Organ-Specific Composition of Epicuticular Waxes of Beech (*Fagus sylvatica* L.) Leaves and Seeds

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Dedicated to Professor Hildegard Debuch on the occasion of her 70th birthday

Fagus sylvatica, Leaves, Seed Shells and Coats, Epicuticular Waxes

The leaf, seed shell and seed coat epicuticular waxes of beech (*Fagus sylvatica* L.) contained the following common wax lipids: alkanes, wax esters, aldehydes, primary alcohols and fatty acids, all occurring in a homologous series of very long chained and saturated components with variations in the maxima in each homologous series. In addition, small amounts of benzylacyl esters in leaves, and ketones in seed shells and coats were also found in homologous series. β-Sitosterol was present only in seed shells and coats. Free primary alcohols (34.8%) were found to be the major class in the leaves whereas free fatty acids were predominant in seed shells (39.5%) and coats (42.1%). The beech organs studied showed always a specific wax composition.

Introduction

The function of the cuticular membrane is essentially conditioned by its wax layer. Very little information is known about the composition of epicuticular waxes of leaves and seeds from deciduous broadleaved trees [1]. We are now reporting the epicuticular wax composition of beech (*Fagus sylvatica* L.) leaves and seeds. From the waxes of beech leaves, only the hydrocarbon composition was studied earlier by Jacob [2]. The complete lipid composition of the beech seed oil has been just reported [3]. *Fagus sylvatica,* popularly known as Common or European Beech is an important forest tree in Central and South Europe. Beech trees are known in several cultivated forms. These trees grow up to 30–45 m with leaves of ovate to elliptic forms in length of 5–10 cm. Beech fruits contain an ovoid-triangular nut in groups of 1 to 3, surrounded by the accrescent cupule [4–6].

Materials and Methods

Leaves of beech were harvested in May and September 1988 from a tree cultivated in the garden of Botanical Institute, University of Cologne. Beech seeds were harvested in 1986 and obtained from "Staatliches Forstamt Oerrel" in Münster-Oerrel. Shells and coats were separated from seeds. Epicuticular waxes were extracted from leaves (345 g), seed shells (216.6 g) and seed coats (26.6 g) with chloroform. They were dipped consecutively into three beakers of chloroform for 3 min.

The crude wax was redissolved in pentane and fractionated by successive elution with pentane, 2-chloropropane and methanol as described previously [7]. These fractions were separated again by preparative TLC on silica gel precoated plates (Merck 60, Darmstadt) with the following solvents: 1. toluene (R_f 1) and 2. dichloromethane : ethyl acetate (24:1) (R_f 2). The isolated compounds were identified by chemical reactions such as methanolysis, ethanolysis, hydrogenation and also by TLC, GC and GC-MS with authentic samples [8]. GC was carried out with a Hewlett Packard 5710 unit fitted with a FID and an integrator 3380A on a capillary column OV 1 (10 m). The temperature was programmed from 140 °C to 340 °C as required. GC-MS detection was done by Finnigan MAT 4510, 70 eV, EI with a fused silica capillary column DB-11 (15 m).

Results and Discussion

Epicuticular waxes of the leaves, seed shells and seed coats of beech were found to be 0.53, 0.12 and 0.29% respectively of the dry weight. The leaves contained an extractable epicuticular wax layer of about 15 μg/cm^2. Epicuticular waxes from leaves, seed shells and coats constituted homologues of alkanes, wax esters, aldehydes, free fatty acids and primary alcohols (Table I). Free primary alcohols were the major components in the leaves whereas free fatty acids were found to be the predominant

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Table I. Composition and yield of epicuticular waxes from *Fagus sylvatica* leaves, seed shells and coats (weight, per cent).

Components	Leaves		Seed shells		Seed coats	
	[mg]	[%]	[mg]	[%]	[mg]	[%]
Hydrocarbons	119.0	17.0	13.0	5.4	4.0	6.0*
Wax esters	121.7	17.4	47.3	19.5	13.6	20.2
Aldehydes	71.8	10.3	14.3	5.9	6.6	9.9
Ketones	–	–	0.4	0.2	0.8	1.2
Benzylacyl esters	6.4	0.9	–	–	–	–
Free fatty acids	56.6	8.1	95.8	39.5	28.2	42.1
Free primary alcohols	242.3	34.8	24.1	10.0	4.1	6.1
Sterols	–	–	8.0	3.3	4.1	6.1
Unidentified (including lost on the column)	80.2	11.5	39.1	16.2	5.6	8.4

* 3.8% Hydrocarbons and 2.2% squalene.

class in seed shells and coats. In addition to the common lipid components, the epicuticular waxes of the leaves contained a homologous series of benzylacyl esters, and on seed shells and coats ketones and β-sitosterol were constituted in small amounts. The results presented for the leaves were the mean values of three preparations.

Hydrocarbons: Hydrocarbons were separated from the crude wax with pentane and found to be only saturated *n*-alkanes (R_f 1, 0.70) in a series of 13 homologues ranging from C-21 to C-33 (Table II). The predominant component was heptacosane (C-27) in all the organs of beech. The hydrocarbons from the leaves of the beech were reported to contain C-27 as the main component by Jacob [2]. Eventhough C-27 was the main alkane in seed shells and coats, the other *n*-alkanes were also present in more quantities unlike in leaves. The alkanes of the leaves showed a very steep distribution pattern with a predominant main component of heptacosane with 89.5%. The seed shells and coats have also the main component of C-27 but in lower amounts (42.3% and 23.5%) and in contrast to leaves these organs exhibited a random distribution of alkanes. In addition to the *n*-alkanes, seed coats hydrocarbon fraction contained squalene (2.2% of crude wax) which is an

Table II. Composition of hydrocarbons, aldehydes, free alcohols and free fatty acids from epicuticular waxes of *Fagus sylvatica* leaves, seed shells and coats (peak area %).

Carbon No.	Leaves				Seed shells				Seed coats			
	Hydrocarbons	Aldehydes	Alcohols	Acids	Hydrocarbons	Aldehydes	Alcohols	Acids	Hydrocarbons	Aldehydes	Alcohols	Acids
14			+	7.8			+	2.0			+	1.3
16			1.2	7.8			4.8	8.7			11.7	11.3
18			1.7	4.3			+	19.3			+	42.5
20		0.1	48.5	4.2		+	3.6	2.3		+	7.5	3.0
21	+		0.3	4.1	1.2		+	1.2	1.6		2.2	0.5
22	+	0.5	9.9	10.1	1.8	3.8	15.1	3.8	2.1	+	14.9	4.7
23	0.3	1.2	0.1	0.4	15.0	+	+	1.6	13.2		+	1.5
24	+	0.6	7.6	8.0	2.7	7.2	11.2	16.7	2.6	34.2	12.6	12.8
25	3.2	3.8	0.1	0.8	14.1	+	0.9	5.7	13.7	2.4	2.2	2.9
26	1.7	6.8	3.6	8.4	4.4	5.1	4.2	18.5	4.0	15.6	5.6	9.9
27	89.5	5.1	+	+	42.3	+	2.0	3.1	23.5	1.8	2.8	1.4
28	0.5	73.4	23.8	39.7	8.8	82.5	42.5	7.9	4.2	28.0	27.5	4.2
29	4.5	3.6	0.6	2.0	5.7	+	+	+	13.7	2.4	+	+
30	+	4.9	2.6	2.4	1.1	1.4	15.7	9.2	4.5	15.6	13.0	4.0
31	0.3				1.6				11.3			
32	+				0.7				2.9			
33	+				0.6				2.7			

+ = traces < 0.1%.

unusual component in epicuticular waxes. The presence of squalene was confirmed by carbazole positive reaction on TLC plate and also by GC-MS data.

Wax esters: The second major component in the crude waxes of all the examined organs of beech was wax esters (R_f 1, 0.64) which was also the main lipid class in the chloropropane eluate. All the wax esters were found in homologous series ranging from C-36 to C-52 (Table III). The main components of the wax esters were not found in higher amounts (only in 20 to 30%) and differ in leaves (C-42), shells (C-48) and coats (C-50). The amounts of the higher homologues (C-46, C-48 and C-50) were more in shells and coats than in leaves.

Table III. Composition of wax esters from epicuticular waxes of *Fagus sylvatica* leaves, seed shells and coats (peak area %).

Carbon No.	Leaves	Seed shells	Seed coats
36	+	+	+
38	2.3	1.8	2.8
39	+	0.4	0.5
40	15.6	5.6	4.3
41	1.3	0.6	0.6
42	23.8	12.3	6.3
43	1.5	1.2	1.6
44	18.7	17.9	19.1
45	1.6	2.2	3.7
46	12.8	18.4	17.5
47	1.5	2.1	0.8
48	10.3	19.7	11.8
49	1.0	2.0	1.7
50	6.5	15.8	29.3
51	+	+	+
52	3.1	+	+

+ = Traces < 0.1%.

Ethanolysis of the esters resulted in primary alcohols and saturated fatty acids in the form of their ethyl esters (FAEE). FAEE ranged from C-14 to C-28. The major component was C-22 in leaves (36.4%) and shells (29.5%) whereas C-28 was the predominant in coats (26.1%). Primary alcohols were found in series from C-18 to C-28 comprising C-20 as the main component in leaves (38%) and shells (29.7%) and C-22 in coats (29.9%).

Aldehydes: The chloropropane eluate was also consisted of aldehydes (R_f 1, 0.45) with 10.3, 5.9 and 9.9% of the crude waxes of leaves, shells and coats, in homologous series ranging from C-20 to C-30. The predominant aldehyde was C-28 in leaves (73.4%) and shells (82.5%) (Table II). But the seed coat aldehydes exhibited a different pattern of distribution with C-24 (34.2%) as the main component followed by C-28 (28%), C-30 (15.6%) and C-26 (15.6%). The presence of aldehyde homologues were confirmed by GC-MS data.

Benzylacyl esters: In addition to the wax esters and aldehydes, the chloropropane fraction of the leaves was found to contain another homologous series of esters. This fraction was separated by TLC (R_f 1, 0.5) and subjected to GC-MS analysis and identified as benzylacyl esters with a characteristic fragment ion of *m/z* 91. The predominant ester in the series was C-35 (64%) followed by C-33 (24.4%) and C-31 (11.6%) with a fatty acid chain length of C-28, C-26 and C-24 respectively. GC retention times and GC-MS data of these esters were the same as those of synthetic benzylacyl esters and of jojoba leaves which were reported earlier [9].

Ketones: The chloropropane fractions of seed shells and coats were found to contain a homologous series of long-chain ketones (R_f 1, 0.53) in small amounts of 0.2 and 1.2% of crude wax respectively. The main ketone was palmitone (C-31, 77.3 and 81.9%) in both cases followed by C-29 (22.7 and 13.6%) and C-27 (traces and 4.5%). Long-chain ketones were also found in the leaves of *Asparagus officinalis* [10]. The presence of ketones was confirmed by GC-MS data.

Fatty acids: Fatty acid methyl esters (FAME) were separated from methanol fraction by silica gel column chromatography after esterification of fatty acids. FAME were eluted with 2-chloropropane and alcohols with methanol. Fatty acids were the major components in shells (39.5% of crude waxes) and coats (42.1%) whereas leaves constituted only 8.1% of crude waxes as fatty acids. Fatty acids in homologous series ranging from C-14 to C-30 were found in all the organs in different compositions. C-28 (39.7%) was the main fatty acid in leaves. The content of C-18, C-24 and C-26 were similar in seed shell fatty acid fraction and C-18 (42.5%) was the predominant fatty acid in seed coats followed by C-16, C-24 and C-26 in similar amounts. Fatty acids from leaves were found to be saturated but in seed shells and coats C-18 unsaturated fatty acids were also present with a more content in the latter (Table II).

Primary alcohols: Alcohols were the major components in leaves (34.8% of crude wax). In seed shells (10.0%) and coats (6.1%) they were found in

smaller amounts. Homologue alcohols ranging from C-14 to C-30 were found in all organs. The major alcohols in leaves were C-20 (48.5%) and C-28 (23.8%). In seed shells and coats, C-28 (42.5% and 27.5%) was the main alcohol. The content of C-30 alcohol was more in seed shells (15.7%) and coats (13%) than in leaves (2.6%) (Table II).

Sterols: β-Sitosterol was found in the methanol fraction of seed shells and coats in 3.3% and 6.1% of crude waxes. The presence of β-sitosterol was confirmed (R_f 2, 0.25) with a positive carbazole reaction on a TLC plate [11] and also by GC-MS data. β-Sitosterol was previously reported in the leaf epicuticular waxes of *Citrus halimii* [8], *Simmondsia chinensis* [12] and *Tilia tomentosa* and *T. europaea* [1].

The epicuticular waxes of beech organs analyzed in the present investigation exhibited an organ specific composition. All the organs contained indeed the same homologous series of the common wax lipids with variations in amounts and specific distribution pattern. But qualitative differences were found such as benzylacyl esters in leaves, ketones and β-sitosterol in seed shells and coats, and squalene only in seed coats. The homologous series in leaf waxes namely hydrocarbons, aldehydes, fatty acids and alcohols were found in a most steep distribution pattern with saturated and long-chain components. The wax components in seed shells and more in seed coats exhibited a random distribution pattern without a predominant component in higher amounts with a trend towards shorter chain lengths (except for seed shell aldehydes). Unsaturated fatty acids were found only in seed shells and coats with a more content in the latter. Similar results were found in the earlier studies on organ waxes of *Simmondsia chinensis* [13] and *Cistus albidus* [14]. The presence of benzylacyl esters is a characteristic observation for beech leaf waxes just like β-amyrin acetate in lime leaves [1].

The developmental variations and seasonal factors of beech leaves are under progress.

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