] Diluent: pure methyl oleate.

refined oils, crude oils and esters were analyzed by the procedure of Harris et al. and by this procedure.

Feuge et al. (7) reported that HBr catalyzes the acetylation of the cyclopropene moiety, resulting in a mixture of brominated and acetylated products when HBr-HOAc is used as titrating reagent. They suggested toluene as solvent for HBr. Attempts to use HBr-toluene on a micro scale were not successful. The indicator precipitates, leaving too little color to see in the small volume. As an alternative the side reactions with acetic acid were examined as to extent and reproducibility. Several samples of cyclopropenoids whose purity had been established by GLC and high resolution NMR spectroscopy were analyzed repeatedly by this proposed HBr-HOAc titration method. The data shown in Table II demonstrate that 85% of the cyclopropene is hydrobrominated. The reaction is sufficiently reproducible to justify use of a factor, 1.175, to arrive at the true cyclopropene content. This procedure can be recommended for routine use where sample size is limited.

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