

Terpenes of *Persea tolimanensis* (Lauraceae), an Ancestor of the Guatemalan Avocados

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Persea tolimanensis is a recently described species from the Guatemalan highlands. It is considered to be an ancestor of the Guatemalan criollos and edible avocados together with *Persia americana* ssp. *nubigena* and *steyermarkii*. Its essential leaf oils, including the late eluting avocado furans, are here described for the first time. Of 63 components eluted on a 30-m $R_{\alpha-1}$ capillary column, 48 were identified through mass spectroscopy, and the MS data are given for the remaining 15 unknowns. The largest sesquiterpene was β -caryophyllene with 23%, the largest monoterpene was sabinene with 21%, and the largest furan component was avocadenofuran with 4.2%. It is evident from the data that *P. tolimanensis* does not belong to the Mexican or Lowland (West Indian) group of avocados but firmly belongs to the Guatemalan assemblage and may very well be an ancestor to these.

INTRODUCTION

The edible cultivated avocados of the species *Persea americana* Mill. arose from tertiary-quaternary antecedents in Meso-America. *P. americana*, together with *Persea zentmyeri* Schieber and Berg, *Persea parviflora* Williams, *Persea primatogena* Williams and Molina, *Persea schiedeana* Nees, and the recently described *Persea tolimanensis* Schieber, belongs to the subgenus *Persea* whose species and subspecies are all graft-compatible with each other but not with the taxa of subgenus *Eriodaphne*.

In the polymorphic species *P. americana* we recognize three wild subspecies, namely *nubigena* (Williams) Kopp, *steyermarkii* Allen, and *floccosa* Mez., and three cultivated subspecies, namely *americana*, the lowland avocado (often named West Indian); *drymifolia* (Schlecht. and Cham.) Blake, the Mexican avocados; and *guatemalensis* Williams, the Guatemalan avocados.

The latter widely cultivated subspecies evolved most probably in the highlands of Guatemala. Williams (1977) believed that ssp. *nubigena* was one of its ancestors, and Schieber and Zentmyer (1978) suggested ssp. *steyermarkii* to be the other. Such a relationship of *steyermarkii* to *guatemalensis* was supported by cpDNA data and a relationship to *nubigena* by rDNA (Fournier et al., 1989). In 1987 Schieber and Zentmyer widened their concept of the ancestry of ssp. *guatemalensis* to include additionally *P. steyermarkii* aguacate de montaña and *P. tolimanensis* aguacate de mico.

It is this latter wild species, one of the proposed ancestors of the edible Guatemalan avocados, that we investigated by GC-MS for its essential leaf oils which are reported here for the first time.

While Schieber and Zentmyer reported *P. tolimanensis* as a bridge between ssp. *steyermarkii* and *nubigena* to the more primitive Guatemalan criollos, Berg [Department of Botany (Emeritus), UCR, 1993, personal communication] regards the widespread *P. tolimanensis* to be more primitive than ssp. *steyermarkii* or *nubigena*. We believe *nubigena* to be closest to the criollos.

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MATERIALS AND METHODS

Two hundred grams of healthy mature intact leaves was harvested from *P. tolimanensis* growing on its own rootstock at the University of California South Coast Field Station near Irvine, CA, and steam distilled in an inert atmosphere in a Clevenger unit for oils lighter than water. The oil was collected for 1 h after the first drop of distillate eluted from the cold finger. The essential leaf oils were immediately injected into a Shimadzu GC-14A. The column used was a 30-m nonpolar capillary $R_{\alpha-1}$ column (Restek Co.) cross-bonded 100% dimethylpolysiloxane. The carrier gas used was helium 1.0, H₂ 0.6, and air 0.5 kg/cm². The injector temperature was 150 °C, and the temperature program was as follows: 50 °C for 5 min, increase at 2 °C/min to 200 °C, held for 2 min, and increased at 5 °C/min to 260 °C, and held for 10 min. The percentages of the 0.5- μ L injections were calculated with a Beckman 427 integrator.

For GC-MS analysis a Hewlett-Packard 5989A instrument was operated at 70-eV electron energy and 200 °C source temperature. Chromatographic separation was carried out as above with an identical column under the same parameters.

The identification of the gas chromatographic components was affected by GC retention time information based on our own standard library and by comparison of the GC-MS spectra with published spectra (Adams, 1989) and those of the GC-MS library as well as by background subtraction of coeluting compounds.

RESULTS AND DISCUSSION

The essential leaf oils of *P. tolimanensis* were investigated by GC and GC-MS. Of 63 components separated, 48 were identified, representing 76% of the leaf oil. This leaf oil contains about 52% monoterpenes. The largest oil components were sabinene with 21%, β -pinene with 8%, 1,4-cineole with 6%, and α -pinene also with 6%.

By far the largest component of the sesquiterpenes was β -caryophyllene with 23% of the total leaf oil. Another group was the C₁₇, C₁₉, and C₂₁ avocado furans which comprised about 7% of the total leaf oils. Its largest component was avocadenofuran with 4.2%. These furans substituted in the 2-position are typical for the genus *Persea* (Weyerstahl et al., 1993).

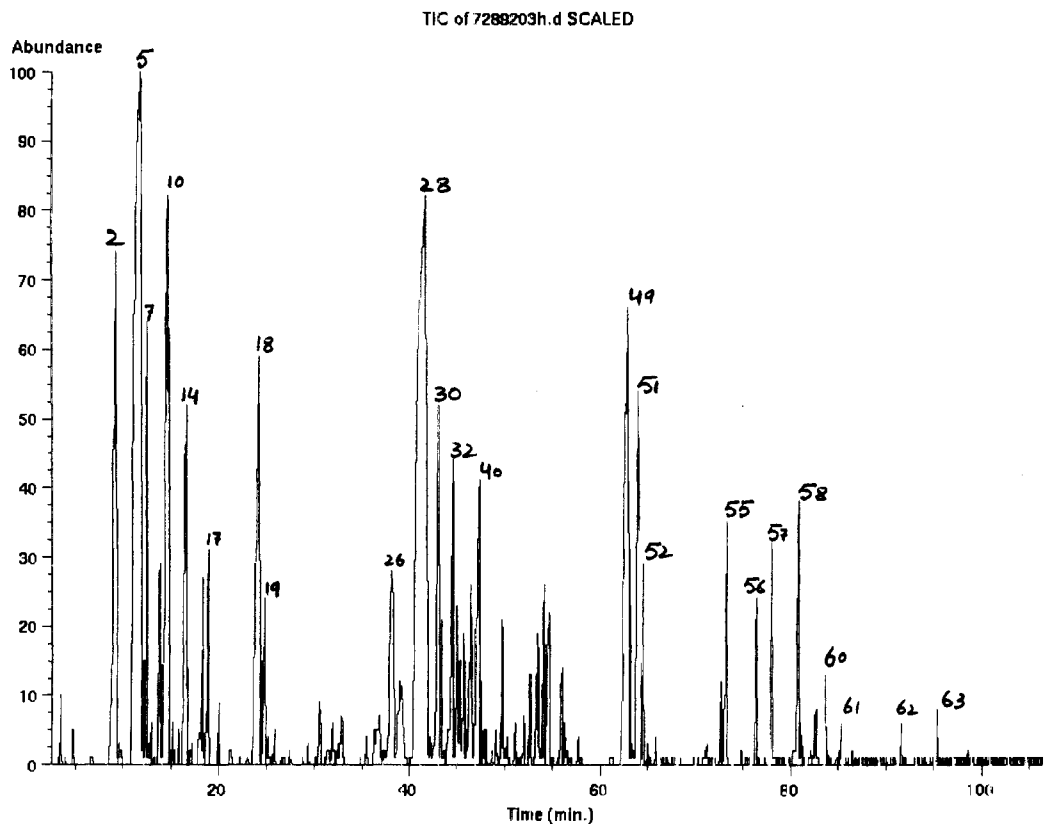


Figure 1. GC-MS chromatogram of the essential leaf oil of *P. tolimanensis*.

Table I. Volatile Leaf Components of *P. tolimanensis*

peak	component	GC RT	MS RT	% composition	peak	component	GC RT	MS RT	% composition
	tricyclene	9.72	8.804	0.51		valencene	47.51	45.011	0.22
2	α -pinene	10.06	9.189	5.66		viridiflorine	47.83	45.286	0.21
	camphene	10.65	9.774	0.10		germacrene B	47.95	45.432	0.29
4	sabinene	12.23	11.85	20.99		α -muurolene	48.39	45.761	0.38
5	β -pinene	12.36	11.933	7.96		unknown 1	48.43	46.072	0.22
	2-pentylfuran	13.13	12.225	0.08		unknown 2	49.07	45.456	0.66
7	β -myrcene	13.38	12.572	1.40		α -farnesene	49.43	46.584	0.07
	α -phellandrene	13.98	12.993	0.06	40	γ -cadinene	49.80	47.169	2.12
	δ -3-carene	14.81	13.870	1.09		δ -cadinene	50.24	47.609	0.12
10	1,4-cineole	15.48	14.675	6.24		globulol	52.63	49.856	0.45
	limonene	15.64	14.858	0.92		cubanol	55.70	52.687	0.21
	<i>cis</i> -ocimene	16.37	15.187	0.05		T-muurolol	56.44	53.492	0.33
	γ -terpinene	17.09	15.845	0.06		T-cadinol	56.61	53.620	0.24
14	terpinolene	17.62	16.612	2.15		α -cadinol	57.10	54.169	0.48
	linalool	17.86	16.704	0.14		unknown 3	57.66	54.608	0.27
	4-terpinenyl acetate	19.65	18.348	0.61		unknown 4	57.79	54.736	0.16
17	nonanal	20.24	18.896	0.27	49	avocadenofuran	66.02	62.939	4.20
18	terpinen-4-ol	25.58	24.231	3.02		avocadofuran	66.49	63.214	0.18
19	α -terpineol	26.41	24.853	0.41	51	avocadynofuran	67.29	64.147	2.11
	<i>cis</i> -piperitol	26.99	25.218	0.02	52	unknown 5	67.92	64.568	0.26
	carvacrol	34.90	32.579	0.03		avocadienofuran	69.53	65.883	0.03
	2,4-nonadienol	35.19	32.944	0.06		unknown 6	76.56	72.718	0.13
	δ -elemene	38.76	35.520	0.16	55	unknown 7	77.07	73.341	0.59
	α -cubebene	39.10	36.396	0.19	56	unknown 8	80.36	76.476	0.35
	neryl acetate	39.81	36.798	0.13	57	unknown 9	83.26	78.126	0.35
26	α -copaene	40.46	38.223	1.64	58	unknown 10	84.80	80.949	0.98
	β -cubebene	41.30	39.120	0.68		unknown 11	86.37	80.949	0.98
28	β -caryophyllene	43.33	41.572	23.11	60	unknown 12	87.17	83.773	0.16
	β -gurjunene	44.58	41.902	0.06	61	unknown 13	88.57	85.423	0.04
30	α -humulene	45.25	43.146	2.25	62	unknown 14	94.17	91.638	0.02
	γ -muurolene	45.67	43.402	0.27	63	unknown 15	98.61	95.470	0.03
32	germacrene D	46.98	44.664	1.75					98.05

The identified components are listed in Table I, and the mass spectra of 15 unidentified components are given in Table II. The GC-MS chromatograph of essential oils is shown as Figure 1.

Being part of the Guatemalan complex, the essential leaf oil does not contain estragole, anethole, or methyl-eugenol which characterize the Mexican complex as well as partially *P. americana* ssp. *nubigena* and *steyermarkii*.

Table II. Mass Spectra of Unidentified Components

component	GC RT	MS RT	% composition	MS ID	comparative abundance
unknown 1	48.43	46.072	0.22	204	161 (100), 105 (70), 41 (69), 119 (62), 134 (51), 81 (46)
unknown 2	49.07	46.456	0.66	?	43 (100), 161 (85), 41 (75), 105 (57), 93 (54), 55 (44), 207 (46) ^a
unknown 3	57.66	54.608	0.27	?	43 (100), 79 (62), 41 (41), 80 (39), 67 (36), 55 (19), 179 (4) ^b
unknown 4	57.79	54.736	0.16	?	43 (100), 79 (57), 41 (42), 91 (31), 67 (29), 55 (19), 173 (5) ^b
unknown 5	67.92	64.568	0.26	204	69 (100), 43 (75), 41 (64), 81 (34), 93 (29), 136 (19)
unknown 6	76.56	72.718	0.13	276	94 (100), 81 (85), 41 (54), 95 (47), 107 (37), 43 (41)
unknown 7	77.07	73.341	0.59	278	81 (100), 95 (62), 82 (51), 43 (47), 41 (37), 123 (24)
unknown 8	80.36	76.476	0.35	276	94 (100), 105 (54), 79 (23), 41 (21), 43 (19), 135 (15)
unknown 9	83.26	78.126	0.35	138	71 (100), 43 (49), 41 (37), 57 (37), 55 (32), 81 (28)
unknown 10	84.80	80.949	0.98	302	81 (100), 67 (60), 41 (52), 94 (43), 55 (41), 121 (18)
unknown 11	86.37	82.654	0.04	306	81 (100), 95 (64), 43 (57), 83 (54), 41 (34), 123 (31)
unknown 12	87.17	83.773	0.16	300	94 (100), 79 (91), 41 (82), 107 (78), 67 (65), 55 (56)
unknown 13	88.57	85.423	0.04	304	94 (100), 107 (53), 43 (34), 79 (24), 81 (22), 135 (18)
unknown 14	94.17	91.638	0.02	?	57 (100), 43 (84), 71 (71), 85 (43), 41 (34), 99 (19), 141 (6) ^c
unknown 15	98.91	95.470	0.03	?	57 (100), 37 (79), 71 (68), 85 (35), 55 (24), 99 (21), 381 (5) ^c

^a Possibly cyclic hydrocarbon. ^b Unknown, but chemically related. ^c Hydrocarbon.

As the Guatemalan avocado leaf oils contain an appreciable amount of avocado furans, these components could have been derived from *P. tolimanensis* ancestry, since *ssp. nubigena* has only minute amounts and *ssp. steyermarkii* only a few.

It is our opinion that *P. tolimanensis* belongs firmly to the Guatemalan complex of avocados and that it may be ancestral to *ssp. nubigena* and *steyermarkii* and the primitive Guatemalan criollos.

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LITERATURE CITED

Adams, R. P. *Identification of Essential Oils by Ion Trap Mass Spectroscopy*; Academic Press: San Diego, 1989; 302 pp.

Furnier, S. R.; Cummings, M. P.; Clegg, M. T. Evolution of the Avocados as revealed by DNA Restriction Fragment Variation. *J. Hered.* 1991, 81, 183-188.

Schieber, E.; Zentmyer, G. A. Hunting for *Persea steyermarkii* in the mountains of Guatemala. *Calif. Avocado Soc. Yearb.* 1978, 62, 67-71.

Schieber, E.; Zentmyer, G. A. Exploring for sources of resistance among *Persea americana* var. *guatemalensis* and *Persea schiedeana* in Middle America. *S. Afr. Avocado Yearb.* 1987, 10, 20-21.

Weyerstahl, P.; Marchall, H.; Scora, R. W. Constituents of the Essential Leaf Oil of *Persea indica* (L.) K. Spreng. *Flavour Fragrance J.* 1993, 8, 201-207.

Williams, L. O. The Avocados, A synopsis of the Genus *Persea*, subg. *Persea*. *Econ. Bot.* 1977, 31, 315-320.

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