# FATTY ACIDS OF *THALICTRUM REVOLUTUM* FRUIT LIPID

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ABSTRACT.—The fatty acids of *Thalictrum revolutum* fruit lipid have been determined and found to contain mainly the unsaturated acids with trans-unsaturation at 5-position. The major component acids found were trans-5-hexadecenoic (5%), trans-5-octadecenoic (30%), trans-5, cis-9-octadecadienoic (7%), cis-9, cis-12-octadecadienoic (20%), and trans-5, cis-9, cis-12-octadecatrienoic (30%) acids. In addition, myristic, palmitic, stearic and linolenic acids were found as minor constituents.

The *Thalictrum* genus (Runanculaceae family) is presently the only natural source of a unique class of fatty acids having isolated trans- double bonds at 5-position. The first report of *Thalictrum* fatty acids was by Bagby et al. (1) in which the characterization of trans-5-octadecenoic and trans-5, cis-9, cis-12-octadecatrienoic acids from *Thalictrum polycarpum* seed oil was reported. Subsequently, trans-5-hexadecenoic and trans-5, cis-9-octadecadienoic acids were characterized from *Thalictrum venulosum* seed oil by Bhatty et. al. (2). More recently, Rankoff et. al. (3) reported the presence of all four of these trans-5 series of fatty acids in five species of *Thalictrum*. A preliminary investigation of the fatty acids of *Thalictrum revolutum* DC. fruit lipids which indicated the presence of trans-unsaturation and components with unusual gas chromatographic retention times prompted us to undertake the present study.

## EXPERIMENTAL[1]

SOURCE.—Fruit of *Thalictrum revolutum* DC. were collected from plants growing in the Ohio State University College of Pharmacy Medicinal Plant Garden. Herbarium specimens of this species are in the herbarium of the Division of Pharmacognosy and Natural Products Chemistry.

EXTRACTION AND SAPONIFICATION OF THE OIL.—The *Thalictrum revolutum* fruits were oven dried at 40° and then ground (mesh number 20). The ground material was extracted in a Soxhlet extractor with light petroleum ether (30°–60°). When the solvent was removed on a rotary evaporator, a light green oil was obtained. Five grams of oil (10 parts by weight) was saponified (4) by refluxing with potassium hydroxide (3 parts by weight) in methanol (50 parts by volume) for 3 hours in an atmosphere of nitrogen gas. After saponification, the methanol was removed by distillation. The resulting soap was dissolved in water and extracted four times with diethyl ether. The combined ethereal extract was washed with water and dried over anhydrous sodium sulfate. Removal of the ether by evaporation left a nonsaponifiable residue (670 mg). The aqueous layer remaining after the extraction of nonsaponifiables was acidified with 4N sulfuric acid in an atmosphere of carbon dioxide gas. The free fatty acids

[1]The uv and ir spectra were taken on a Beckman UV5260 and Beckman IR4230 spectrophotometers, respectively. The ms were recorded on a DuPont 21-491 low resolution mass spectrometer. All the analytical gas chromatography was done on a Hewlett-Packard model 402 gas chromatograph, equipped with a flame ionization detector. Glass U-tube columns (6 ft x 3 mm id) were packed with 10% DEGS liquid phase on Gas-chrom Q (80–100 mesh). Column oven temperature was 170° for most of the runs. For short chain monocarboxylic acids, it was programmed from 100°–170° at the rate of 3°/minute. Injection port and detector temperatures were maintained at 220°. Helium flow rate was 45 ml/minute at 40 psi. The preparative gas chromatograph used was Prepkomatic 750 by Perkin Elmer with a thermal conductivity detector. Stainless steel preparative columns (biwalled, 3 ft x 0.5 inch each) of a total length of 6 ft (two sections) were packed with 5% DEGS on Gas-chrom Q (60–80 mesh). Oven temperature was programmed from 100° to 200° at the rate of 3°/minute. Helium flow rate was 200 ml/minute and 50 ml/minute through the column and the reference cell, respectively, at a pressure of 35 psi. The solid support, liquid phase and authentic fatty acid methyl esters were obtained from Applied Science Laboratories, Inc., State College, Pa., U.S.A.

were extracted four times with diethyl ether and petroleum ether alternately. The combined extracts were dried over anhydrous sodium sulfate, and the solvent was then removed by evaporation; 2 g of free fatty acids were obtained.

METHYLATION OF FREE FATTY ACIDS.—The methyl ester of the free fatty acid mixture was obtained by refluxing of the fatty acids (2 g) with 200 ml of dry methanol, containing 0.5% of sulfuric acid. The refluxing was continued for 4 hours in an atmosphere of nitrogen. The resultant mixture was cooled and diluted with water; the fatty acid methyl esters were recovered by extraction with diethyl ether and petroleum ether (30°–60°), respectively, 3 times each. The combined extract was dried over anhydrous sodium sulfate; when the solvent was evaporated, 2.3 g of fatty acid methyl esters were obtained.

FRACTIONATION OF MIXED METHYL ESTERS.—The mixed methyl esters were fractionated in a silver nitrate treated silicic acid column. The coating of silver nitrate on silicic acid and the elution technique was the same as reported elsewhere (5). A column (67 cm x 4 cm) was packed with 200 g of coated silicic acid. After the column was packed, its height was 57 cm. The sample (1 g) was applied on the column and then was eluted with light petroleum ether (30°–60°) containing increasing amounts of diethyl ether. Fractions of 20 ml each were collected in tubes and were analyzed by gas chromatography on a 10% DEGS (diethylene glycol succinate polyester) column. The fractions were pooled on the basis of their compositions. The saturated, monoenoic, dienoic and trienoic esters were eluted by 2, 5, 10 and 20 percent of ether, respectively.

ANALYSIS OF THE SATURATED ESTER FRACTION.—The fraction (300 mg) eluted by 2% ether in petroleum ether was analyzed by gas chromatography. The component peaks were matched with peaks of authentic saturated fatty acids and found to contain myristic ($C_{14}$), palmitic ($C_{16}$), and stearic ($C_{18}$) acids.

ANALYSIS OF THE MONOENOIC ESTER FRACTION.—The monoenoic ester fraction (ca 200 mg after pooling) which was eluted by 5% ether was found to contain two components in the ratio of 1:6 by gas chromatography. The equivalent chain lengths (ECL) of these two fatty acids were found to be 16.23 and 18.25 on a DEGS column, for the minor and major components, respectively. The two components were separated by preparative gas chromatography on a 5% DEGS column. The tlc of these two compounds was done on a silver nitrate (10%) coated plate (0.25 mm) (6). The plate was developed twice to 15 cm with a mixture of petroleum ether (30°–60°) and diethyl ether in the ratio of 9:1. The plate was sprayed with a 0.2% solution of 2', 7', dichlorofluorescein in ethanol and visualized under short uv light. The $R_f$ values of these two acids were the same and were identical with that of elaidic acid ($R_f$=0.62). The ir spectrum of both acids showed absorption at 966 $cm^{-1}$ (10.35$\mu$), characteristic of isolated trans-double bonds (6). Catalytic reduction (7) of 1 mg of each of the acids with platinum oxide in the presence of methanol produced palmitic ($C_{16}$) and stearic ($C_{18}$) acids from the acids with lower and higher retention times, respectively. The ms of the two methyl esters gave molecular ion peaks at $m/e$ 268 and $m/e$ 296 for the 16-carbon chain and 18-carbon chain esters, respectively. The two acids (1 mg each) were oxidized with permanganate-periodate reagent (8). The products of oxidation were methylated with diazomethane (9), and the methyl esters were gas chromatographed. The mono- and dicarboxylic acid esters were identified by comparison of their retention times with authentic standards. The 16-carbon chain acid produced glutaric ($C_5$) and undecanoic ($C_{11}$) acids, whereas the 18-carbon chain acid produced glutaric ($C_5$) and tridecanoic ($C_{13}$) acids.

ANALYSIS OF THE FRACTION CONTAINING DIENOIC ESTERS.—The fractions (125 mg) eluted by 10% ether from the silver nitrate column were pooled on the basis of gas chromatographic analyses; and two compounds, with some contamination, were isolated. These acids were further purified by preparative tlc on silver-nitrate-impregnated silica gel plates. The ECL values for the compounds were found to be 18.55 and 18.75 on a 10% DEGS column. The ir spectrum of the acid (ECL=18.55) had an absorption band at 10.35$\mu$ and did not show any uv absorption. The ms showed a molecular ion peak at $m/e$ 294. This acid ester (35 mg) was hydrolyzed with methanolic potassium hydroxide, and the free fatty acid was recovered (30 mg). The fatty acid was dissolved in 50% aqueous ethanol and partially reduced (10) with hydrazine hydrate (99%) by agitation for two hours at 50°. The reduction product was worked up and methylated with diazomethane. The tlc of the mixed methyl esters of the reduction product was done on a silver nitrate coated plate as described previously. The esters were separated on the tlc plate into four bands which corresponded with the $R_f$ values of stearic, elaidic, oleic acid esters and the unreduced starting material. The bands corresponding to elaidic and oleic acids were scraped from the plate and were oxidized by permanganate-periodate reagent. The oxidized products were methylated by diazomethane and identified by gas chromatography. The trans- acid produced glutaric ($C_5$) and tridecanoic ($C_{13}$) acids, whereas the cis- acid produced azelaic ($C_9$) and nonanoic ($C_9$) acids. The other dienoic acid (ECL= 18.75) did not show any ir (at 10.35$\mu$) or uv absorptions. The ms gave a molecular ion peak at $m/e$ 294. On catalytic reduction, it produced stearic acid ($C_{18}$). Partial reduction of this

acid with hydrazine hydrate and the tlc of the products after methylation showed three bands corresponding to stearic acid, oleic acid and unreduced starting material. The band corresponding to oleic acid was scraped from the plate and was oxidized by permanganate-periodate. The products of oxidation were identified as hexanoic ($C_6$, traces), nonanoic ($C_9$), azelaic ($C_9$), and dodecanedioic ($C_{12}$) acids.

ANALYSIS OF THE FRACTION CONTAINING TRIENOIC ESTERS.—This fraction (150 mg) was eluted by 20% ether. The major compound (90 mg) after purification had an ECL of 20.00 on a DEGS column. The compound showed ir absorption band at 10.35$\mu$ but did not have any uv absorption. It was converted to stearic acid by catalytic hydrogenation. The ms of the compound showed a molecular ion peak at *m/e* 292. The compound (60 mg) was partially reduced by heating with hydrazine hydrate for three and a half hours. The tlc of the methyl esters on a silver nitrate plate showed six bands. The monoenoic bands corresponding to elaidate and oleate were scraped from the plate and were oxidized by permanganate-periodate reagent. The trans- monoene produced glutaric ($C_5$) and tridecanoic ($C_{13}$) acids, whereas the cis- monoene band produced hexanoic ($C_6$, traces), nonanoic ($C_9$), azelaic ($C_9$) and dodecanedioic ($C_{12}$) acids. The other trienoic acid (ECL=20.30) was contaminated by the major component and only 3 mg could be isolated after purification by silver nitrate tlc. This acid did not show any ir (at 10.35$\mu$) or uv absorption. On catalytic reduction, it produced stearic acid. The ms of this compound showed a molecular ion peak at *m/e* 292. The retention time of this compound was identical with that of methyl linolenate on DEGS polyester and SE-30 nonpolar columns. The $R_f$ value of this compound was identical to that of methyl linolenate ($R_f$=0.20) on a silver nitrate tlc plate.

## RESULTS AND DISCUSSIONS

The yield of oil, iodine value and amount of nonsaponifiable material are presented in table 1. The high iodine value is indicative of the presence of an

TABLE 1. Oil content, nonsaponifiable content and iodine value of *Thalictrum revolutum* oil.

| Oil Content (% fruit) | Iodine Value (Hanus') | Nonsaponifiable content (% oil) |
|---|---|---|
| 15.1 | 142.0 | 13.4 |

appreciable amount of unsaturated acids. The analytical data for the determination of the absolute structures of the unsaturated acids is summarized in table 2. The chain lengths of the unsaturated acids were determined by gas chromatography of the hydrogenated acids and authentic saturated fatty acid esters. The number of double bonds in each acid were determined by the ms of the parent acids. The fact that trans- isomers of unsaturated fatty acids travels faster than the cis- isomers through a silver nitrate treated column or tlc has been established by a number of workers (11–14). In the present study, fatty acids having a particular number of double bonds were eluted by a solvent system of definite composition (7), and the trans- isomers were eluted in earlier fractions, followed by the cis- isomers. The monoenoic acids eluted through the column were all trans- , as revealed by their ir spectra and tlc mobility on silver nitrate coated plates. They were directly oxidized to produce mono- and dicarboxylic acids. Both the 16- and 18-carbon chain monoenes produced glutaric ($C_5$) acid, indicating that the trans-unsaturation in both the acids were at the 5- position. The trans-monoenoic acids obtained by the partial reduction of the di- and trienoic acids also produced glutaric acid, indicating that all the trans-unsaturated acids of *T. revolutum* contained their trans- double bonds at the 5- position. The cis-monoenoic acid obtained by the partial reduction of the diene (containing a trans-double bond) yielded azelaic ($C_9$) and nonanoic ($C_9$) acids proving that the diene contained a cis- double bond at the 9- position. The diene containing two cis-

TABLE 2. Analytical data for the structures of the unsaturated fatty acids of *T. revolutum*.

| Component acids[a] | Molecular ion peak of the methyl ester (m/e) | Chain length of the acid produced after hydrogenation[b] | Nature of double bonds in the monoenes[c, e] | Mono- and dicarboxylic acids obtained by the oxidation of cis- and trans- monoenes | | | | Position and configuration of the double bonds in the parent acids |
|---|---|---|---|---|---|---|---|---|
| | | | | Cis- monoene[d] | | Trans- monoene | | |
| | | | | M | D | M | D | |
| $C_{16:1}$.......... | 268 | $C_{16}$ | t | | | $C_{11}$ | $C_5$ | 5t |
| $C_{18:1}$.......... | 296 | $C_{18}$ | t | | | $C_{13}$ | $C_5$ | 5t |
| $C_{18:2}$.......... | 294 | $C_{18}$ | t, c | $C_9$ | $C_9$ | $C_{13}$ | $C_5$ | 5t, 9c |
| $C_{18:2}$.......... | 294 | $C_{18}$ | c, c | $C_6$, $C_9$ | $C_9$, $C_{12}$ | | | 9c, 12c |
| $C_{18:3}$.......... | 292 | $C_{18}$ | t, c, c | $C_6$, $C_9$ | $C_9$, $C_{12}$ | $C_{13}$ | $C_5$ | 5t, 9c, 12c |

[a]The first and second figures represent, chain length: number of double bonds.
[b]Determined by gas chromatography.
[c]The monoenes were obtained by the partial reduction of the polyenes.
[d]M and D represents, mono- and dicarboxylic acids respectively.
[e]c and t represents the cis- and trans-unsaturations respectively.

TABLE 3. Fatty acid composition of *T. revolutum* fruit lipids.[a]

| Component acids[b] | Double bond position and configuration[c] | Percent (w/w) | RRT[d] | ECL[e] |
|---|---|---|---|---|
| $C_{14:0}$......... | | 0.4 | 0.26 | 14.00 |
| $C_{16:0}$......... | | 3.0 | 0.47 | 16.00 |
| $C_{16:1}$......... | 5t | 5.0 | 0.55 | 16.23 |
| $C_{18:0}$......... | | 2.6 | 0.86 | 18.00 |
| $C_{18:1}$......... | 5t | 30.5 | 1.00 | 18.25 |
| $C_{18:2}$......... | 5t, 9c | 7.0 | 1.20 | 18.55 |
| $C_{18:2}$......... | 9c, 12c | 20.0 | 1.35 | 18.75 |
| $C_{18:3}$......... | 5t, 9c, 12c | 30.2 | 1.55 | 20.00 |
| $C_{18:3}$......... | 9c, 12c, 15c | 1.3 | 1.85 | 20.3 |

[a]Calculated from the analysis on 10% DEGS column.
[b]The first and second figures represent, carbon chain length: number of double bonds.
[c]c and t represents cis- and trans- configurations respectively.
[d]Relative retention times, with respect to methyl oleate.
[e]Equivalent chain lengths.

double bonds was found to be linoleic acid. The triene fraction also contained two isomeric acids, one with a trans- double bond, the other being the all cis-isomer. Partial reduction of the triene (ECL = 20.00) with hydrazine hydrate yielded three monoenes. One of them was trans-monoene and the other two were cis- isomers as revealed by their ir spectra (a trans- isomer has an ir absorption band at 10.35$\mu$, whereas a cis- isomer lacks this band) and mobility on a silver nitrate tlc plate. Oxidation of the two cis- monoenes produced two mono-carboxylic ($C_6$, hexanoic; $C_9$, nonanoic) and two dicarboxylic ($C_9$, azelaic; $C_{12}$, dodecanedioic) acids. Oxidation of the trans- monoene, on the other hand, gave glutaric ($C_5$) and tridecanoic ($C_{13}$) acids. This indicated that the triene contained two cis- double bonds, one at the 9- position and the other at the 12- position, and a trans- double bond at the 5- position.

The other triene isolated from the same origin, (ECL = 20.30), did not show any ir (at 10.35$\mu$) and uv absorption which indicated that the three double bonds were all cis. It proved to be linolenic acid (18:3, 9c, 12c, 15c) by direct comparison of the methyl ester's retention time on DEGS Polyester and SE–30 non-polar columns, $R_f$ ($R_f$ = 0.2) value on a silver nitrate tlc plate, ir and ms spectral properties. On catalytic hydrogenation it produced stearic acid.

The fatty acid composition and their gas chromatographic retention data have been presented in table 3. The major components were octadeca-trans-5, cis-9, cis-12 trienoic and octadec-trans-5-enoic acids, each being about 30%. This trienoic acid (5t, 9c, 12c) was found in this study to be less in amount than reported in earlier studies for other *Thalictrum* species (1–3). Rankoff (3) found this acid to constitute over 40% in four species and as high as 56.7% in *T. aquilegifolium* seed oil. The other major component, trans-5-octadecenoic acid, is considerably higher than that found in the other species studied by Rankoff (3). Oleic acid was not found in the present study, whereas it was present in considerable amounts in the other species (3). The trans-5-hexadecenoic acid found in *T. revolutum* is considerably higher than the amounts reported in other species of *Thalictrum* (3). The total unsaturated acids of *T. revolutum* was found to be 94% which correlates with the high iodine value and is in agreement with previous reports (3).

## ACKNOWLEDGMENT

We wish to thank the U.S. Public Health Service for the grant (HL–07502) from the National Institutes of Health which supported this study.

*Received 10 September 1979.*

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