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EPICUTICULAR WAXES OF *ABIES BALSAMEA* AND *PICEA GLAUCA*: OCCURRENCE OF LONG-CHAIN METHYL ESTERS

ALEXANDER P. TULLOCH

Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, Canada S7N OW9

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Key Word Index—*Abies balsamea*; Picea glauca; Pinaceae; epicuticular wax composition; hexyl triacontanoate; methyl triacontanoate; triacontanol; nonacosan-10-ol; nonacosane-5,10-diol.

Abstract—Epicuticular wax from *Abies balsamea* contains methyl esters (7 %), hexyl and octyl esters (9 %), nonacosan-10-ol (14 %), free acids (20 %), diterpene acids (3 %) and free acids and diols (2 %) and wax from *Picea glauca* has methyl esters (6 %), hexyl and octyl esters (5 %), aldehydes (3 %), nonacosan-10-ol (30 %), free acids (3 %), diterpene acids (10 %) and free alcohols and diols (2 %). The major methyl ester is methyl triacontanoate; the long-chain esters are C_{30}–C_{44} hexyl and octyl esters of C_{24}–C_{34} acids; the major aldehyde is triacontanal. Major free acids and alcohols are C_{16}–C_{32} and the principal diol is nonacosane-5,10-diol.

INTRODUCTION

Larvae of the eastern spruce budworm, *Choristoneura fumiferana*, a serious forest pest in eastern Canada, prefer to feed on newly developed needles of balsam fir, *Abies balsamea* (L.) Mill., and white spruce, *Picea glauca* (Moench) Voss. It has been proposed that components of the needle surface are responsible for this feeding preference [1]. Epicuticular waxes of these two conifer species have now been analysed to determine whether wax components are responsible for feeding preferences.

RESULTS AND DISCUSSION

The amount of wax on the needles is slightly greater than that previously reported for other conifers, 0.5–0.7 % of dry weight compared to 0.2–0.4 % [2–4]. The compositions of the waxes are shown in Table 1. Hydrocarbon amounts were too small for estimation and identification. Two types of ester are present, methyl esters of long-chain acids and long-chain esters of C_6 and C_8 alcohols.

Table 2 shows the compositions of the long-chain esters. Balsam fir esters range from C_{30} to C_{44} and white spruce esters from C_{36} to C_{42}. The C_{36} ester and C_{38} ester are the major esters of balsam fir and C_{40} and C_{38} are those of white spruce. Structures of these esters were clearly shown by GC/MS. An ester RCO_2R^1 gives ions $[R^1-1]^+$ and $[CO_2R^1]^+$ indicating the alcohol chain length and $[RCO_2H_2]^+$ showing the acid chain length [5]. Thus the C_{30} ester has ions at m/z 84, $[R^1-1]^+$, and 129, $[CO_2R^1]^+$, showing that the alcohol component is hexanol and an ion at m/z 453, $[RCO_2H_2]^+$, showing that the acid component is triacontanoic acid. The C_{40} ester from white spruce has corresponding ions at m/z 112, 157 and 481 showing that it is octyl dotricontanoate.

The mass spectra of most of the esters from balsam fir wax also have an ion at m/z 145, $[RCO_2H_2]^+$, suggesting

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Table 1. Composition of epicuticular waxes from *Abies balsamea* and *Picea glauca*

	Abies balsamea	*Picea glauca*
Methyl esters	7	6
Long-chain esters	9	5
Aldehydes	—	3
Nonacosan-10-ol	14	30
Free acids	20	3
Diterpene acids	3	10
Free alcohols	1	1
Diols	1	1
Unidentified	45	41
Yield (% dry wt)	0.7	0.5

Table 2. Composition of long-chain esters from waxes of *Abies balsamea* and *Picea glauca*

	Abies balsamea		*Picea glauca*	
Ester carbon no.	Ester (%)	Chain lengths of component acids and alcohols	Ester (%)	Chain lengths of component acids and alcohols
30	4	24, 6	—	—
32	5	26, 6	—	—
34	9	28, 6	—	—
36	27	30, 6	13	30, 6
38	22	32, 6	28	32, 6 + 30, 8 (17:11)
40	17	34, 6	39	32, 8
42	11	36, 6 + 34, 8 (1:1)	20	34, 8
44	5	34, 10	—	—

the presence of esters of octanoic acid. The mass spectrum of the C_{38} ester from white spruce contained in addition an ion at *m/z* 173 indicating the presence of esters of decanoic acid as well, the corresponding $[R^1-1]^+$ ions were not observed, however *cf.* [6]. It was not possible to estimate the amounts of these esters since $[RCO_2H_2]^+$ ions are generally much more intense than $[R^1-1]^+$ ions and also more intense at low *m/z* values.

These types of esters have been observed in waxes from other conifers. Wax from *Juniperus scopulorum* contained octyl and decyl esters of long-chain acids [4] and wax from *Pinus radiata* contained C_6–C_{10} alcohol esters of long chain acids and in addition long-chain alcohol esters of C_6–C_{12} acids [6]. The compositions are, however, relatively simple compared with those from angiosperm waxes in which a greater range of long-chain alcohols is present, often randomly esterified [7].

Rather unexpectedly long-chain methyl esters were also identified in the ester fraction and comprised 6–7% of the waxes. The structures were determined by GC/MS all relevant ions being observed. Their composition is shown in Table 3. In both waxes methyl triacontanoate is the major component followed by methyl dotriacontanoate. Methyl esters have not, apparently, been previously observed as components of epicuticular waxes of higher plants except in the case of waxes from some eragrostoid grasses in which methyl esters formed just 0.25% of the ester fraction [8]. These findings suggest that methyl esters may be present in wax of other plants, perhaps particularly in those of conifers but have been overlooked so far because only small amounts were present.

Long-chain aldehydes, identified by GC/MS, were also present in the ester fraction of white spruce wax but none were observed in wax from balsam fir. The chain length distribution is similar to that of the methyl esters in that C_{30} is the major component and C_{24} prominent but C_{26} and C_{28} are larger in the aldehydes and C_{32} is much smaller. Nonacosan-10-ol is an important component of both waxes, it has been found in waxes from many conifer species and also in waxes from some dicotyledonous families [4, 7].

Long-chain free acids are also present, particularly in wax from balsam fir where they are major components, the chain lengths are shown in Table 4. Compositions are not very similar for the two waxes, though C_{24} is the major component in each, C_{26}–C_{30} acids are much more prominent in wax from white spruce. For white spruce wax composition of the free acids resembles that of the aldehydes as C_{24}–C_{30} components are fairly similar. A resemblance between the compositions of free acids and aldehydes was noted previously [8]. Diterpene acids are present in moderate amounts, dehydroabietic acid is the major component accompanied by small amounts of abietic and isopimaric acids. These acids were also observed in wax from *P. radiata* [2].

Free alcohols (Table 4) and diols are present in both waxes in very small amounts. Compositions of free alcohols and free acids differ, except that a C_{24} component is prominent in each. Generally it can be concluded that combined acids, as long-chain or methyl esters, are similar but that free acids, alcohols and aldehydes are appreciably different in composition. The principal diol is nonacosane-5,10-diol along with lesser amounts of the 4,10-diol and possibly other diols. These diols have previously been reported in wax from *P. radiata* [2] and in waxes from a number of *Juniperus* species [4].

Both waxes contained over 40% of unidentified material but it is likely that much of this is assorted estolides which are very common in waxes from conifers [2–4]. The composition of these fractions was not investigated.

The results of examination of wax fractions as spruce bud worm antifeedants will be published elsewhere. The composition of the long-chain esters suggests one possibility. Since combined alcohols of esters are generally at least partly similar to free alcohols, free hexanol and octanol may have been present in the original wax on the needles. Such short-chain alcohols could well have been lost during solvent evaporation. If such free alcohols exist in the wax they could act as antifeedants in proportion to the quantity present.

Table 3. Composition of methyl esters and aldehydes from waxes of *Abies balsamea* and *Picea glauca*

Chain length	Methyl esters *Abies balsamea*	Methyl esters *Picea glauca*	Aldehydes *Picea glauca*
22	5	5	3
24	10	19	19
26	1	7	17
28	2	6	18
30	40	30	30
32	35	23	9
34	7	10	4

Table 4. Composition of free acids and alcohols from waxes of *Abies balsamea* and *Picea glauca*

Chain length	Free acids *Abies balsamea*	Free acids *Picea glauca*	Free alcohols *Abies balsamea*	Free alcohols *Picea glauca*
16	3	10	—	—
18	6	10	28	—
20	8	—	—	—
22	14	5	11	22
24	55	22	27	29
26	8	16	3	34
28	3	15	21	15
30	3	19	10	—
32	—	3	—	—

EXPERIMENTAL

Wax was extracted from recently developed needles by Dr. P. J. Albert, Department of Biology, Concordia University, Montreal, with redistilled hexane. TLC was carried out as previously described [4]. Wax was chromatographed on silica gel and fractions eluted with hexane containing increasing proportions of Et_2O. The ester fraction was eluted with hexane–Et_2O (99:1), nonacosan-10-ol with hexane–Et_2O (19:1), free acids with hexane–Et_2O (10:1), free alcohols and unidentified material with hexane–Et_2O (3:1) and diols and unidentified material with hexane–Et_2O–EtOH (5:4:1). Fractions were analysed by GC/MS after trimethylsilylation, or diazomethane treatment (free acids and diterpene acids), using a 15 m × 0.25 mm column coated with DB-1, linear velocity of He was 70 cm/sec, samples were injected at 40°, the temp. immediately raised to 125° and programmed at 4°/min to 300°.

GC/MS analysis of the long-chain ester fraction from balsam fir showed [70 eV, *m/z* (rel. int.)] C_{30}: 452 [M]$^+$ (6), 369 (17), 145 (9), 129 (5), 84 (61); C_{32}: 480 [M]$^+$ (5), 397 (11), 145 (8), 129 (9), 84 (51); C_{34}: 508 [M]$^+$ (6), 425 (12), 145 (10), 129 (10), 84 (50); C_{36}: 536 [M]$^+$ (5), 453 (8), 145 (9), 129 (11), 84 (46); C_{38}: 564 [M]$^+$ (6), 481 (8), 145 (12), 129 (11), 84 (46); C_{40}: 592 [M]$^+$ (6), 509 (8), 145 (12), 129 (10), 84 (42); C_{42}: 620 [M]$^+$ (16), 537 (3), 509 (7), 173 (7), 157 (5), 145 (4), 129 (9), 112 (23), 84 (28); C_{44}: 648 [M]$^+$ (7), 140 (21). Analysis of the ester fraction from white spruce showed long-chain esters C_{36}: 536 [M]$^+$ (6), 453 (10), 145 (10), 129 (11), 84 (47); C_{38}: 564 [M]$^+$ (7), 481 (6), 453 (8), 173 (6), 157 (6), 145 (7), 129 (11), 112 (25), 84 (37); C_{40}: 592 [M]$^+$ (8), 481 (10), 173 (7), 157 (4), 112 (27); C_{42}: 620 [M]$^+$ (6), 509 (9), 173 (8) 157 (4), 112 (26).

Methyl ester components of both waxes had the expected MS; thus methyl triacontanoate had the MS [70 eV, *m/z* (rel. int)]: 466 [M]$^+$ (13), 423 (3), 367 (2), 311 (1), 255 (1), 199 (4), 143 (20), 129 (7), 87 (76), 74 (100). Aldehydes in the ester fraction of white spruce also had the expected MS, that of triacontanal was: 436 [M]$^+$ (0.3), 418 [M $-H_2O$]$^+$ (5), 96 (48), 82 (71).

Nonacosan-10-ol, as the TMSi ether, had MS very similar to that reported previously [4]. Free acids were identified by MS of their methyl esters and free alcohols by that of their TMSi ethers. Diterpene acids were identified by comparison of the MS of their methyl esters with those previously reported [9]. Nonacosane-4,10-diol, and other diols had MS, as TMSi ethers, very similar to those reported earlier [4].

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