Epicuticular Waxes from Leaves of Maple (*Acer pseudoplatanus* L.)

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The epicuticular waxes from the leaves of maples (*Acer pseudoplatanus* L.) contained hydrocarbons (6.9%), wax esters (5.5%), aldehydes (38.1%), primary alcohols (10.2%) and fatty acids (17.1%). In addition to these common wax lipids, benzyl acyl esters (2.1%) and triterpenoids were also present. β-Sitosterol, β-amyrin and 24-methylene-cycloartenol were found in the form of acetates (14.4%). β-Amyrin was also present as free alcohol (4.9%) and esterified with long chain fatty acids (~0.7%).

Introduction

Acer, commonly called maple, is ranging over almost all the northern temperate region and extending into the tropics [1]. *Acer pseudoplatanus* L. is spread all over Europe up to Caucasus mostly in and around the mountains and cultivated since ancient times, as a valuable forest tree and also suitable for park and street trees [2–4]. We are now reporting the chemical composition of the epicuticular waxes of maple leaves.

Materials and Methods

Leaves of maples were harvested in June and July from a tree cultivated in the Garden of Botanical Institute, University of Cologne. The surface waxes (0.306 g) were extracted from the fresh leaves (96.4 g) by immersion (2 min) twice in $CHCl_3$. Waxes were identified and quantified by column chromatography (CC) on silica gel, resulting in three fractions: pentane, 2-chloropropane and methanol, TLC (Rf_1: Toluene, Rf_2: CH_2Cl_2/EtoAc, 24/1) and GC methods as described previously [5, 6]. The results in Table II were the mean values of two preparations.

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

The surface waxes extracted represent 1.0% of the dry weight of the leaves. The leaves contained an extractable epicuticular wax layer of 34 μg/cm^2. Aldehydes (38.1%) were the predominant lipid class in the waxes and other lipids present in less than 15% quantities. In addition to the common wax lipids, benzyl acyl esters (2.1%), and triterpenoids in the form of alcohols (4.9%), acetates (14.4%) and esters (< 1%) were also present (Table I).

The composition of the individual lipid classes are given in Table II. Hydrocarbons (Rf_1, 0.70) constituted of homologous *n*-alkanes with chain lengths ranging from C_{23} to C_{35}. Nonacosane (59.3%) was found to be the major component. As there was no change in the composition of the hydrocarbons after the hydrogenation over Pd as a catalyst, it was concluded that unsaturated hydrocarbons were not present and traces of branched alkanes were observed.

Wax esters (Rf_1, 0.64) contained even numbered homologous series (C_{38} to C_{54}) with C_{42} (29.0%) as

Table I. Composition and yields of surface waxes from leaves of *A. pseudoplatanus*.

	[mg]	% Wax	% Dry wt.
Hydrocarbons	21.0	6.9	0.069
Wax esters	16.7	5.5	0.055
Benzyl acyl esters	6.6	2.1	0.021
Triterpenol acetates	44.2	14.4	0.144
Aldehydes	116.5	38.1	0.381
Primary alcohols	31.2	10.2	0.102
Triterpenols	15.1	4.9	0.049
Fatty acids	52.2	17.1	0.171
Lost on column and unidentified	2.5	0.8	0.008
	306.0	100.0	1.000

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Table II. Composition of hydrocarbons, aldehydes, benzyl acyl esters, primary alcohols, fatty acids and wax esters from surface waxes of *A. pseudoplatanus* leaves (peak area per cent).

Carbon no.	Hydro-carbons	Aldehydes	Benzyl acyl esters	Primary alcohols	Fatty acids	Carbon no.	Wax esters
14				0.7	1.7	38	4.6
16				0.5	8.0	40	19.0
18				6.4	4.3	42	29.0
20				3.1	2.3	44	19.1
22		+		4.0	3.8	46	13.5
23	0.4			+	+	48	10.3
24	0.3	1.2		37.4	23.8	50	3.1
25	3.0	0.3		0.4	0.4	52	1.4
26	1.1	7.7		27.1	11.3	54	+
27	13.2	0.4		0.5	0.5		
28	3.2	39.2		13.7	24.2		
29	59.3	+		0.5	0.5		
30	0.8	47.4		3.8	17.8		
31	14.7	+	9.0	+	+		
32	0.9	3.8		1.9	1.4		
33	2.5		34.1				
34	0.2						
35	0.4		56.9				

a predominant component followed by C_{44} (19.1%), C_{40} (19.0%), C_{46} (13.5%) and C_{48} (10.3%) in considerable amounts. This fraction also contained triterpenol esters. The GC analysis of the hydrolysis products revealed that the wax esters were formed mainly from C_{24} (34.5%), C_{26} (20.1%), C_{18} (17.3%) and C_{20} (10.3%) alcohols esterified with C_{16} (25.2%), C_{18} (21.4%), C_{24} (19.6%) and C_{28} (10.9%) fatty acids. In addition β-amyrin was found to contain in wax ester fraction esterified with long chain fatty acids (~0.7%). 24-Methylene-cycloartenol could not be detected in these esters by GC-MS, but β-sitosterol was analyzed in traces after hydrolyses of the esters.

Aldehydes (Rf_1, 0.45), the major lipid class of maple surface waxes were found to contain C_{30} (47.4%) and C_{28} (39.2%) as predominant individual components. The primary alcohol (Rf_1, 0.06) fraction represented with a homologous series ranging from C_{14} to C_{32} with C_{24} (37.4%), C_{26} (27.1%) and C_{28} (13.7%) as dominating alcohols.

The fatty acids were also present in a homologous series (C_{14} to C_{32}) like alcohols but with a different distribution pattern. C_{28} (24.2%) was the major fatty acid followed by C_{24} (23.8%), C_{30} (17.8%) and C_{26} (11.3%).

The 2-chloropropane fraction of the CC was found to contain benzyl acyl esters (Rf_1, 0.53), triterpenol acetates (Rf_1, 0.33) and triterpenol esters (Rf_1, 0.72) in addition to wax esters and aldehydes. The benzyl acyl ester series consisted of fatty acids with chain lengths of C_{28}, C_{26} and C_{24}, respectively. These esters were found earlier in jojoba [7] and beech leaves [8] also.

The triterpenol acetates were separated by TLC in noticeable amounts of 14.4% of waxes and found to contain β-amyrin, 24-methylene-cycloartenol and also β-sitosterol acetates in 24, 33 and 43% quantities, respectively. The presence of these components was confirmed by GC-MS data of acetates and their corresponding alcohols [5, 9] after hydrolysis with MeOH/HCl. β-Amyrin acetate was previously found in the leaf waxes of *Tilia* species [10] and in *Euphorbia aphylla* [11], too.

Free triterpenol namely β-amyrin was found in MeOH fraction of maple leaf waxes together with alcohols and fatty acids. β-Sitosterol and 24-methylene-cycloartenol could not be identified, not even in traces, in this fraction by GC-MS. The free triterpenols, their acetates and their esters respectively showed a positive colour reaction with carbazole.

All the lipid classes identified in the present investigation were found to contain saturated and long chain components with aldehydes as the major lipid class. The chemical compositions revealed

that only hydrocarbon fraction exhibited a steep distribution pattern with C_{29} as a major component and the other classes represented a random distribution pattern without any predominant component in higher amounts. Triterpenol acetates are not very common in surface waxes, especially β-sitosterol acetate and 24-methylene-cycloartenol acetate have not been described before.

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