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n-ALKANES AND ω-HYDROXYALKANOIC ACIDS FROM THE NEEDLES OF TWENTY-EIGHT *PICEA* SPECIES

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Key Word Index—*Picea*; Pinaceae; estolide waxes; chemotaxonomy; *n*-alkanes; w-hydroxyalkanoic acids; GC–MS.

Abstract—The needle wax of twenty-eight species of *Picea* has been investigated. The quantitative patterns of the *n*-alkanes and ω-hydroxyalkanoic acids isolated from these waxes support the view that the genus should not be divided into sections.

INTRODUCTION

The epicuticular wax of conifer needles contains normal monomeric wax esters [1], in addition to the characteristic polymeric esters, the estolides [2], formed from a series of ω-hydroxyalkanoic acids either by self-esterification or by esterification with long-chain diols [3]. The quantitative variation in the individual ω-hydroxy acids from these estolides have been successfully used as taxonomic discriminants in the genus *Pinus* (Pinaceae) and in the Cupressaceae [4]. The composition of the wax from *Picea* (Pinaceae) has been investigated to a limited extent [5–7] but no previous attempt has been made to use the wax components as aids in the taxonomy of this genus.

RESULTS AND DISCUSSION

In the present study, the composition of the wax from needles of twenty-eight species of *Picea* was investigated. Each species yielded two types of wax: non-estolide wax which was extracted with *n*-hexane, and the chloroform-soluble estolide wax. The estolide wax was saponified to yield the individual ω-hydroxyalkanoic acids. These were identified by GC–MS of their acetoxymethyl esters. The results of the quantitative analysis of the ω-hydroxy acids and of the *n*-alkanes are given in Tables 1 and 2 respectively. The species are listed in order of their most abundant homologue. The ω-hydroxy acid patterns closely resemble those obtained for the Cupressaceae and Pinaceae [4]. The proportion of even-numbered alkanes is high and there is no obvious dominant homologue. A similar situation was noted with *Pinus* species, whereas in *Cupressus* species the C_{33} alkane was strikingly dominant.

It was not possible to ensure that the composition of the wax from each of the twenty-eight species did not represent an extreme of intraspecific variation. However, a pilot study using two trees from each of three species showed little variation in the percentage ω-hydroxyalkanoic acids: *Picea bicolor* $C_{12} = 22\%$; $C_{14} = 29\%$; $C_{16} = 46\%$. *P. koyami*, $C_{12} = 23\%$; $C_{14} = 34\%$; $C_{16} = 43\%$. *P. likiangensis* $C_{12} = 67\%$; $C_{14} = 16\%$; $C_{16} = 17\%$. A variation of % hydroxy acids from the geographical standpoint is negligible. For example the values for *P. pungens* (Table 1) compare favourably with values reported for the species from Canada [6] and Kew [4].

The published taxonomic treatments of *Picea* were subjected to a process of evaluation by comparing them with both the ω-hydroxy acid patterns (Table 1) and the *n*-alkane composition (Table 2). Some authorities divide the genus into two [8–12] and others into three sections [13–17]. Wright [18] studied morphological, distributional and genetic data for *Picea* and could find no support for such sectional divisions; the present results agree with this conclusion. Our data however support the view of Wright [18] that *P. obovata* is intermediate between *P. abies* and *P. asperata* and also that that taxa referred to as *P. asperata*, *P. gemmata* and *P. meyeri* all belong to the same species complex [14]. In addition, the fact that *P. balfouriana* and *P. likiangensis* have similar hydroxy acid composition agrees with morphological evidence indicating that these two taxa are either varieties [16] of the same species or two very closely related species [18].

EXPERIMENTAL

Needles were collected in early May from named collections in the National Botanic Gardens, Dublin. Needles were collected from three levels (30 cm, 1 m and 2 m) and from four different aspects of each tree and pooled. Needles from the current years growth were rejected.

Extraction of needle wax. The air-dried needles (40–100 g) for each species were dipped in *n*-hexane (400 ml) for 1 min, dried in a current of air, and then dipped in $CHCl_3$ (400 ml) for 1 min. The *n*-hexane and $CHCl_3$ extracts were evaporated *in vacuo*.

Analysis of n-*alkanes*. *n*-Alkanes were obtained from the *n*-hexane extract by prep TLC (Si gel/*n*-hexane). The individual alkanes from each species were identified by GLC (3% SE-30 on chromosorb G. AW. DMCS. N_2 at 25 ml/min. temp. 230°) by comparison of their retention data with those of standard

Table 1. Mole percentage of ω-hydroxyalkanoic acids in the needle wax of *Picea* species

Picea species	Classification of Pilger [10]	Classification of Harrison [16]	ω-Hydroxyalkanoic acids C_{12}	C_{14}	C_{15}	C_{16}
C_{12} Dominant	*Section*					
P. balfouriana Rehder & Wilson	Eupicea	—	54	11	5	28
P. likiangensis (Franch.) Pritzel	Eupicea	Casicta	57	17	2	24
P. morrisonicola Hay.	Eupicea	Eupicea	36	19	10	34
P. yezoensis (Sieb et Zucc.) Carr.	Omorica	Casicta	35	27	13	25
P. schrenkiana Fisch. & Meyer	Eupicea	Eupicea	39	24	17	20
C_{14} Dominant						
P. abies (L.) Karst.		Eupicea	20	60	—	20
P. breweriana S. Wats.	Omorica	Omorika	20	37	10	33
P. glauca (Moench) Voss.	Eupicea	Eupicea	18	51	—	31
P. omorika Purkyně	Omorica	Omorika	27	35	24	14
P. pungens Engelm.	Eupicea	Casicta	22	54	—	24
P sitchensis (Bong.) Carr	Omorica	Casicta	13	56	—	31
C_{16} Dominant						
P. bicolor (Maxim). Mayr	Eupicea	Eupicea	18	34	—	48
P. gemmata Rehder & Wilson	Eupicea	—	36	18	5	41
P. koyamai Shiras.	Eupicea	Eupicea	25	33	—	42
P. mariana (Mill.) B.S.P.	Eupicea	Eupicea	5	45	—	50
P. meyeri Rehder & Wilson	Eupicea	—	20	22	18	40
P. montigena Masters	Eupicea	—	23	25	6	46
P. obovata Ledeb.	—	Eupicea	26	34	—	40
P. orientalis (L.) Link.	—	Eupicea	1	13	2	84
P purpurea Masters	Eupicea	—	35	21	—	44
P. sargentiana Rehder & Wilson	Omorica	—	19	37	—	44
P. smithiana (Lamb.) Boiss.	Eupicea	Eupicea	9	15	3	73
P. spinulosa (Griff.) Henry	Omorica	Omorika	15	23	—	62
P. wilsonii Masters	Eupicea	Eupicea	29	29	—	42
P asperata Masters	Eupicea	Eupicea	40	17	—	43
No acid dominant						
P. maximowiczii (Regel) Masters	Eupicea	Eupicea	35	35	—	30
P. brachytyla (Franch.) Pritzel	Omorica	Omorika	39	21	—	40
P. engelmannii Parry ex Engelm.	Eupicea	Casicta	30	22	18	30

* (—) not recognized in this classification.

alkanes and from the straight line plot of log *Tr* vs hydrocarbon number [19]. The relative amount of each alkane was determined from digital integrator data.

Analysis of ω-hydroxyalkanoic acids. The $CHCl_3$ extract for each species was saponified with ethanolic KOH (2N) at 100° for 3 hr. Non-saponifiables were removed with *n*-hexane. The fatty acids were removed by acidification (HCl) and extraction with Et_2O. The fatty acid mixtures were methylated and the methyl esters removed by extraction with Et_2O. The methyl ω-hydroxyalkanoates were obtained by PLC (Si gel/$CHCl_3$–EtOAc, 7:3). The band (*ca* R_f 0.58) when eluted with $CHCl_3$ and conc co-chromatographed (Si gel, $CHCl_3$–EtOAc, 7:3) with a sample of authentic ω-16-methylhydroxyhexadecanoate. The ω-methylhydroxyalkanoates were acetylated (Ac_2O–Py) and the resulting products co-chromatographed (Si gel, $CHCl_3$–*n*-hexane, 3:1) with authentic ω-16-methylacetoxyhexadecanoate.

Identification. The individual ω-methylacetoxyalkanoates were identified by a comparison of the retention data with those of standard compounds (3% SE 30. on chromosorb G.AW. DMCS. N_2 at 25 ml/min temp. 205°). The identifications were confirmed by GC–MS (Finnigan 3200F series GC–MS system under the control of a 6110 data system; conditions; OV-1, 3%, temp. 180–240° at 2°/min, ionizing voltage 70 eV mass range 35–400 AMU.). The relative amount of each methylacetoxy alkanoate in the mixture from each species was determined from digital electronic integrator data.

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Table 2. Mole percentages of n-alkanes in the needle wax of *Picea* species

Picea species	*Classification* of Pilger [10]	*Classification* of Harrison [16]	n-Alkanes C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}
P. morrisonicola Hay.	Eupicea	Eupicea	5	8	12	11	12	5	12	8	4	2	2	1	3	2	7	—	6
P. bicolor (maxim.) Mayr.	Eupicea	Eupicea	6	8	8	3	6	3	9	2	15	7	8	3	6	2	8	—	4
P. breweriana S. Wats.	Omorica	Omorika	9	8	12	4	13	T*	11	T	14	7	7	2	5	1	5	T	T
P. gemmata Rehder & Wilson	Eupicea	—	3	2	T	T	8	T	3	T	39	9	13	5	5	6	6	—	T
P. koyamai Shiras.	Eupicea	Eupicea	4	4	5	5	10	T	6	5	18	3	13	4	13	5	6	T	1
P. likiangensis (Franch.) Pritzel	Eupicea	Casicta	3	3	3	3	6	T	7	—	4	5	13	7	13	6	11	1	3
P. montigena Masters	Eupicea	—	3	4	2	T	6	T	5	2	16	7	12	7	9	7	13	T	3
P. obovata Ledeb.	—†	Eupicea	6	7	7	2	4	T	7	1	16	6	10	3	13	3	15	—	—
P. sargentiana Rehder & Wilson	Omorica	—	3	3	4	5	1	T	10	T	36	4	9	1	8	2	12	—	T
P. yezoensis (Sieb et Zucc.) Carr.	Omorica	Casicta	5	3	6	4	7	2	5	2	14	6	10	6	12	5	11	—	1
P. brachytyla (Franch.) Pritzel	Omorica	Omorika	6	7	12	2	10	2	7	T	9	4	15	7	5	1	8	—	T
P. engelmannii Parry ex Engelm.	Eupicea	Casicta	1	3	4	—	3	—	1	—	8	11	17	8	13	6	16	—	T
P. purpurea Masters	Eupicea	—	T	T	2	2	4	T	5	T	9	5	16	8	15	10	15	—	—
P. schrenkiana Fisch. & Meyer	Eupicea	Eupicea	8	7	8	4	9	3	7	5	9	7	11	5	6	1	7	—	T
P. spinulosa (Griff.) Henry	Omorica	Omorika	5	8	9	T	8	1	11	2	14	8	14	2	5	1	7	—	2
P. maximowiczii (Regel) Masters	Eupicea	Eupicea	3	4	7	5	5	3	7	2	8	5	8	4	12	2	11	—	5
P. wilsonii Masters	Eupicea	Eupicea	4	6	7	2	4	T	5	1	12	8	13	6	15	7	9	—	T
P. abies (L.) Karst.	—	Eupicea	T	T	2	2	2	1	3	2	6	4	14	7	15	7	26	T	8
P. asperata Masters	Eupicea	Eupicea	8	9	6	4	10	T	9	T	12	5	5	2	8	2	13	1	1
P. balfouriana Rehder & Wilson	Eupicea	—	3	4	5	3	7	T	6	T	12	6	10	6	13	5	18	—	T
P. glauca (Moench) Voss.	Eupicea	Eupicea	3	2	3	2	3	T	10	T	14	10	15	3	8	3	16	—	3
P. mariana (Mill.) B.S.P.	Eupicea	Eupicea	1	1	2	2	3	2	4	2	9	3	10	6	10	6	26	3	8
P. meyeri Rehder & Wilson	Eupicea	—	1	2	1	2	4	T	4	T	11	5	13	6	19	6	22	T	2
P. omorica Purkyně	Omorica	Omorika	7	6	8	7	9	—	9	—	9	4	8	T	5	7	17	—	—
P. orientalis (L.) Link.	—	Eupicea	5	4	5	7	6	—	4	3	11	6	11	3	9	2	20	—	—
P. pungens Engelm.	Eupicea	Casicta	2	3	3	3	2	2	2	T	6	3	13	13	14	14	18	—	T
P. sitchensis (Bong.) Carr.	Omorica	Casicta	5	2	4	1	4	T	2	2	3	3	12	10	14	10	19	—	7
P. smithiana (Lamb.) Boiss.	Eupicea	Eupicea	2	3	4	4	2	1	4	1	7	8	11	9	9	9	14	2	8

* T trace less than 1 %. † Not recognized in this classification.

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