The leaf essential oil of *Abies grandis* (Doug. ex D. Don) Lindl. *(Pinaceae)*: revisited 38 years later

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ABSTRACT

The first report on the volatile leaf terpenoids of *Abies grandis* was in 1976 by Ernst von Rudloff. Using new GCMS technology that has been developed in the interim, a comprehensive analysis was performed on the leaf terpenoids of *A. grandis* and is presented. The oil of *A. grandis* is dominated by β-pinene (20.3 - 31%), bornyl acetate (12.7 - 26.2%), β-phellandrene (13.7 - 25.2%) and camphene (8.3 - 11.5%), with moderate amounts of α-pinene (4.4 - 7.4%), α-terpinene (1.1 - 2.2%), terpinolene (1.3 - 2.9%) and α-terpineol (1.1 - 3.6%). Fifty nine compounds were identified encompassing monoterpenes, sesquiterpenes and one diterpene (abietadiene). Only 1-epi-cubenol appeared to differ between coastal (0.5 - 0.7%) and inland grand fir (0 - trace). Otherwise, just as von Rudloff (1976) and Zavarin et al. (1977) concluded, the volatile oils (leaf and wood) do not differentiate coastal and inland grand fir. The von Rudloff (1976) analysis, using packed GC columns, agreed closely with the present analysis.
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KEY WORDS: *Abies grandis,* terpenes, composition, volatile leaf oils, Pinaceae.

In 1976, I (RPA) was on a sabbatical with Ernst von Rudloff in Saskatoon, Canada when his paper on the volatile leaf oil of grand fir (*Abies grandis*) was published (von Rudloff, 1976). As was his usual procedure, he reported on within- and between-tree variation before reporting on inland and coastal populations. von Rudloff (1976) reported quantitative data for thirteen monoterpenes, alcohols and acetates, with combined values for the sesquiterpene hydrocarbons and alcohols using a packed GC column. This was before the widespread use of fused-quartz capillary columns. My (RPA) work developing a computerized mass spectra library of volatile oil components was began in von Rudloff's lab and with the gracious sharing of his large stock of known terpenoids (Adams, et al. 1979; Adams, 2007). After 38 years of progress in the identification of terpenoids, those days of trapping individual components from packed columns and then running IR and NMR, pale in comparison to present-day technology enabling one to run GCMS and identify 95 - 98% of more than 100 components in two hours.

von Rudloff (1975, 1976) reported only minor differences in the volatile leaf oils between inland and coastal *A. grandis* and turned his attention to analysis of other conifer oils. Zavarin et al. (1977) examined quantitative variation in eight monoterpenes in wood oleoresin in 395 samples from 48 populations of *A. grandis*. They concluded "..noteworthy is the absence of any difference between coastal and Rocky Mountain populations...". The reports from von Rudloff and Zavarin's labs, appear to have deterred any additional research on the leaf essential oils of *A. grandis*. A search of the literature revealed only two more recent papers on the leaf oils of *A. grandis*. Muzika et al. (1989) examined the effects of nitrogen fertilization on 10 leaf terpenes and Muzika et al. (1990) reported a comparison of extraction methods on yields of monoterpenes from *A. grandis*.

This paper, using high resolution capillary GCMS, presents the first detailed report on the composition of the volatile leaf oil of *A. grandis* since the original report of von Rudloff (1976).

MATERIALS AND METHODS

Leaf samples were collected from *Abies grandis,* Kane Creek, OR, 42° 21' 39" N, 123° 01' 16" W, 2268 ft., *Adams (F. Callahan) 14343-14347*; 14 mi e of Coeur d'Alene, ID, on I90, 47° 37' 16" N, 116° 31' 10" w, 3100 ft., *Adams 14363-14364*; Mt. Spokane Park, WA, 47° 54' 13" N, 117° 06' 09" W, 4528 ft., *Adams 14358-14362*; 3 mi. se of Sequim, WA, 48° 2.08" N, 123° 1.36" W, 72 ft., *Adams 12913-12917*; Del Norte Co., CA, 41° 45' 27.99" N, 124° 08' 41.84" W, 289 ft., *Adams (M. Kauffmann) 12970-12974*, Humboldt Co., CA, 40° 45' 57.95" N, 124° 07' 18.31" W, 310 ft., *Adams (M. Kauffmann) 12975-12979*. Voucher specimens are deposited in the herbarium, Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The leaf oil of *Abies grandis* was clear in color. The oil of *A. grandis* (Table 1) is dominated by β-pinene (20.3 - 31%), bornyl acetate (12.7 - 26.2%), β-phellandrene (13.7 - 25.2%) and camphene (8.3 - 11.5%), with moderate amounts of α-pinene (4.4 - 7.4%), α-terpinene (1.1 - 2.2%), terpinolene (1.3 - 2.9%) and α-terpineol (1.1 - 3.6%). Fifty nine compounds were identified encompassing monoterpenes, sesquiterpenes and diterpenes (Table 1). Only 1-epi-cubenol (Table 1) appeared to differ between coastal (0.5 - 0.7%) and inland grand fir (0 - trace). Otherwise, just as von Rudloff (1976) and Zavarin et al. (1977) concluded, the volatile oils (leaf and wood) do not differentiate coastal and inland grand fir. The von Rudloff (1976) analysis, using packed GC columns thirty eight years ago, agreed closely with the present analysis (Table 1).

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Table 1. Comparison of leaf oil compositions of coastal and inland *Abies grandis* populations. Only 1-epi-cubenol (in bold face) appears to separate coastal and inland groups. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index based a linear calculation on DB-5 column. Sqm,WA = Sequim, WA; sw OR = sw of Central Point, OR; Hmb, CA = Humboldt Co. CA; CdA, ID = Coeur d'Alene, ID. The data for V.I., BC (Vancouver Island, BC, EvR 1976) and CF,ID (Clark Fork, ID, EvR 1976) were taken from von Rudloff (1976, Table 3),

		Coastal				inland	
		2014 collections			1976	1976	2014
KI	compound	Sqm,WA	sw OR	HMB,CA	V.I.,BC	C.F.,ID	CdA,ID
846	(E)-hexenal	0.3	0.2	0.1			0.3
884	santene	t	t	0.3			0.2
921	tricyclene	0.6	0.4	1.0	1.3	1.2	0.7
924	α-thujene	0.4	t	t			0.1
932	α-pinene	4.7	5.0	7.4	6.0	8.1	4.4
946	camphene	11.5	8.3	10.5	13.8	14.0	10.7
969	sabinene	t	t	t			t
974	β-pinene	30.8	31.1	20.3	21.4	25.2	20.3
988	myrcene	1.0	1.4	1.0	1.0	1.1	0.9
1002	α-phellandrene	1.0	1.9	0.8			0.7
1008	δ-3-carene	0.1	t	0.5			0.7
1014	α-terpinene	2.0	2.1	1.4			1.1
1020	p-cymene	0.2	t	t			t
1024	limonene	1.1	0.8	1.3	2.0	2.5	2.1
1025	β-phellandrene	13.7	15.9	25.2	11.3	16.0	18.9
1032	(Z)-β-ocimene	t	t	-			t
1054	γ-terpinene	0.7	0.6	0.4			0.3
1086	terpinolene	2.9	2.8	1.3			1.3
1095	linalool	t	t	t			t
1114	endo-fenchol	0.3	-	t			0.3
1118	cis-p-menth-2-en-1-ol	0.2	t	0.2			t
1122	α-campholenal	0.2	t	t			t
1136	trans-p-menth-2-en-1-ol	0.4	t	0.2			t
1141	camphor	0.4	0.3	0.3	0.7	0.6	0.5
1145	camphene hydrate	t	0.2	0.1			0.3
1148	citronellal	0.5	0.4	0.6			0.4
1148	iso-isopulegol	t	t	0.2			t
1165	borneol	0.4	0.2	0.5	0.7	0.5	0.2
1174	terpinen-4-ol	0.2	0.2	0.2			0.2
1186	α-terpineol	3.6	2.5	1.1	0.5	0.5	1.8
1195	cis-piperitol	-	-	t			-
1207	trans-piperitol	-	-	t			-
1223	citronellol	0.3	0.7	1.0			0.4
1264	geranial	-	t	-			t
1287	bornyl acetate	17.0	12.7	17.6	18.5	18.8	26.2
1298	2-undecanone	t	t	t			t
1345	α-cubebene	t	t	t			t
1350	citronellyl acetate	0.2	0.9	0.5	0.9	0.6	0.2
1374	α-copaene	t	t	0.1			t
1379	geranyl acetate	1.2	0.3	0.4	0.3	0.7	0.3

Table 1 (contd.)

		Coastal				inland	
		2014 collections			1976	1976	2014
KI	compound	Sqm,WA	sw OR	HMB,CA	V.I.,BC	C.F.,ID	CdA,ID
1417	(E)-caryophyllene	t	t	t			t
1451	trans-muurola-3,5-diene	t	t	t			t
1475	trans-cadina-1(6),4-diene	t	0.3	t			0.1
1493	trans-muurola-4(14),5-diene	0.2	0.6	0.2			0.2
1493	epi-cubebol	0.2	0.2	0.3			0.3
1500	α-muurolene	0.2	0.2	0.1			t
1514	cubebol	0.3	0.6	0.5			0.6
1522	δ-cadinene	0.9	1.2	0.7			1.0
1528	zonarene	t	0.2	t			t
1533	trans-cadina-1,4-diene	0.1	0.4	0.1			0.2
1561	(E)-nerolidol	t	t	t			t
1614	(selina-6-en-4α-ol)	t	0.2	0.1			t
1627	**1-epi-cubenol**	**0.6**	**0.7**	**0.5**			**t**
1645	cubenol	0.4	0.5	t			0.6
1644	α-muurolol	t	t	t			t
1652	α-cadinol	0.2	0.3	t			0.3
1665	intermedeol	-	1.2	-			-
1722	(2Z,6E)-farnesol	-	t	-			t
1864	benzyl salicylate	-	0.1	-			-
2087	abietadiene	-	-	0.1			-