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# ESTERS OF *PICEA ABIES* NEEDLE CUTICULAR WAX

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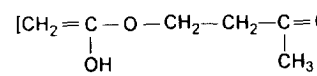
**Key Word Index**—*Picea abies*; Pinaceae; cuticular wax; wax esters; fatty acid *iso*pentenyl esters.

**Abstract**—A new class of wax ester has been isolated from the needle cuticular wax of *Picea abies*. Identification of 2-methyl-but-1-en-4-yl triacontanoate, -dotriacontanoate, -tetratriacontanoate and -hexatriacontanoate was carried out by gas chromatography, mass spectroscopy, $^1$H NMR and chemcial studies.

## INTRODUCTION

Wax esters are common constituents of cuticular waxes of higher plants [1, 2]. Conifers, however, usually have only minor quantities of these compounds and up to now methyl esters and various alkyl esters of long chain *n*-fatty acids and *n*-alcohols with numerous homologues have been reported [3–7]. The work presented herein describes the identification of a new class of wax esters in *Picea abies* formed by *n*-fatty acids and *iso*pentenyl alcohol. Although the literature was thoroughly checked, no reports concerning the occurrence of such compounds were found.



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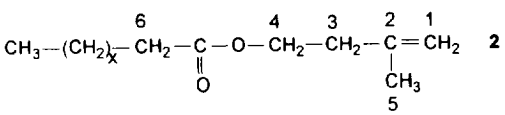

2

x = 27, 29, 31, 33

## RESULTS AND DISCUSSION

Besides the already well known methyl and alkyl esters [3–7], four new compounds could be observed in the ester fraction of *P. abies* cuticular wax. Analysis by GC-MS showed ions of *m/z* 520, 548, 576 and 604, respectively, as $[M]^+$. Ester cleavage gave ions $[RCOOH_2]^+$ [4, 8] for the acid moiety of the ester, the respective ions at *m/z* 453, 481, 509 and 537, indicating the presence of triacontanoic, dotriacontanoic, tetratriacontanoic and hexatriacontanoic acids. Hydrolysis of the esters confirmed the presence of the above mentioned fatty acids. The 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 monounsaturated pentenol as the alcohol component, as well as the fragment *m/z* 128 originating from McLafferty rearrangement (**1**); *m/z* 141 is **1** plus 13 mu, an often obtained fragment also resulting from the McLafferty reaction [9]. Therefore, ester synthesis were carried out with triacontanoic acid and several pentenols. The resulting ester with 2-methyl-but-1-en-4-ol gave the same $R_f$ (TLC) and retention time (GC), as well as an identical mass spectrum compared with the triacontanoic acid ester, so that the structure of the esters could be described according to formula **2**. These results were confirmed by $^1$H NMR spectroscopy. Both the standard substance and the isolated esters gave the same spectra with typical signals at $\delta$4.76 (*d*) indicating a terminal $CH_2$ group and $\delta$1.75 (*s*) for a methyl group adjacent to the double bond. Regarding the occurrence of isopentenyl pyrophosphate (an intermediate in terpene biosynthesis) in conifer needles [10], the possibility of the ester formation between *n*-fatty acids and isopentenyl alcohol seems likely.

Investigations into the cuticular waxes of *Pinus radiata* [3], *Abies balsamea*, *Picea glauca* [4] and *Abies alba* [6] showed, that the composition of the alkyl and methyl ester fractions are very similar regarding the fatty acid moieties of the esters. Despite the involvement of many fatty acid homologues with a broad range of chain lengths, their alkyl and methyl esters are mainly formed by fatty acids with chain lengths of $C_{30}$, $C_{32}$, $C_{34}$ and, to a lesser extent, $C_{36}$. The same chain lengths, regarding the acid moiety, have been found in the new ester fraction described in this paper. The dominance of $C_{30}$–$C_{36}$ chain lengths of the acid moiety seems to be a common feature

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of wax esters of conifers. For these reasons, it is very likely that the isopentenyl esters are part of the cuticular wax of the spruce needles and, therefore, do not originate from the internal tissues of the needles as an artifact of the wax extraction method.

## EXPERIMENTAL

*Plant material.* First and second age-class needles of 6-year-old trees *P. abies* (L.) Karsten were collected at Tharandt, Germany, in June 1993.

*Extraction and purification of components.* Twigs were dipped in liquid $N_2$ and the needles (29 g fr. wt) removed by stripping them off. Cuticular waxes (0.5% fr. wt) were extracted using $CHCl_3$ for 5 min at room temp. The concd solns were applied to 0.25 mm thick TLC silica plates (*ca* 20 mg wax to each) provided with a conc zone and separation was carried out using 1,1,1-trichloroethane. Substances were detected after spraying with primuline in $Me_2CO$–$H_2O$ (4:1) under UV light. The respective ester band with a $R_f$ of 0.7 was scrapped off and substances were recovered by 1 hr extraction in $CHCl_3$ at room temp. The remaining ester fr. (210 $\mu$g) was used for GC and GC-MS investigations and ester hydrolysis. For sample prepn for subsequent $^1$H NMR studies, 530 g needles (fr. wt) were harvested and extracted as described above. The crude wax (2.8 g) was chromatographed on silica gel (20 g) and the ester frs eluted with 1,1,1-trichloroethane. After reconcn, the *iso*pentenyl esters (3.7 mg) were obtained by TLC as described above. Purity was checked by GC. The esters were dissolved in $CDCl_3$ and used for NMR.

*GC.* GC was performed by using a WCOT HP-1 (12 m; 0.32 mm; 0.5 $\mu$m). Injection was done on-column, detection by FID (320°). Oven prog. started at 55° for 2 min, rate A 40° min$^{-1}$ to 200°, rate B 3° min$^{-1}$ to 300° and 300° for 20 min. Inlet pres. was 40 kPa, carrier gas $H_2$.

*GC-MS.* This used a WCOT HP-1 (25 m; 0.32 mm; 0.1 $\mu$m). Injection was done on-column. Oven prog. started at 60° for 2 min, rate A 40° min$^{-1}$, rate B 3° min$^{-1}$ to 300° and 300° for 10 min. Carrier gas was He, inlet pres. 10 kPa. A benchtop instrument was used for MS in the EI made at 70 eV.

*2-Methyl-but-1-en-4-yl triacontanoate m/z* (rel. int.). 520 $[M]^+$ (0.3), 453 $[RCOOH_2]^+$ (0.1), 141 [McLafferty product + *m/z* 13] (15), 128 $[R'\text{–}O\text{–}C(OH)=CH_2^+$, McLafferty product] (16), 68 $[R'-1]^+$ (100). *2-Methyl-but-1-en-4-yl dotriacontanoate m/z* (rel. int.): 548 $[M]^+$ (0.6), 481 $[RCOOH_2]^+$ (0.2), 141 (19), 128 (22), 68 (100). *2-Methyl-but-1-en-4-yl tetratriacontanoate m/z* (rel. int.).576 $[M]^+$ (0.5), 509 $[RCOOH_2]^+$ (0.1), 141 (20), 128 (22), 68 (100). *2-Methyl-but-1-en-4-yl hexatriacontanoate m/z* (rel. int.): 604 $[M]^+$ (0.2), 141 (18), 128 (18), 68 (100).

*Synthesis of 2-methyl-but-1-en-4-yl triacontanoate.* Triacontanoic acid (10 mg), 100 $\mu$l 2-methyl-but-1-en-4-ol and a drop of conc. $H_2SO_4$ were heated (70°) in a Teflon-sealed vial for 4 hr. After addition of $H_2O$, the ester was extracted using $Et_2O$. Purification was achieved by TLC.

*Ester hydrolysis.* After TLC, ester compounds were hydrolysed by adding MeOH–HCl and heating (70°).

*$^1$H NMR.* (300 MHz, $CDCl_3$, int. standard TMS): 2-methyl-but-1-en-4-yl triacontanoate (standard) and extracted ester fr. $\delta$4.76 (2H, *d*, C-1), 4.18 (2H, *t*, C-4), 2.33 (2H, *t*, C-3), 2.28 (2H, *d*, C-6), 1.75 (3H, *s*, C-5), see formula **2**.

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## REFERENCES

1. Holloway, P. J. (1984) in *CRC Handbook of Chromatography. Lipids* (Vol. 1) (Mangold, H. K., Zweig, G. and Sherma, J., eds), p. 347. CRC Press, Boca Raton.
2. Tulloch, A. P. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.), p. 235. Elsevier, Amsterdam.
3. Franich, R. A., Goodin, S. J. and Volkman, J. K. (1985) *Phytochemistry* **24**, 2949.
4. Tulloch, A. P. (1987) *Phytochemistry* **26**, 1041.
5. Tulloch, A. P. and Bergter, L. (1981) *Phytochemistry* **20**, 2711.
6. Güth, S. and Frenzel, B. (1989) *Angew. Botanik* **63**, 259.
7. Günthardt-Goerg, M. S. (1986) *Eur. J. For. Path.* **16**, 400.
8. Gülz, P.-G., Markstädter, C. and Riederer, M. (1994) *Phytochemistry* **35**, 79.
9. Spiteller, G. (1966) *Massenspektrometrische Strukturanalyse organischer Verbindungen.* Akad. Verlagsgesellschaft Geest u. Portig, Leipzig.
10. Gershenzon, J. and Croteau, R. B. (1993) in *Lipid Metabolism in Plants* (Moore, T. S., ed.), p. 339. CRC Press, Boca Raton.