HPLC Analysis of Cyclopropenoid Fatty Acids

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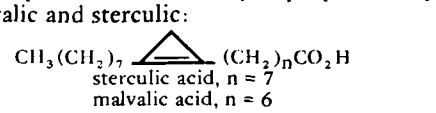
ABSTRACT

Cyclopropenoid fatty acid methyl esters have been analyzed by high pressure liquid chromatography (HPLC). The refractive index detector's linear response to various lipids was studied. Verification of peak identity was by spectroscopy of collected peaks and cochromatography with authentic samples. The HPLC method is simple, convenient and gives precision and absolute, values which are consistent with those from traditional methods.

INTRODUCTION

Plants of the order *Malvales* produce unusual fatty acids containing a cyclopropene ring in the hydrocarbon chain. The seeds and oil preparations from two of these plants, cotton and kapok, contain the cyclopropene fatty acids (CPFA) malvalic and sterculic:



sterculic acid, n = 7
malvalic acid, n = 6

Malvalic and sterculic acids are of particular interest because of the increasing use of the seeds and oils of cotton and kapok in human and animal diets. Numerous physiological disorders in animals have been attributed to CPFA.

In our own laboratory, CPFA, fed as *Sterculia foetida* or *Hibiscus syriacus* oil (as triglycerides) or as methyl esters, were shown to be powerful cocarcinogens with aflatoxin B_1 and other mycotoxins in rainbow trout (1-5). Carcinogenic activity was also demonstrated (6). We investigated the difference in biological activity between methyl malvalate and methyl sterculate (2,7). In order to separate methyl malvalate from methyl sterculate in sufficient quantities for our studies, a spinning band distillation procedure was developed (8). CPFA methyl esters are difficult to purify individually because they readily undergo polymerization (12). Thus the goal of the distillations was to provide individual CPFA methyl ester admixed with straight-chain methyl esters. In the procedure, numerous fractions were collected and monitored by high pressure liquid chromatography (HPLC).

In choosing the fraction monitoring method, traditional approaches were considered. The Halphen reaction is relatively quick, cheap, simple and reasonably sensitive. It has been the mainstay of cyclopropene research. However, the Halphen reaction as a quantitative assay receives much criticism for its deficiencies (9,10), which are reflected in the numerous publications on the subject (11). It is imprecise, especially at low levels, lacks reproducibility, the background varies with the source and processing of the oil, an accurately known standard is necessary, and it will not distinguish malvalic from sterculic acids.

In our laboratory we have relied almost exclusively upon the nuclear magnetic resonance (NMR) method of analysis (12). In situations where the individual amounts of malvalate need be discerned, the silver nitrate-methanol derivatives (13) are formed, but only to determine the ratio of malvalate to sterculate. The total amount of cyclopropenoids can vary by several percent with the silver nitrate-methanol method whereas, with the NMR method, variation between duplicate samples is less than 1%.

The purpose of the work described in this paper was to determine if the HPLC monitoring method was a satisfactory substitute for the traditional methods of analysis.

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EXPERIMENTAL

HPLC was performed with a Water Associates instrument equipped with a U6K injector, Model 6000 solvent delivery system, and a R401 differential refractive index detector. Conditions were as follows: 3.9 mm id × 30 cm μBondapak C_{18} column, acetonitrile/water 9:1 or 85:15 and 0.9-1.0 mL/min flow rate. Sample size of methyl esters from plant oils was ca. 0.2 μg in 5-10 μL acetonitrile. Assignment of peak identities was by cochromatography with authentic compounds, and by NMR and mass spectroscopy (MS) of collected peaks. Detector response was checked by injecting known amounts of the following compounds and comparing peak area per weight of compound: methyl palmitate, methyl oleate, methyl linoleate and methyl dihydrosterculate. Peak areas were determined by multiplying peak height times width at half-height.

The methyl esters used in this study were obtained from *Sterculia foetida* or *Hibiscus syriacus* oil by transesterification. Transesterification was accomplished by adding the oil to ten times its volume of methanol containing 0.02 equivalents of sodium. Methyl esters were extracted in hexane and distilled in vacuo prior to spinning band distillation (8) and HPLC.

RESULTS AND DISCUSSION

The simple chromatographic system used provided adequate resolution (R = 0.8 for acetonitrile/water 9:1 and R = 1.3 for 85:15, oleate-palmitate/sterculate peaks) of the components of interest within a reasonable length of time (less

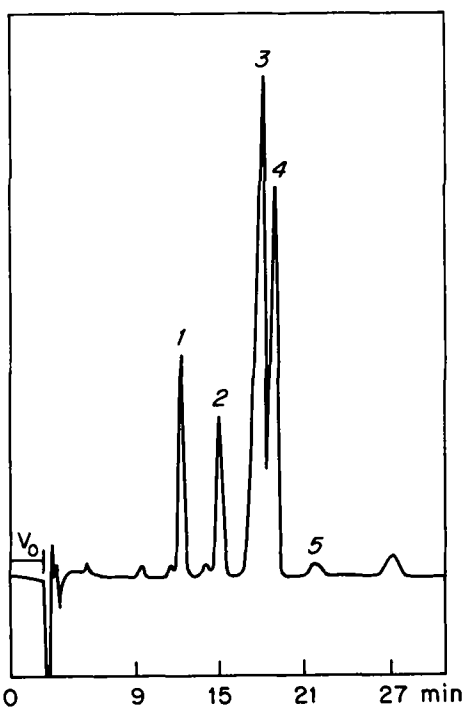

FIG. 1. HPLC of *H. Syriacus* oil methyl esters. Conditions: μBondapak C_{18} (3.9 mm id × 30 cm) column, acetonitrile/water 9:1 at 1.0 mL/min. Sample size was ca. 0.2 μg in 10 μl acetonitrile. Detection was with a Water Associates differential refractometer R 401, attenuation 8 × (¼ × = 6 × 10^{-6} RI units full scale). Peak identity: (1) linoleate, (2) malvalate, (3) palmitate and oleate, (4) sterculate, (5) dihydrosterculate.

than 20 min for sterculate with acetonitrile/water 9:1). Other organic solvent combinations consisting of acetonitrile, tetrahydrofuran and/or methanol were tried, but for equal capacity factors (k'), resolution of sterculate from oleate-palmitate was inferior to the acetonitrile/water mixtures mentioned above (data not shown).

The high pressure liquid chromatogram of *Hibiscus syriacus (H.s.)* oil methyl esters is shown in Figure 1. Methyl esters are analyzed rather than the free acids because of the instability of malvalate and sterculate in the acid form (12). The composition of this oil was previously determined by GLC of silver nitrate-methanol derivatives (13). Verification of peak identity in the liquid chromatogram was by spectral measurements and cochromatography of the collected components.

Peak 2 was identified as methyl malvalate on the basis of the NMR spectrum which gave a singlet at 0.7δ (methylene of cyclopropene ring), and the mass spectrum which gave m/e 294 (P+) and 263 (P-31). Cochromatography of *H.s.* methyl esters with a synthetic sample of methyl sterculate enhanced peak 4. This peak was collected along with peak 3. The NMR of peaks 3 and 4 showed absorption at 0.7δ, indicating that cyclopropene was present. The mass spectrum showed that peak 3 contained palmitate and oleate esters. Cochromatography of authentic methyl palmitate or methyl oleate with the *H.s.* methyl esters caused enhancement of peak 3 in both cases. Peak 2 showed no cyclopropene protons in the NMR, gave m/e 294 (P+), 263 (P-31) and 220 (P-74) and was enhanced by cochromatography with authentic methyl linoleate. Peak 5 was enhanced by cochromatography with synthetic methyl dihydrosterculate. This information is summarized in Table I.

Abundances of individual components in oil mixtures were expressed as percents by weight of total lipid content. Percents were calculated from peak areas of individual components divided by the sum of peak areas in the chromatogram (normalization). The relative sensitivity of the refractive index detector to equal weights of compound was determined for 4 pure authentic methyl esters, including methyl dihydrosterculate (Table I). Values for malvalate or sterculate were not determined because, in pure form, these compounds are labile due to the cyclopropene ring (12). The values obtained show the differences to be small (<4%) and probably not significant. However, relative refractive indices for solutions containing equal weights of methyl esters including malvalate and sterculate were calculated from individual bond refractions (Table I). The calculated values differ from each other by no more than 2.6% (palmitate vs sterculate). We thus feel justified in considering the detector response for sterculate and malvalate to be similar to the other methyl esters. Hence we have ignored any possible differences in detector response in our calculations of percent abundance.

Under the chromatographic conditions used, malvalate is completely resolved from the other components. Sterculate, on the other hand, is only partially resolved from the palmi-

TABLE I

Verification of Identity and Detector Response for Peaks in HPLC of *Hibiscus syriacus* Oil Methyl Esters (Fig. 1).

Methyl ester	k'[a]	Detector response (mm^2/mg)	Relative refractive index (calculated)[d]	Method of assignment of peak identities
Linoleate	3.75	490± 8[c]	1.521	Cochromatography, MS, NMR
Malvalate	4.8	–	1.489	MS, NMR
Palmitate	5.9[b]	472±11	1.521	Cochromatography, MS, NMR
Oleate	5.9[b]	470± 4	1.528	Cochromatography, MS, NMR
Sterculate	6.2	–	1.493	Cochromatography, MS, NMR
Dihydrosterculate	8.2	492± 5	1.492	Cochromatography

[a]k' = true retention time/void volume.
[b]k' is of unresolved mixture.
[c]± SD, n = 2.
[d]Calculated from $R = ([n^2 - 1]M)/([n^2 + 2]\rho)$, where n = index of refraction, M = molecular weight, R = molar refraction = sum of individual bond refractions, and ρ = density, assumed for these calculations to be 1.0000, and from the bond refraction data given in reference 14.

TABLE II

Analysis of *Sterculia foetida* Oil and *Hibiscus syriacus* Oil Cyclopropenoid Fatty Acid Methyl Esters by HPLC and by Conventional Methods

Sample[a]	Method of analysis[b]	% Total cyclopropene	% Malvalate	% Sterculate
S.f. oil methyl esters #1	NMR (n=3)	35.0±0.1	–	–
	$AgNO_3$/GLC (n=4)	34.9±1.1	7.91±0.40	27.0±0.7
	HPLC (n=4)	35±1	8.3±0.4	26±2
S.f. oil methyl esters #2	NMR/GLC (n=1)	25.0	7.0	18.0
	HPLC (n=4)	27±2	6.0±1.1	21±1
H.s. oil methyl esters #1	NMR/GLC (n=1)	23.4	21.6	1.85
	HPLC (n=4)	22.3±0.4	20.3±0.4	1.95±0.1
Distilled *H.s.* oil methyl esters	NMR (n=3)	26.3±0.5	–	–
	HPLC (n=7)	26.4±0.8	26.4±0.8	not detected (<0.04%)

[a]*S.f.* = *Sterculia foetida*, *H.s.* = *Hibiscus syriacus*.
[b]See text for discussion of precision of conventional methods. "NMR/GLC" denotes that total cyclopropene was determined by NMR and malvalate-sterculate ratio by GLC of $AgNO_3$-methanol derivatives.

tate-oleate peak (R = 0.8) with acetonitrile/water 9:1, but nearly completely resolved (R = 1.3) with solvent ratio 85:15. Table II compares the values of percent abundance of malvalate and sterculate in several samples obtained by HPLC, with the values obtained by the NMR-GLC method. As mentioned, NMR is used to obtain the total cyclopropene content of a sample, while the silver nitrate derivative (13) method is used only to determine the sterculate to malvalate ratio. Experience indicates that variation between samples can be considerable for the silver nitrate-GLC method and the mean value may differ from that for the NMR method by as much as 1-2%. Total cyclopropene content from NMR and HPLC analyses consistently agrees fairly well, but HPLC has the obvious advantage of distinguishing between malvalate and sterculate and providing a partial fatty acid profile.

ACKNOWLEDGMENTS

Thomas Eisele performed some of the $AgNO_3$/GLC analyses. This work was supported by Public Health Service Grants CA25766 from the National Cancer Institute and ES00550 from the Division of Environmental Health Sciences. Technical Paper No. 5375, Oregon Agricultural Experiment Station, Oregon State University Corvallis, OR 97331.

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Fatty Acid Composition of the Fat in Selected Food Items with Emphasis on *trans* Components[1]

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ABSTRACT

The fat in 220 samples from 35 food types has been analyzed for component fatty acids by gas liquid chromatography on a 15-m capillary column coated with SP-2340. The methodology permitted the determination of *trans*-octadecenoic fatty acids in the food samples. For food types in which the majority of samples contained *trans* fatty acids, the range (weight percent of methyl esters) of this class of acids arranged by fat content of the food types was: high fat levels (>70% fat) – animal and dairy fats, 0.3-6.6%, stick margarines, 15.9-31.0%, tub margarines, 6.8-17.6%, and vegetable shortenings, 8.7-35.4%; moderately high fat levels (40-70% fat) – diet margarines, 11.3-13.3%; moderate fat levels (10-40% fat) – breading mixes and fried crusts, 8.1-32.7%, cakes, candies and frostings, 3.2-33.2%, cream substitutes, 0.4-11.5%, cookies, 2.5-34.2%, crackers, 1.9-29.0%, pastries and pastry crusts, 0.6-31.2%, corn and mixed grain snack chips, 0.4-30.4%; low fat levels (<10% fat) – breads and rolls, 0.2-23.6%, pretzels, 10.8-29.2%, and puddings, 28.4-35.1%. The majority of samples in the following food types did not contain *trans* fatty acids, except in cases where the label indicated partial hydrogenation of the oil: mayonnaises and salad dressings, salad and cooking oils and potato chips. For samples in these three food types which contained *trans* fatty acids, the range was 0.2-23.2%. None of the peanut butters or pizza crusts analyzed contained *trans* fatty acids.

INTRODUCTION

The level and pattern of fat consumed by Americans has changed considerably during the 20th century. Between 1910 and 1978 there was a 27% increase in the total dietary fat intake with most of the increase being due to vegetable fats (1,2). In recent years the shift from animal fat to vegetable fat consumption has been encouraged with the intention of increasing the levels of dietary polyunsaturated fatty acids at the expense of saturated fatty acids. However, the resulting alterations are more complex than may be recognized, as the majority of vegetable fats consumed by Americans are modified chemically by partial hydrogenation procedures designed to convert the original oils to products with economically desirable physical properties.

These hydrogenation procedures result in the conversion of variable amounts of the naturally occurring *cis* polyunsaturated 18-carbon fatty acids into a variety of isomers of oleic and linoleic acid, including considerable quantities of *trans* fatty acids. Consequently, the fatty acid composition of the processed vegetable fat consumed is quite different from that listed in handbooks for the original oils.

With the advent of glass capillary gas liquid chromatography, it is now possible to obtain more information on the fatty acid composition of foods which contain *trans* fatty acids. Previously reported analyses have included butters, margarines, shortenings and oils (3-7) in addition to some fast foods (8,9) and meats (9). This report extends the data on the *trans* fatty acid content and fatty acid composition of the fats in these foods as well as a variety of foods not previously reported.

MATERIALS AND METHODS

Materials

Unless otherwise noted, all solvents employed in this study were reagent grade or better. Fatty acid methyl ester standards (99% purity) were purchased from Nu Chek Prep. Inc. (Elysian, MN). Food items were purchased over a 24-month period (1978-80) from local supermarkets, stores, bakeries, restaurants and fast-food outlets. An attempt was made to select primarily national brands of items purchased from supermarkets. A total of 220 different food items

[1]Presented in part at the ISF-AOCS World Congress, New York, April 1980.