Composition of the Epicuticular Wax Esters of *Picea abies* (L.) Karst.

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The long-chain alkyl esters homologues of the cuticular needle wax of *Picea abies* consist of a series of isomers, which are formed by different *n*-fatty acids and *n*-alkanols of various chain lengths. Even-numbered esters are dominating. Methyl esters range from C_{27} to C_{37}, even-numbered esters are only minor components. Furthermore, a new class of homologue wax esters was isolated. Examination by gas chromatography, mass spectroscopy and chemical studies resulted in identification of pentenyl esters of triacontanoic, dotriacontanoic, tetratriacontanoic and hexatriacontanoic acid.

Introduction

Long-chain alkyl esters are components of the epicuticular waxes of conifer species, occur however, in most cases, in small quantities only. Two different types of esters, methyl ester and alkyl ester of fatty acids, have been mentioned so far in the literature. Investigations on *Abies balsamea* and *Picea glauca* (Tulloch, 1987) yielded a chain length distribution from 23 to 35 carbon atoms for methyl esters, while for straight chain primary alkyl esters the chain lengths ranged from 30 to nearly 60 carbon atoms (Franich *et al.,* 1985; Tulloch and Bergter, 1981; Günthardt-Goerg, 1986; Riederer, 1989; Tulloch, 1976). Especially for *Picea abies,* there exist only very few findings concerning the composition of these substance classes.

The physiological function of long-chained, lipophilic compounds in the cuticle is believed to be a transport barrier in order to diminish the loss of water by formation of crystalline zones within the cuticle (Reynhardt and Riederer, 1991; Riederer and Schneider, 1990).

A typical feature of the long-chain alkyl esters is a great variety of isomers within a specific single chain length (Franich *et al.,* 1985; Gülz *et al.,* 1994; Holloway, 1984). In this paper, the qualitative composition of the esters of the wax from *Picea abies* as well as the identification of their isomers mainly by gas chromatography/mass spectroscopy is described.

Materials and Methods

Cuticular waxes of needles (28.9 g fresh weight) of *Picea abies* from a research field near Tharandt, Germany, were extracted using $CHCl_3$. After TLC on silica gel with 1,1,1-trichloroethane, the ester bands (R_f 0.9, 0.8 and 0.7, respectively) were scrapped off and reextracted in an excess of $CHCl_3$. Aliquots of the concentrated solutions were injected in a GC/MS system equipped with an on-column injector. A DB-1 WCOT (25 m×0.32 mm i.d.×0.1 µm i.th.) was used for separation. The oven was operated at 50 °C injection temperature for 2 min, rate A 40°/min to 200 °C, rate B 10°/min to 300 °C for 40 min. Carrier gas was helium at a pressure of 10 kPa.

Ester hydrolysis: After TLC, the ester compounds were hydrolyzed by adding of MeOH/HCl (Sigma) and heating (70 °C).

GC-MS: 70 eV, *m/z* (rel. int.): Triacontanoic acid pentenyl ester: 520 $[M]^+$ (0.3), 453 $[RCOOH_2]^+$ (0.1), 141 [McLafferty product + 13 mass units] (15), 128 $[R'-O-C(OH)=CH_2^+$, McLafferty product] (16), 68 $[R'-1]^+$ (100); dotriacontanoic acid pentenyl ester: 548 $[M]^+$ (0.6), 481 $[RCOOH_2]^+$ (0.2), 141 (19), 128 (22), 68 (100); tetratriacontanoic acid pentenyl ester: 576 $[M]^+$ (0.5), 509 $[RCOOH_2]^+$ (0.1), 141 (20), 128 (22), 68 (100); hexatriacontanoic acid pentenyl ester: 604

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Table I. Composition of long chain alkyl esters of the cuticular wax of *Picea abies*.

Ester chain length	$[M]^+$	Relative amount of ester in %[a]	Acid chain length	Acid fragment[b]	Relative amount of isomer in %[c]	Alkanol chain length	Alkanol fragment[d]
34	508	2.2	12	201	4.6	22	308
			14	229	13.9	20	n.d.
			16	257	57.1	18	252
			18	285	20.6	16	224
			24	369	3.8	10	140
36	536	4.7	12	201	18.0	24	n.d.
			14	229	14.1	22	n.d.
			16	257	5.3	20	280
			18	285	47.8	18	252
			20	313	14.9	16	224
			28	425	traces	8	112
38	564	19.9	12	201	3.0	26	n.d.
			14	229	4.2	24	336
			16	257	7.6	22	308
			18	285	2.5	20	280
			20	313	12.1	18	252
			22	341	1.9	16	224
			24	369	0.6	14	196
			28	425	traces	10	140
			30	453	68.1	8	112
40	592	29.7	12	201	6.3	28	n.d.
			14	229	1.7	26	n.d.
			16	257	3.6	24	336
			18	285	1.4	22	308
			20	313	1.8	20	280
			22	341	1.4	18	252
			24	369	0.8	16	224
			30	453	12.8	10	140
			32	481	70.2	8	112
41	606	5.0	12	201	12.3	29	n.d.
			22	341	5.4	19	n.d.
			30	453	48.1	11	154
			32	481	34.1	9	126
42	620	21.4	12	201	18.8	30	n.d.
			14	229	2.9	28	n.d.
			16	257	5.6	26	n.d.
			18	285	2.8	24	336
			20	313	2.8	22	308
			22	341	1.4	20	280
			24	369	1.2	18	252
			32	481	18.6	10	140
			34	509	45.8	8	112
43	634	4.0	20	313	traces	23	n.d.
			22	341	4.8	21	n.d.
			24	369	2.6	19	n.d.
			32	481	92.5	11	154
44	648	10.7	12	201	26.4	32	n.d.
			14	229	4.7	30	n.d.
			16	257	traces	28	n.d.
			18	285	37.8	26	n.d.
			20	313	1.4	24	n.d.
			22	341	1.8	22	n.d.
			24	369	2.3	20	n.d.
			34	509	17.6	10	140
			36	537	7.9	8	112
46	676	2.5	12	201	41.0	34	n.d.
	n.d.		14	229	11.7	32	n.d.
			16	257	traces	30	n.d.
			18	285	traces	28	n.d.
			20	313	traces	26	n.d.
			22	341	3.8	24	n.d.
			24	369	25.8	22	n.d.
			36	537	17.6	10	140
			38	565	traces	8	112

[a] Relative amounts of the individual homologues to total esters obtained by integration of TIC (total ion chromatogram).

[b] *m/z* of fragment $[RCO_2H_2]^+$ (acid moiety).

[c] Relative amounts of the individual isomers to total of the respective homologue.

[d] *m/z* of fragment $[R'-1]^+$ (alcohol moiety).

n.d., not detected due to too low intensity.

$[M]^+$ (0.2), 141 (18), 128 (18), 68 (100). Triacontanoic acid methyl ester: 466 $[M]^+$ (3), 143 (29), 87 (87), 74 (100); dotriacontanoic acid methyl ester: 494 $[M]^+$ (7), 143 (37), 87 (88), 74 (100); tetratriacontanoic acid methyl ester: 522 $[M]^+$ (5), 143 (35), 87 (85), 74 (100) and hexatriacontanoic acid methyl ester: 550 $[M]^+$ (4), 143 (32), 87 (88), 74 (100).

Results and Discussion

After TLC separation, two bands which fit in with the respective standards of methyl and alkyl esters have been observed. The least polar fraction (R_f 0.9) consists of long-chain alkyl esters. The chain lengths of these esters have been determined by their $[M]^+$ (Table I).

A semiquantitative estimation has been carried out by integrating the ester peaks in the total ion chromatogram (TIC). Dominant chain lengths are 38, 40 and 42. Esters formed by an odd-numbered carbon skeleton have been found in minor quantities, with the alcohol moiety odd-numbered. The composition of the isomers of each homologue has been detected by use of the typical fragments of ester cleavage $[RCO_2H_2]^+$ (acid moiety) and $[M]^+$. Due to their low intensity, especially the fragment $[R'-1]^+$ (alcohol moiety) of the higher alcohols has been difficult to detect (Table I). The homologues consist of alcohols and acids with a wide range of chain lengths. The shorter esters are formed mainly quite symmetrically, *i.e.* the differences in chain lengths are relatively small when comparing acids with alcohols. With increasing ester lengths, molecules with short acids and long alcohols dominate and *vice versa*. Similar results exist for *Pinus radiata* (Franich *et al.,* 1985). An approximate estimation of relative amounts of isomers to the total of a homologue has been made by integration of the respective $[RCO_2H_2]^+$ ion, because the method according to Aasen may not be applicable straight away and can be modified this way (Franich *et al.,* 1985; Bianchi *et al.,* 1989). The results are given in Table I and confirm the already mentioned distribution of isomers. Octyl esters have been proven to dominate in quantity especially with increasing chain length of the fatty acids up to a chain length of 42. Similar statements were made for alkyl esters of *Pinus radiata* (Franich *et al.,* 1985) and *Abies alba* (Güth and Frenzel, 1989). Generally, for each chain length a single dominant isomer may be observed, which represents about 50% or more of the total sum of all isomers.

The most polar fraction of esters after TLC separation (R_f 0.7) consists of methyl esters of fatty acids. The range of the homologues is shown in Table II. Identification has been possible by $[M]^+$ and the typical fragments *m/z* 74, 87 and 143. Integration of *m/z* 74 and 143 shows the dominant role of the chain lengths of 31, 33 and 35, while the even-numbered esters occurring in small quantities only.

Besides the already well-known methyl and alkyl esters (Tulloch, 1987; Franich *et al.,* 1985; Tulloch and Bergter, 1981; Günthardt-Goerg, 1986; Güth and Frenzel, 1989), four new compounds could be observed in the ester fraction of *Picea abies* cuticular wax. The R_f value after TLC was 0.8. Analysis by GC/MS showed *m/z* 520, 548, 576 and 604, respectively, as $[M]^+$. Ester cleavage gives ions $[RCOOH_2]^+$ (Tulloch, 1987; Gülz *et al.,* 1994) for the acid moiety of the ester, the respective *m/z* 453, 481, 509 and 537, indicating triacontanoic, dotriacontanoic, tetratriacontanoic and hexatriacontanoic acid, were found in the mass spectra. Hydrolysis of the esters confirmed the presence of the mentioned fatty acids. Base peak of all spectra was *m/z* 68, which is assumed to be the mass of $[R'-1]^+$, the alcohol moiety of the ester molecule. This mass fragment goes with a sum formula of $[C_5H_8]^+$ indicating a monounsatu-

Table II. Qualitative and semiquantitative composition of cuticular wax methyl ester fraction of *Picea abies.*

Chain length of methyl esters	$[M]^+$	Relative amount to total esters in %[a]
27	410	0.73
28	424	traces
29	438	1.79
30	452	traces
31	466	29.62
32	480	2.04
33	494	37.59
34	508	1.47
35	522	24.77
36	536	0.33
37	550	1.64

[a] Obtained by integration of typical ester fragments *m/z* 74 and 143, mean value.

rated pentenol as alcohol component as well as the fragment *m/z* 128 originating from the McLafferty rearrangement and *m/z* 141, an often obtained fragment also resulting from the McLafferty reaction (Spiteller, 1966). Regarding the presence of isopenten or isopentenyl pyrophosphate in conifer needles (Gershenzon and Croteau, 1993), the possibility of the ester formation by *n*-fatty acids and isopentenol seems likely. It is remarkable, that in contrast to the methyl esters, which occur in a chain length range from C_{27} to C_{37}, only four fatty acids participate in the formation of this kind of esters.

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