THE VOLATILE TWIG AND LEAF OIL TERPENE COMPOSITIONS OF THREE WESTERN NORTH AMERICAN LARCHES, *LARIX LARICINA, LARIX OCCIDENTALIS,* AND *LARIX LYALLII*[1]

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Analysis of the steam-volatile leaf oils of larch species for possible chemosystematic studies (1,2) presents some problems because of the deciduous nature of this genus. Hence, we investigated whether the twigs could serve as a suitable source of volatile terpenes. We found that the twigs could be transported without apparent loss of volatile oil and that the yields of oil were higher than those from the leaves. This communication describes the terpene composition and quantitative variation of the twig oils of tamarack, *Larix laricina* (Du Roi) K. Koch, western larch, *Larix occidentalis* Nutt., and alpine larch, *Larix lyallii* Parl, as well as that of the leaves of tamarack and western larch.

MATERIALS AND METHODS

PLANT MATERIAL.—Fresh leaves and twigs (up to 25 cm long, 150-200 g) were cut from most parts of the crown (1,3,4) from five trees each from the following locations: *L. laricina*-Prince Albert area, Saskatchewan, Wolf Creek (near Hinton) and Nordegg area, Alberta, and along the Demster Highway, Yukon Territory (km 109, 226, and 356); *L. occidentalis*-Silver Star Mountain (near Vernon), Rossland, and Johnson Creek (near Rock Creek), British Columbia, and Tiger and Sherman Creek Pass, Washington; *L. lyallii*-Lake Agnes, Alberta. The foliage was placed under snow or ice into cooler boxes for transport and was stored at −20° until steam-distillation could be carried out. Notwithstanding these standard precautions, loss of volatile oil was encountered in some leaf samples on longer collecting trips. The identity of the specimens were confirmed by comparison of leaf, twig, and cone samples with authenticated samples at the herbarium of the Pacific Forest Research Centre, Canadian Foresty Service, 506 W. Burnside Rd., Victoria, B.C.

For the chemical analyses, the leaves were detached from the twigs, the twigs cut into small pieces and macerated, and steam-distilled separately for 5-6 h. The recovered oils were analyzed on four packed gc columns of different polarity as used earlier (3,4), and the identity of individual components was confirmed by the on-line, computerized gc-ms method described before (5). A 30-m long SP 2100 surface-coated glass capillary column was used for the latter analysis, which separated all compounds satisfactorily. Components were considered as positively identified when the retentions and mass spectra agreed with those of known compounds. The four diterpenes that could be identified were thunbergene, thunbergol, manoyl oxide, and manool, but Mills (6) reported that epimeric labda-13-ols were not separated by gc, and, hence, *epi*-manool and its oxide may be present as well. The relative percentages listed in Tables 1-3 were obtained by averaging those recorded on the four packed and glass capillary columns. The percentages obtained for well-resolved peaks on the packed columns and thermal conductivity detectors agreed well with those obtained by total ion count on the capillary column attached to the mass spectrometer. The diterpenes were eluted satisfactorily only on this glass capillary column. Germacrene-D tended to decompose or rearrange on the packed columns (3). The cadinene isomers and corresponding alcohols were γ- and δ-cadinene, α- and γ-muurolene, cadinol, torreyol, and τ-muurolol commonly found in conifer leaf oils (1). The coefficient of variation was derived from $\nu=(100\times\sigma)/\Sigma$, where σ=standard deviation and Σ=mean.

RESULTS

The mean percentages (Σ) and coefficients of variation (ν) of the twig oil terpenes of five trees each of tamarack, western larch, and alpine larch, as well as the yields of volatile oil, are shown in Table 1. The tree-to-tree variability (as indicated by ν) was similar for most terpenes, and only that of car-3-ene and some of the sesquiterpenes was high ($\nu>50$). The three species differ markedly in their relative amounts of the major (above 2%) terpenes: α- and β-pinene, car-3-ene, sabinene, β-phellandrene, limonene, terpinolene, camphene, bornyl acetate, cadinene and cadinol isomers, nerolidol, farnesol isomers, and manool. Fenchone, α-fenchol, fenchyl acetate, methyl thymol, and thymol were found in trace to 0.4% amounts in western larch only, and longifolene could not be detected in this species. Germacrene-D and calamenene were absent in the twig oil of tamarack, and sabinol and myrtenal were absent in that of the alpine larch. Thus, minor qualitative differences were present as well. Minor components common to all three larch species were tricyclene, myrcene, α-phellandrene, *p*-cymene, *cis*-ocimene, linalool, camphene

[1]NRCC No. 25859

TABLE 1. The Means and Variation of the Terpene Percentages of the Twig Oils of Five Trees each of Tamarack (*Larix laricina*), Western Larch (*Larix occidentalis*) and Alpine Larch (*Larix lyallii*)

	Tamarack Dempster Highway (Yukon)		Western Larch Johnson Creek (British Columbia)		Alpine Larch Lake Agnes (Alberta)	
Monoterpenes	Σ	ν	Σ	ν	Σ	ν
α-pinene	20.78	18.54	23.86	27.38	39.20	16.06
β-pinene	15.38	21.53	24.10	23.13	14.06	27.24
car-3-ene	21.30	40.93	13.62	60.65	6.70	22.39
sabinene	2.36	35.40	0.62	44.76	7.48	41.76
myrcene	1.50	25.39	2.12	16.81	1.42	20.43
limonene	1.38	11.91	1.66	13.87	2.14	10.75
β-phellandrene	0.92	20.91	2.10	38.54	3.82	15.39
camphene	4.00	25.50	1.20	42.49	0.48	16.73
borneol	0.67	29.85	0.18	53.19	0.12	37.26
bornyl acetate	14.24	21.05	1.44	38.54	1.14	19.91
γ-terpinene	0.76	25.65	0.60	31.18	1.12	33.00
terpinolene	1.80	25.46	1.54	29.61	3.42	42.29
terpinen-4-ol	1.76	29.41	1.14	47.08	1.88	33.16
sabinene hydrate	0.06	—	0.33	54.55	0.24	54.17
sabinol	0.56	41.11	1.26	47.60	—	—
myrtenal	0.14	—	0.40	39.53	—	—
myrtenol	0.42	19.92	1.16	42.06	trace	—
α-terpineol	1.42	23.57	1.84	29.42	0.27	40.74
α-terpineol acetate	0.11	—	0.98	26.53	0.37	27.03
piperitone	trace	—	0.82	26.83	trace	—
citronellol	0.08	—	0.52	34.61	0.14	—
geranyl acetate	0.21	85.42	trace	—	trace	—
others	1.6	—	0.8	—	0.7	—
Sesquiterpenes						
β-elemene	trace	—	0.83	42.41	0.25	36.06
longifolene	0.46	32.97	—	—	1.09	31.10
caryophyllene	0.10	—	0.49	51.43	0.21	66.83
humulene	0.36	66.19	0.52	63.07	trace	—
germacrene-D	—	—	0.78	31.82	1.30	28.23
cadinene isomers	0.08	—	3.78	30.59	3.96	42.42
calamene	trace	—	1.08	30.99	?	—
cadinol isomers	trace	—	6.30	34.92	3.12	42.95
nerolidol	5.00	53.24	0.46	32.97	4.14	21.78
farnesol isomers	1.60	30.63	0.36	31.67	0.09	—
unidentified	0.3	—	0.1	—	0.1	—
Diterpenes						
thunbergene	0.13	—	0.42	82.97	0.34	58.63
other hydrocarbons	trace	—	0.08	—	0.08	—
manool	0.69	32.02	2.58	51.93	0.41	51.24
other oxides	0.46	—	0.10	—	0.28	—
Yield of volatile oil (%)	1.10	—	0.86	—	2.78	—

hydrate, myrtenol, borneol, geraniol, citronellyl acetate, β-elemene, and the non-terpenoid artifact hex-2-en-1-al (1); trace components were α-thujene, *o*-cymene, *trans*-ocimene, linalool, *trans*-sabinene hydrate, and 2-undecanone. Two trace diterpenes could not be identified. Mills (6) reported that the epimeric labda-13-ols were not separated by gc; hence, it is possible that *epi*-manool and its oxide were also present in these larch twig oils. The yield of volatile oil was particularly high from the twigs of alpine larch.

The twig oils from several different populations of tamarack and western larch were analyzed to determine possible geographical differences (see Tables 2 and 3). From the limited data of this study it appeared that, just as with the leaf oils of Douglas fir, *Pseudotsuga menziesii*, (7,8) and lodgepole pine, *Pinus contorta*,

TABLE 2. Mean Twig and Leaf Oil Terpene Composition of Tamarack, *Larix laricina*, from Different Locations in Western Canada

Compound	Twig oil: Yukon Territory, km 356	Twig oil: Yukon Territory, km 226	Twig oil: Alberta, Wolf Creek	Twig oil: Alberta, Nordegg	Twig oil: Saskatchewan, Prince Albert	Leaf oil (Saskatchewan area)
Monoterpenes						
α-pinene	20.9	20.3	30.5	22.7	20.8	17.9
β-pinene	16.2	17.1	7.7	17.6	6.8	5.9
car-3-ene	23.5	19.8	21.6	17.7	28.6	7.6
sabinene	2.9	4.1	1.8	2.6	1.7	0.8
myrcene	1.3	1.6	1.5	1.2	4.0	11.3
limonene	1.4	1.3	4.9	4.4	0.9	2.4
β-phellandrene	0.9	0.8	1.1	1.0	1.1	0.5
camphene group[a]	17.5	20.7	9.9	18.7	3.7	18.5
terpinene group[b]	4.6	4.6	3.4	6.0	6.5	2.4
sabinene hydrate	0.05	0.05	0.2	0.1	0.1	0.1
sabinol	0.5	0.3	0.6	0.2	0.2	—
α-terpineol	0.4	0.6	1.6	1.5	1.6	0.3
geranyl acetate	0.3	0.4	0.2	0.6	0.7	0.7
others	0.7	0.8	1.1	0.4	1.2	5.8
Sesquiterpenes						
longifolene	0.4	0.3	0.4	0.6	3.5	4.5
caryophyllene	0.2	0.2	0.2	0.2	5.5	3.8
humulene	0.6	0.5	0.6	0.2	1.8	1.2
germacrene-D	—	—	—	—	—	11.7
cadinene isomers[c]	0.1	0.1	0.4	0.5	4.8	1.4
cadinol isomers[d]	0.1	0.1	0.2	0.2	0.9	2.4
nerolidol	5.0	3.6	10.3	2.2	0.8	0.1
farnesol isomers	1.1	0.9	0.2	0.3	0.7	0.1
Diterpenes						
thunbergene	0.1	0.2	0.1	0.2	0.05	0.05
other hydrocarbons	0.05	0.05	0.05	—	—	0.05
manool	0.8	0.6	0.7	0.6	3.0	0.3
other oxides[e]	0.2	0.7	0.5	0.2	0.8	0.05
Yield of volatile oil (%)	1.11	1.26	0.86	0.98	0.93	0.31

[a]Mainly camphene and bornyl acetate.
[b]Mainly terpinolene and terpinen-4-ol.
[c]Mainly γ- and δ-cadinene and γ-muurolene.
[d]Mainly cadinol.
[e]Mainly thunbergol.

(3,9), the relative amounts of camphene, borneol, bornyl acetate, and perhaps tricyclene and camphene hydrate, as well as α- and γ-terpinene, terpinolene, and terpinen-4-ol correlated positively, and, hence, their percentages were combined into the "camphene" and "terpinene" groups, respectively, in Tables 2 and 3.

The variation in the major terpene percentages in different tamarack populations is shown in Table 2. Whereas those of the two stands along the Dempster Highway, Yukon Territory, near km 356 and 226 were similar to those near km 109 (see Table 1), those of the populations from Alberta and Saskatchewan differed noticeably, in particular the relative percentages of α and β-pinene, limonene, the camphene group, the sesquiterpenes, and manool. The leaf oil composition from a site in Saskatchewan (Table 2, last column) differed not only quantitatively from that of the twigs, but thujone (4.5%) and isothujone (0.6%) were recorded (listed under "others"). The latter were not found in any of the other larch oils obtained in

TABLE 3. Mean Twig Terpene Composition of Western Larch, *Larix occidentalis,* from Four Different Locations in Western Canada and the Northwestern United States, and the Leaf Oil Terpene Composition from Two Locations

Compound	Twig Oil				Leaf Oil	
	British Columbia		Washington		British Columbia	Washington
	Silver Star	Rossland	Tiger	Sherman Creek	Johnston Creek	Sherman Creek
Monoterpenes						
α-pinene	21.0	28.1	22.7	22.8	28.2	29.5
β-pinene	24.5	26.9	31.6	25.3	35.5	22.8
car-3-ene	19.3	7.6	6.9	13.1	11.6	10.4
sabinene	1.6	1.1	1.3	2.8	0.6	2.1
myrcene	1.8	1.2	1.4	1.1	2.1	1.2
limonene	1.6	2.1	2.0	1.8	1.3	1.5
β-phellandrene	1.1	0.8	1.1	1.0	1.2	0.9
camphene group[a]	2.5	4.1	3.4	3.3	2.2	10.5
terpinene group[b]	4.7	6.4	4.3	6.8	4.1	3.3
sabinene hydrate	0.1	0.6	0.7	0.5	0.2	0.6
sabinol	2.1	2.8	2.6	1.4	0.3	0.6
myrtenal	0.4	0.4	1.2	0.9	0.1	0.2
myrtenol	1.4	1.8	1.8	1.6	0.3	0.6
α-terpineol	1.9	3.2	5.5	2.8	3.0	0.8
α-terpineol acetate	0.3	0.8	0.2	1.2	—	—
piperitone	0.4	1.1	0.5	0.6	0.1	0.3
citronellol	0.8	1.4	1.3	2.2	0.4	0.2
geranyl acetate	0.1	0.1	0.3	0.2	0.7	0.4
others	1.6	2.2	1.9	1.4	0.8	0.4
Sesquiterpenes						
β-elemene	0.2	0.5	0.2	0.1	0.1	0.05
caryophyllene	0.4	0.2	0.3	0.2	1.2	0.5
humulene	0.7	0.3	0.1	0.4	2.5	3.4
germacrene-D	0.4	0.1	0.4	0.3	0.8	2.1
cadinene isomers[c]	1.1	0.7	1.1	0.9	0.8	2.7
cadinol isomers[d]	1.8	0.6	1.4	1.8	1.3	0.6
nerolidol	5.3	1.2	0.3	1.3	0.2	2.7
farnesol isomers	1.9	0.3	2.1	0.8	0.3	1.3
Diterpenes						
thunbergene	0.3	0.4	0.7	0.4	?	?
other hydrocarbons	0.05	0.2	0.05	0.1	—	—
manool	0.8	2.0	0.2	1.1	0.1	0.2
other oxides[e]	0.6	0.5	2.2	1.0	—	0.1
Yield of volatile oil (%)	0.85	0.76	0.44	1.17	0.28	0.21

[a]Mainly camphene and bornyl acetate.
[b]Mainly terpinolene and terpinen-4-ol.
[c]Mainly γ- and δ-cadinene and γ-muurolene.
[d]Mainly cadinol.
[e]Mainly thunbergol.

this investigation, and larger percentages of these two bicyclic terpenes are common only in *Thuja* species (1).

The western larch twig oil composition also varied quantitatively from one site to another (Table 3), but in this species the most variable terpenes were car-3-ene, sabinene, α-terpineol and its acetate, citronellol, the cadinene-cadinol isomers, nerolidol, the farnesol isomers, and manool. The leaf oil composition (Table 3, last column) appears to be fairly similar to that of the twigs, except that the camphene group and germacrene-D percentages were somewhat higher, and those of the diterpenes lower. The leaf oil composition of the alpine larch (not shown) also appeared to be fairly similar to that of the twigs, but it was evident that losses had occurred during transport and/or storage.

The above data show that just as with the larch resins (6) quantitative as well as some minor qualitative differences between these three western larch species exist in their twig oils, and that regional or geographic variation within tamarack and western larch can be found. Thus, chemosystematic studies[2] based on the twig oil compositions appear to be feasible. Yields of volatile oil varied from 0.5 to 3.1%, which was larger than those of the leaf oils. The leaf oils appear to be less suitable for such studies because of possible loss of volatiles during transport when longer field trips are involved. Also, the time of collecting the mature larch leaves before abscission is much shorter than for those of the nondeciduous conifers which can be collected from fall through winter to early spring in northern latitudes (1). However, whereas leaf oils are derived from a single plant organ [and possibly a single biosynthetic site (1)], the twig oils are comprised of cortical and xylem terpenes. The complex compositions and the presence of characteristic diterpenes demands the use of the more efficient capillary gc columns.

ACKNOWLEDGMENTS

The excellent technical assistance by M. Granat and L.R. Hogge, and the collection of tamarack foliage samples from the Dempster Highway by E.T. Oswald, Pacific Forest Centre, Canadian Forest Service, Environment Canada, are gratefully acknowledged.

LITERATURE CITED

1. E. von Rudloff, *Biochem. Syst. Ecol*, **2**, 131 (1975).
2. A.E. Squillace, in: "Modern Methods in Forest Genetics." Ed. by J.P. Miksche, 1976, pp. 126-157.
3. E. von Rudloff, M.S. Lapp, and R.G. McMinn, *Can. J. For. Res.*, **15**, 801 (1985).
4. M.S. Lapp and E. von Rudloff, *Can. J. Bot.*, **60**. 2762 (1982).
5. R.P. Adams, M. Granat, L.R. Hogge, and E. von Rudloff, *J. Chromatog. Sci.*, **17**, 75 (1979).
6. J.S. Mills, *Phytochemistry*, **12**, 2407 (1973).
7. E. von Rudloff and G.E. Rehfeldt, *Can. J. Bot.*, **58**, 546 (1980).
8. E. von Rudloff, *Silvae Gen.*, **33** 198 (1984).
9. E. von Rudloff and M.S. Lapp, *Can. J. For. Res.*, **17**, (1987).

Received 16 June 1986

[2]Such studies are not considered at this laboratory owing to the retirement of the author.