TABLE I

Reactivity between Rabbit Antisera to Cotton Dust and Bract, Normal Rabbit Serum and Extracts of Cotton Dust, Cotton Tissues and House Dust

Antigen	AD[a]	AB	NS
Cotton dust	+	+	–
Cotton bract	+	+	–
Cotton stem	+	+	–
Cotton leaf	+	+	–
Baled cotton	+	+	–
Gin trash	+	+	–
Cottonseed hulls	–	–	–
Cottonseed proteins	–	–	–
Cotton lint (clean)	–	–	–
House dust	–	–	–

[a](AD), antisera to dust; (AB), antisera to bract; (NS), normal serum; (+), positive reaction; (–), no reaction.

indicate that this dust possesses relatively little physiological activity, as measured by the capacity to induce in vitro release of histamine from pig lung tissue (19).

Cotton dust very effectively activates in vitro the human complement cascade (13,14). Extracts from cotton plant tissues are active immunologically with antisera to cotton dust, and extracts from cottonseed tissues are inactive. If this cotton dust antigen is proven to be associated with the symptoms of byssinosis, the results of these tests could provide a basis for the reported low prevalence of the disease in oil mills. Although the cottonseed kernels contain proteins, these data suggest that the seed proteins are not associated with antigens found in cotton dust from textile mills, dust that has been implicated in the symptoms/causes of byssinosis.

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[Received September 2, 1980]

Separation of Methyl Malvalate from Methyl Sterculate

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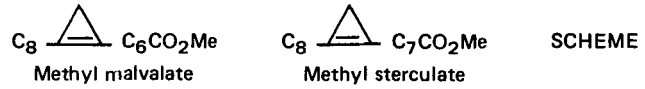
ABSTRACT

Methyl malvalate and sterculate, labile and differing by a single methylene, are difficult to separate completely. They have been separated in our laboratory by high vacuum spinning band distillation. Each of these fatty esters has been prepared completely free of the other.

The unique fatty acids, malvalic and sterculic, contain a cyclopropene ring in the center of their carbon chains. Cottonseed and kapok oils, which contain these acids, are consumed in large amounts by the world's population and cottonseed flour or meal is finding increasing use as a source of protein for human consumption. These cyclopropenoid fatty acids are responsible for several physiological disorders in farm and laboratory animals (1-10), including cocarcinogenic (11-13) and carcinogenic activity in rainbow trout (14). Their mechanisms of action remain for the most part a mystery. Previous work in these laboratories (12,15) has indicated that the biological activity of malvalic acid may differ from that of sterculic acid, and a complete separation of these two fatty acids, never before achieved, would be desirable.

Research with cyclopropenoid fatty acids is complicated by their high ground-state energy which renders them unstable, or quite reactive. Methyl malvalate and sterculate differ by a single methylene group (Scheme I) and have nearly identical chemical and physical properties. Both instability and similarity create a major task for their separation.



SCHEME I

Nunn (16) and Kircher (17) purified sterculic acid from *Sterculia foetida* oil via crystallization of the urea inclusion complexes. This technique provides a reasonably pure sterculic acid, but not one that is totally free of malvalic acid. Shenstone and Vickery, and Fogerty and coworkers (18,19) have separated the cyclopropenoid fatty acids by reverse-phase column chromatography. This method is quite laborious and in our hands did not clearly separate the two acids.

Surprisingly, cyclopropenoid fatty esters can be distilled under vacuum. Methyl malavate has a slightly lower boiling point and faster distillation rate than sterculate which is the basis of our separation. The low pressure of oxygen during distillation abates free radical initiated polymerizations and while higher temperatures enhance decomposition

TABLE I

Fatty Acid Composition of Distillate from *Hibiscus syriacus*[a] Methyl Esters

Volume distilled[b]	16:0 (%)	Malvalate (%)	18:2 (%)	Sterculate (%)	Other (%)	Pot temp (C)	Head temp (C)
4 ml	94.5	trace	trace	0	5.5[c]	132	92
2	32.5	34.2	25.5	0	7.7[d]	135	94
2	9.8	51.9	29.9	0	8.4[d]	136	94
1.25	7.7	57.1	35.2	0	–	137	95
8	9.1	31.7	59.2	0	–	139	96
1	1.4	1.0	97	trace	–	142	98

[a]Original oil: 14% palmitate; 9% oleate; 47% linoleate; 22% malvalate; 2.7% sterculate, analysis by high pressure liquid chromatography.

[b]Starting pot volume, 40 ml.

[c]Low boiling esters 14:0, 16:1 and 16:2.

[d]18:1.

reactions, they also eliminate intermolecular associations (20) between cyclopropene rings, decreasing the entropy of dimerization and polymerization.

PROCEDURE AND APPARATUS

Transesterified *Hibiscus syriacus* oil was subjected to a simple vacuum distillation to provide a clean source of methyl malvalate for further purification. This prior simple vacuum distillation extends the pot life during spinning band distillation by a factor of eight. Methyl sterculate was obtained in essentially the same manner from *S. foetida* seed extracts. With a short path distillation head, these transmethylated oils distill at 105-110 C and 105-118 C, respectively (0.05 mm Hg) with an oil bath temperature 20 C higher.

Methyl esters were rectified on a 7 mm x 46 cm stainless steel spinning band column under a vacuum of 0.004 mm Hg. The pot was stirred rapidly with a magnetic stirrer and heated with a silicone oil bath. So that the distillation would remain adiabatic, the oil bath, column, head and all interfaces were wrapped with insulation, and a column and a head heater were applied to suppress heat loss from the low density vapor. Thermocouples in the head, column and pot provided temperature data.

Vacuum was obtained with a Welch Duo-Seal pump (broken in by running four weeks under a total vacuum, and changing oil whenever metal particles became visible). The pump oil was also changed after each simple distillation or whenever the system failed to achieve 0.004 mm Hg. After each oil change, the oil and vacuum lines were degassed by running the system for 24 hr before applying heat. With a pot temperature of 135 C, the column and head at 114 C and 97 C, distillation commenced. Starting with 40 ml of methyl esters, 20 ml of material was distilled over 4-5 days, while slowly raising the temperature of all components to maintain a reasonable distillation rate of ca. 0.25-0.5 ml/hr. Due to a low heat capacity and low vapor density, condensation occurs primarily on the walls of the distillation head. An accurate reflux ratio cannot be determined.

DISCUSSION

A typical spinning band distillation of *H. syriacus* methyl esters is shown in Table I. These data show that fractions are collected which are rich in methyl malvalate and completely free from sterculate. The distillate, collected over 4 days, contains about two-thirds of the malvalate initially present. The remaining cyclopropenoid fatty acids, after 4-5 days at 140 C, are lost as a thick polymer in the still-pot.

Malvalate co-distills with at least two other methyl fatty esters which is not only unavoidable, but necessary. Malvalate, sterculate and other 1,2-dialkylcyclopropenes do not store well when concentrated because intermolecular associations (11) facilitate dimerization and other reactions. Dilution with an inert solvent decreases these intermolecular adhesions and greatly extends the storage life of these reactive esters. In this case, other fatty esters serve as a solvent which is compatible with the vacuum.

Sterculate can be prepared in a similar manner from *S. foetida* methyl esters as shown in Table II. More heat is applied and the early fractions are collected more quickly. Only the later fractions of sterculate are completely free of malvalate. Distillate containing as high as 90% methyl sterculate can be collected.

TABLE II

Fatty Acid Composition of Distillate from *Sterculia foetida*[a] Methyl Esters

Fraction volume distilled[b]	16:0 + 18:1 (%)	Malvalate (%)	18:2 (%)	Sterculate (%)	Other (%)	Pot temp (C)	Head temp (C)
2-1/4	25.5	18.5	27.8	22.7	–[c]	151	110
4	21.1	8.1	29.7	36.5	4.5[d]	154	113
4-3/4	18.7	2.1	22.4	49.4	7.3[d]	155	114.5
1-1/4	2.8	1.0	6.6	79.8	9.7[d]	154	116
2-1/2	e	e	e	88.6	8.4[d]	157	116-119

[a]Original oil: 65% sterculate, 4.5% malvalate after simple distillation, analysis by HPLC.

[b]Starting pot volume, 33 ml; pressure was 0.005 mm Hg throughout the distillation.

[c]Dihydrosterculate, 1.8%; 3.8% unconfirmed 20:0.

[d]Dihydrosterculate, 1%; remainder unconfirmed 20:0.

[e]Less than 1%.

Should 100% methyl malvalate or sterculate be desired, further separation from the accompanying common fatty esters can be achieved by standard procedures. Both cyclopropenoid esters can be precipitated from linoleate during a low temperature crystallization and palmitate can be separated by the urea complex technique (16,17).

ACKNOWLEDGMENTS

This work was supported by Public Health Service Grants ES00550 and ES01926 from the Division of Environmental Health Sciences and CA25766 from the National Cancer Institute. Technical paper no. 5374, Agricultural Experiment Station, Oregon State University, Corvallis, OR 97331.

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[Received January 7, 1980]

Detection of Adulteration of Olive Oil with Seed Oils by a Combination of Column and Gas Liquid Chromatography[1]

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ABSTRACT

Samples of virgin olive oil and refined seed oils, as well as mixtures of olive oil with 10 and 5% seed oils were fractionated by column chromatography on silicic acid impregnated with ammoniacal silver nitrate. It was possible to isolate a characteristic fraction enriched in polyunsaturated triglycerides. Its linoleic acid content in pure olive oil never exceeds 9.3%, whereas in pure seed oils, it varies between 38.1 and 70.1%; in mixtures of olive oil with 10 and 5% of seed oils, the respective values are 22.3-38.2% and 15.6-32.1%. The oleic-to-linoleic acid ratios of the same fraction are more than 7.6 (olive oil), 0.2-0.8 (seed oils), 1.1-2.0 (olive oil with 10% seed oils) and 1.4-3.6 (olive oil with 5% seed oils). These analytical values may be used as a safe criterion for the eventual adulteration of olive oil with seed oils.

INTRODUCTION

Olive oil is frequently adulterated by other vegetable oils of a lower commercial price. Official methods for purity control of olive oil include the old classic criteria (physical and chemical constants [1]) in combination with the determination of the specific extinction coefficient in the ultraviolet (UV) (2) and the fatty acid composition by gas liquid chromatography (GLC) (3-5). However, it is largely recognized that when the level of the adulteration is lower than 10-15%, these criteria are insufficient to give reliable conclusions.

The GLC analysis of the fatty acid composition of a natural oil does not always lead to safe indications regarding its purity, since most natural oils—mainly the vegetable oils—contain the same fatty acids in variable amounts. Thus, in adulterating an oil with low proportions of one or more other oils, the determination of the overall fatty acid composition is not sufficient to reveal the admixture.

For this reason, a deeper insight into the structural features of the natural oils is required to solve this problem, based on the composition of certain characteristic glyceride fractions (6). This approach is based on the "even" (7) and the "restricted random" (8) distribution theories which are believed to describe with a satisfactory approximation the rules governing the distribution of individual fatty acids into glycerides' molecular species in most natural oils.

On the other hand, the degree of unsaturation of triglycerides (i.e., the presence in their molecule of one or more double bonds) is related to their polarity and therefore, by the use of suitable analytical techniques they may be separated into groups containing triglycerides of several types, e.g., saturated (of the type SSS), monounsaturated (of the type SSO), diunsaturated (of the types SOO and SSL), where S, O, L, denote, respectively, saturated acyl-, oleoyl- and linoleoyl- moieties of triglycerides. These groups have a characteristic and almost constant relative amount of fatty acids, which is very different from oil to oil.

For the separation of triglycerides on the basis of their degree of unsaturation most suitable seems to be the thin layer chromatographic (TLC) technique on silica gel impregnated with silver nitrate, initially used by Barret et al. (9) in order to study their structure.

[1] This work was taken in part from the doctoral dissertation of S. Passaloglou-Emmanouilidou.

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