



Research Article

Chemical composition of the foliar essential oil of *Tsuga mertensiana* (Bong.) Carrière

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Abstract

Tsuga mertensiana (mountain hemlock, Pinaceae) is a species of hemlock that is native to the west coast of North America, from the Sierra Nevada Mountains of California to the Kenai Peninsula of Alaska. In this work, foliage samples of *T. mertensiana* were obtained from four individual trees located on Mt. Hood, Oregon. The essential oils were obtained by hydrodistillation and analyzed by gas chromatography, including enantioselective gas chromatography. The essential oils were dominated by monoterpene hydrocarbons (68.8-80.1%) and oxygenated monoterpenoids (6.4-15.3%). The major components were α -pinene (24.5 \pm 3.2%, predominantly (+)- α -pinene), β -phellandrene (16.3 \pm 2.1%, predominantly (+)- β -phellandrene), limonene (9.8 \pm 6.2%, predominantly (-)-limonene), benzoic acid (6.4 \pm 1.5%), β -pinene (5.9 \pm 1.0%, predominantly (-)- β -pinene), (-)- α -phellandrene (5.8 \pm 1.1%), and bornyl acetate (5.7 \pm 3.5%). This is the first report on the essential oil composition, including enantiomeric distribution, of *T. mertensiana* collected from its natural habitat and complements our understanding of the phytochemistry of the genus. Chiral analyses of other *Tsuga* species would further help in chemically characterizing the genus.

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1. Introduction

Tsuga mertensiana (Bong.) Carrière (mountain hemlock) is an Alpine member of the Pinaceae. A coastal population is found in western North America from coastal Alaska and British Columbia, extending south into Sequoia National Park, California. There is also an inland population in the northern Rocky Mountains of British Columbia, south into northern Idaho and northwestern Montana (Fig. 1) [1–3]. The northern populations (Alaska, British Columbia, Washington, Oregon, Idaho, Montana) are generally *T. mertensiana* subsp. *mertensiana*, while the Sierra Nevada, California, population is *T. mertensiana* subsp. *grandicona* Farjon [4]. The trees grow up to 55 m tall; the leaves (needles) spread out from the branches in all directions, 7-20 mm long and 1-1.5 mm

wide, bearing stomata on both surfaces; the trunk bark is thick, rough, and longitudinally furrowed, dark gray or dark reddish brown [4] (Fig. 2). Several *Tsuga* species, including *Tsuga mertensiana*, have been classified as threatened species and sensitive to climate change [5]. As part of our interest in essential oils of *Tsuga* species [6–8], we have examined the essential oils composition, including enantiomeric distribution of chiral monoterpenoids, of *T. mertensiana* subsp. *mertensiana* from the Cascade Range of Oregon. The volatile composition of *T. mertensiana* (a cultivated specimen, subspecies not indicated) has been reported [9]. The present report, however, presents, for the first time, the essential oil composition of *T. mertensiana* collected from its native



Figure 1. Geographical range of *Tsuga mertensiana* (Bong.) Carrière (mountain hemlock) [3]. This image is in the public domain of the United States because it only contains material that originally came from the United States Geological Survey, an agency of the United States Department of the Interior.



Figure 2. *Tsuga mertensiana* (Bong.) Carrière (mountain hemlock). **A:** Leaves. **B:** Bark. Photographs by K. Swor at the time of collection. **C:** Scan of pressed plant material.

habitat.

2. Materials and methods

2.1 Plant Material

The fresh foliage of *T. mertensiana* was collected from four different individual trees located on the southern slope of Mt. Hood, Oregon, on 25 June 2024 (Table 1). The trees were identified in the field by W.N. Setzer using a field guide [10] and later verified by comparison with samples from the New York Botanical Garden [11]. A voucher specimen (WNS-Tm-0262) has been deposited into the herbarium at the University of Alabama in Huntsville. The fresh foliage from each tree was frozen ($-20\text{ }^{\circ}\text{C}$) and stored frozen until distillation. The fresh/frozen foliage of each sample was chopped and hydrodistilled for four hours using a Likens-Nickerson apparatus [12–14] with continuous extraction of the distillate with dichloromethane (Table 1).

2.2 Gas Chromatographic Analysis

The *T. mertensiana* foliar essential oils were analyzed by gas chromatography (GC-MS, GC-FID, and chiral GC-MS) as previously reported [15]. Retention indices were calculated using the method of van den Dool and Kratz [16]. Essential oil components were identified by comparison of mass spectral fragmentation patterns and retention indices found in the Adams [17], FFNSC3 [18], NIST20 [19], and Satyal [20] databases.

3. Results and discussion

The foliage was collected from four different trees growing near Mt. Hood, Oregon. The foliage was hydrodistilled to give colorless essential oils in yields of 3.10–4.32%. Analysis of the essential oils resulted in a total of 87 identified components, representing more than 99% of the essential oil compositions (Table 2). Monoterpene hydrocarbons (68.8–80.1%) and oxygenated monoterpenoids (6.4–15.3%) were the most abundant chemical classes in *T. mertensiana*, consistent with previous reports of *Tsuga* volatiles [6–9]. The monoterpene hydrocarbons α -pinene (19.9–26.9%), β -phellandrene (13.3–18.0%), limonene (3.4–15.4%), β -pinene (4.6–6.9%), and α -phellandrene (4.8–7.5%) dominated the essential oils. Benzoic acid (4.8–7.9%) and bornyl acetate (0.7–8.8%) were also relatively abundant. The compositions obtained in

this work are similar to those obtained by Lagalante and Montgomery, who analyzed foliage from trees cultivated at the University of Rhode Island using solid-phase microextraction with gas chromatography-mass spectrometry (SPME/GC-MS) [9]. In this previous work, the major components were α -pinene (26.6%), β -phellandrene (19.9%), α -phellandrene (7.3%), β -pinene (7.0%), and germacrene D (21.7%). Limonene concentration was lower (0.9%), while benzoic acid was not observed. In the present study, germacrene D was relatively low in concentration (0.5–2.9%).

The geographical range of *T. mertensiana* closely matches the range of *Tsuga heterophylla* Sarg [8]. There are some notable similarities and differences between the foliar essential oils of these two *Tsuga* species. The major components of *T. heterophylla* were α -pinene ($18.0 \pm 4.7\%$), myrcene ($18.3 \pm 7.4\%$), β -phellandrene ($13.6 \pm 5.5\%$), β -pinene ($10.4 \pm 3.2\%$), and (*Z*)- β -ocimene ($6.0 \pm 3.6\%$) [8]. α -Phellandrene ($2.0 \pm 2.1\%$), benzoic acid ($2.5 \pm 1.1\%$), and bornyl acetate ($0.1 \pm 0.1\%$) concentrations were lower in *T. heterophylla* compared to *T. mertensiana*, while myrcene ($2.5 \pm 0.2\%$) was lower in *T. mertensiana* compared to *T. heterophylla*.

Enantioselective GC-MS was carried out in order to evaluate the enantiomeric distribution of chiral monoterpene components (Table 3). The major enantiomers in *T. mertensiana* essential oil were (+)- α -pinene ($66.3 \pm 1.7\%$), (–)-camphene ($77.6 \pm 13.5\%$), (–)-sabinene ($85.2 \pm 10.3\%$), (–)- β -pinene ($81.8 \pm 0.9\%$), (–)- α -phellandrene (100%), (–)-limonene ($86.0 \pm 9.9\%$), and (+)- β -phellandrene ($91.9 \pm 0.5\%$). Neither terpinen-4-ol nor α -terpineol showed consistent distributions of the enantiomers. The enantiomeric distributions in *T. mertensiana* show notable differences compared to those of *Tsuga heterophylla* Sarg. [8]. In *T. heterophylla*, α -pinene was virtually racemic, (+)-sabinene was the major enantiomer ($91.9 \pm 16.6\%$), (+)- α -phellandrene dominated ($93.6 \pm 3.8\%$), (–)- β -phellandrene was dominant ($80.7 \pm 22.5\%$), and both (–)-terpinen-4-ol ($62.5 \pm 6.7\%$) and (–)- α -terpineol ($79.1 \pm 9.4\%$) were the major enantiomers. (–)-Camphene ($74.3 \pm 7.8\%$), (–)- β -pinene ($96.4 \pm 2.4\%$), (–)-limonene ($80.3 \pm 8.5\%$), and (+)- β -phellandrene ($80.7 \pm 22.5\%$) were the major enantiomers in *T. heterophylla*, in agreement with the distributions in *T. mertensiana*.

Table 1. Plant collection and hydrodistillation details for *Tsuga mertensiana* from Mt. Hood, Oregon.

Sample	Geographical location	Mass foliage (g)	Mass essential oil (g)	Yield (%)
A	45°19'18" N, 121°42'19" W, 1663 m asl	94.92	4.1026	4.322
B	45°19'18" N, 121°42'19" W, 1663 m asl	91.62	2.8413	3.101
C	45°19'18" N, 121°42'20" W, 1664 m asl	101.39	3.8286	3.776
D	45°19'18" N, 121°42'20" W, 1664 m asl	135.41	4.9372	3.646

Table 2. Foliar essential oil composition (percentage) of *Tsuga mertensiana* from Mt. Hood, Oregon.

Compounds	RI _{calc}	RI _{db}	Tree samples			
			A	B	C	D
(3Z)-Hexenal	799	797	0.1	tr	0.1	0.1
Hexanal	801	801	tr	tr	tr	tr
(2E)-Hexenal	849	849	1.3	0.8	1.4	1.0
(3Z)-Hexenol	851	853	0.3	0.3	0.3	0.1
Tiglic acid	919	911	0.1	0.1	0.1	0.1
Tricyclene	922	923	2.4	1.7	2.4	0.2
α -Thujene	925	925	0.1	0.1	tr	tr
α -Pinene	932	932	19.9	26.9	26.5	24.9
α -Fenchene	947	948	0.1	0.1	0.1	0.1
Camphene	949	950	2.2	1.6	1.9	0.3
Thuja-2,4(10)diene	952	953	tr	tr	tr	tr
Benzaldehyde	961	960	tr	tr	tr	tr
Sabinene	971	971	0.3	0.2	0.3	0.1
β -Pinene	977	978	4.6	6.5	5.6	6.9
Myrcene	988	989	2.4	2.5	2.2	2.7
α -Phellandrene	1007	1007	5.3	5.8	4.8	7.5
δ -3-Carene	1009	1009	0.1	0.1	0.1	0.1
α -Terpinene	1017	1017	0.4	0.2	0.3	0.3
<i>p</i> -Cymene	1024	1025	0.7	2.7	1.4	1.1
Limonene	1030	1030	14.9	3.4	5.6	15.4
β -Phellandrene	1031	1031	13.3	18.0	16.2	17.6
(Z)- β -Ocimene	1035	1034	0.7	1.3	1.5	1.8
(E)- β -Ocimene	1045	1045	0.7	0.9	0.2	0.6
γ -Terpinene	1057	1057	0.3	0.2	0.2	0.1
Pinol	1071	1072	0.1	tr	0.1	tr
Terpinolene	1085	1086	0.6	0.5	0.5	0.5
<i>p</i> -Cymenene	1089	1091	0.1	0.1	tr	0.1
α -Pinene oxide	1099	1097	-	0.1	0.1	tr
Linalool	1099	1101	tr	-	-	tr
(E)-4,8-Dimethylnona-1,3,7-triene	1112	1113	tr	tr	tr	tr
<i>cis-p</i> -Menth-2-en-1-ol	1124	1124	0.5	0.9	0.5	0.7
α -Campholenal	1126	1126	0.1	0.2	0.1	0.1
(4E,6Z)- <i>allo</i> -Ocimene	1127	1127	tr	tr	0.1	0.1
<i>trans-p</i> -Menth-2-en-1-ol	1142	1142	0.4	0.7	0.4	0.5
Camphene hydrate	1154	1155	0.1	0.1	0.1	tr
<i>p</i> -Mentha-1,5-dien-8-ol	1171	1171	0.1	tr	0.1	0.1
Benzoic acid	1180	1167	7.5	7.9	5.5	4.8
Terpinen-4-ol	1180	1180	0.6	0.5	0.5	0.2
Cryptone	1187	1187	0.1	0.6	0.3	0.1
<i>p</i> -Cymen-8-ol	1188	1188	0.1	-	-	0.1

Table 2. (Continued)

Compounds	RI _{calc}	RI _{db}	Tree samples			
			A	B	C	D
α -Terpineol	1196	1195	2.7	3.1	2.6	2.1
Verbenone	1208	1208	0.1	0.3	0.1	0.2
Thymyl methyl ether	1229	1229	0.1	0.1	tr	tr
Bornyl acetate	1284	1285	8.8	6.5	6.8	0.7
(E)-Anethole	1286	1288	-	-	-	0.1
Methyl myrtenate	1295	1296	-	-	tr	-
<i>trans</i> -Pinocarvyl acetate	1295	1296	-	-	-	tr
Myrtenyl acetate	1322	1322	0.5	0.1	0.4	0.5
<i>cis</i> -Piperityl acetate	1335	1335	-	0.1	0.1	0.1
α -Longipinene	1351	1352	-	-	0.1	0.2
Neryl acetate	1359	1361	0.1	0.1	0.1	0.1
α -Copaene	1376	1375	0.1	tr	tr	0.1
Geranyl acetate	1378	1378	1.0	0.7	0.8	0.9
β -Bourbonene	1384	1382	tr	tr	tr	0.1
<i>trans</i> - β -Elemene	1390	1390	0.1	tr	0.1	0.1
Sibirene	1403	1399	tr	-	-	-
Longifolene	1410	1411	-	-	tr	0.1
β -Ylangene	1419	1422	tr	tr	tr	tr
(E)- β -Caryophyllene	1420	1424	0.1	0.1	0.3	0.1
β -Copaene	1430	1430	tr	tr	tr	0.1
(E)- β -Farnesene	1453	1452	tr	tr	tr	tr
α -Humulene	1456	1454	0.1	tr	0.2	0.1
γ -Muuroolene	1475	1475	0.2	0.1	0.2	0.2
Germacrene D	1481	1480	2.8	0.5	2.2	2.9
β -Selinene	1489	1489	tr	0.1	0.1	tr
<i>trans</i> -Muurolo-4(14),5-diene	1492	1492	0.1	0.1	0.1	0.1
α -Selinene	1496	1497	tr	tr	0.1	0.1
α -Muuroolene	1499	1497	0.1	0.1	0.3	0.2
(E,E)- α -Farnesene	1504	1504	0.1	0.1	tr	tr
γ -Cadinene	1513	1512	0.2	0.2	0.4	0.3
δ -Cadinene	1518	1518	0.5	0.5	1.2	0.8
<i>trans</i> -Cadina-1,4-diene	1533	1533	tr	tr	tr	tr
α -Cadinene	1537	1538	tr	tr	0.1	tr
Dodecanoic acid	1560	1559	tr	-	-	-
(E)-Nerolidol	1562	1561	0.1	0.2	0.2	0.1
Salvial-4(14)-en-1-one	1593	1593	tr	tr	tr	tr
1- <i>epi</i> -Cubenol	1628	1628	0.1	tr	0.1	tr
τ -Cadinol	1643	1643	0.1	0.2	0.5	0.2
τ -Muurolol	1645	1645	0.2	0.2	0.7	0.2
α -Muurolol (= δ -Cadinol)	1648	1651	0.1	0.1	0.3	0.1
α -Cadinol	1656	1655	0.5	0.6	1.9	0.7
Benzyl benzoate	1766	1769	0.1	0.1	0.1	tr
Benzyl salicylate	1868	1869	0.2	0.1	0.2	tr
Palmitic acid	1958	1958	0.3	0.3	tr	tr
Manool	2053	2053	0.1	0.2	0.2	0.2
Palustrinal	2229	2245	0.1	0.2	tr	0.1
Dehydroabietal	2261	2266	tr	0.1	tr	tr

Table 2. (Continued)

Compound classes	Tree samples			
	A	B	C	D
Monoterpene hydrocarbons	68.8	72.5	69.7	80.1
Oxygenated monoterpenoids	15.3	13.9	12.9	6.4
Sesquiterpene hydrocarbons	4.2	1.9	5.4	5.3
Oxygenated sesquiterpenoids	1.0	1.0	3.5	1.2
Diterpenoids	0.2	0.5	0.2	0.3
Benzenoid aromatics	7.8	8.1	5.8	4.9
Others	2.0	1.5	1.8	1.2
Total identified	99.5	99.4	99.3	99.3

RI_{calc} = Retention index calculated with respect to a homologous series of *n*-alkanes on a ZB-5ms column [16]. RI_{db} = Reference retention index from the databases [17–20]. tr = trace (< 0.05%).

Table 3. Enantiomeric distribution (percent) of chiral monoterpenoids in the foliar essential oil of *Tsuga mertensiana*.

Enantiomers	RI _{calc}	RI _{db}	Tree samples			
			A	B	C	D
(-)- α -Pinene	977	976	34.8	35.1	33.7	31.3
(+)- α -Pinene	980	982	65.2	64.9	66.3	68.7
(-)-Camphene	1001	998	85.5	83.1	84.5	57.4
(+)-Camphene	1005	1005	14.5	16.9	15.5	42.6
(+)-Sabinene	1021	1021	22.8	4.2	24.4	7.7
(-)-Sabinene	1029	1030	77.2	95.8	75.6	92.3
(+)- β -Pinene	1026	1027	17.7	18.3	19.4	17.2
(-)- β -Pinene	1030	1031	82.3	81.7	80.6	82.8
(-)- α -Phellandrene	1051	1050	100.0	100.0	100.0	100.0
(+)- α -Phellandrene	nd	1053	0.0	0.0	0.0	0.0
(-)-Limonene	1074	1073	94.0	72.3	85.6	92.2
(+)-Limonene	1082	1081	6.0	27.7	14.4	7.8
(-)- β -Phellandrene	1084	1083	8.7	7.4	8.2	8.2
(+)- β -Phellandrene	1087	1089	91.3	92.6	91.8	91.8
(+)-Terpinen-4-ol	1295	1297	36.8	43.9	36.4	60.9
(-)-Terpinen-4-ol	1298	1300	63.2	56.1	63.6	39.1
(-)- α -Terpineol	1348	1347	0.0	26.8	0.0	67.8
(+)- α -Terpineol	1356	1356	100.0	73.2	100.0	32.2

RI_{db} = Retention index from our in-house database based on commercially available compounds available from Sigma-Aldrich and augmented with our own data. RI_{calc} = Calculated retention index based on a series of *n*-alkanes on a Restek B-Dex 325 capillary column. nd = not detected.

4. Conclusions

This work presents, for the first time, the foliar essential oil of *Tsuga mertensiana* collected from its natural habitat in the Cascade Mountain Range of Oregon. In addition, the enantiomeric distributions of chiral monoterpenoids have been determined. The major components in *T. mertensiana* essential oils were (+)- α -pinene, (-)- β -pinene, (-)- α -phellandrene, (-)-limonene, (+)- β -phellandrene, benzoic acid, and bornyl acetate. There are quantitative differences in

the volatile components of *T. mertensiana* from this work and those of *T. mertensiana* cultivated in Rhode Island. In addition, there are notable differences in enantiomeric distributions in *T. mertensiana* compared to *T. heterophylla*. In addition to *T. mertensiana* and *T. heterophylla*, there are 10 additional *Tsuga* species. It would be interesting to determine the enantiomeric distributions in these other *Tsuga* species for comparison.

Authors' contributions

Conceptualization, W.N.S.; Methodology, A.P., P.S., and W.N.S.; Software, P.S.; Validation, W.N.S., Formal Analysis, A.P., and W.N.S.; Investigation, K.S. A.P., P.S., and W.N.S.; Resources, P.S. and W.N.S.; Data curation, W.N.S.; Writing – original draft preparation, W.N.S.; Writing – review & editing, K.S., A.P., P.S. and W.N.S.; Project administration, W.N.S.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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