# Cyclopropenoid Fatty Acids in Seed Oils of *Sida acuta* and *Sida rhombifolia* (Malvaceae)

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## ABSTRACT

Seed oils of *Sida acuta* and *Sida rhombifolia* were found to contain sterculic (11.0, 10.8%) and malvalic (1.7, 2.0%) acids respectively, in addition to the normal fatty acids. Co-occurrence of these acids was established by gas liquid chromatography of the silver nitrate-methanol-treated methyl esters using *Sterculia foetida* esters as a reference standard. This gas liquid chromatography technique of quantitation was found most suitable to estimate these acids in low level cyclopropenoid acid-containing seed oils.

## INTRODUCTION

Recently cyclopropenoid fatty acids have been the subject of much investigation due to their profound biological effects on animals (1-3) and cocarcinogenic properties (4-5). Fatty acids containing cyclopropene ring have been found in seed oils (6-8) of the genus *Sida* (Malvaceae). In a recent report in this journal, Rao et al. (9) observed that *S. acuta* seed oil contains the usual fatty acids in its glycerides. These authors found no cyclopropene acids because they made no effort to look for these acids and used laboratory procedures which destroyed cyclopropene acids. As a part of a screening programe aimed at the search for biologically active cyclopropene acids in herbaceous seed oils, it was found that *S. acuta* seed oil gave positive test (Halphen) for cyclopropenoid acids. Seed oil from another species of this genus, *S. rhombifolia*, also responded to Halphen test. Therefore these two cyclopropenoid acid-containing seed oils were thoroughly studied and the present paper describes the results of their fatty-acid analysis.

## EXPERIMENTAL PROCEDURES

Oils were extracted from crushed seeds with petroleum ether (40-60 C) in a soxhlet apparatus, and the solvent was evaporated under vacuum in a rotary evaporator. The fatty acid methyl esters were prepared by transmethylation of 1 g of oil in 50 ml of absolute methanol that contained 1% sodium methoxide. The reaction was allowed to proceed by refluxing for 20 min, and the methyl esters were extracted with ether as usual.

The methyl esters of each oil (200 mg) were treated with 60 ml of absolute methanol saturated with silver nitrate (10). The reaction was allowed to proceed at room temperature with stirring for 24 hr. The normal methyl esters and the reaction products from cyclopropenes were recovered from the reaction mixture by adding 100 ml of distilled water and extracting with ether. The extracts were dried over anhydrous sodium sulfate and the solvent evaporated in the stream of nitrogen.

Infrared (IR) spectra were determined in $CCl_4$ using Perkin-Elmer model 521 Spectrophotometer. Ultraviolet (UV) spectra were measured on a Beckman-DU-Spectrophotometer using a methanolic solution. Nuclear magnetic resonance (NMR) spectra were run in $CDCl_3$ on EM-360 60 MHz spectrometer with tetramethyl silane as the internal standard.

The esters of each oil were examined qualitatively by direct, reversed-phase and argentation thin layer chromatography (TLC) using *S. foetida* esters as the cyclopropenoid acid reference. Direct TLC showed only non-oxygenated acids. The reversed-phase TLC, using acetonitrile-acetic acid-water (70:10:20, v/v) as the solvent system, revealed a spot near the starting point corresponding to the spot exhibited by *S. foetida* esters. Clear spots of usual critical pairs were also obtained. Argentation TLC showed spots of saturates; monoene and diene parallel to those obtained from *S. foetida* esters resolved alongside.

Gas liquid chromatography (GLC) was done on F & M-720 GLC unit provided with flame ionization detector using EGSS-X Column (8 ft x 3/16 in.). The separations were carried out isothermally at 200 C. The temperatures at the injection port and detector block were 300 C. Nitrogen at a flow rate of 360 ml/hr was the carrier gas, and chart speed was 15 in./hr.

Freshly prepared *S. foetida* esters were treated with silver nitrate-methanol. The esters containing sterculate and malvalate derivatives thus obtained were used in GLC analysis as reference standard. Comparison of the relative retention times of the derivatives of *S. foetida* esters as well as those of *S. acuta* and *S. rhombifolia* esters clearly established the presence of sterculic and malvalic acids in these seed oils. Peak areas were calculated by triangulation method.

## RESULTS AND DISCUSSION

Light petroleum extraction of the crushed seeds yielded 12% oil in *S. acuta* and 14% in *S. rhombifolia.* Oil characteristics (Table I), iodine value, saponification value, refractive index, nitrogen (crude protein) and moisture percent-

TABLE I

Analytical Data on Seeds and Oils

| Species | Seed Analysis | | | Oil Properties | | |
|---|---|---|---|---|---|---|
| | Oil content % | Protein content Nx6.25, % | Moisture % | I.V.[a] | S.V.[b] | Ref. Index $N_D^{28}$ |
| Sida acuta | 12 | 26.8 | 6 | 76 | 184 | 1.4628 |
| Sida rhombifolia | 14 | 21.8 | 7.5 | 85 | 212 | 1.4669 |

[a]Iodine value (I.V.)
[b]Saponification value (S.V.)

age were determined by AOCS methods (11).

Both oils responded to Halphen test (12), thereby indicating the presence of cyclopropenoid acid. The oils showed the typical NMR signal at 9.28τ for the cyclopropene moiety. The methyl esters of each oil had the characteristic IR band for the cyclopropene moiety at 1008 $cm^{-1}$. There was no indication in the spectrum of a hydroxyl or terminal acetylenic group. The UV spectra indicated no conjugation in the oils. Quantitation of total cyclopropenoid fatty acid by the method of HBr titration (13) showed the presence of 11.92% and 12.51% by weight of cyclopropenoid acid in *S. acuta* and *S. rhombifolia* seed oils respectively.

GLC of methyl esters was done after treatment with silver nitrate in absolute methanol to form stable derivatives of cyclopropenoid acid according to the method of Schneider et al. (10). GLC compositional data is given in Table II.

GLC data of the cyclopropenoid acids in two seed oils were found to agree with those obtained by the method of HBr titration. As compared to the hydrogenation method (14-16) and methyl mercaptan derivatization technique (17) for the quantitation of cyclopropenoid fatty acids, the silver nitrate method produced a clear resolution of sterculate and malvalate derivatives in the GLC chromatogram. Further, this method has the additional advantage of not reacting with the other unsaturated acids present in the oil.

TABLE II

Gas Liquid Chromatography Analysis of the $AgNO_3$-Methanol Reacted Methyl Esters

| Fatty Acids | *Sterculia foetida* Wt % | *Sida acuta* Wt % | *Sida rhombifolia* Wt % |
|---|---|---|---|
| Palmitic | 26.0 | 35.8 | 26.6 |
| Palmitoleic | 1.0 | 5.7 | 3.5 |
| Stearic | 3.4 | 7.1 | 3.3 |
| Oleic | 9.4 | 20.3 | 38.1 |
| Linoleic | 1.3 | 18.4 | 15.7 |
| Linolenic | 0.6 | – | – |
| Malvalic | 7.1 | 1.7 | 2.0 |
| Sterculic | 51.2 | 11.0 | 10.8 |

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