J Am Oil Chem Soc (2008) 85:353–356
DOI 10.1007/s11746-008-1196-z

ORIGINAL PAPER

Santalum insulare Acetylenic Fatty Acid Seed Oils: Comparison within the *Santalum* Genus

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Received: 7 April 2007 / Revised: 4 December 2007 / Accepted: 7 January 2008 / Published online: 24 January 2008

Abstract The sandalwood kernels of *Santalum insulare* (Santalaceae) collected in French Polynesia give seed oils containing significant amounts of ximenynic acid, *E*-11-octadecen-9-oic acid (64–86%). Fatty acid (FA) identifications were performed by gas chromatography/mass spectrometry (GC/MS) of FA methyl esters. Among the other main eight identified fatty acids, oleic acid was found at a 7–28% level. The content in stearolic acid, octadec-9-ynoic acid, was low (0.7–3.0%). An inverse relationship was demonstrated between ximenynic acid and oleic acid using 20 seed oils. Results obtained have been compared to other previously published data on species belonging to the *Santalum* genus, using multivariate statistical analysis. The relative FA *S. insulare* composition, rich in ximenynic acid is in the same order of those given for *S. album* or *S. obtusifolium.* The other compared species (*S. acuminatum, S. lanceolatum, S. spicatum* and *S. murrayanum*) are richer in oleic acid (40–59%) with some little differences in linolenic content.

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Keywords *Santalum insulare* · Santalaceae · Ximenynic acid · Stearolic acid · French Polynesia · Multivariate statistical analysis

Introduction

The Polynesian sandalwood, *Santalum insulare* Bertero ex A. DC. (Santalaceae) is a small tree endemic to Eastern Polynesia and has been used in fragrances, as medicine and for religious purposes [1]. Studies on unusual acetylenic fatty acids (FA) of *Santalum* seed oil genus began in the 1930s and most of them were identified by comparison with those found in seed oils of the *Ximenia* genus (Olacaceae), such as ximenynic acid, *E*-11-octadecen-9-ynoic acid, a long chain acetylenic FA [2–4]. This rare ximenynic acid previously named santalbic acid, was then identified and reported in various genera of Santalaceae [4–9]. Proximate and fatty acid composition changes in developing sandalwood (*S. spicatum*) seeds and the separation and identification of ximenynic acid isomers in this seed oil as their 4,4-dimethyloxazoline derivatives was achieved by Liu et al. [10, 11]. Some works on triacylglycerols were also conducted on *S. album* and *S. spicatum* [12–14].

In continuation of our study of the chemical variability of *S. insulare* [1, 15], we focused on the FA profiles, since an inverse relationship was demonstrated between the relative proportions of oleic and ximenynic acids in the case of *S. spicatum* [16], *S. acuminatum* [4, 12, 17] or *S. murrayanum* [4, 16]. In this paper, as a part of our research project on sandalwood, development and extension in the Pacific islands, the FA composition of the *S. insulare* seed oils are described for the first time. Results obtained from twenty seed oils, have been compared, using multivariate statistical analyses, with other *Santalum* species seed oils.

Table 1 Geographical origin and physical characteristics of *S. insulare* seeds investigated

Location	Number of seeds	Length (mm)	Width (mm)	Weight (g)
Nuku Hiva (800–1000 m elevation)	15	25.9	25.1	6.73
Nuku Hiva (670 m elevation)	1	19	18	2.73
Tahuata (200–400 m elevation)	4	20.2	19.2	3.28
Sum or mean	20	24.5	23.6	5.8

Experimental Procedures

Ripe fruits of *S. insulare* were collected on Nuku Hiva Island at two locations (670 and 800–1,000 m elevation) and Tahuata Island (Marquesas archipelago) in 2002 and 2003. The dried seeds (Table 1) were ground and extracted in a Soxhlet apparatus for 3 h with *n*-hexane. After extraction, the solvent was removed by vacuum distillation at 30 °C and FA methyl esters (FAME) were immediately prepared by transesterification using 0.5 mol L^{-1} sodium methoxide in anhydrous methanol at room temperature overnight in a sealed tube under nitrogen [18].

Infrared spectra (IR) of FAME were recorded on a Jasco FT-IR 460 Plus spectrophotometer.

A Hewlett Packard 5890 Series gas chromatograph equipped with a flame ionisation detector (FID) and a fused silica capillary column (25 m long, 0.25 mm i.d.) coated with Carbowax (CW20M, 0.2 µm phase thickness) was used for analyses. Temperatures were 220 °C for column and 260 °C for inlet and detector ovens. Available FAME were used for quantitative external standards. Identifications of fatty acids were carried out using mass spectrometry of their FAME.

Gas chromatography–mass spectrometry was performed using the same GC as described above coupled to a mass selective detector (MSD) HP 5970 B Series. Helium was used as the carrier gas, ion source 220 °C, ionising voltage 70 eV. Results were compared to the NBS75K data bank and to published results for ximenynic acid [11].

Principal component analysis was performed using the dataset composed of the eight samples (mean of results published on each *Santalum* species and mean of this work on *S. insulare*) and seven variables (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and ximenynic acids) transformed into centered and reduced variables (standardized PCA). Data were processed with XLSTAT program version 7.5 (Addinsoft, France).

Results and Discussion

The geographical origin and the physical characteristics of *S. insulare* seeds collected on two islands of French Polynesia are given in Table 1. Among the ten FAMEs, nine were identified by GC/MS. The mass spectra of the main peak gave a molecular ion (M^+ *m/z* 292) in agreement with the molecular formula of ximenynic acid methyl ester $C_{19}H_{32}O_2$. Furthermore the occurrence of a significant fragment (50%) *m/z* 150 $[CH_2{=}C{=}CH{-}CH{=}CH{-}(CH_2)_5{-}CH_3]^+$ and a basic peak at *m/z* 79 $[CH_2{=}C{=}CH{-}CH{=}CH{-}CH_2]^+$ confirmed unambiguously the 11-octadecen-9-ynoic acid methyl ester (Fig. 1). An IR absorption of the oil at

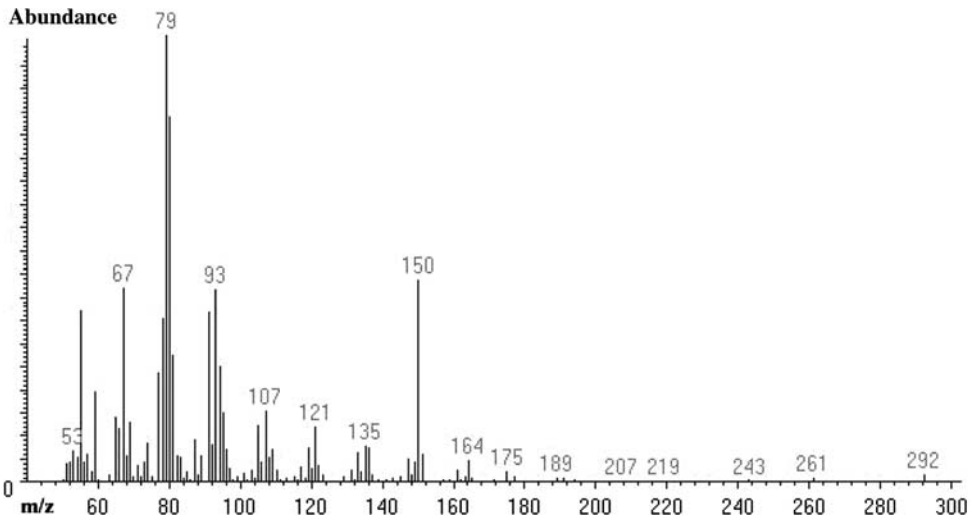

Fig. 1 Mass spectra of the ximenynic methyl ester obtained by GC/MS of the *S. insulare* FAME seed oil

Table 2 Range of fatty acid composition of *S. insulare* kernels investigated from Marquesas Islands

Fatty acid	Mean[a]	SD	Min.	Max.
Palmitic	1.0	0.2	0.8	1.4
Palmitoleic	0.6	0.1	0.4	0.8
Stearic	1.0	0.3	0.5	1.7
Oleic	18.1	5.4	7.1	27.8
Linoleic	0.5	0.1	0.3	0.8
Linolenic	1.0	0.2	0.6	1.3
Stearolic	1.4	0.5	0.7	3.0
Arachidic	0.4	0.1	0.3	0.5
unknown	1.0	0.2	0.7	1.7
Ximenynic	74.5	5.9	64.1	86.1

[a] Mean of 20 samples

955–956 cm^{-1} indicated the presence of the *trans* double bond in the enyne system. Other small fragments were in agreement with results previously published [11]. The *cis*-ximenynic isomer previously characterized in minute amount in *S. spicatum* [11] was not detected in these *S. insulare* seed oils. Another acetylenic FA was identified as stearolic acid (octadec-9-ynoic acid). One peak in small amounts (0.7–1.7%) remained unidentified. Among the nine FA identified (Table 2), two of them, ximenynic and oleic acids represented about 95% of the relative percentage of these oils. The relative proportion of ximenynic acid presents a large variation within the 20 *S. insulare* samples from 64.1 up to 86.1% with an average of 74.5%. In the case of oleic acid, the average was 18.1% with a minimum of 7.1 and a maximum of 27.8%. A high negative correlation (0.99) between these two acids was observed and the sum of these two acids was remarkably stable around 92–94%. Such inverse correlation was previously observed in the case of *S. spicatum* [16] and no significant change was observed within elevation or between the two islands investigated.

Since some results have been done on the FA composition of various *Santalum* species seed oils, we used multivariate statistical analyses for *S. insulare* classification with the other species. Table 3 give the FA content of *S. album* [5, 12, 14], *S. acuminatum* [4, 12, 17], *S. lanceolatum* [12], *S. murrayanum* [4, 12], *S. obtusifolium* [9], and *S. spicatum*, [4, 5, 12, 13, 16]. The data set used was composed of 8 samples (the mean of results published on each *Santalum* species and means of this work on *S. insulare*) and 7 variables (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and ximenynic acids). A graphic representation of the projection of variables and samples onto the two first principal components is given in Fig. 2, using principal component analysis. Axis 1, which represents 48.0% of the total information, is positively loaded with linoleic acid (0.91), linolenic acid (0.77) and oleic acid (0.84) and negatively loaded with ximenynic acid (−0.72) and palmitoleic acid (−0.16). Differentiation of *S. spicatum* and *S. murrayanum* from the other species is done on this axis. On axis 2 (36.0% of the total information), which is positively loaded with palmitoleic acid (0.91) and oleic acid (0.44) and negatively with ximenynic acid (−0.67), the two species containing lower amounts of ximenynic acid namely *S. lanceolatum* and *S. acuminatum* are well differentiated from the three species rich in ximenynic acid, *S. album*, *S. obtusifolium* and *S. insulare*. Differentiation of *S. murrayanum* and *S. spicatum* from *S. lanceolatum* and *S. acuminatum* may be explained by a higher content of linolenic acid.

Acetylenic acids such as ximenynic acid are known to interfere with fatty acid metabolism in a variety of tissues [19, 20]. Therefore the seed oil of *S. insulare* may be a good source of ximenynic acid for cyclo-oxygenase and lipoxygenase enzyme studies [21].

Table 3 Comparative composition of oil and main fatty acids of various *Santalum species* kernel oils

Species	*album*			*acuminatum*			*lanceolatum*	*murrayanum*		*obtusifolium*	*spicatum*				*insulare*	
Ref	[11]	[13]	[4]	[11]	[3]	[17]	[11]	[11]	[3]	[8][a]	[12]	[16]	[11]	[3]	[4]	This work[b]
Palmitic	0.8	0.8		2–2.9	3	2.4	2.3	2.4		0.6	3.9–5.7	3.2	3.5			1.0
Palmitoleic	0.5	0.6		0.3–2.7			1.3	0.3		0.4	0.6–1.3	0.1	0.7			0.6
Stearic	1	0.4		1.1–2.3	1	1.7	2.7	2.1		1.2	1.8–3.3	1.9	1.9			1.0
Oleic	12.3	18		43.8–57.7	50	49.9	26	54.8	[c]	14.3	50.6–58.7	49.1	54.4			1.8
Linoleic		0.7		0.3–1.4		1.3		1.4		0.7	0.9–1.7	1.0	0.6			0.5
Linolenic	0.8	0.5		0–2.5	2	2.4		2.3		3.2	2.4–3.8	3.5				1.0
Ximenynic	82.8	79	75	32.2–46.2	40	39.8	45.5	35.5	45	71.5	27.9–37.3	40.3	33.4	36.3	34	74.5

[a] Other minor FA identified in this species: dihydrosterculic acid 0.1, gondoic 0.3, eicosadienoic 2.5, eicosatrienoic 0.2, eicosapentaenoic 4.3

[b] Mean of 20 samples

[c] Presence confirmed

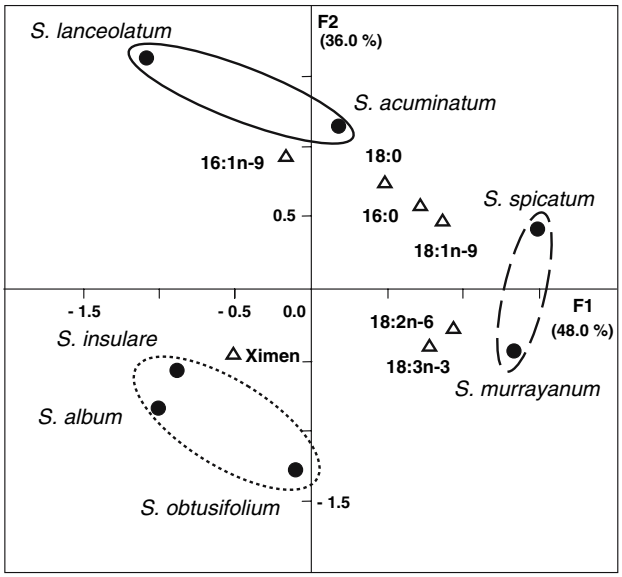

Fig. 2 Two dimensional plot of the FAME profiles of *Santalum* genus kernel oils investigated by PCA. The amounts of variance relative to each axis and the FAME providing contribution are given

Acknowledgments We wish to thank the Rural Development Service (SDR) of French Polynesia for its logistical support and for its participation in this study. We thank the Ministère de l'Ecologie et du Développement Durable (France) for a grant for this research project included in the "Ecosystèmes Tropicaux" program.

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