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The Fatty Acid Composition of Seed Oils from Ten Plant Families with Particular Reference to Cyclopropene and Dihydrosterculic Acids

J.R. VICKERY, CSIRO Division of Food Research, North Ryde, NSW 2113, Australia

ABSTRACT

Oil contents and fatty acid compositions of 40 seed oils of the plant families Elaeocarpaceae, Thymelaeceae, Malvaceae, Sterculiaceae (order Malvales); Anacardiaceae, Celestraceae, Sapindaceae (Sapindales); Ebenaceae, Sapotaceae (Ebenales) and Rhamnaceae (Rhamnales) are presented. Cyclopropene fatty acids (CPFA) occur in two families in the order Malvales not hitherto assayed. CPFA contents of seed oils of 12 Australian and Pacific species of Malvaceae and Sterculiaceae are given. CPFA occur randomly in small amounts in at least six families not in the order Malvales. Dehydrosterculic acid (DHS) occurs in small amounts in many species of Anacardiaceae, Celestraceae, Elaeocarpaceae, Malvaceae, Sapindaceae, Sapotaceae and Sterculiaceae. Linoleic acid was predominant in 28 of 40 seed oils, being as high as 63.9% in two species. The sum of 18:1 and 18:2 esters exceeded 70% in 20 oils.

INTRODUCTION

The occurrence of cyclopropene fatty acids (CPFA), malvalic and sterculic, in many species of the plant families Bombacaceae, Malvaceae, Sterculiaceae and Tiliaceae was summarized up to 1969 by Phelps et al. (1), Recourt et al. (2) and Christie (3). Subsequently, 30 other species in these families have been analyzed for CPFA (4-10).

Assays of seed oils for CPFA in other families in the order Malvales have not recently been reported. Analyses on some species of Thymelaeaceae and Elaeocarpaceae are reported in this paper as well as a number of Australian and New Caledonian species of Malvaceae and Sterculiaceae not hitherto studied. In their study of seed oils of species from 113 plant families, Earle and Jones (11) reported the occurrences of CPFA in plants not in the order Malvales. These included *Styrax americana* (order Ebenales, family Styracaceae), a *Randia* sp. (order Rubiales, family Rubiaceae) and *Astragalus brazoensis* (order Rosales, family Leguminosae). Assays were conducted on some seed oils in Ebenaceae and Sapotaceae (order Ebenales), in Anacardiaceae, Celastraceae, and Sapindaceae (order Sapindales) and in Rhamnaceae (Rhamnales).

CPFA were detected in the seed oils by the Halphen color test (12). The assays on 20 Halphen-positive and 20 Halphen-negative oils are reported.

The cyclopropane fatty acid, dihydrosterculic (DHS), frequently occurs in oils containing CPFA (8). It also occurs as a major component (41%) in *Litchi chinensis* seed oil (13) and comprises 17% in the oil of *Euphoria longan* (14), both species being in the family Sapindaceae. In the present investigations DHS was assayed in each oil.

This work is part of a continuing survey of seed oils from the Australian and South Pacific regions.

EXPERIMENTAL PROCEDURES

Material

Most seeds were collected by officers of the Royal Botanic Gardens, Sydney, H.S. McKee of New Caledonia and the author.

Treatment of Oils

After drying at room temperature, the seeds were disintegrated and extracted with cold hexane (b.p. 65-67 C) in an "Omni-Mixer." The methyl esters were prepared by the rapid method of Glass and Christopherson (15) using sodium methoxide. The esters were purified by dissolving them in hexane and placing up to 100 mg on a column comprising 12 g Florisil containing 7% water, and eluting with 40 ml hexane. The methyl esters were eluted with 70 ml diethyl ether in hexane (5% v/v).

Halphen Color Test on Oils

The AOAC method (12) was used, but, when the amount of oil available was less than 0.5 g, only 1-2 ml of reagent was used; less than 50 mg of two oils were available and dilutions of 1 in 20 and 35 were necessary. Dilution rather than large reduction of the reagent volume was chosen, because at least 1 ml of reagent was necessary to perceive the development of a faint pink color.

Argentation of Methyl Esters

Samples of esters from oils having a positive Halphen Color test were analyzed for cyclopropene and other acids by the method of Schneider et al. (16), which involves the reaction of silver nitrate in methanol with CPFA esters to form ether and ketone derivatives. The recovered esters and ether and ketone derivatives were submitted to analysis by gas chromatography.

When CPFA concentrations were less than ca. 3%, the derivatives were separated from the methyl esters by column chromatography with neutral alumina (16). The methyl ester fraction did not contain derivatives, but the derivative fraction usually contained small amounts of methyl esters. In the subsequent analysis of the reaction products by gas chromatography, a known weight of the methyl ester 20:0 was added as an internal standard.

TABLE I

Comparative Composition of Methyl Esters of *Heritiera actinophylla* Seed Oil Using Two Stationary Phases – Mass Percent

Saturated			Unsaturated			Cyclopropenes		
Ester	1[a]	2[a]	Ester	1	2	Ester	1	2
14:0	0.3	0.8	16:1	0.7	0.9	Malvalic	2.0	2.0
16:0	17.1	17.5	18:1	17.8	17.9	Sterculic	0.8	0.6
16 BC[b]	0.4	0.4	18:2	41.6	43.5			
18:0	2.8	2.8	18:3	1.1	2.4			
20:0	0.9	0.0	20:1	8.7	8.4			
DHS	2.7	0.0	20:3	0.9	1.3			
			22:1	2.2	1.4			

[a]1 = SP-222-PS phase; 2 = Silar-10 C phase.
[b]BC, branched chain ester.

Gas Chromatography

In a Pye series 104 chromatograph, 10% Silar-10 C on Gas Chrom Q was packed in a 12 ft glass column having 0.125 in. i.d. In a Packard chromatograph, the stationary phase was 10% SP-222-PS packed in a 6 ft glass column having 0.125 in. i.d. Both chromatographs had flame ionization detectors; the first column was operated isothermally at 190 C and the second at 151 C. When there was uncertainty in the identification of the unsaturated esters, the sample was fully hydrogenated, using Adams catalyst, and gas chromatography of the hydrogenated esters conducted. Two phases were used because (a) they had different McReynolds Constants and therefore gave a check on accuracy of analysis, (b) Silar-10 C separated *cis* and *trans* esters provided that the column temperature did not exceed 192 C and (c) SP-222-PS, but not Silar-10 C, separated DHS from linoleic ester when the latter occurred in high concentration.

The retention times of the ether and ketone reaction products were checked against those derived from the esters of *Sterculia foetida* oil which contained 58% and 10% of sterculic and malvalic acids, respectively. The retention times were also checked after the addition of 20% (w/w) *Sterculia foetida* esters to the esters from five seed oils which gave positive Halphen tests. The accuracy of CPFA assays in five oil samples containing more than 3% CPFA was tested by adding as a "marker" 15% (w/w) pure methyl ester of 20:0.

In order to check the retention time of DHA, 10% (w/w) DHS ester was added to the methyl esters of three seed oils just prior to submitting them to gas chromatography. Using 10% SP-222-PS as the stationary phase, the average equivalent chain lengths (E.C.L.) of DHS and linoleic esters at a column oven temperature of 151 C and a fixed N_2 gas flow rate of 37 cm^3/min were 19:28 and 19:08, respectively. Similar values for the E.C.L.s were obtained when a standard mixture of 14:0, 16:0, 18:0, 18:2 and DHS esters was submitted to similar conditions of gas chromatography.

RESULTS AND DISCUSSION

The results of the methyl ester assays with the two phases used in the g.c. equipment did not differ appreciably, except that with Silar-10 C the DHS peak was "buried" in that of 18:2. Comparative assays for the seed oil of *Heritiera actinophylla* are given in Table I.

Reasonable separation of the ether and ketone derivatives of both CPFA from each other and from the methyl esters was achieved. An example of such separation is illustrated in Figure 1, which shows the g.c. of the chloroform fraction of *Radyera farragei* esters and derivatives after separation on an alumina column: this oil contains less than 1% total CPFA. Relative to the methyl ester of 20:0, the retention times of the two ether derivatives were 1.39 and 1.52, 2.53 and 2.81 for the two ketone derivatives. The concentrations of the constituents were: 16:0, 7.1%; 18:1, 4.7%; 18:2, 11.4%; 20:0, 35.0%; malvalic ether 26.9%; sterculic ether 10.9%; malvalic ketone 2.9%; sterculic ketone 1.1%. As the chloroform fraction contained about 23% methyl esters (excluding 20:0), clear separation of the shorter chain esters from the CPFA derivatives was not obtained.

Oil Contents

The oil contents of the seeds without testa are shown in Tables II and III. They range from very low (*Litchi chinensis*) to values exceeding 46% (*Jagera pseudorhus* and *Harpephyllum caffrum*). Oil contents within families tend to be very variable.

Cyclopropene Fatty Acids

CPFA contents of 20 Halphen-positive seed oils are given in Table II. CPFA were present in moderate amounts in the two species of Thymelaeaceae, but present in only one species of Elaeocarpaceae of four assayed. These results, together with those reported by previous workers (1-10), indicate that CPFA are present in most species of Bombacaceae, Malvaceae and Sterculiaceae. The results also show that CPFA may occasionally occur in minor amounts in Celastraceae, Sapindaceae and Sapotaceae, thus widening the range of their occurrence to six families not in the order Malvales. Earle and Jones (11), however, obtained negative

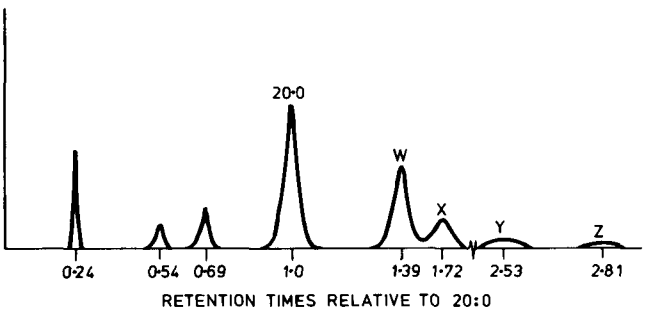

FIG. 1. Gas chromatograph of chloroform fraction containing CPFA ether and ketone derivatives of *Radyera farragei* seed oil. Methyl ester of 20:0 (36%) used as marker. Peaks w and x, malvalic and sterculic ethers; peaks y and z, corresponding ketones.

TABLE II

Halphen Positive Seed Oils. Oil Contents and Methyl Esters, Mass Percent

Family	Species	Oil content	Saturated				Unsaturated						D.b. S.	Malvalic	Sterculic	Other esters
			14:0	16:0	18:0	20:0	16.1	18.1	18.2	18.3	20:1	20:2				
Thymelaeaceae	*Pimelea decora Domin.*	4.2	2.2	17.5	5.9	1.1	2.2	26.8	12.7	25.4	--	---	--	2.1	1.0	14:1, 2.2 22:0, 1.0
	P. linifolia Sm.	12.9	1.4	18.8	4.2	18.8	2.5	16.7	10.8	10.0	---	0.9	—	1.9	13.9	
Elaeocarpaceae	*Elaeocarpus reticulatus* Sm.	2.9	0.4	13.9	5.8	---	4.2	47.3	27.0	0.5	---	---	--	0.2	0.7	
Malvaceae	*Abutilon auritum* (Wall. ex Link) Sweet	13.7	0.1	13.4	2.6	1.0	0.4	16.0	49.3	0.7	10.6	0.4	1.3	1.5	1.2	17:0, 0.1, 16 BC, 0.1 (a) 22:1, 1.3
	Gossypium sturtianum J.H. Willis	11.3	0.3	19.0	2.9	--	0.4	14.9	59.4	---	0.1	0.3	1.2	0.8	0.4	16 BC, 0.3
	Hibiscus trionum L.	19.0	0.3	16.5	4.1	--	1.1	13.0	60.1	1.3	—	---	--	3.0	0.6	
	H. diversifolius Jacq.	14.2	0.1	21.8	2.4	--	0.8	22.6	50.0	---	---	---	1.2	0.6	0.3	17:0, 0.2
	Lagunaria patersonia (Andr.) G. Don	16.0	--	21.7	2.4	—	8.1	28.7	30.5	0.7	---	---	--	5.3	2.5	
	Lavatera plebeia Sims	14.5	0.8	16.0	3.0	0.7	0.8	11.6	52.6	1.4	3.6	—	--	6.9	1.6	17:0, 1.0
	Pavonia hastata Cav.	13.5	—	20.0	1.9	—	0.8	13.8	52.9	0.6	0.5	4.0	1.9	2.1	1.3	16 BC, 0.2
	Radyera farragei (F. Muell.) Fryxell & Hashmi	16.4	0.2	13.2	2.4	--	0.3	15.1	65.8	---	---	---	2.0	0.5	0.2	16:2, 0.3
Sterculiaceae	*Brachychiton gregorii* F. Muell.	18.3	0.1	18.7	3.0	0.2	1.1	28.1	42.6	0.2	---	---	—	5.3	0.4	16 BC, 0.3
	Heritiera actinophylla (F.M. Bail.) Kostermans	2.1	0.3	17.1	2.8	0.9	0.7	17.8	41.6	1.1	8.7	---	2.7	2.0	0.8	17:0, 0.1 16 BC, 0.3 20:3, 0.9 22:1, 5.4
	Lasiopetalum macrophyllum Grah.	2.7	4.6	16.1	2.9	18.0	0.8	10.3	24.4	0.2	2.7	---	4.4	0.7	0.2	12:0, 6.1 15:0, 0.6 16 BC, 0.7 20:3, 1.9 22:1, 5.4
	Rulingia corylifolia Grah.	9.5	--	12.2	3.4	0.8	1.6	9.5	61.4	0.8	---	—	--	5.9	3.5	
Sapotaceae	*Pouteria wakere* (Panch. & Sebert) Baehni	15.9	0.2	17.8	10.3	—	0.3	42.8	27.3	0.2	0.4	---	—	tr.	0.2	15:0, 0.1
Celastraceae	*Elaeodendron australe* Vent.	16.5	—	17.8	4.4	—	0.6	23.9	52.5	0.7	—	—	--	tr.	0.1	
Sapindaceae	*Dodonaea triquetra* Wendl.	17.8	--	13.7	2.6	0.6	0.7	22.6	54.4	2.0	0.9	---	1.8	0.6	0.1	
	Litchi chinensis Sonner. cv Kwai Mi	0.6	0.9	12.1	5.1	0.6	1.9	27.7	1.2	4.5	---	0.4	40.9	tr.	0.1	15:0, 0.3 20:3, 0.3 9-10 Methylenehexadecanoic, 4.0
	Do cv Bengal	0.6	0.5	14.7	6.1	0.4	2.0	31.0	1.4	6.2	--	0.3	31.9	tr.	0.2	15:0, 0.3 22:1, 0.1 9-10 Methylenehexadecanoic, 4.9
	Koelreuteria elegans (Seem.) A.C. Sm.	29.1	--	6.1	1.0	3.0	0.2	25.7	9.2	2.6	45.3	0.7	---	0.4	5.8	

[a]BC = Branched chain.

[b]= Dihydrosterculic ester.

TABLE III

Oil Contents and Fatty Acid Composition of Halphen-Negative Oils, Mass Percent

Family	Species	Oil %	Saturated esters						Unsaturated esters						Other esters
			14:0	16:0	18:0	20:0	22:0	DHS[b]	16:1	18:1	18:2	18:3	20:1	20:2	
Elaeocarpaceae	*Elaeocarpus persicifolius* Brong. et Gris	23.2	0.7	22.0	6.0	—	—	—	1.7	51.8	17.3	—	0.5	—	
	E. rotundifolius Brong. et Gris	27.9	0.2	25.2	5.8	—	—	0.5	13.9	29.9	25.0	—	—	—	
	E. alaternoides Brong. et Gris	34.3	—	21.7	4.3	—	—	1.6	7.6	32.6	33.8	—	—	—	
Sapotaceae	*Mimusops commersonii* (G. Don) Engler	9.1	0.1	23.1	7.7	0.8	—	—	0.1	56.0	11.5	0.1	0.4	—	17:0, 0.2
	Planchonella australis (R.Br) Pierre	31.2	0.1	15.1	5.0	0.2	0.1	2.6	0.3	27.2	51.3	0.4	0.3	—	
	P. myrsinoides (A. Cunn. ex Benth) W.D. Francis	7.6	0.4	22.7	5.9	0.2	0.1	0.9	0.4	32.1	36.1	1.0	0.8		12:0, 0.1 15:0, 0.1 17:0, 0.1
	Pyriluma sphaerocarpum (Baill.) Aubrév.	8.2	1.6	22.3	5.5	—	—	—	1.4	32.6	35.7	0.3	0.2	—	15:0, 0.4
Ebenaceae	*Diospyros australis* (R. & Br.) Hiern	3.0	1.4	22.6	3.7	1.1	0.7	—	1.0	24.5	38.9	2.2	2.2	0.1	12:0, 0.3 15:0, 0.9 14:1, 0.3
Rhamnaceae	*Emmenosperma pancheranum* Baill.	13.2	0.4	6.0	3.3	3.7	1.1	—	0.4	1.3 trans 20.0 cis	55.7	6.3	1.7	—	
Anacardiaceae	*Harpephyllum caffrum* Bernh. ex Krauss	46.7	0.6	15.7	5.0	—	—	1.3	0.6	13.2	63.9	0.4	0.2		
	Melanorrhoea pubescens Wall	2.3	2.8	21.6	6.4	—	—	1.9	3.7	32.0	29.8	—	1.8	—	
	Schinus molle L.	13.3[a]	0.3	13.0	2.4	—	—	—	1.3	23.3	57.0	0.2	—	—	12:0, 1.7 12:1, 0.6 17:0, 0.2
	Pistacia chinensis Bunge	5.6	0.3	21.0	1.8	—	—	—	1.4	41.1	31.4	2.9	—	—	17:0, 0.1
Sapindaceae	*Dodonaea boroniifolia* G. Don	13.9	—	11.1	2.7	2.3	0.3	1.4	0.3	19.3	63.0	0.5	0.4	—	17BC, 0.2[c]
	D. petiolaris F. Muell.	11.7	—	12.4	6.4	1.4	—	4.1	0.8	24.7	48.3	0.6	—	1.3	
	D. triangularis Lindl.	6.9	0.3	9.6	3.3	3.6	1.9	—	0.6	21.0	57.1	1.7	0.6	0.2	
	D. truncatiales F. Muell.	18.7	—	8.6	4.2	1.8	—	2.6	1.0	18.8	63.9	0.9	—	0.8	
	D. viscosa L.	14.3	—	10.3	3.7	4.5	—	1.5	2.0	21.9	53.9	2.3	—	1.4	
	Jagera pseudorhus (A. Rich.) Radlk	46.9	0.1	3.7	1.6	—	—	2.1	0.2	28.3	16.9	44.1	—	0.9	14:1, 0.1
Celastraceae	*Elaeodendron melanocarpum* B. Gray 1494	40.5	—	21.9	6.9	1.8	—	0.6	1.0	23.6	44.2	—	0.9	—	

[a]Oil contains 19% unsaponifiable material.

[b]Dihydrosterculic ester.

[c]Branched - chain.

Halphen color tests in two seed oils from Sapotaceae, one Symplocaceae and three of four species of Ebenaceae (order Ebenales), and 22 species in Anacardiaceae, Aquifoliaceae, Celastraceae, Limnanthaceae and Sapindaceae (order Sapindales).

Results in Table II and those of other workers show that the concentrations of malvalic acid may usually be greater than those of sterculic acid in Malvaceae. In Bombacaceae and Sterculiaceae either malvalic or sterculic acid may predominate in about equal frequency.

Dihydrosterculic Acid

The findings of Bohannon and Kleiman (8), and those given in Tables II and III, show that small amounts of DHS occur in a large number of species of Anacardiaceae, Bombacaceae, Celastraceae, Elaeocarpaceae, Malvaceae, Sapotaceae and Sterculiaceae. DHS may occur in low to high concentrations in Sapindaceae.

Evidence obtained by Yano et al. (17) suggests that the CPFA biosynthetic pathway involves the initial formation of DHS from oleic acid, with subsequent desaturation to sterculic acid, and α-oxidation to malvalic and dihydromalvalic acid (DHM). The frequent association of DHS with CPFA in plant families within and without the order Malvales suggests that a wider search for DHS and perhaps DHM in many other species may be profitable.

Other Esters

Amongst the saturated esters, there were relatively high occurrences of 20:0 in *Pimelea linifolia* and *Lasiopetalum macrophyllum* (Table II). In Sapindaceae species, 16:0 is generally low (Tables II and III).

In 28 of 40 oils, 18:2 was predominant, with a maximum of 63.9% in *Harpephyllum caffrum* and *Dodonaea truncatiales.* This ester was consistently high in the genus *Dodonaea,* and high in many species of Celastraceae, Malvaceae and Sterculiaceae. Hilditch and Williams (18) had noted high levels in other Sapindaceae species. *Dodonaea viscosa* oil was studied by Badami et al. (19); an analysis of Australian seeds (Table III) showed that there were no appreciable differences from those grown in India.

The sum of the concentrations of 18:1 and 18:2 exceeded 70% in 20 of 40 oils and were over 60% in 30.

Jagera pseudorbus and *Pimelea decora* oils had high concentrations (>25%) of 18:3, the former also having a high seed oil content. In all other species, 18:3 was low or absent.

Koelreuteria elegans is notable for its high content of 20:1 (45%).

Within genera, the pattern of fatty acid contents tends to be rather uniform, but, in *Diospyros australis* (Table III) the values of 16:0 (23%), 18:1 (24%) and 18:2 (39%) differ from those found by Tandon et al. (20) for *D. perigrina* where they are 8%, 47%, and 15%, respectively.

The only *trans* ester detected was in the 18:1 fraction of *Emmenosperma pancheranum* (Rhamnaceae), Table III, where it comprised 6% of the total 18:1 esters.

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