Chemical Composition and Surface Structures of Epicuticular Leaf Waxes from *Castanea sativa* and *Aesculus hippocastanum*

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Epicuticular leaf waxes of *Castanea sativa* and *Aesculus hippocastanum* contain the same lipids in form of homologous series of hydrocarbons, wax esters, aldehydes, primary alcohols and fatty acids in similar concentrations without any main component dominating. In *Ae. hippocastanum* wax acetates are present, additionally. Both waxes are found to contain triterpenols and triterpenol esters in remarkable amounts. β-Amyrin, α-amyrin and lupeol are present in both plant waxes, in *Ae. hippocastanum* wax friedelanol and friedelanone, additionally.

The epidermis of both plants are covered with a thin continuous wax layer without crystalloids. But the adaxial leaf surface of *C. sativa* shows granular wax sculptures and therefore a different micromorphological ultrastructure for both leaf sides.

Introduction

In continuation of our studies on epicuticular leaf waxes of deciduous trees, two not very closely related genera were now analyzed, the trees *Castanea sativa* Miller (Sweet chestnut) and *Aesculus hippocastanum* L. (Horse chestnut).

C. sativa is a member of the plant family Fagaceae (Fagales), like *Fagus* and *Quercus* species. *Ae. hippocastanum* is arranged to the family Hippocastanaceae (Sapindales). Both trees have seeds of similar shapes and therefore are named with the same trival term chestnut. But they differ very much in the seed compounds. Seeds of sweet chestnuts are edible and form an article for diet. Seeds of horse chestnuts are applied often for pharmaceutical products and are not edible. The trees of both species are native in eastern Mediterranean countries and also in the northern hemisphere including middle Europe [1–4]. The composition of the epicuticular leaf waxes are correlated with the surface structures of these deciduous trees in the following paper.

Abbreviations: SEM, scanning electron microscopy; CC, column chromatography; TLC, thin layer chromatography; GC, gas chromatography; RT, retention time; MS, mass spectrometry.

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Materials and Methods

Leaves of *C. sativa* Miller and *Ae. hippocastanum* L. were harvested in August 1990 from trees cultivated in the garden of the Botanical Institute, University of Cologne. The epicuticular waxes were extracted from fresh leaves by two-fold immersion in $CHCl_3$ (1 min each). The waxes were separated by CC on silica gel. Three fractions were obtained with *n*-pentane, 2-chloropropane and methanol as solvents. Subsequently the constituents were analyzed by TLC with toluene as solvent and GC as described previously [5, 6]. The quantitative composition is listed in Tables I–II as mean values of two preparations.

Air dried leaves for SEM were prepared by sputtering with gold using an Emscope sputter coater and examined in a Hitachi S-405A scanning electron microscope at 10 to 25 kV, respectively.

Results

Wax composition

C. sativa leaves (120 g) contained an extractable epicuticular wax (577 mg) of about 1.12% dry wt., which calculated for 48 μg wax per cm^2 leaf surface area or 4421 μg wax per one leaf. This wax of mature leaves consisted of homologous series of wax lipids and greater amounts of triterpenoids (32% of the wax). Main lipid classes are fatty acids (25% wax), wax esters (25%), primary alcohols (14%), hydrocarbons (4%) and aldehydes (2%). Compo-

sition and yield of the individual lipid classes are listed in Table I. The compositions of the homologous series are summarized in Table II.

The hydrocarbon fraction consisted of *n*-alkanes with chain lengths ranging from C_{19} to C_{35} and nonacosane as dominating component (67.6%), followed by heptacosane and hentriacontane.

Homologous wax esters of long chain fatty acids with long chain primary alcohols were present

Table I. Composition and yield of epicuticular leaf waxes from *Castanea sativa* and *Aesculus hippocastanum*.

Components	R_f	*C. sativa*			*Ae. hippocastanum*		
		mg	% wax	% dry wt.	mg	% wax	% dry wt.
Hydrocarbons	0.7	22	4	0.042	11	5	0.031
Triterpenol esters	0.65	14	2	0.027	2	1	0.005
Wax esters	0.6	110	19	0.212	17	7	0.040
Aldehydes	0.4	10	2	0.020	30	13	0.080
Acetates	0.3	–			12	5	0.030
Alcohols	0.06	79	14	0.153	17	7	0.046
Triterpenols	0.06	175	30	0.338	131	53	0.345
β-Amyrin			5.6			20	
α-Amyrin			12.2			15	
Lupeol			12.2			15	
Friedelan-3-ol		–	–			1.5	
Friedelan-3-one		–	–			1.5	
Fatty acids	0.02	141	25	0.272	18	8	0.048
lost on column		26	4	0.050	3	1	0.008
		577		1.114	241		0.633

Table II. Composition (peak area %) of hydrocarbons, aldehydes, acetates, primary alcohols, fatty acids and wax esters of epicuticular leaf waxes of *Castanea sativa* and *Aesculus hippocastanum*.

Carbon No.	Hydrocarbons		Aldehydes		Acetates	Primary alcohols		Fatty acids		Carbon No.	Wax ester	
	C. s.	*Ae. h.*	*C. s.*	*Ae. h.*	*Ae. h.*	*C. s.*	*Ae. h.*	*C. s.*	*Ae. h.*		*C. s.*	*Ae. h.*
14								4.1	1.0	36	0.3	5.7
16						+		7.6	7.7	38	1.5	22.1
18			+	+		0.3	1.0	8.0	2.9	39	0.1	0.8
19	+	+				+	+	+	+	40	3.3	30.7
20			2.0	0.3	+	0.5	3.6	18.4	1.9	41	0.2	1.3
21	0.2	0.2	+	0.3	1.1	+	+	0.8	+	42	8.2	18.2
22	0.2	0.5	4.8	0.7	1.1	11.5	11.7	13.2	6.3	43	1.2	1.1
23	2.0	3.3	4.8	0.9	2.2	1.9	+	0.5	2.4	44	30.3	9.6
24	0.3	1.5	22.6	23.3	8.8	67.3	20.0	10.4	23.6	45	3.0	0.9
25	1.3	8.7	3.6	+	+	1.0	+	0.5	6.3	46	32.9	8.6
26	0.3	1.3	7.3	16.8	52.4	5.1	28.7	5.6	13.0	47	2.0	+
27	14.1	17.1	2.0	+	5.0	2.6	1.0	+	3.8	48	13.5	0.9
28	2.7	2.1	14.8	13.9	14.7	3.2	9.3	6.7	11.5	49	0.8	+
29	67.6	58.5	4.0	+	7.0	+	+	+	+	50	1.6	+
30	1.4	1.6	13.6	40.9	6.4	1.0	21.5	21.6	12.5	51	0.3	+
31	8.9	4.6	2.8	+	+	+	+	+	+	52	0.8	+
32	0.8	0.1	15.0	2.8	1.0	5.6	2.7	1.8	6.3			
33	0.2	0.5	2.0	+		+	+	+	+			
34			0.6	0.3	0.5	+	0.4	0.7	0.8			
35	+	+										

with chain lengths from C_{36} to C_{52}. Esters with the carbon numbers C_{44} und C_{46} are predominant in similar concentrations of about 30%.

Aldehydes were found in chain lengths ranging from C_{20} to C_{34} with no main component dominating.

Primary alcohols formed homologous series with chain lengths ranging from C_{16} to C_{34}. Main component is the alcohol tetracosanol (67.3%).

Fatty acids were present in homologous series from C_{14} to C_{34} with no main component dominating. Long chain fatty acids C_{20}, C_{22}, C_{24}, and C_{30} are found in concentrations of more than 10%.

Triterpenols were found in the leaf wax of *C. sativa* in amounts of 30%. β-Amyrin, α-amyrin and lupeol were identified. These triterpenols show a positive colour reaction with carbazol, have discrete RT values in GC and characteristic fragments in the MS spectra [5–7]. We were not able to separate α-amyrin and lupeol by GC. They were, unambiguously, identified by their mixed mass spectra [7]. α-Amyrin and lupeol are present in nearly equal amounts. The relationship of the three triterpenol are found to 1:2.2:2.2 (see Table I). These triterpenols are found also esterified with fatty acids (2% of the wax).

Ae. hippocastanum leaves (102 g) contained an extractable epicuticular wax (241 mg) of about 0.63% dry wt., which calculated for 22 µg wax per cm^2 leaf surface area or 4540 µg wax per one leaf. This wax of mature leaves consisted, like the wax of *C. sativa,* of homologous series of wax lipids and in addition greater amounts of triterpenoids (54% of the wax). Main lipid classes are aldehydes (13%), fatty acids (8%), primary alcohols (7%), wax esters (7%), hydrocarbons (5%) and additionally acetates (5%). Composition and yield of the individual lipid classes are listed in Table I. The compositions of the homologous series are summarized in Table II.

All these lipid classes have the same chain lengths as found for *C. sativa* and show no main component dominating. The distribution patterns of these lipids are therefore very even.

Triterpenols were found to be the dominating wax compounds. β-Amyrin, α-amyrin and lupeol were again predominant in a relative ratio of 3:2.2:2.2. These triterpenols were present also esterified with fatty acids (2% of the wax). Furthermore, friedelanol and friedelanone were identified by GC-MS and comparison with authentic samples [6, 7]. The latters were found in concentrations of about 1.5% of the wax, each (see Table I).

Surface structures

SEM figures of mature *C. sativa* leaves show a dense arrangement of stellate hairs, singular glandular trichomes and numerous stomata on the lower (abaxial) epidermis. The abaxial epidermal cells are covered with a continuous wax layer without wax sculptures or crystalloids (Fig. 1, A and C). The upper (adaxial) epidermis has no trichomes. This adaxial leaf surface is also covered with a continuous wax layer, but superimposed by numerous granular wax sculptures without a definite shape (Fig. 1, B and D). The abaxial and adaxial leaf surfaces of this plant are found to develop different wax ultrastructures.

The SEM figures of mature leaves of *Ae. hippocastanum* show no trichomes on the abaxial as well as on the adaxial epidermis. Both leaf surfaces are characterized by numerous cuticular lamellae (Fig. 2, A and B). The cuticular lamellae are found to be linear (Fig. 2, C) and also wavelike (Fig. 2, D). All epidermal cells are covered with a continuous wax layer without any wax sculptures or crystalloids. After washing the leaves with chloroform all wax was extracted. The remaining cuticular lamellae are clearly visible on the abaxial and adaxial epidermal cells (Fig. 2, E and F).

Discussion

C. sativa and *Ae. hippocastanum* are systematically not very closely related plant genera. However, they have a rather similar wax composition. They contain the same homologous series of wax lipids, but with individual distribution patterns. No one of these lipid classes has one main component dominating. In addition to the wax lipids, both plant waxes contain mixtures of triterpenols. In *C. sativa* wax β-amyrin, α-amyrin and lupeol are found. In *Ae. hippocastanum* wax the same triterpenols are analyzed and additionally friedelanol and friedelanone. Both leaf waxes contain wax lipids and triterpenols with no main component in a dominating concentration. Therefore, wax crystalloids on the leaf surfaces of these two plants are

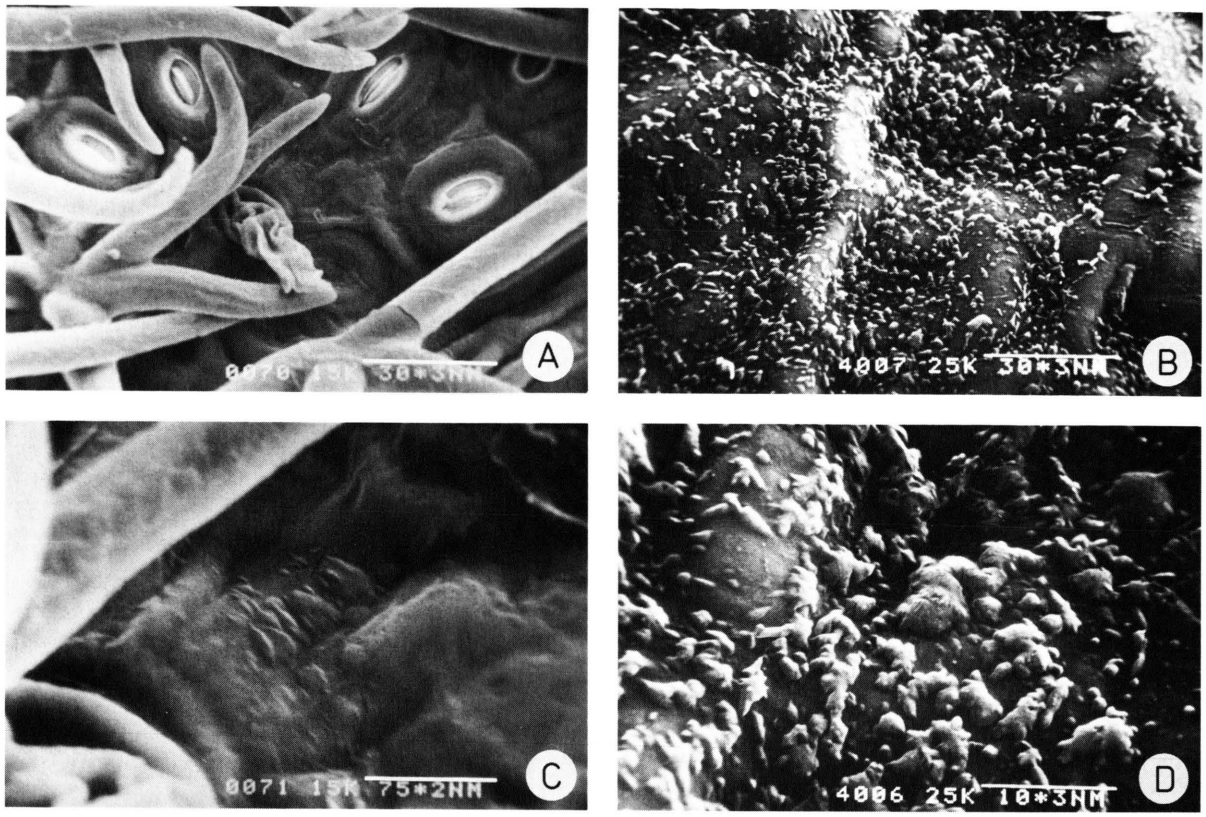

Fig. 1. *Castanea sativa* surface structures of a mature leaf.
A. Abaxial epidermis with stellate hairs, singular trichomes and numerous stomata. Bar = 30 μm.
B. Adaxial epidermis without any trichomes, covered with numerous wax sculptures. Bar = 30 μm.
C. Abaxial epidermis shows a continuous wax layer without any wax sculptures. Bar = 7.5 μm.
D. Adaxial epidermis, the continuous wax layer is superimposed with numerous granular wax sculptures. Bar = 10 μm.

not expected. Indeed, on the abaxial and adaxial epidermis of both plants no wax crystalloids are found, but granular wax sculptures are observed on the adaxial leaf surface of *C. sativa*. This observation may be an indication, that the wax composition on the abaxial and adaxial leaf sides is not always the same. Different surface structures on both leaf sides are found also for *Acer* [8], *Juglans* [8], *Tilia* [9], Fagus [10], Magnolia [7] and Liriodendron [7]. These different surface ultrastructures on both leaf sides of the same plant may be resulted of variable enzyme activities for lipids on both leaf surfaces.

C. sativa is belonging to the family Fagaceae (Fagales), just as the species of *Fagus* and *Quercus* [3]. All these genera have a different wax composition and in consequence different surface wax ultrastructures.

F. sylvatica wax [11] contains only wax lipids without any triterpenoids. No lipid class shows a main component dominating. Therefore the surface wax ultrastructures show no crystalloids, but on the adaxial leaf surface wax sculptures are observed [10].

Similar granular wax sculptures are found also on the adaxial epidermis of *C. sativa*. Concerning the usual wax lipids, *C. sativa* wax contains the same lipid classes as *F. sylvatica* with even distribution patterns. But in addition this wax contains also several triterpenols (30% of the wax).

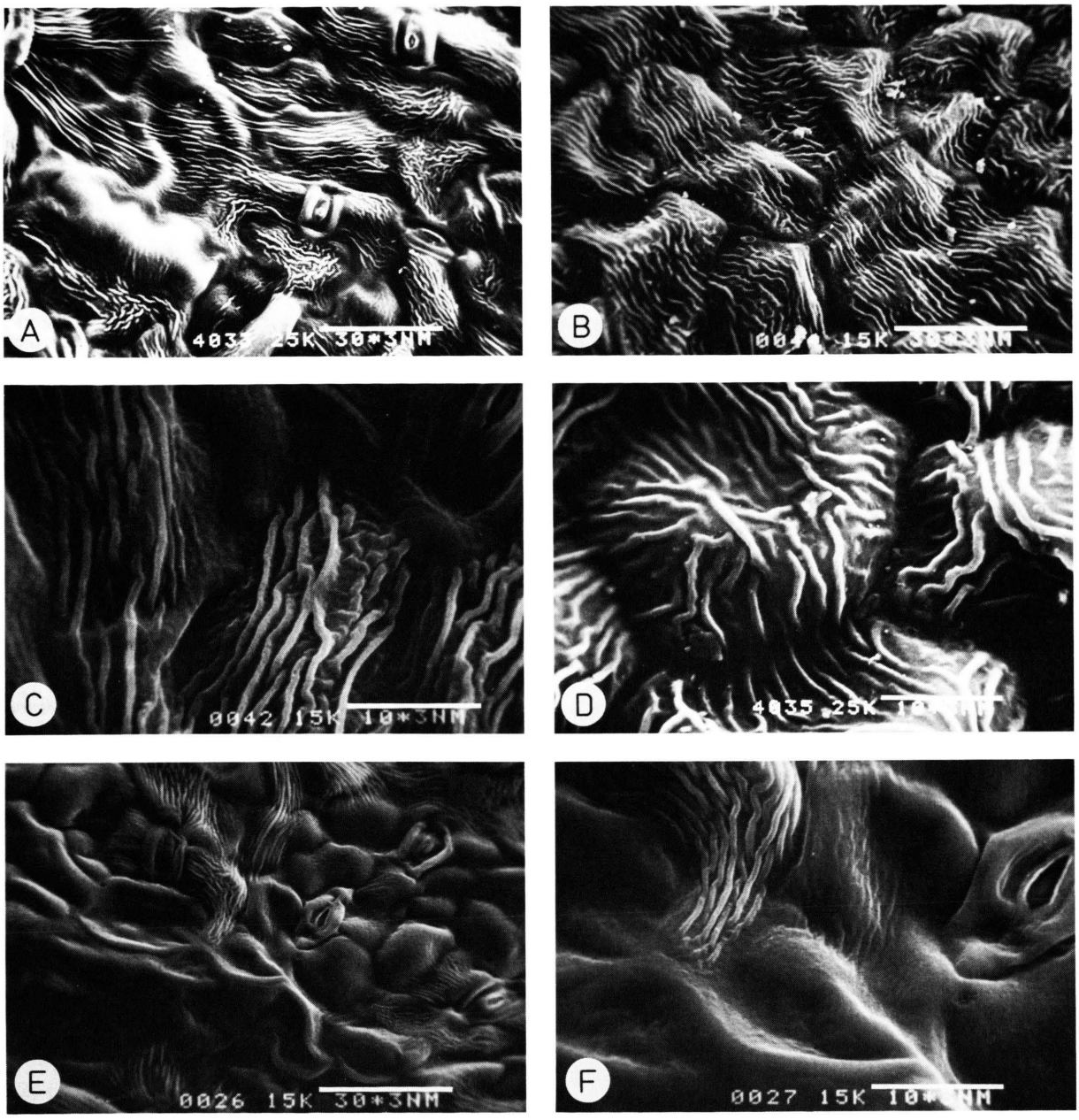

Fig. 2. *Aesculus hippocastanum* surface structures of a mature leaf.
A. Abaxial epidermis without trichomes, but with numerous stomata and linear or wavelike cuticular lamellae and cuticular papillae. Bar = 30 μm.
B. Adaxial epidermis with linear and wavelike cuticular lamellae and cuticular papillae. Bar = 30 μm.
C. Abaxial surface with a continuous wax layer without any wax sculptures, but with cuticular lamellae. Bar = 10 μm.
D. Adaxial surface with a continuous wax layer without any wax sculptures, but with cuticular lamellae. Bar = 10 μm.
E. Abaxial epidermis washed with chloroform, the cuticular lamellae remained visible. Bar = 30 μm.
F. Abaxial epidermis washed with chloroform. Bar = 10 μm.

Q. rubor leaf wax has the usual wax lipids and in addition several triterpenoids (4.1% of the wax). Already one lipid class is predominant in this wax. Alcohols amounted to about 40% with tetracosanol as main component dominating to about 95%. This tetracosanol forms fringed edged platelets on both leaf surfaces of *Q. robur* [8].

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