The Determination of Lactic Acid in Shortening Containing Lactylated Glycerides, by Liquid-Liquid Partition Chromatography[1]

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Abstract

Shortenings containing ca. 1% lactic acid added as lactylated glycerides are analyzed for total or water insoluble combined lactic acid (WICLA) by a liquid-liquid partition chromatographic procedure. The sample is saponified, acidified with H_2SO_4, dispersed on silicic acid, slurried with chloroform and transferred to the top of a chromatographic column composed of a 0.5 N sulfuric acid stationary phase supported on silicic acid. After elution of the fatty acids by chloroform, 15% *n*-butanol removes the excess H_2SO_4 followed by a well resolved lactic acid fraction. Titration of the latter fraction with standard methanolic sodium hydroxide has shown the lactic acid recovery to be 94%. The WICLA determination requires 7 hr and has a standard deviation of ±0.020%.

Introduction

GLYCEROL LACTOPALMITATE (GLP) has been used as a shortening emulsifier in prepared mixes for several years. Analytical techniques for lactylated shortening are necessary to insure uniformity in prepared mixes and to control product cost, since GLP is considerably more expensive than the other emulsifiers used in mixes. Fett (1) recommends the water washing of lactylated shortening to remove such non-emulsifiers as mono-, di-, and trilactin before appraising the active GLP content on the basis of the "combined" lactic acid concn. Fett (1) has adapted Barker and Summerson's (2) colorimetric procedure using *p*-phenylphenol to the analysis of lactylated shortenings. Although Fett reported only the analysis of shortenings, the present author and probably a number of others have used this method on GLP as well. The method is complicated by the extreme sensitivity of the color forming reaction which necessitates great dilution of sample solutions with the result that a fraction of a μg of lactic acid contamination can cause a serious error. Barker and Summerson (2) have shown that troublesome amounts of lactic acid can be found on the operator's hands and Neidlinger et al. (5) have recommended elaborate precautions against accidental addition of the acid from this source.

In two similar titrimetric procedures the samples are saponified and acidified before extraction with an organic solvent to remove the fatty acids. The lactic acid contents are calculated from the differences between the titrations of the remaining water layers and similar water layers obtained from blank determinations. Acidulation in the Stetzler and Andress (3) procedure for the analysis of GLP is accomplished by adding the exact mineral acid equivalent of the alkali used for saponification. In the second method, Pohle et al. (4) when analyzing either lactylated monoglycerides or shortening containing the latter, add an excess of mineral acid which is titrated along with the lactic acid in the water layer. The usual volumetric errors are important because relatively concd alkaline and acid solutions are metered in the presence of a small amt of lactic acid. The measure of lactic acid, sample titration minus blank, is adequate for the determination of lactic acid in GLP, but it falls to about one ml when the Pohle et al. (4) procedure is applied to shortening lactated at the usual rate of ca. 0.9%. This report describes the separation of lactic acid from the large amt of fatty acids and mineral acid by liquid-liquid partition chromatography before titration. The titrimetric procedures determine any water soluble acid whereas the chromatographic method is specific for lactic acid because of its characteristic retention volume.

Experimental

To determine WICLA, a dichloromethane solution of the sample is washed with water; the solvent is evaporated and the sample is saponified with alcoholic potassium hydroxide. After acidification with H_2SO_4, the sample is dispersed on silicic acid, slurried with chloroform and transferred to the top of a chromatographic column composed of a 0.5 N H_2SO_4 stationary phase supported on silicic acid. The fatty acids are eluted by chloroform and then 15% *n*-butanol in chloroform removes the excess H_2SO_4 followed by lactic acid.

Special Apparatus:

- Extraction cylinders, 25 ml, with siphon.
- Hypodermic syringe, 2 ml, with No. 24 needle.
- Erlenmeyer flasks, 10 ml.
- Condenser, air, with rubber sleeve to fit 10 ml Erlenmeyer.
- Microburet, 5 ml, calibrated to 0.01 ml.
- Chromatographic tube, 10 mm I.D. and 300 mm long, with coarse fritted disc.
- Technicon fraction collector with drop counter.
- Automatic buret, 10 ml, calibrated to 0.05 ml with reservoir and soda-lime trap.

Reagents:

- Alcoholic potassium hydroxide. Dissolve 150 g reagent grade KOH (85–87%) in one liter 95% ethanol (Formula 3A). Moderate discoloration does not affect the determination.
- Sulfuric acid solution, 16% v/v, dilute 160 ml concd reagent grade H_2SO_4 to one liter.
- Methyl red solution, 0.02 g dissolved in 100 ml 60% ethanol.
- Mallinckrodt silicic acid No. 2847. Remove small particles by repeated suspension in distilled

[1] Presented in part at the AOCS Meeting, New Orleans, 1962.

TABLE I

Analysis of Synthetic Samples

	% Total lactic acid		Recovery %
	Added	Found	
Shortening	None	None	.....
Shortening + reagent grade lactic acid	0.918	0.860	93.7
Shortening + reagent grade lactic acid	0.918	0.866	94.3
Shortening + glycerol lactates	1.00	0.941	94.1
Shortening + glycerol lactates	1.00	0.957	95.7

water and decantation. Dry overnight at 110C.

H_2SO_4, ca. 0.5 N. Dilute 13.9 ml concd reagent grade H_2SO_4 to one liter.

Chloroform, U.S.P. Water wash twice and equilibrate with 0.5 N H_2SO_4.

Methanolic sodium hydroxide, 0.025 N accurately standardized.

Phenol red solution 0.1 g mixed with 11.4 ml 0.025 N methanolic NaOH in a mortar and diluted to one liter with ethanol.

Butanol—Chloroform solution, 15 volumes *n*-butanol and 85 volumes water washed U.S.P. chloroform are equilibrated with 3.0 volumes 0.5 N H_2SO_4.

Procedure

Weigh accurately 0.40–0.44 g lactylated shortening into a 25-ml graduated cylinder and dissolve it in 15 ml dichloromethane. Add 10 ml distilled water, invert the cylinder 25 times and sipon off the water layer when it has separated from the dichloromethane. After a total of three water washings pour the dichloromethane solution into a 100-ml beaker. Wash the cylinder once with acetone and twice with dichloromethane, adding the washings to the 100-ml beaker. Evaporate the solvents on a steam bath under an air stream. Removal of the last droplets of water is aided by the addition of a few ml acetone to the concd sample on the steam bath. Using dichloromethane in a 2-ml hypodermic syringe quantitatively transfer the sample to a 10-ml Erlenmeyer flask and then remove the dichloromethane by placing the flask on the steam bath under an air jet. Following the recommendation of Stetzler and Andress (3) for the complete vaporization of dichloromethane, the sample is treated on the steam bath until no solvent odor is detectable, then 1.0 ml ethanol is added and heating is continued until the solvent odor again disappears. (The dichloromethane must be completely removed because acidification is based on a blank titration of KOH used and the loss of KOH by reaction with traces of dichloromethane causes an excess of H_2SO_4 which is difficult to separate chromatographically from lactic acid). Pipette 1.0 ml alcoholic KOH into the sample flask and into two small beakers. Attach the air condenser to the 10-ml Erlenmeyer flask and place it on the steam bath for one-half hr. During the saponification, titrate the alcoholic KOH blanks to methyl red end points using 16% v/v H_2SO_4. To prepare the chromatography column mix 2.7 ml 0.5 N H_2SO_4 with 4.0 g silicic acid, slurry the mixture with chloroform and transfer it to the chromatography tube using a powder funnel temporarily attached to the top of the chromatography tube to facilitate the transfer. The packed column is ca. 11 cm in depth with a holdup of 5.4 ml. When the saponification is complete, remove the condenser, and place the flask under an air jet on the steam bath until the sample gels. Add 0.1 ml ethanol and an amt of 16% v/v H_2SO_4 equal to the blank titration determined above.

TABLE II

Precision of WICLA Determination on a Commercial Lactated Shortening

	Per cent WICLA
	.850
	.863
	.849
	.797
	.834
	.809
	.839
	.826
	.837
	.819
Average	.832
Standard deviation	±0.0200%
Total lactic acid	1.01 and 1.03%

After warming the sample mixture slightly on the steam bath to decompose the soaps, mix in 0.6 g silicic acid and quickly add ca. 2 ml $CHCl_3$. Stir the mixture with a rubber policeman attached to a small glass rod bent to fit the Erlenmeyer flask until the solid phase is homogeneous. Using $CHCl_3$ from a wash bottle transfer the sample mixture to the chromatography tube being careful to keep the lumps of silicic acid moist at all times. Wipe the sides of the column by pressing a tight fitting filter paper disc into the tube to produce a sample section ca. 2.3 cm deep and then remove the powder funnel from the top of the tube. Carefully wash the $CHCl_3$ soln. of fatty acids into the column and continue elution with $CHCl_3$. Transfer the chromatography tube to a fraction collector equipped for drop counting and begin collection of 2.6 ml (ca. 110 drops) fractions. To each test tube there is added 0.5 ml methanol and 1 drop phenol red solution before fitting the test tube with a nitrogen bubbler and titrating the fractions with 0.025 N methanolic NaOH. When the complete elution of fatty acids is indicated by a low and constant titration, the last of the $CHCl_3$ is allowed to run into the silicic acid bed and elution with butanol-chloroform is started immediately at a rate of 0.5–0.6 ml/min. If the test tube filling at this time is numbered zero, two H_2SO_4 peaks will be found with max at tubes 2 and 7 followed by the lactic acid peak with a max at tube 14 or 15. The water insoluble combined lactic acid content is calculated from the total titration of the latter peak, usually fractions 12–19 inclusive, after subtraction of the product (reagent blank) of the number of tubes in the peak and the small and constant titration found before and after the peak.

Total lactic acid may be determined by eliminating the water washing step and weighing the sample directly into the 10-ml Erlenmeyer flask for saponification.

Results and Discussion

A blank determination on unlactylated shortening revealed two H_2SO_4 peaks but no peak was observed at the retention volume corresponding to lactic acid. The first H_2SO_4 peak was associated with a portion of cloudy eluate formed by the mixing of wet chloroform in the column with the newly added wet butanol-chloroform solution. A second H_2SO_4 peak of much smaller size was then eluted. During twelve analyses of lactylated shortening the third peak, due to lactic acid, was well separated from the second H_2SO_4 peak and it exhibited a max at either fraction 14 or 15. The total time required for the WICLA determination was 7 hr and for the total lactic acid determination 5.5 hr.

Because a standard sample of lactylated shortening was not available, the accuracy of the method was determined by analysis of synthetic mixtures for total

lactic acid. The esterification of 3 moles reagent grade lactic acid with 2 moles glycerol yielded a mixture of glycerol lactates that were added to shortening on the basis of their saponification equivalent. Reagent grade lactic acid was also added to shortening in relation to the lot analysis. The results show in Table I.

Precision. The estimation of precision given in Table II was obtained from ten WICLA determinations on a commercial lactylated shortening.

Determination of Similar Acids. As new fat emulsifiers which contain low mol wt hydroxylated acids appear and require analysis, the present method might be adapted for this purpose. For instance, in a preliminary experiment, 0.2% citric acid in shortening has been eluted qualitatively from this system by a mixture of 55 volumes *n*-butanol and 45 volumes chloroform.

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[Received January 22, 1964—Accepted March 23, 1964]

Search for New Industrial Oils. XI. Oils of Boraginaceae

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Abstract

Analysis of seed oils from 29 species of the family Boraginaceae revealed widespread occurrence of 6,9,12-octadecatrienoic and C_{18} nonconjugated tetraenoic acids in addition to linolenic and other common C_{16} and C_{18} acids. The 6,9,12-octadecatrienoic acid ranged in amount from 0–27%, tetraene from 0–17%, and linolenic acid from 0.3–50%. Iodine values of the oils ranged from 88–225.

Introduction

ANALYSIS of seed oils from an extensive sampling of the plant kingdom reveals that the family Boraginaceae is unique in the frequency with which nonconjugated tetraenoic acids occur. In the first paper (2) from this continuing program two species in the family were reported to contain small amounts of tetraene on the basis of ultraviolet absorption after isomerization by alkali. In later samples, oil was analyzed by gas-liquid chromatography (GLC), and the tetraene was identified as an 18-carbon acid. Furthermore, the trienoic fraction was shown to include two components. One had the equivalent chain length (ECL) of the usual linolenic acid and the other the ECL of the 6,9,12 isomer (4), long known only in oil from the genus *Oenothera,* family Onagraceae but recently reported from *Humulus lupulus,* family Moraceae (5). The tetraenoic acid has been identified as 6,9,12,15-octadecatetraenoic acid in oils from *Echium plantagineum* (6) and *Onosmodium occidentale* (1), and our GLC identification of the 6,9,12-octadecatrienoic acid has been confirmed.

This paper contains the analyses of seeds and oils from 29 species in the borage family.

Materials and Methods

The plant family Boraginaceae includes some 100 genera and 2000 species, mostly herbaceous and often perennial. There are five subfamilies, one of which is divided into five tribes (7). Four of the subfamilies and all five tribes are represented in this paper. Numerous species are grown as ornamentals, but we do not know of any studies directed toward large-scale seed production for industrial use. Seed samples were provided by the U. S. Dept. of Agriculture's Crops Res. Div. as obtained by staff botanists from wild plants, by botanists under contract in various parts of the world, or by purchase from commerical seed suppliers.

Seeds were cleaned and analyzed as previously described (2). The components analyzed included seed plus pericarp in all instances except two, *Paracaryum angustifolium* and *Lappula redowskii,* for which seed only was analyzed. Methyl esters were prepared from the oils by HCl-catalyzed methanolysis.

Each ester preparation was analyzed by GLC at least three times in equipment described previously (3). A rapid exploratory analysis was made in a 125 x 0.3 cm column to identify the slowest moving components. Then each ester preparation was analyzed on two different columns: a 200 x 0.6 cm glass column containing 20% Apiezon L on 60–80 mesh Celite 545 at 258C with a helium flow of 90 ml/min and a 200 x 0.6 cm glass column containing 20% LAC-2-R 446 on the same support at 196C with flow rate of 120 ml/min. In the polar column the peak of the C_{18} tetraene (ECL 20.1) overlapped the C_{20} esters. However, in the nonpolar column as operated, the C_{18} tetraene (ECL 17.4) was widely separated from the C_{20} esters but coincided with the 6,9,12-triene (4) and was not fully separated from the usual C_{18} unsaturates. The percentage of tetraene was determined by subtracting the C_{20} methyl esters (ECL 19.7–20.0) from the Apiezon L chromatogram from the methyl esters in the C_{20} region from the LAC-2-R 446 column (ECL 20.0–20.4). The percentage of 6,9,12-triene (ECL 19.3 LAC-2-R 446 column) was determined from the LAC-2-R 446 chromatogram where separation was complete. When the nonpolar column was operated at 210C with a flow rate of 116 ml/min, the peak representing the tetraene and 6,9,12-triene was separated from that of the usual C_{18} unsaturates and the amount agreed with the sum of these two components determined by the procedure described.

Results and Discussion

In all but three of the samples analyzed (Table I), GLC shows the presence of C_{18} tetraene, C_{18} 6,9,12-triene, or both. The exceptions are *Lithospermum officinale, Ehretia acuminata,* and *E. aspera,* whose

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