Separation and Identification of Triximenynin from *Santalum spicatum* R. Br.

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ABSTRACT: Seed lipids of Western Australian sandalwood (*Santalum spicatum*) were separated using preparative thin-layer chromatography. The oil contains about 49% oleic acid and about 40% ximenynic acid. The individual triacylglycerol bands were characterized by high-performance liquid chromatography and gas chromatography. The oil consisted of three major triglycerides: triximenynoyl-glycerol (triximenynin), an oleoyl-diximenynoyl-glycerol, and a dioleoyl-ximenynoyl-glycerol, as demonstrated by gas chromatography with mass selective detection.
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KEY WORDS: Gas chromatography, high-performance liquid chromatography, sandalwood (*Santalum spicatum*), thin-layer chromatography, triximenynin, ximenynic acid (santalbic acid).

Sandalwood (*Santalum spicatum* R. Br.*)* is an important commercial tree species in Western Australia, where it has been harvested to provide volatile oil used in the manufacture of perfumes and joss (incense) sticks. Present wild stocks of sandalwood are greatly reduced, and current programs are in place to study growth and propagation of the tree. While the tree may take 50 yr or more to reach a harvestable size in arid natural habitats, a significant annual crop of large fruit and associated seed may be achieved under irrigated cultivation conditions within about 7 yr. The seed is contained within a hard-shelled endocarp, inside a softer pericarp, and yields about 50–60% of a drying fixed oil (1).

The pale yellow fixed oil contains a number of long-chain acetylenic fatty acids in triacylglycerols with ximenynic acid [*trans*-11-octadecen-9-ynoic acid (santalbic acid)], here called X, as the major component. Other significant fatty acids detected in the oil include stearolic (octadec-9-ynoic acid) and oleic (here called O) acids. A general analysis of the fixed oil demonstrates a fatty acid profile containing 40.31% X and 49.15% O. Stearolic acid occurs only in low concentration (0.74%). We now present basic information on the separation and characterization of the triacylglycerol occurring in the fixed oil.

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EXPERIMENTAL PROCEDURES

Materials All solvents and reagents were analytical or high-performance liquid chromatographic grade; silica gel G 60 F254 precoated sheets (E. Merck, Darmstadt, Germany) were used for thin-layer chromatography (TLC).

Oil extraction. Mature seeds of *S. spicatum* were harvested in late October 1993 from 12-yr-old trees, which had been grown under irrigation in the Curtin University Field Trial Area. The seeds were maintained at about 20°C before analysis. Approximately 2-g samples of seeds were finely ground using a mortar and pestle and extracted with hexane (200 mL) in a Soxhlet apparatus for 2 h. The solution was dried using anhydrous sodium sulfate and the solvent removed by using a Buchi rotary evaporator under reduced pressure at 60°C, to yield a viscous pale yellow oil.

TLC. TLC was performed by streaking a 5% solution of seed oil in chloroform/methanol (1:1, vol/vol) onto precoated plates (20 × 20 cm, 0.2-mm thick). The plates were developed using hexane/diethyl ether/acetic acid (70:30:1, vol/vol/vol) (2). The plates were air-dried and the triacylglycerol bands visualized under ultraviolet light (at 254 nm) as dark bands. Five bands were present after TLC development, two minor (R_f = 0.26, 0.90) and three major (R_f = 0.58, 0.67, and 0.75). Each major band was scraped off, eluted using chloroform/methanol solvent mixture (10 mL, 1:1 vol/vol) and the extracts concentrated under a stream of nitrogen at 50°C.

Triacylglycerol analysis. The seed oil was examined using high-performance liquid chromatography (HPLC) according to the procedure of El Hamdy and Perkins (3). The apparatuses used for HPLC were a μ-Bondapak™ C18 column (10 μ, 3.9 × 300 mm; Waters Associates, Milford, MA), a Waters Associates 501 pump, fitted with a Rheodyne 7125 injec-

TABLE 1
Fatty Acid Composition of *Santalum spicatum* Seed Oil and Isolated Fractions

	Percentage content of fatty acids							
Band	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{18:1}$[a]	XMY[a]
Band 4	5.76	1.31	3.14	55.93	3.47	4.09	1.85	24.45
Band 3	3.06	trace	1.65	33.30	1.36	3.03	2.01	55.62
Band 2	trace	trace	trace	trace	trace	trace	n.d.	99.90
Whole oil	3.22	0.10	1.90	49.15	0.99	3.49	0.74	40.31

[a]$C_{18:1}$ = stearolic acid; XMY = ximenynic acid; n.d. = not detected.

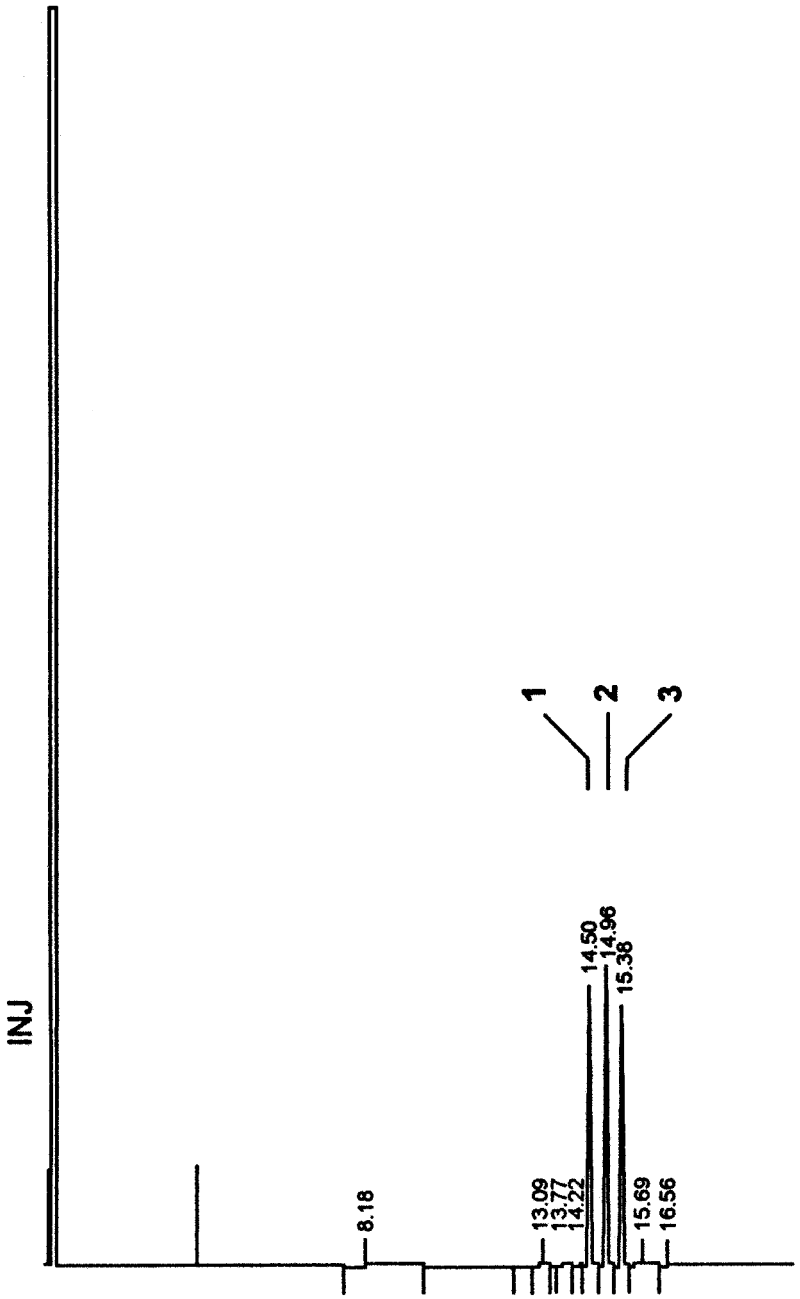

FIG. 1. Gas chromatography of sandalwood seed oil. Triacylglycerol: 1 = XXO or XOX (37.1 relative %); 2 = OOX or OXO (34.9%); 3 = XXX (28.1%), where X = ximenynic acid and O = oleic acid.

tor and 20 µL loop (Cotati, CA), a Waters Associates 401 differential refractometer, and a Hewlett-Packard 3396 integrator (Palo Alto, CA).

Gas chromatography (GC). The original oil and the three principal eluted bands from TLC (at R_f = 0.58, 0.67, and 0.75) were also analyzed as solutions in hexane by GC with flame-ionization detection (FID), using an HT5 column (cyclocarboborane-modified polysiloxane; 6 m × 0.53 mm i.d., 0.10-µm film thickness; SGE, Ringwood, Victoria, Australia). Helium was used as carrier gas at a head pressure of 20 kPa. The injector and detector temperatures were set at 300 and 420°C, respectively, and the oven temperature was programmed from 200 to 370°C, rising at 10°C/min and then maintained at 370°C for 5 min. The sample solutions were approximately 1% wt/vol in hexane and 2-µL volumes were injected.

Fatty acid analysis The fatty acid methyl esters (FAME) were prepared by acid catalyzed transmethylation (H_2SO_4–CH_3OH method) with heptadecanoic acid (0.5 mg) as an internal standard (4). FAME were quantified by GC and mass spectrometry using standard procedures as described previously (5).

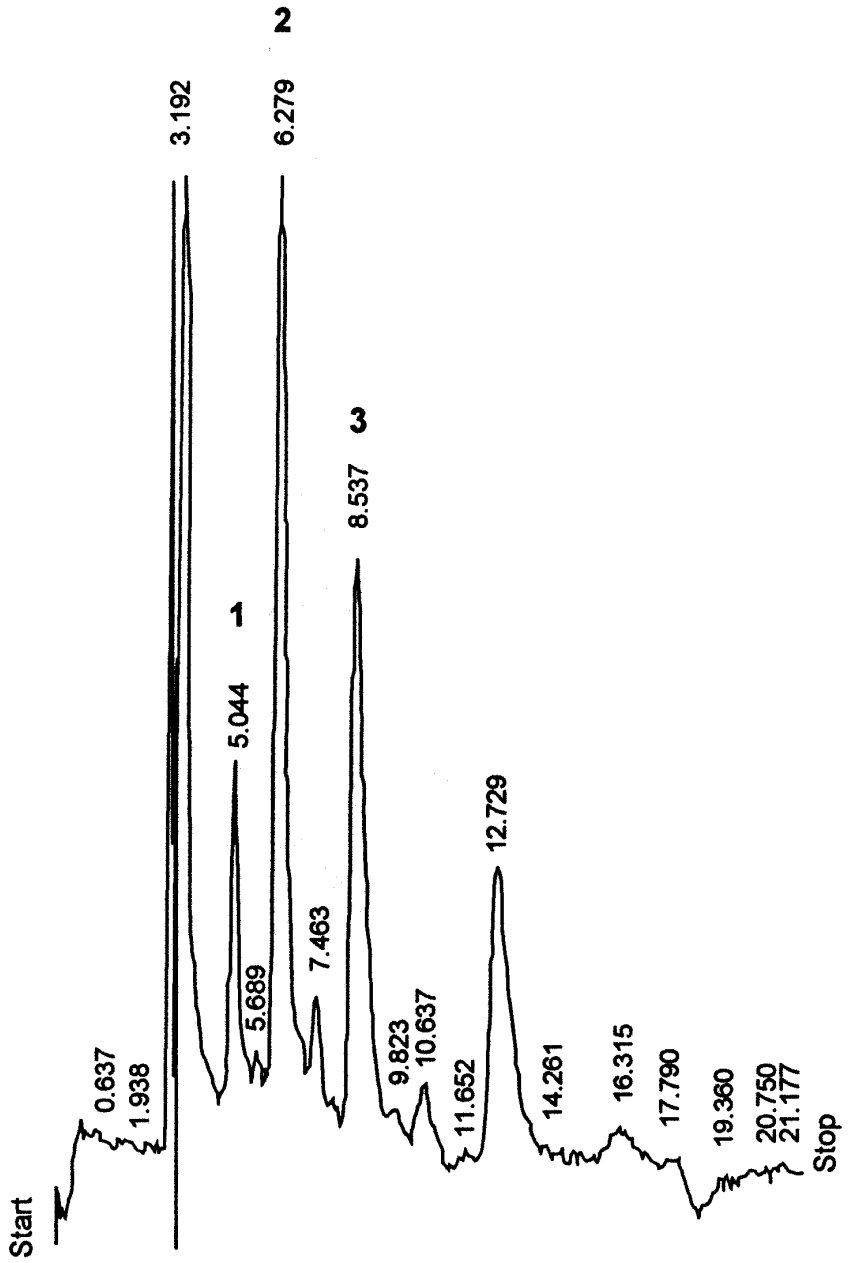

FIG. 2. High-performance liquid chromatography of sandalwood seed oil. Triacylglycerol: 1 = XXX (13.3 relative %); 2 = XXO or XOX (51.4 %); 3 = OOX or OXO (35.3 %). See Figure 1 for abbreviations.

RESULTS AND DISCUSSION

The results of FAME analysis of the lipids present in triacylglycerol bands isolated by TLC are summarized in Table 1. It was determined that O and/or X were the important constituents and that, although other fatty acids were detected, they were present in low concentrations only, probably representing other "contaminant" minor triacylglycerol species.

The co-occurrence of O and X as the major constituents of the fixed oil may be indicative of a biosynthetic relationship between the two acids. Previous studies on a variety of sandalwood seed oils obtained from different locations in Western Australia have indicated (6) that, although no major pattern of fatty acid percentage content can be ascribed to geographical location, there is an inverse hyperbolic relationship ($r = 0.9702$) between the O and X contents of each fixed oil sample. It has been speculated (6,7) that this relationship may indicate that X arises from O as its precursor. Thus the relative decrease in proportion of O may indicate uptake in the form of the new product X. We have previously demonstrated (8) that the relative concentrations of O and X increase during the development of the sandalwood seed oil while a corresponding decrease in $C_{16:0}$, $C_{18:2}$, and $C_{18:3}$ occurs. These changes occur particularly at about 105 d after flowering.

Examination of the FAME results indicated that TLC band 4 (R_f = 0.75) was principally composed of two oleic residues and one ximenynic residue, thus being the triacylglycerol OOX and/or OXO. Band 3 (R_f = 0.67) was composed of two ximenynic residues and one O residue, suggesting the triacylglycerol XXO and/or XOX. Finally, band 2 (R_f = 0.58) was shown to be solely composed of X and was therefore XXX, triximenynin. Stearolic acid, a normal constituent of *S. spicatum* (at *ca.* 1.5%), was only detected at low concentrations in bands at R_f = 0.75 and R_f = 0.67. Band 2 (R_f = 0.58), showed only faint traces of other fatty acids. A summary of the relative percentage fatty acid composition is shown in Table 1.

These findings are in contrast to those of D.E. Rivett and coworkers (9) on the related Quandong, *S. acuminatum,* which contains *ca.* 40% X. They determined the significant triacylglycerols to be XOX (or XXO), OOX, and OXO (two peaks). The presence of triximenynin (XXX) was not demonstrated in *S. acuminatum*. A study of the triacylglycerol occurring in *S. album* seed oil using similar preparative TLC procedures has also demonstrated the presence of three principal triacylglycerol species (10). The seed oil from *S. album* contains more X (79%) and less O (18%), and in particular it was also shown that triximenynin (trisantalbin) was the major triacylglycerol (79%). Thus, it would appear that the three *Santalum* species discussed differ markedly in the occurrence of X in the triacylglycerol, and these differences would appear to characterize the species.

In this study triacylglycerol analysis by GC/FID of the seed oil and the three major bands at R_f = 0.58, R_f = 0.67, and R_f = 0.75 isolated by TLC showed that the seed oil consisted principally of three triacylglycerol species with only minor contributions from other compounds. Each isolated band was further analyzed by GC/FID and demonstrated to be relatively pure, providing identification of the peaks in the original chromatogram, after FAME analysis. The chromatographic results for triacylglycerol by GC/FID analysis are shown in Figure 1.

HPLC analysis of the oil showed several peaks, of which three could be equated with the isolated triacylglycerol fractions, as shown in Figure 2, when each isolated band was analyzed using the HPLC procedure. The triacylglycerols containing the remaining fatty acids have not yet been characterized but represent only minor constituents. It is intended that further studies will be performed on larger scale isolation of triximenynin as a pure compound.

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REFERENCES

1. Hatt, H.H., and R. Schoenfeld, Some Seed Fats of the *Santalaceae* Family, *J. Sci. Food. Agric. 7*:130–133 (1956).
2. Sajbidir, J., M. Certik, and J. Grego, Lipid Analysis of Baker's Yeast, *J. Chromatogr. A 665*:191–195 (1994).
3. El-Hamdy, A.H., and E.G. Perkins, High-Performance Reversed-Phase Liquid Chromatography of Natural Triglyceride Mixtures, *J. Am. Oil Chem. Soc. 58*:49–53 (1981).
4. Christie, W.W., *Gas Chromatography and Lipids*, The Oily Press, Ayr, 1989, 68 pp.
5. Liu, Y.D., R.B. Longmore, and J.E.D. Fox, Separation and Identification of Ximenynic Acid Isomers in the Seed Oil of *Santalum spicatum* R.Br. as Their 4,4-Dimethyloxazoline Derivatives, *J. Am. Oil Chem. Soc. 73*:1729–1731 (1996).
6. Liu,Y., R.B. Longmore, J.E.D. Fox, and S.G. Kailis, A Comparison of Kernel Compositions of Sandalwood (*Santalum spicatum*) Seeds from Different Western Australian Locations, *Mulga Research Centre Journal* (Perth, Western Australia) *12*:15–21 (1995).
7. Bu'Lock, J.D., The Biogenesis of Natural Acetylenes, in *Comparative Phytochemistry*, edited by T. Swain, Academic Press, London, 1966.
8. Liu, Y.D., R.B. Longmore, and S.G. Kailis, Proximate and Fatty Acid Composition Changes in Developing Sandalwood (*Santalum spicatum*) Seeds, *J. Sci. Food Agr. 74* (in press).
9. Rivett, D.E., G.P. Jones, D.J. Tucker, and M. Sedgley, The Chemical Composition of Kernels of *Santalum* Species, *Proceedings of a Colloquium on the Food Potential of Seeds from Australian Native Plants*, edited by G.P. Jones, Deakin University, Victoria, Australia, March 7, 1984, pp. 75–92.
10. Lie Ken Jie, M.S.F., M.K. Pasha, and F. Ahmed, Ultrasound-Assisted Synthesis of Santalbic Acid and a Study of Triacylglycerol Species in *Santalum album* (Linn.) Seed Oil, *Lipids 31*:1083–1089 (1996).

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