Epicuticular Wax Compositions of Predominant Conifers of Western North America

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Z. Naturforsch. **54c,** 17–24 (1999); received June 25/October 6, 1998

Gymnosperms, Epicuticular Wax Composition, *n*-Alkanes, *n*-Alkanoic Acids, *n*-Alkanols

The compositions of epicuticular waxes from conifers constituting the predominant species of western North America were determined by GC and GC-MS. The primary components identified include alkanes, fatty acids, fatty alcohols, aldehydes, ketones, phytosterols, triterpenoids and wax esters. Average chain lengths (ACL) for alkanes in Oregon conifers decreased with increasing distance away from the Coastal range which suggests an adaptation by conifers to humid climate conditions. Differences in the chemical compositions make this information useful for chemotaxonomic purposes, for identifying natural organic aerosol input sources to the atmosphere, for comparison with the tracers in smoke emissions from burning of these biomass fuels, and for monitoring in assessment of global climate change.

Introduction

Epicuticular plant waxes consist mainly of aliphatic compounds such as higher molecular weight *n*-alkanes, *n*-alkanals, *n*-alkanols, *n*-alkanoic acids and wax esters (Eglinton *et al.*, 1962; Kolattukudy, 1970, 1976). The identification of plant wax constituents have been of utility for chemotaxonomic purposes (Nishimoto, 1974; Tulloch, 1981; Salasoo, 1987; Zygaldo *et al.*, 1994; Maffei, 1996), as indicators for determining pollutant exposure (Lutz *et al.*, 1990; Percy and Baker, 1990; Kerfourn and Garrec, 1992; Percy *et al.*, 1993; Burkhardt *et al.*, 1995), and in studies of environmental influences on plant development (Hadley and Smith, 1990; Jagels, 1991; Cape and Percy, 1993; Pfeifhofer, 1995). Plant waxes are major components of the particulate organic matter of aerosols in urban, rural and remote areas (Simoneit and Mazurek, 1982; Simoneit *et al.*, 1988; Simoneit, 1989; Rogge *et al.*, 1993). They have been used for source reconciliation studies of urban, rural and remote aerosols (Simoneit, 1977; Gagosian *et al.*, 1982; Mazurek and Simoneit, 1984; Simoneit *et al.*, 1991a,b; Rogge, *et al.*, 1993; Chen and Simoneit, 1994; Schauer *et al.*, 1996), and for characterization of fuel sources in biomass burning (Standley and Simoneit, 1987; Rogge *et al.*, 1994; Abas *et al.*, 1995).

Here we report the chemical composition of epicuticular waxes for conifers constituting the dominant species of western North America. Samples were collected from forested areas of Oregon, USA and Durango, Mexico away from urban areas and major roads (Standley, 1987): Coastal Range, Columbia Basin, Umatilla National Forest, Willamette National Forest and a forest reserve in the Sierra Madre Occidental (Table I). The samples represent a variety of conifers from areas with different climate conditions. Conifer needles were randomly selected from individual tree canopies and composited into a single sample, thus the abundances of the chemical components reported here reflect average values. This random selection minimizes any chemical bias from physiological factors such as differences in needle age and water content, and from environmental influences such as pollutant and fog exposure. The primary components identified in the soluble lipid fractions include the alkanes, fatty acids, fatty alcohols, aldehydes, ketones, phytosterols, triterpenoids and wax esters. It is these compounds as such and their thermal alteration products which are used as tracers for tracking emissions from biomass burning (Mazurek and Simoneit, 1997).

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0939–5075/99/0100–0017 $ 06.00

Materials and Methods

Extracts of 15 vegetation samples were obtained by briefly dipping (3–5 sec, 3 times each) needle fronds into chloroform ($CHCl_3$) to dissolve the external waxes. Solvent to sample contact was kept brief to minimize the extraction of significant amounts of internal cellular lipids (intra-cuticular waxes). Extracts were filtered through annealed glass wool and concentrated under aspirator vacuum to approximately 2 ml. A 500 µl aliquot of the total extract was then taken for derivatization. Alkanoic acid and phenolic moieties were methylated using diazomethane in diethyl ether prepared from the precursor N-methyl-N'-nitro-N-nitrosoguanidine (Pierce Chemical Co.) (Schlenk and Gellerman, 1960).

The methylated extracts were separated into four fractions by preparative thin layer chromatography on silica gel plates (Analtech, Inc.) with a mobile phase mixture of hexane and chloroform (19:1 v/v). The four fractions contained the following classes of compounds: (1) *n*-alkanes and saturated and unsaturated cyclic di- and triterpenoid hydrocarbons, (2) *n*-alkanones and *n*-alkanals, (3) *n*-alkanoic acids (as methyl esters) and saturated and unsaturated di- and triterpenoid ketones, and (4) *n*-alkanols, terpenols and polar organics. An aliquot of the fourth fraction was then converted to trimethylsilyl derivatives by reaction with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) plus 1% trimethylchlorosilane:anhydrous pyridine (1:1) for approximately 30 minutes at 70 °C under a nitrogen atmosphere.

The extract fractions were analyzed by capillary gas chromatography (GC, Hewlett-Packard Model 5890A) with a 25 m x 0.20 mm i.d. fused silica capillary column coated with DB-5 (J&W Scientific) which was temperature programmed as a hold at 65 °C for 5 min, ramped to 130 °C at 15 °C/min, then at 6 °C/min to 310 °C, with an isothermal hold at 310 °C for 60–120 min. Selected samples were also analyzed by capillary gas chromatography-mass spectrometry (GC-MS) using a Finnigan 4000 or Hewlett-Packard 6890 MSD quadrupole mass spectrometer operated in the electron impact mode at 70 eV and coupled to a GC. The GC was equipped with a 30 m x 0.25 mm i.d. capillary column coated with DB-5 (J&W Scientific) and was temperature programmed as follows: 65 °C for 6 min, to 310 °C at a rate of 4 °C/min, then held isothermal at 310 °C for 60–120 min.

The homologous compound series were quantified by comparison of the GC peak areas with that of a co-injected known standard, hexamethylbenzene. Molecular markers were quantified in the GC-MS data by comparison of peaks with the same standard.

Results and Discussion

The compositions of the lipid constituents in the conifer waxes are given in Table I. The polar lipids for all the conifers sampled make up an overall

Table I. Composition of lipid constituents in the epicuticular waxes of predominant conifers of western North America.

Samples				Lipid Compositions (%)					
Common name	Code	Scientific name	Region collected	*n*-Alkanes	Wax esters	*n*-Alkanoic acids	*n*-Alkanones	*n*-Alkanols	Polar lipids
Apache Pine	AP	*Pinus engelmannii*	Sierra Madre Occidental, Mex	1.4	1.8	15.3	0.1	0.5	80.9
Big-Cone Douglas Fir	BDF	*Pseudotsuga macrocarpa*	Willamette National Forest, OR	0.1	1.4	0.7	2.6	2.2	93.0
Brewer Spruce	BS(CR)	*Picea brewerana*	Coastal Range, OR	0.1	1.7	0.9	4.3	4.4	88.6
Brewer Spruce	BS(WNF)	*Picea brewerana*	Willamette National Forest, OR	1.1	0.8	1.1	2.8	4.4	89.8
California Redwood	CR	*Sequoia sempervirens*	Jedidiah Smith State Park, CA	4.4	0.5	3.3	0.1	0.3	91.4
Douglas Fir	DF(CR)	*Pseudotsuga menziesii*	Coastal Range, OR	0.1	0.9	1.1	8.3	1.4	88.2
Douglas Fir	DF(UNF)	*Pseudotsuga menziesii*	Umatilla National Forest, OR	0.1	0.4	1.2	1.5	5.4	91.4
Montezuma Pine	MP	*Pinus montezumae*	Sierra Madre Occidental, Mex	9.8	6.5	20.3	1.2	0.4	61.8
Mountain Hemlock	MH	*Tsuga mertensiana*	Willamette National Forest, OR	3.5	3.3	0.3	23.7	1.5	67.7
Norway Spruce	NS	*Picea abies*	Coastal Range, OR	0.9	bd	0.1	2.3	2.1	94.6
Pacific Silver Fir	PSF	*Abies amabilis*	Umatilla National Forest, OR	0.1	0.3	0.8	0.6	5.8	92.4
Ponderosa Pine	PP	*Pinus ponderosa*	Umatilla National Forest, OR	1.5	3.3	2.9	4.2	4.7	83.4
Sitka Spruce	SS	*Picea sitchensis*	Coastal Range, OR	1.1	bd	4.7	0.1	0.3	93.8
Western Juniper	WJ	*Juniperus occidentalis*	Columbia Basin, OR	0.1	0.1	0.1	0.5	5.6	93.6
White Fir	WF	*Abies procera*	Umatilla National Forest, OR	bd	0.1	0.4	0.1	7.2	92.2

bd = below detection limit.

average of 87% of the total wax extracts. The highest polar lipid content was found in Norway Spruce (94.6%) while the lowest was in Montezuma Pine (61.8%). Mountain Hemlock which has a low polar lipid content at 68% shows the highest amount of nonpolar lipids as aldehydes and ketones (24%), which is 12 times greater than the average content of aldehydes and ketones in the other wax samples. This may reflect the degree of biochemical coupling between the enzymes acyl-CoA reductase and aldehyde reductase which are required for the biosynthesis of fatty alcohols from fatty acids. Lack of a tight coupling mechanism results in the accumulation of aldehydes as are present in this conifer wax (Kolattukudy *et al.*, 1976).

Analytical data for the lipid characteristics of the conifer epicuticular waxes are given in Table II. The carbon number range (C_{range}), carbon number maximum (C_{max}) and the carbon preference indices (CPI) (Mazurek and Simoneit, 1984) for the homologous series of *n*-alkanes, *n*-alkanoic acids and *n*-alkanols are listed. The average chain length (ACL) parameters for higher plant *n*-alkanes and *n*-alkanols are also given in Table II. The ACL parameter may be used as an additional indicator of source composition (Poynter and Eglinton, 1990). The ACLs were derived by using the percent composition values of individual lipid components present in conifer waxes.

Hydrocarbons: The conifer wax extracts exhibited *n*-alkanes ranging from C_{16} to C_{35} and C_{max} values ranging from 23 to 33. The most common C_{max} values for the *n*-alkanes were C_{25} and C_{29} each characteristic of four conifers. Brewer Spruce (WNF) and Apache Pine waxes exhibited the lowest C_{max} at 23 and three conifer waxes showed a C_{max} at 33. All C_{max} are odd carbon numbered *n*-alkanes. The CPIs for the *n*-alkanes ranged from 2.6 to 17.0 with an average of 5.8 (all >>1.0). The lowest CPI was found in the wax of California

Table II. Analytical data of the lipid constituents of epicuticular waxes from predominant conifers of western North America.

Sample	*n*-Alkanes[1]			*n*-Alkanoic Acids[1]			*n*-Alkanols[1]			*n*-Alkane ACL[2]	*n*-Alkanol ACL[2]
	C_{range}	C_{max}	$CPI_{C_{20}-C_{36}}$	C_{range}	C_{max}	$CPI_{C_{15}-C_{37}}$	C_{range}	C_{max}	$CPI_{C_{12}-C_{34}}$	$C_{23}-C_{35}$	$C_{22}-C_{34}$
Apache Pine	16–33	23	3.2	7–34	20	7.4	14–28	24	1.5	26.8	25.1
Big-Cone Douglas Fir	22–31	25	7.4	16–28	24	7.8	16–30	26	4.0	25.8	25.3
Brewer Spruce (CR)	21–31	29	4.7	16–30	24	17.0	14–32	24	3.0	27.3	26.9
Brewer Spruce (WNF)	21–31	23	6.7	22–26	24	14.0	16–30	24	4.0	25.6	25.4
California Redwood	19–33	27	2.6	13–32	32	19.3	14–26	26	9.7	28.1	25.0
Douglas Fir (CR)	24–31	29	7.4	16–26	24	15.0	16–32	26	3.0	28.0	26.6
Douglas Fir (UNF)	21–31	25	6.0	16–26	24	10.4	16–30	26	5.6	26.5	26.5
Montezuma Pine	18–33	29	3.2	8–34	20	5.5	12–28	22	4.0	27.7	24.0
Mountain Hemlock	24–33	31	17.0	16–30	28	2.3	16–30	28	6.5	29.9	27.3
Norway Spruce	25–35	33	5.9	14–24	16	∞	16–30	26	4.0	33.3	26.5
Pacific Silver Fir	21–31	25	3.6	16–28	24	4.8	12–30	24	3.2	26.0	25.2
Ponderosa Pine	23–35	33	4.9	–	–	–	12–32	24	10.6	29.5	26.0
Sitka Spruce	18–31	29	2.8	7–32	22	5.6	18–28	20	2.9	28.0	23.0
Western Juniper	21–31	25	5.3	–	–	–	16–30	26	4.4	26.1	26.0
White Fir	21–35	33	6.0	–	–	–	16–30	22	8.2	28.4	24.1
Range:	16–35	23–33	2.6–17	7–34	16–28	2.3–19.3	12–32	20–28	1.5–10.6	25.6–33.3	23.0–27.3

[1]: Determined by GC/MS; C_{max} = Carbon number maximum as defined in Mazurek and Simoneit (1984); CPI = Carbon preference index is the sum of the odd carbon number homologs divided by the sum of the even carbon number homologs for *n*-alkanes and the inverse for *n*-alkanoic acids and *n*-alkanols (Mazurek and Simoneit, 1984).

[2]: ACL = Average chain length is the average number of carbon atoms per molecule based on the abundance of the odd alkanes from $C_{23}-C_{35}$ or the even alkanols from $C_{22}-C_{34}$ (Poynter and Eglinton, 1990):

$$n\text{-Alkane ACL} = \frac{23 \times [C_{23}] + 25 \times [C_{25}] + 27 \times [C_{27}] + 29 \times [C_{29}] + 31 \times [C_{31}] + 33 \times [C_{33}] + 35 \times [C_{35}]}{[C_{23}] + [C_{25}] + [C_{27}] + [C_{29}] + [C_{31}] + [C_{33}] + [C_{35}]}$$

$$n\text{-Alkanol ACL} = \frac{22 \times [C_{22}] + 24 \times [C_{24}] + 26 \times [C_{26}] + 28 \times [C_{28}] + 30 \times [C_{30}] + 32 \times [C_{32}] + 34 \times [C_{34}]}{[C_{22}] + [C_{24}] + [C_{26}] + [C_{28}] + [C_{30}] + [C_{32}] + [C_{34}]}$$

Redwood and the highest for that of Mountain Hemlock. Although the homologous series of *n*-alkanes are useful for chemotaxonomic purposes, their source information is not always definitive because of their relatively simple assemblage and the complexity of other components present in waxes (Kolattukudy, 1976). Care must be taken if the CPI is used solely for chemotaxonomic and source correlation purposes.

The average of the ACLs for *n*-alkanes in Oregon conifer waxes decreases significantly by two carbon numbers with increased distance away from the coastal range. The average *n*-alkane ACL in the Coastal Range was 29.2 (n=4) while the inland region values average 27.2 (n=8). Since the mean daily temperatures of the Coastal Range and inland regions were similar during the sampling period (16.5 °C) and the regional altitudes were the same (<1 km), this observation suggests an adaptation by conifers to a more humid climate present at the coastal range rather than a temperature dependence. This observation also occurs within species, as for example, the coastal conifers Douglas Fir and Brewer Spruce both have higher *n*-alkane ACLs than their inland relations, i.e., 28.0 to 26.5 and 27.3 to 25.6, respectively. The difference in *n*-alkane ACLs observed within species also supports the finding reported by Percy *et al.* (1993) which showed that environmental conditions, such as microclimate influences (coastal fog exposure), may influence conifer wax composition. It has also been reported that aging of conifer needles induces a shift towards longer chain length in some conifer species (Lutz *et al.*, 1990). However, since conifer needles were selected at random and composited, in order to minimize wax composition differences due to age, this should not be a factor. Further research on the observed humidity adaptation is warranted. It is further suggested that *n*-alkane ACL determinations be used as indicator tools to monitor the effects of global climate change on declining forest populations. No other regional trends are apparent from the *n*-alkane ACL data.

Alcohols: The *n*-alkanol series present in the wax extracts displayed a C_{range} from C_{12} to C_{32}

Table III. Analytical data for the ω-hydroxyalkanoic acids, *n*-alkan-10-ones, unsaturated aldehydes and wax esters in conifer waxes.

Sample	ω-Hydroxy-alkanoic acids		*n*-Alkan-10-ones		Unsaturated aldehydes		Wax esters[1]			
	C_{range}	C_{max}	C_{range}	C_{max}	C_{range}	C_{max}	C_{range}	C_{max}	$CPI_{C_{18}-C_{50}}$	$ACL_{C_{18}-C_{50}}$[2]
Apache Pine	–	–	19	19	–	–	24–28	26	∞	26.1
Big-Cone Douglas Fir	–	–	–	–	–	–	34–41	40	43.0	38.5
Brewer Spruce (CR)	14–16	16	–	–	–	–	34–38	36	20.4	35.6
Brewer Spruce (WNF)	–	–	29	29	30–34	30	30–42	38	107.7	37.8
California Redwood	–	–	–	–	–	–	26–30	28	∞	27.8
Douglas Fir (CR)	14–16	14	–	–	–	–	36–38	38	15.8	37.4
Douglas Fir (UNF)	14–16	14	17–29	29	28–30	30	36–42	38	83.0	39.6
Montezuma Pine	–	–	19	19	–	–	18–30	26	∞	26.6
Mountain Hemlock	–	–	–	–	–	–	–	–	–	–
Norway Spruce	–	–	29	29	28–29	28	29–50	42	2.6	41.9
Pacific Silver Fir	14–16	16	–	–	–	–	–	–	–	–
Ponderosa Pine	14–16	14	–	–	–	–	–	–	–	–
Sitka Spruce	–	–	19	19	–	–	–	–	–	–
Western Juniper	14–16	16	29–31	29	28–32	30	28–46	38	21.9	39.6
White Fir	14–16	14	17–29	29	28–32	30	34–40	38	8.3	37.2
Range:	14–16	14–16	17–31	19–29	28–34	28–30	18–50	26–42	2.6–107.7	26.1–41.9

[1]: Determined by GC-MS as *n*-alkyl-*n*-alkanoate moieties; C_{max}=Carbon number maximum as defined by Mazurek and Simoneit (1984); CPI = Carbon preference index for wax esters is the sum of the even carbon number homologs divided by the sum of the odd carbon number homologs (Mazurek and Simoneit, 1984).
[2]: ACL = Average chain length is the average number of carbon atoms per molecule based on the abundance of the even wax esters from C_{18}–C_{50}.

$$\text{Wax ester ACL} = \frac{< 26 \times [C_{26}] \ldots + 28 \times [C_{28}] + 30 \times [C_{30}] + 32 \times [C_{32}] + \ldots 50 \times [C_{50}]}{< [C_{26}] \ldots + [C_{28}] + [C_{30}] + [C_{32}] + \ldots [C_{50}]}$$

(Table II). The C_{max} ranged from 20 to 28 with 20 predominant in the extract of Sitka Spruce and 28 in Mountain Hemlock (all even carbon number homologs). The CPI values of the *n*-alkanols ranged from 1.5 to 10.6 with an average of 5.0 (all >>1.0 reflecting their biochemical origin). Ponderosa Pine wax had the highest CPI of 10.6 while the lowest CPI of 1.5 was found in Apache Pine wax (Table II). There are no apparent trends in the *n*-alkanol ACLs.

Fatty Acids: The *n*-alkanoic acids ranged from C_7 to C_{34} with C_{max} values from 16 to 28 (Table II). The most common C_{max} at 24 was identified among six of the twelve species where *n*-alkanoic acids were present. All alkanoic acids had a strong even carbon numbered predominance, characteristic of their biogenic origin. The CPIs for the *n*-alkanoic acids were high and ranged from 2.3 to 19.3 with an average of 9.9, not including the sample with a CPI of infinity. California Redwood wax displayed the highest CPI while Mountain Hemlock wax had the lowest. Since free *n*-alkanoic acids are relatively minor wax components and intermediary in the production of other wax constituents, concentrations may be influenced significantly by processes occurring in the needles and by degradation of wax esters, which can hydrolyze to alkanoic acids and alkanols (Tulloch, 1976). Thus, information from *n*-alkanoic acid and *n*-alkanol homologs must be viewed cautiously due to the variable processes which produce them.

Free ω-hydroxyalkanoic acids ranging from C_{14} to C_{16} are present in the conifer waxes (Table III). The C_{12}, C_{14} and C_{16} ω-hydroxyalkanoic acids are found in the estolide fraction of cuticular waxes of the *Cupressaceae* and *Pinaceae* (Herbin and Robins, 1968; Herbin and Sharma, 1969). Estolides, as neutral polyesters of 4–6 molecules of C_{12}, C_{14}, C_{16} and C_{18} ω-hydroxyalkanoic acids, have also been described for gymnosperms, which contain ω-hydroxyalkanoic acids in the cutin (Caldicott and Eglinton, 1973; Tulloch, 1976) and in epicuticular waxes (Schulten *et al.*, 1986).

Carbonyl compounds. Homologous carbonyl compounds were identified as *n*-alkan-10-ones in the wax extracts and ranged from C_{17} to C_{31}, with an odd carbon number predominance and C_{max} at 19 and 29 (Table III). The *n*-alkan-10-ones in Brewer Spruce (WNF) and Norway Spruce showed the presence of only C_{29}, while Apache Pine, Montezuma Pine and Sitka Spruce had only C_{19}. Western Juniper displayed a C_{range} of C_{29} to C_{31}, while Douglas Fir (UNF) and White Fir had a C_{range} from C_{17} to C_{29}. The *n*-alkanones were not detected in the other conifer waxes.

Unsaturated aldehydes (double bond location not defined) were found as minor components in some samples with a C_{range} from C_{28} to C_{34} (Table III). Norway Spruce has C_{28} and C_{29} (C_{max} at C_{28}), while in Brewer Spruce (WNF) wax contains C_{30} to C_{34} with a C_{max} at 30. Saturated aldehydes (n-alkanals) were not detected in any of the wax extracts.

Wax esters. Wax esters have been previously reported in conifer cuticular waxes (Tulloch, 1987; Sümmchen *et al.*, 1995). These compounds form crystalline zones in the cuticle that act as transport barriers to diminish the loss of water (Riederer and Schneider, 1990). Here in the epicuticular lipid extracts, the wax esters range mainly from C_{24}-C_{50} (total carbon number of compounds) and have exclusively saturated fatty acid and alcohol moieties (Table III). The major homolog and predominant C_{max} is 38 in five of the samples where wax esters are present. Acid moieties range from C_6 to C_{36} and alcohols from C_6 to C_{32}, with common combinations of acid and alcohol moieties of C_{12} to C_{14}, C_{14} to C_{14} and C_{24}, C_{16} to C_{22} and C_{26}, C_8 to C_{10} and C_{30}, and C_6 to C_{32} predominating. The compositions of the acid and alcohol moieties vary considerably from species to species, thus these compounds may be useful source indicators for plant species in environmental samples.

The averages of the wax ester ACLs of the Coastal Range conifers (38.3, n=3) are lower than that of the Inland conifers (38.8, n=5) (Table III). This difference is especially apparent within species where the coastal conifers Douglas Fir and Brewer Spruce both exhibit significantly lower wax ester ACLs than their inland relations, 37.4 to 39.6 (difference of 2.2) and 35.6 to 37.8 (difference of 2.2), respectively. This observation suggests the presence of a plant or microbial enzymatic mechanism in the cuticle which is specific for the humidity adaptation. The proposed enzymatic reaction mechanism would include the cleavage of long chain alkyl esters into methyl esters and *n*-alkanes with two less carbon atoms. Methyl esters have been previously identified in epicuticular waxes of

conifers (Tulloch, 1987) and the increase in n-alkane concentrations supports the increased *n*-alkane ACL observations.

Molecular markers: Phytosterol (C_{28}, C_{29}) and triterpenoid (C_{30}) molecular markers were detected in 10 of the 15 conifer waxes sampled and the results are given in Table IV. Of the four phytosterols identified, the two most common were brassicasterol (present in 8 samples), followed by campesterol (present in 7). The other phytosterols present were β-sitosterol and stigmasterol. A trace of cholesterol was found in Ponderosa Pine wax and may represent adsorption of smoke particles from meat grilling (campground) near the sampling site (Rogge *et al.*, 1991).

Cyclic terpenoids are produced by higher plants and are useful as chemotaxonomic tracers or molecular markers due to their molecular complexity and structural specificity (Simoneit, 1986; Hemmers and Gülz, 1989a, 1989b). For the triterpenoids, α-amyrin, accompanied by β-amyrin, was encountered most among the conifer waxes (Table IV). Other triterpenoids in the waxes include taraxerone in Big-Cone Douglas Fir, 22-hopanol in Norway Spruce, and ursonic and morolic acids both in Mountain Hemlock wax.

Conclusions

This work reports the lipid and molecular marker components of epicuticular waxes from predominant conifers of western North America. The average chain length (ACL) values determined for both *n*-alkanes and wax ester compositions suggests a humidity adaptation by coastal conifers which is evident by a two carbon number decrease in ACL for these compounds. The mechanism may be of plant or microbial origin and remains to be determined. Because only single composite samples of each vegetation type were taken at different climate locations, future work should include a more systematic analysis of the reproducibility of epicuticular plant wax signatures. However, the gross wax composition data is of utility for assessing direct particle emission signatures from biomass and secondary emission compositions from biomass fuels during burning. The full data set of the wax homolog compositions is available from the corresponding author.

Acknowledgements

This work was funded in part by the U. S. Environmental Protection Agency (Grant R-823990–01).

Table IV. The composition and yield of phytosterol and triterpenoid molecular markers in epicuticular waxes.

Compound			Sample (Region)* and Yield (% normalized to C_{max} of *n*-alkanols)									
Name	M.W.	Composition	BDF	BS(CR)	BS(WNF)	DF(UNF)	MH	NS	PSF	PP	WJ	WF
Phytosterols												
Brassicasterol	398	$C_{28}H_{46}O$	19.3	2.2	29.9	3.1	–	–	2.4	5.9	9.9	1.1
Campesterol	400	$C_{28}H_{48}O$	–	8.8	25.0	1.5	–	–	3.1	0.8	12.5	2.2
Stigmasterol	412	$C_{29}H_{48}O$	8.2	9.3	40.5	–	–	–	–	–	–	–
β-Sitosterol	414	$C_{29}H_{50}O$	–	–	–	–	–	1.0	1.4	–	10.0	0.2
Triterpenoids												
Taraxerone	424	$C_{30}H_{48}O$	4.9	–	–	–	–	–	–	–	–	–
α-Amyrin	426	$C_{30}H_{50}O$	–	–	–	–	10.4	0.5	–	–	–	0.2
β-Amyrin	426	$C_{30}H_{50}O$	–	0.1	–	–	9.1	0.2	–	–	–	0.04
22-Hopanol	428	$C_{30}H_{52}O$	–	–	–	–	–	1.7	–	–	–	–
Ursonic acid	454	$C_{30}H_{46}O_3$	–	–	–	–	1.4	–	–	–	–	–
Morolic acid	456	$C_{30}H_{48}O_3$	–	–	–	–	0.2	–	–	–	–	–

*Sample and locale codes as in Table I (accuracy of data ± 10% of each value).

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