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A SURVEY OF THE SARCOLAENACEAE FOR CYCLOPROPENE FATTY ACIDS

EMILE M. GAYDOU* and ARMAND RENÉ P. RAMANOELINA

Département des Industries Agricoles et Alimentaires, Etablissement d'Enseignement Supérieur des Sciences Agronomiques, Université de Madagascar, B.P. 175, Antananarivo, Democratic Republic of Madagascar

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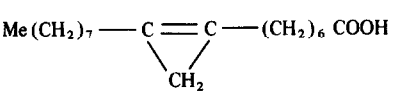
Key Word Index—Sarcolaenaceae; Chlaenaceae; cyclopropene fatty acids esters; malvalic acid; sterculic acid; chemotaxonomy.

Abstract—An investigation of the fatty acid composition of 22 representative species of the 10 genera of the family Sarcolaenaceae yielded some results of chemotaxonomic interest. Two cyclopropene fatty acids, malvalic and sterculic acids, were detected and quantitated in the seed oil of most species. The occurrence of cyclopropene fatty acids shows that this family has more biochemical affinities with the order Malvales than with the order Parietales.

INTRODUCTION

The Sarcolaenaceae is a small family of ornamental shrubs or trees including 33 species, located in Madagascar [1]. A key for the 10 genera of the family is given by Capuron [2]. Reports on the botanical classification [1, 2] and anatomy of leaves [3] and pollen [4] reveal that this plant family has some affinities with the orders Malvales, Theales, Guttiferales, Terebintales and Parietales. We know virtually nothing of the chemistry of this little family [5, 6]. No chemotaxonomic study has been performed hitherto.

The occurrence of cyclopropene fatty acids (CPEFA), such as malvalic (8,9-methylene-heptadec-8-enoic) **1** and sterculic (9,10-methylene-octadec-9-enoic) **2** acids, in



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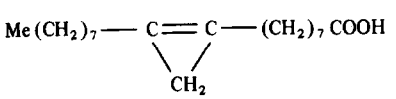

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many species of Bombacaceae, Malvaceae, Sterculiaceae and Thymelaeceae (Malvales) has been reported [7–10]. These CPEFA are not widespread in other plant orders and their detection in seed oils can represent a useful tool for plant classification.

Due to our interest in the distribution of CPEFA in plant seed oils [10, 11], we have undertaken the screening of 22 Sarcolaenaceae species for malvalic and sterculic acids.

*Present address: Ecole Supérieure de Chimie de Marseille, Université de Droit, d'Economie et des Sciences, Centre de Saint-Jérôme, rue Henri Poincaré, 13397 Marseille Cédex 4, France.

RESULTS

The 22 seed samples investigated represent the 10 genera described by Capuron [2]. Extraction of the dried seeds of the various Sarcolaenaceae species yielded 0.5–5% of neutral lipids. Only one species, *Rhodolaena altivola*, contained more than 17% neutral lipids (Table 1). The seed oils obtained are yellow fluids which crystallize at 4–5°. Among the 22 oils investigated, 16 gave a positive red-pink Halphen colour test [12], showing the presence of a cyclopropenic group. This fact was confirmed by a characteristic IR band at 1009–1010 cm^{-1} for the cyclopropene moiety. There was no UV maximum, indicating the presence of conjugated unsaturation. Fatty acid methyl esters were prepared by base-catalysed transmethylation [13]. GC of the methyl esters was done using a Carbowax 20 M WCOT glass capillary column. Equivalent chain length (ECL) values obtained for normal fatty acids were in good agreement with those given by Flanzy *et al.* [14] and Mordret *et al.* [15]. Methyl malvalate (C_{18}:CE) and methyl sterculate (C_{19}:CE) have ECL 17.94 and 18.90 respectively [10, 11]. Treatment of fatty acid methyl esters with silver nitrate–methanol, showed the presence of malvalic and sterculic ester derivatives [11] confirming, therefore, the identity of the CPEFA. Cyclopropanic fatty acids (CPAFA), such as methyl dihydrosterculate (C_{19}:CA) was also detected at ECL 19.21 [11]. Among saturated fatty acids, myristic (0.2–1.4%), pentadecanoic (0.1–0.9%), palmitic (16–30%) and stearic (2–11%) acids were observed in all species. Arachidic (C_{20}:0) acid (0.7–12%) was not detected in the two *Perrierodendron* species. Among the monounsaturated fatty acids, palmitoleic acid (0.1–1.1%) and its isomer 16:1 ω 7 (0.2–1.3%) were observed in all species. Heptadecenoic acid (0.1–0.7%) was not characterized in the genera *Eremolaena* and *Sarcolaena*. Heptadecadienoic acid (0.2–1%) was not detected in the genera *Eremolaena*, *Mediusella*, *Pentachlaena* and *Rhodolaena*. Oleic acid (7.6–49%) and vaccenic: 18:1ω7 (1.1–2%) were observed in most species. Linoleic (16–47%) and oleic acids constituted the main fatty acids of these lipids. The content of gadoleic (C_{20}:1ω9) acid ranges from 0 to 6.8%.

Table 1. Neutral lipid contents and methyl ester compositions of Sarcolaenaceae seed oils

Genus Species	Oil content (%)	Fatty acid (%)																		
		Saturated					Unsaturated							Cyclopropenic*			19:CA† + 18:3ω3	Unknowns‡		
		14:0	15:0	16:0	18:0	20:0	16:1ω9	16:1ω7	17:1	17:2	18:1ω9	18:1ω7	18:2ω6	20:1ω9	18:CE	19:CE		19.86	21.36	Others
Eremolaena rotundifolia Ger.	5.0	0.2	0.1	25.3	3.2	1.2	0.3	0.4	—	—	26.7	1.6	38.5	0.8	—	—	0.5	1.2	—	—
Leptolaena multiflora Dup.-Thou.	2.0	0.4	0.2	17.6	2.1	0.7	0.4	0.4	0.3	0.2	14.8	1.4	42.6	—	—	0.3	1.2	1.4	11.0	5.0
pauciflora Bak.	1.0	0.6	0.3	21.6	2.7	1.9	0.7	1.3	—	0.8	17.1	1.5	43.1	—	—	—	0.7	3.1	4.6	—
Mediusella bernieri Cav.	1.2	0.4	0.3	31.5	4.4	1.2	0.7	1.0	0.4	—	21.5	1.9	26.5	—	—	tr.	6.8	3.4	—	—
Pentachlaena latifolia H. Perr.	4.0	0.1	tr.	16.2	11.6	3.3	0.1	0.3	0.2	—	49.0	—	16.6	—	—	—	1.4	0.7	—	0.5
Perrierodendron boinense H. Perr.	0.5	0.9	0.9	29.8	4.7	—	1.1	0.8	0.6	2.6	11.9	1.2	23.8	—	—	—	2.1	2.7	6.5	12.4§
orientale R. Cap.	0.5	1.4	0.4	29.5	3.8	—	1.0	0.9	—	1.0	7.6	1.3	37.1	—	—	—	7.0	5.6	1.2	1.8
Rhodolaena altivola Dup.-Thou.	17.3	0.5	tr.	25.5	2.0	2.3	0.2	0.4	0.1	—	17.4	1.9	41.1	6.8	tr.	tr.	0.6	1.2	—	—
Sarcolaena codonochlamus Bak.	1.6	0.4	0.2	25.0	3.6	2.5	0.3	0.3	—	0.2	27.5	1.3	35.3	0.9	0.7	0.4	1.1	0.3	—	—
grandiflora Dup.-Thou.	2.1	0.3	0.1	22.8	2.9	9.0	0.2	0.2	—	—	22.3	1.1	36.2	1.8	—	—	1.2	—	—	—
microphylla R. Cap.	0.9	0.5	0.2	20.6	4.8	1.9	0.4	0.4	—	0.4	24.2	1.4	38.7	1.1	0.2	0.3	3.5	1.4	—	—
multiflora Dup.-Thou.	1.6	0.4	0.2	24.2	3.2	2.7	0.3	0.4	—	0.6	28.6	1.6	32.7	1.6	1.2	0.5	1.4	0.4	—	—
oblongifolia Ger.	1.5	—	0.2	21.3	3.2	2.6	0.3	0.3	—	0.4	24.4	1.5	42.4	1.4	tr.	tr.	1.5	0.6	—	—
Schizolaena elongata Dup.-Thou.	1.6	0.5	0.2	22.8	5.2	2.3	0.3	0.4	0.2	0.3	26.0	1.7	35.3	1.0	—	—	2.1	1.7	—	—
exinvolucrata Bak.	2.2	0.4	0.2	25.0	4.6	1.9	0.3	0.5	0.5	0.4	26.2	2.0	29.3	0.8	0.6	0.4	2.3	1.7	—	2.9
hystrix R. Cap.	1.9	0.4	0.2	21.3	4.9	1.6	0.4	0.4	0.7	0.3	24.3	1.2	32.4	—	0.4	—	3.2	3.4	2.6	2.3
pertinata R. Cap.	2.1	0.5	0.1	24.5	4.8	3.1	0.3	0.3	0.2	0.3	24.8	1.6	33.4	0.7	0.1	0.2	1.6	3.2	—	0.3
rosea Dup.-Thou.	2.6	0.6	0.5	17.4	2.3	12.3	0.8	0.8	0.5	0.8	18.9	1.1	32.2	0.4	0.8	0.4	6.5	—	—	3.8
viscosa Ger.	1.9	0.4	0.2	21.1	4.2	2.7	0.4	0.4	0.3	0.4	26.4	1.4	30.9	0.7	—	0.5	2.0	2.6	—	5.4
Xerochlamys diospyroidea R. Cap.	3.4	—	0.1	21.6	3.6	2.1	0.1	0.3	0.2	0.2	27.2	1.5	40.5	1.0	tr.	tr.	1.0	0.6	—	—
Xyloolaena perrieri Ger.	1.9	0.4	0.2	20.9	2.9	2.6	0.4	0.7	0.2	0.3	17.4	1.7	46.7	1.0	tr.	—	1.0	0.6	—	3.0
richardii H. Bn.	5.1	0.4	0.1	19.5	2.2	1.7	0.3	0.5	—	0.5	24.0	1.3	41.1	1.5	0.3	—	1.3	3.8	—	1.5

*18:CE, malvalic acid; 19:CE, sterculic acid.
†19:CA, dihydrosterculic acid.
‡ECL values are 19.86 and 21.36.
§ECL, 21.47.
tr, Trace.

CPEFA were only detected in the oils giving a positive Halphen test. The content was variable (Table 1) but in all cases CPEFA did not exceed 2%. Dihydrosterculic (C_{19}:CA) acid was observed in most oils but with the Carbowax 20 M column used, a poor separation of this acid (ECL 19.21) from linoleic (C_{18}:3ω3) acid (ECL 19.23) was obtained (Table 1). Unknown compounds with ECL 19.86 were noted in all samples, together with ECL 21.36 (1.2–11%) in the genera *Leptolaena, Schizolaena* and *Perrierodendron.*

DISCUSSION

The fatty acid composition of the various species of Sarcolaenaceae reveals homogeneity of seed oils which are characterized by a high content of palmitic, oleic and linoleic acids. The presence of odd-numbered fatty acids, such as pentadecanoic, heptadecanoic, heptadecenoic and heptadecadienoic acids was recently observed in some families belonging to the order Malvales [8–10]. The occurrence of CPEFA has been detected in small amounts in all seed oils giving a positive Halphen test. Three genera (*Eremolaena, Pentachlaena* and *Perrierodendron*) do not contain CPEFA. Capuron [2], in his systematic treatment of Sarcolaenaceae taxa, subdivided the family into four main groups of genera: (A) *Xyloolaena, Sarcolaena, Xerochlamys, Mediusella, Leptolaena*; (B) *Schizolaena*; (C) *Rhodolaena*; and (D) *Pentachlaena, Eremolaena* and *Perrierodendron.* They correspond to four evolutionary branches. One can remark that only group D does not contain CPEFA. Furthermore, these CPEFA were not detected in one species of *Sarcolaena* (*S. grandiflora*) and one species of *Schizolaena* (*S. elongata*). We have attempted to summarize the occurrence of CPEFA in higher plants (Table 2). It should be noted that two plant family types occur. The first type, where CPEFA and CPAFA occur, being the Bombacaceae, Malvaceae, Sterculiaceae, Tiliaceae and Thymelaeaceae (order Malvales). Some of their species contain high percentages of malvalic or sterculic acids. An appreciable amount of CPEFA was also found recently by Berry [24] in the seed oil of *Gnetum gnemon* (Gnetaceae) which belongs to the gymnosperms. The second plant family type includes taxa in the orders Sapindales, Ebenales and one family of Malvales (Elaeocarpaceae). They sometimes contain CPEFA in small amounts but contain, in most cases, CPAFA. It was noted by Bohannon and Kleiman [8] and Vickery [17] that the amounts of dihydromalvalic acid are generally much lower than those of dihydrosterculic acid. Sapindaceae seed oils are characterized by a high content in CPAFA [9, 23, 25], particularly *Litchi sinensis* seed oil which contains more than 40% of dihydrosterculic acid [9, 23]. The Sarcolaenaceae offer difficulties for classification. Most authors, such as Cavaco [1], Capuron [2], Burnett [26], Caruel [27], Takhtajan [28], Emberger [29] and Gibbs [6] have associated the Sarcolaenaceae with the order Malvales. Thorne [30] and Cronquist [31] have put this family in the Theales, Hutchinson [32] in the Ochnales, and Boivin [33] in the Guttiferales or its equivalent. The presence of CPEFA has not been observed so far in the Theales, Ochnales and Guttiferales. It seems therefore, more logical to include the Sarcolaenaceae with the Malvales. Research on the occurrence of CPEFA in plants appears to provide an interesting tool for the chemotaxonomic purposes. It should remove the doubts concerning classification of a few small families, such as the Scytopetalaceae and Gonystylaceae.

EXPERIMENTAL

Material. Seeds were collected by officers of the Department Recherches Forestières, Ambatobe, Antananarivo, and the authors. Oils were extracted from ground seeds with petrol (bp 30–60°). Methyl esters were prepared from the oils with MeOH and NaOMe as catalyst, using the technique of ref. [13].

Halphen colour test. The method of ref. [12] was used for characterisation of CPEFA. Equal vols. of oil, amyl alcohol and CS_2 containing 1% S were placed in a test tube and warmed at 100° for 10–15 min. A characteristic red-pink colour appeared for 16 oils. The oils giving a negative Halphen test were: *Eremolaena rotundifolia, Pentachlaena latifolia, Perrierodendron boinense, P. orientale, Sarcolaena grandiflora* and *Schizolaena elongata.*

Argentation of methyl esters. The methyl esters containing CPEFA were reacted with MeOH saturated with $AgNO_3$ [34]. The reaction was carried out as described previously [11].

GC. Methyl esters were analysed on a 40 m × 0.35 mm

Table 2. Cyclopropene and cyclopropane fatty acid distribution in higher plants.

Order	Family	Halphen colour test*	Fatty acid (%)				Ref.
			Malvalic	Sterculic	Dihydromalvalic	Dihydrosterculic	
Malvales	Bombacaceae	+	1–16	4–38	tr–0.5	1–4	[8, 10, 16]
	Elaeocarparceae	±	0–0.2	0–0.7	0.5	0	[9, 17]
	Malvaceae	+	1–18	1–5	tr–0.4	tr–2	[8, 9, 17]
	Sterculiaceae	±	0–26	0–56	tr–1.2	tr–4.4	[9, 17–19]
	Tiliaceae	+	tr–2.4	tr–5.0	?	?	[20–22]
	Thymelaeaceae	+	1–2	1–14	tr–0.2	0	[9, 17]
	Sarcolaenaceae	±	0–0.8	0–0.7	?	tr–1.5	
Sapindales	Anacardiaceae	–	0	0	0–0.2	0–2	[9, 17]
	Celastraceae	±	0–tr	0–0.1	0	0–0.6	[9, 17]
	Sapindaceae	±	0–0.4	0–6	0–0.6	2–40	[9, 23]
Ebenales	Ebenaceae	–	0	0	0	0	[9, 17]
	Sapotaceae	±	0–tr.	0–0.2	0.1–0.4	0–2.6	[9, 17]
Gnetales	Gnetaceae	+	38.6	13.0	0.5	6.1	[17, 24]

* ±, Positive with some species, negative in other species; tr., trace.

Carbowax 20 M WCOT glass capillary column (170°, split ratio 50:1, sample vol. 1–2 μl) using He as carrier gas (1 ml/min). Ester identification were made as reported previously [10, 11]. Quantitative analyses were performed with an electronic integrator.

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