Chemical Composition and Surface Structures of Epicuticular Leaf Waxes of *Ginkgo biloba, Magnolia grandiflora* and *Liriodendron tulipifera*

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Epicuticular leaf waxes of *Ginkgo biloba, Magnolia grandiflora* and *Liriodendron tulipifera* contain homologous series of hydrocarbons, wax esters, benzyl acyl esters, aldehydes, primary alcohols, and fatty acids. None of these lipid classes is found to contain any main component dominating. In addition to these usual wax lipids, in *G. biloba* leaf wax a secondary alcohol namely nonacosan-10-ol (15.0%), γ-tocopherol (1.7%) and acetates (0.3%) is also present. The wax of *L. tulipifera,* however, contains hentriacontan-16-one (23%) and several triterpenols (10%), additionally.

On *G. biloba* leaves a dense arrangement of tubular wax crystalloids are found on the lower as well as on the upper leaf surface. The openings of the tubules can be seen very well in the SEM figures at a magnification of 20000. The small tubules are a clear indication for the presence of nonacosan-10-ol as also reported previously for conyferyl waxes.

Leaves of *M. grandiflora* have an abaxial epidermis with a continuous wax layer without any crystalloids or sculptures. The adaxial epidermis also shows a continuous wax layer but with little irregular granular sculptures.

L. tulipifera leaves show an abaxial epidermis with a continuous wax layer superimposed by a dense arrangement of crystalloids in shape of angular rodlets which are composed of several piled up layers. The adaxial leaf surface is also superimposed with wax crystalloids, the rodlets of which, however, are not sculptured in such definite way. They usually appear melted up and also form granular sculptures. The wax crystalloids in shape of angular rodlets on the abaxial surface are formed by hentriacontan-16-one. The abaxial and adaxial leaf surfaces show most different micromorphological wax ultrastructures, as shown for all trees studied.

Introduction

The maidenhair tree (*Ginkgo biloba* L.) is the only living representative of a large ancient order of conifer-like trees. The genus *Ginkgo* enjoyed a world wide distribution and the species were important constituents of the flora during the Mesozoic era. The leaves are of special interest, having a wedgeshaped leaf blade borne on a long stalke (petiole). The venation is an open forking system with numerous dichotomously branching veins [1, 2]. Leaf wax of *G. biloba* was first described by Ageta (1959) to contain about 10% free fatty acids, 15% esters, 75% paraffine and wax alcohols [3–5]. The main component, called "Ginnol", is the secondary alcohol nonacosan-10-ol.

Plants belonging to the family of Magnoliaceae are trees or shrubs with alternate, simple, deciduous leaves and actinomorphic, hermaphrodite large and solitary blossoms. The genera *Magnolia* and *Liriodendron* are closely related and show many primitive marks. They form the basis of the

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

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developmental steps in the group of the Angiosperms. The Magnoliaceae are native in southeast Asia and America, and today species of the genera *Magnolia* and *Liriodendron* are cultivated as most popular ornamental trees in the northern Hemisphere [1, 6, 7].

In the present investigation, the epicuticular waxes of mature leaves are analyzed in detail and correlated with the surface structures of these waxes by scanning electron microscopy. This way, the systematic relationship of these plant genera shall be proved by comparison of their epicuticular wax lipids.

Materials and Methods

Leaves of *Ginkgo biloba* L., *Magnolia grandiflora* L. and *Liriodendron tulipifera* L. were harvested in August 1990 from trees cultivated in the garden of the Botanical Institute, University of Cologne. The surface waxes were extracted from fresh leaves by immersion in $CHCl_3$ (twice for 1 min). Waxes were separated by CC on silica gel, eluting in three fractions with *n*-pentane, 2-chloropropane and methanol as solvents. Subsequently the constituents were analyzed by TLC with toluene as solvent and by GC as described previously [8, 9]. The quantitative composition of the waxes is listed in Tables I–III as mean values of two preparations.

GC-MS analyses were performed on a DB-1 fused silica capillary column (15 m). This way, the following substances were identified:

Nonacosan-10-ol, *m/z* 424 (M^+) [10];
γ-Tocopherol, *m/z* 416 (M^+) [11];
Hentriacontan-16-one, *m/z* 450 (M^+) [12–15];
β-Sitosterol, *m/z* 414 (M^+) [9];
β-Amyrin, *m/z* 426 (M^+) [8, 9];
α-Amyrin and Lupeol, *m/z* 426 (M^+) [8, 9],
Friedelanol, *m/z* 428 (M^+) [9];
Friedelanone, *m/z* 426 (M^+) [9];

Peak A (scan 966), *m/z* (rel. int.), 428 (1, M^+); 410 (4, M^+-15); 395 (1); 339 (2); 321 (2); 307 (2); 189 (10).

Peak B (scan 983), *m/z* (rel. int.), 440 (5, M^+); 425 (1, M^+-15); 273 (40); 232 (50); 217 (10); 207 (10), 189 (1); 175 (20).

Peak C (scan 1002), *m/z* (rel. int.), 442 (5, M^+); 427 (2, M^+-15); 251 (1); 234 (40); 221 (20); 219 (20); 207 (50); 189 (20); 177 (20).

Peak D (scan 1025), *m/z* (rel. int.), 448 (M^+); 399 (2.5); 321 (5); 307 (2.5); 239 (10).

Air dried leaves for SEM were prepared by sputtering with gold using an Emscope sputter coater and examined in a Hitachi S-405 A, a Hitachi S-520 or a Zeiss DSM 940 scanning electron microscope at 15 or 20 kV, respectively.

Results

Wax composition of G. biloba

From fresh leaves (188 g) of *G. biloba* were extracted epicuticular waxes (293 mg) in a concentration of about 0.84% of the leaf dry wt. Thus, 36.4 μg wax per cm^2 leaf surface area or 1477 μg per one leaf was found. This wax consisted only of wax lipids and of no triterpenoids. Main components were fatty acids (38.6%), primary alcohols (25.9%) and nonacosan-10-ol (15.0%). The yield and composition of the individual lipid classes are listed in Table I. The compositions of the homologous series are summarized in Table II.

The hydrocarbon fraction (1%) consisted of homologous series of *n*-alkanes ranging from C_{19} to C_{35} without any dominating main component. Heptacosane (38.1%), pentacosane (17.4%) and tricosane (12.5%) were present in higher amounts. The remarkable feature is the presence of even numbered *n*-alkanes up to 22.5%. Traces of branched or unsaturated alkanes were also observed by GC.

Homologous wax esters of long chain fatty acids with long chain alcohols were present only in 0.7% of wax with chain lengths ranging from C_{36} to C_{50}. The wax esters with chain lengths of C_{44} (30.5%), C_{42} (23.5%), C_{40} (19.5%) and C_{38} (16.7%) are the major constituents.

Esters of benzyl alcohol with the very long chain fatty acids C_{24}, C_{26}, C_{28}, and C_{30} were found in an amount of 2.4%. Similar homologous series of benzyl acyl esters were discovered earlier in leaf waxes of Jojoba [16], *Fagus* [17], and *Acer* [18]. These esters were isolated by preparative TLC with toluene as solvent and identified by their mass spectra showing the characteristic fragments *m/z* (rel. int.) 91 (100) and 108 (70).

Aldehydes (4.8%) were detected in a homologous series with chain lengths ranging from C_{20} to C_{32}, with C_{28} as main component in a concentration of only 42%. Mass spectra of aldehydes

Table I. Composition and yield of epicuticular leaf waxes from *Ginkgo biloba, Magnolia grandiflora* and *Liriodendron tulipifera.*

Components	R_f	*G. biloba*			*M. grandiflora*			*L. tulipifera*		
		mg	% Wax	% dry wt.	mg	% Wax	% dry wt.	mg	% Wax	% dry wt.
Hydrocarbons	0.7	3	1.0	0.009	12	7	0.009	24	8	0.07
Wax esters	0.6	2	0.7	0.006	7	4	0.004	20	7	0.06
Benzyl acyl esters	0.55	7	2.4	0.020	3	2	0.002	3	1	0.01
Hentriacontan-16-one	0.5	–	–	–	–	–	–	67	23	0.21
Aldehydes	0.4	14	4.8	0.040	22	13	0.017	36	12	0.11
Acetates	0.3	1	0.3	0.003	–	–	–	–	–	–
Nonacosanol-10-ol	0.2	44	15.0	0.126	–	–	–	–	–	–
γ-Tocopherol	0.15	5	1.7	0.014	–	–	–	–	–	–
Alcohols	0.06	76	25.9	0.217	49	28	0.037	41	13	0.12
Triterpenols	0.06	–	–	–	–	–	–	30	10	0.09
β-Sitosterol	0.06	–	–	–	–	–	–	3	1	0.01
Fatty acids	0.02	113	38.6	0.323	61	35	0.046	69	23	0.21
Not identified and		17	5.8	0.049	–	–	–	–	-	–
Lost on column		11	3.8	0.031	19	11	0.014	10	3	0.03
		293	100.0	0.838	173	100.0	0.129	305	100.0	0.92

Table II. Composition (peak area %) of hydrocarbons, benzyl acyl esters, aldehydes, acetates, primary alcohols, fatty acids and wax esters of epicuticular leaf wax from *Ginkgo biloba.*

Carbon No.	Hydro-carbons	Benzyl acyl esters	Aldehydes	Acetates	Primary alcohols	Fatty acids	Carbon No.	Wax esters
14						0.5	36	+
16					0.6	8.7	38	16.7
18					+	2.2 ×	40	19.5
19	0.2						41	+
20	0.3		0.5		0.8	1.1	42	23.5
21	0.8		+		0.3	0.9	43	5.0
22	1.7		0.5	+	1.1	3.3	44	30.5
23	12.5		1.5		0.3	1.1	45	+
24	8.6		3.8	4.7	4.6	16.2	46	4.8
25	17.4		5.6	+	1.0	3.2	47	
26	7.8		18.4	12.6	19.5	20.0	48	+
27	38.1		11.8	+	7.0	4.2	49	
28	2.3		42.9	73.1	46.8	25.0	50	+
29	6.4		8.4	+	2.9	1.5		
30	1.3		6.6	9.6	10.8	11.8		
31	1.9	14.2			0.7	0.2		
32	0.4		+	+	3.6	1.1		
33	0.2	33.6						
34	0.1							
35	+	36.5						
36								
37		15.7						

× Contains unsaturated fatty acids.

showed characteristic fragments at *m/z* (rel. int.) 82 (50) and M^+-18.

Acetates (0.3%) of long chain alcohols are found in small amounts with chain lengths from C_{22} to C_{32}. The acetate of the alcohol C_{28} is predominant in this lipid class. In their mass spectra the acetates show typical fragments at *m/z* (rel. int.) 83 (40) and M^+-60.

Nonacosan-10-ol is present in the high amount of 15% of the wax. This secondary alcohol was isolated by TLC with toluene as solvent in a purity of about 95%, showing a R_f-value of 0.2. The com-

pound was identified by GC-MS, the fragments at *m/z* (rel. int.) 157 (20) and 297 (10) are characteristic for the position of the hydroxy group in position Δ 10 [10].

γ-Tocopherol is a very unusual component of waxes and here its identification in leaf waxes is described for the first time. This substance was also isolated by preparative TLC with toluene as solvent (R_f-value 0.15) in a high purity of more than 95%. The mass spectra showed the fragments *m/z* (rel. int.) 151 (100), 191 (20), and 416 (40, M^+) identical with literature values of γ-tocopherol [11].

Primary alcohols (25.9%) form a homologous series with chain lengths ranging from C_{16} to C_{34} and the main component is C_{28} in a concentration of 46.8%. Triterpenols or their derivatives were not found in this wax. None of the fractions showed a positive reaction with carbazole [19].

Remarkably, free fatty acids account for 38.6% of the wax. They were found in homologous series with chain lengths from C_{14} to C_{34}, but no main component is dominating. Fatty acids with C_{24}, C_{26}, and C_{28} were found in equal amounts of about 20%. Interestingly, the C_{18}-acids were present in saturated and unsaturated form (Table II).

Wax composition of M. grandiflora

The extractable epicuticular wax (173 mg) of *M. grandiflora* leaves (301 g) amounted to 0.13% of the dry wt, which calculates for 10.6 μg wax per cm^2 surface area or 1383 μg wax per leaf. This wax of mature leaves consisted only of homologous series of wax lipids. Main lipid classes were long chain fatty acids (35% of the wax) and long chain primary alcohols (28%) followed by aldehydes (13%), hydrocarbons (7%), wax esters (4%) and benzyl acyl esters (2%). Yield and composition of these lipids are listed in Table I. The compositions of the homologous series are summarized in Table III.

The hydrocarbon fraction consisted of *n*-alkanes possessing chain lengths ranging from C_{19} to C_{33} with no main component dominating. Nonacosane and heptacosane are most abundant with about 33% and 21%, respectively. Even numbered *n*-alkanes reach the remarkable value of about 21%.

Homologous wax esters of long chain fatty acids with long chain alcohols were present showing chain lengths from C_{36} to C_{52}. Esters with carbon numbers C_{46} and C_{48} are predominant in similar concentrations of about 30%.

Table III. Composition (peak area %) of hydrocarbons, benzyl acyl esters, aldehydes, primary alcohols, fatty acids, and wax esters of epicuticular leaf waxes of *Magnolia grandiflora* and *Liriodendron tulipifera*.

Carbon No.	Hydrocarbon		Benzyl acyl esters		Aldehydes		Primary alcohols		Fatty acids		Carbon No.	Wax esters	
	M. g.	L. t.	M. g.	L. t.	M. g.	L. t.	M. g.	L. t.	M. g.	L. t.		M. g.	L. t.
14									0.5	1.3	36	+	+
16							0.4	0.9	3.4	4.5	38	2.6	2.5
18							0.2	+	4.3	1.3	39	0.5	0.7
19	0.1	+							0.6		40	3.0	11.9
20	0.1				0.5	+	+	+	1.4	3.0	41	0.5	1.2
21	0.5	0.1			0.9	0.4	0.2		1.2	0.2	42	4.5	25.9
22	2.0	0.1			1.3	0.8	2.7	1.9	3.9	2.5	43	1.0	1.5
23	7.9	1.5			2.9	0.6	0.1	0.5	2.9	1.2	44	15.0	27.6
24	4.1	1.3			9.8	5.3	35.3	9.3	21.2	15.1	45	2.0	1.2
25	10.7	6.2			6.9	3.5	1.2	2.5	4.2	3.5	46	30.0	14.7
26	5.3	2.4			13.0	20.5	27.8	28.8	12.6	17.4	47	3.2	1.0
27	20.8	34.9			5.8	5.7	0.5	3.9	3.8	3.9	48	29.0	10.7
28	6.7	3.8			19.5	26.0	25.9	33.4	15.4	22.0	49	+	+
29	32.8	42.9			9.4	2.9	0.5	2.7	3.5	+	50	8.0	1.2
30	2.6	1.8			20.6	22.7	4.7	12.5	16.5	17.2	51		
31	6.1	4.7	39.1	48.8	+	5.7	0.2	0.3	1.2	+	52	+	+
32	0.1	0.2			6.1	5.9	0.3	3.2	3.4	5.3			
33	0.2	0.1	37.7	21.6	+	+			+	+			
34					3.2	+	+	+	0.2	0.4			
35			23.2	21.6									
37			+	8.0									

Esters of benzyl alcohol with the very long fatty acids C_{24}, C_{26}, C_{28}, and C_{30} were also present.

Aldehydes were found in homologous series with chain lengths ranging from C_{20} to C_{34}. The main components with chain lengths of C_{24}, C_{26}, C_{28}, and C_{30} were present in similar concentrations of about 10 to 20%, each.

Primary alcohols formed a homologous series with chain lengths ranging from C_{16} to C_{34} without any dominating component. Main compounds were the C_{24}, C_{26}, and C_{28} alcohols, each present with more than 20%. No triterpenols or their derivatives were found in the wax of *M. grandiflora.*

Free fatty acids amounted to 35% of the wax, much more than usual. Again no dominating component was present. However, in the homologous series from C_{14} to C_{34} the acids C_{24}, C_{26}, C_{28}, and C_{30} accounted for more than 10%, each.

Wax composition of L. tulipifera

The epicuticular wax (305 mg) extracted from fresh leaves (103 g) of *L. tulipifera* was found in a concentration of about 0.92% of the dry wt. Thus, 30.6 µg wax per cm^2 leaf surface area or 5073 µg wax per one leaf are present. The wax of *L. tulipifera* contained, like the wax of *M. grandiflora*, homologous series of the same long chained wax lipids in identical chain lengths and similar concentrations: fatty acids (23% of the wax), alcohols (23%), aldehydes (12%), hydrocarbons (8%), wax esters (7%) and benzyl acyl esters (1%). All these lipid classes, again, have not dominating main component (see Tables I and III). In addition to these usual wax lipids, in the epicuticular wax of *L. tulipifera* hentriacontan-16-one (23% of the wax), called palmitone, and several triterpenols (11%) were found.

Hentriacontan-16-one was isolated by preparative TLC in a purity of more than 95%. This substance was identified by GC-MS and comparison with an authentic sample (palmitone). The mass spectrum showed the characteristic fragment *m/z* 239, which is an indication for the keto group in position Δ 16. Palmitone has been found in several plants before *e.g.* in waxes of several *Brassica* species [13–15] leaves of *Annona muricata* (25%, of the wax), *Aristolochia arborea* (35%) and *Umbellaria californica* (20%) [20].

Of the triterpenols present in the wax of *L. tulipifera* β-amyrin, α-amyrin and lupeol were dominating in a relative ratio of 1:2:2. We are not able to separate α-amyrin and lupeol by GC. They were, however, unambiguously identified by their mixed mass spectrum. These triterpenols show a positive colour reaction with carbazol, have discrete RT in GC and characteristic fragments in the mass spectra [8, 9]. Furthermore, friedelanol and friedelanone were identified by GC-MS and comparison with authentic samples [9]. The latter were found in concentrations of about 1% of the wax. Traces of four as yet unidentified triterpenols with longer RT than friedelanone were also found. The fragments of their mass spectra are listed in materials and methods.

In small amounts (1%) β-sitosterol was present in the wax of *L. tulipifera,* which has been shown to be constituent of the waxes of *Citrus* [9], *Tilia* [21] and *Acer* [18].

Wax ultrastructures

SEM figures of a mature *Ginkgo* leaf showed a fan shaped foliage with an open dichotome venation. A continuous wax layer could be observed on the lower (abaxial) as well as on the upper (adaxial) leaf surface with a SEM at a magnification of 250. This wax layer was washed off with $CHCl_3$ and analyzed as described above. Stomata and many cuticular foldings were only observed on the abaxial side of the leaf (Fig. 1 A), whereas the adaxial epidermal cells appeared long stretched and not as wrinkled as the abaxial epidermis (Fig. 1 B). A higher magnification revealed that the wax layer was superimposed with a dense arrangement of crystalloids on both leaf sides (Fig. 1 C and D). At a magnification of 10,000 the shape of these crystalloids appeared round and rodlike but were identified to be tubules at a magnification of 20,000. At this rather high magnification the openings of the tubules could be clearly demonstrated on both leaf surfaces (Fig. 2, A and B). The tubules were 500 to 1000 nm long and 100 nm wide (outer diameter).

SEM figures of mature *Magnolia* leaves show a dense arrangement of singular trichomes and stomata on the lower (abaxial) surface. The epidermal cells are covered with a continuous wax layer without any sculptures (Fig. 3, A and C). On the upper (adaxial) leaf side no trichomes exist. The

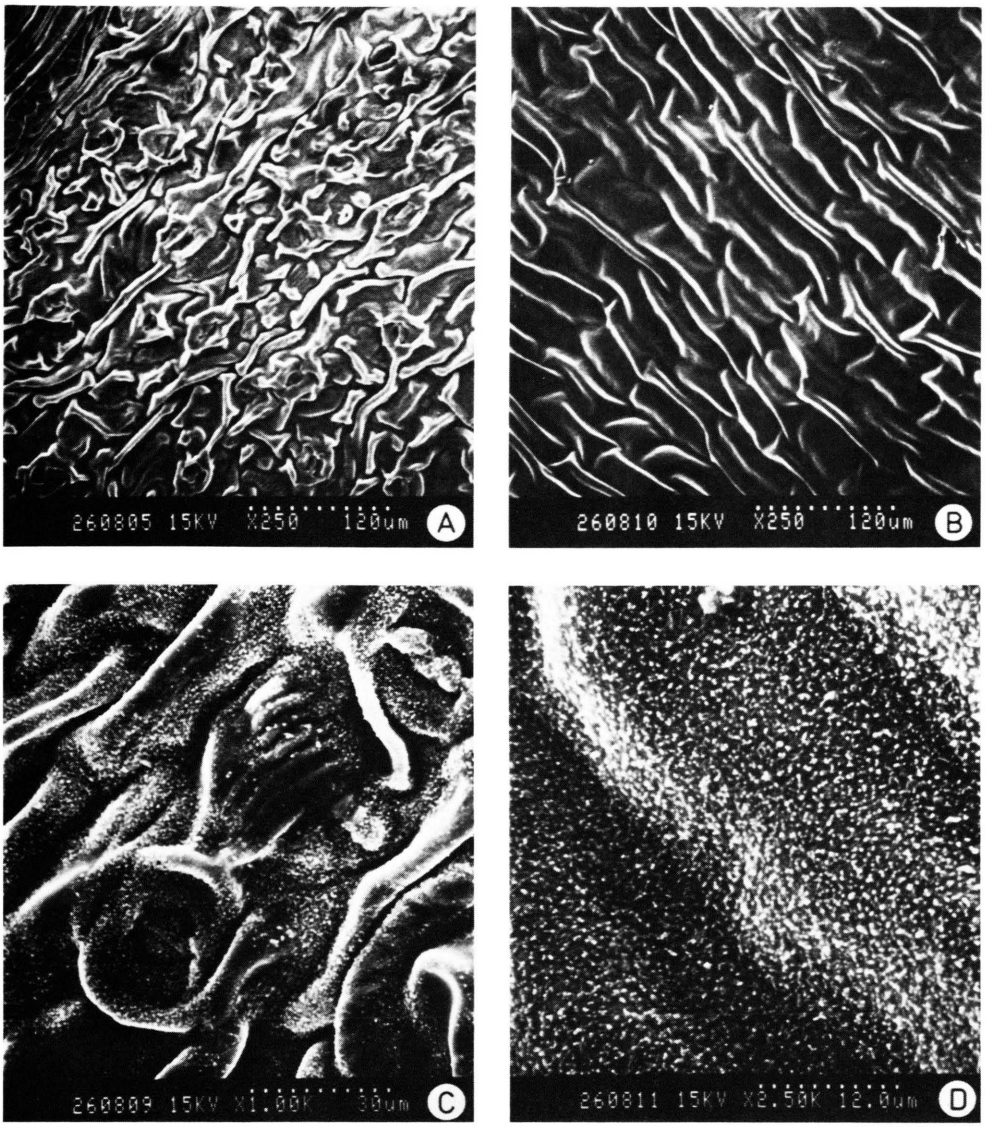

Fig. 1. *Ginkgo biloba* surface structures from a mature leaf.
A. Abaxial epidermis with a continuous wax layer, stomata and cuticular lamellae. Bar = 120 μm.
B. Adaxial leaf epidermis covered with a continuous wax layer on long stretched epidermal cells. Bar = 120 μm.
C. Abaxial leaf epidermis covered with a dense arrangement of wax crystalloids. Bar = 30 μm.
D. Arrangement of wax crystalloids on the adaxial leaf surface. Bar = 30 μm.

continuous wax layer is superimposed with irregular scales or granular sculptures (Fig. 3, B and D). The abaxial and adaxial leaf surfaces were found to develop different surface wax structures.

SEM figures of mature *Liriodendron* leaves show many cuticular papillae and stomata on the lower (abaxial) leaf surfaces. The continuous wax layer shows a dense arrangement of crystalloids, which are also distributed over the stomata (Fig. 4, A and B). The ultrastructure of these crystalloids becomes visible with a magnification of 20,000-fold. They form rather small angular shaped rod-

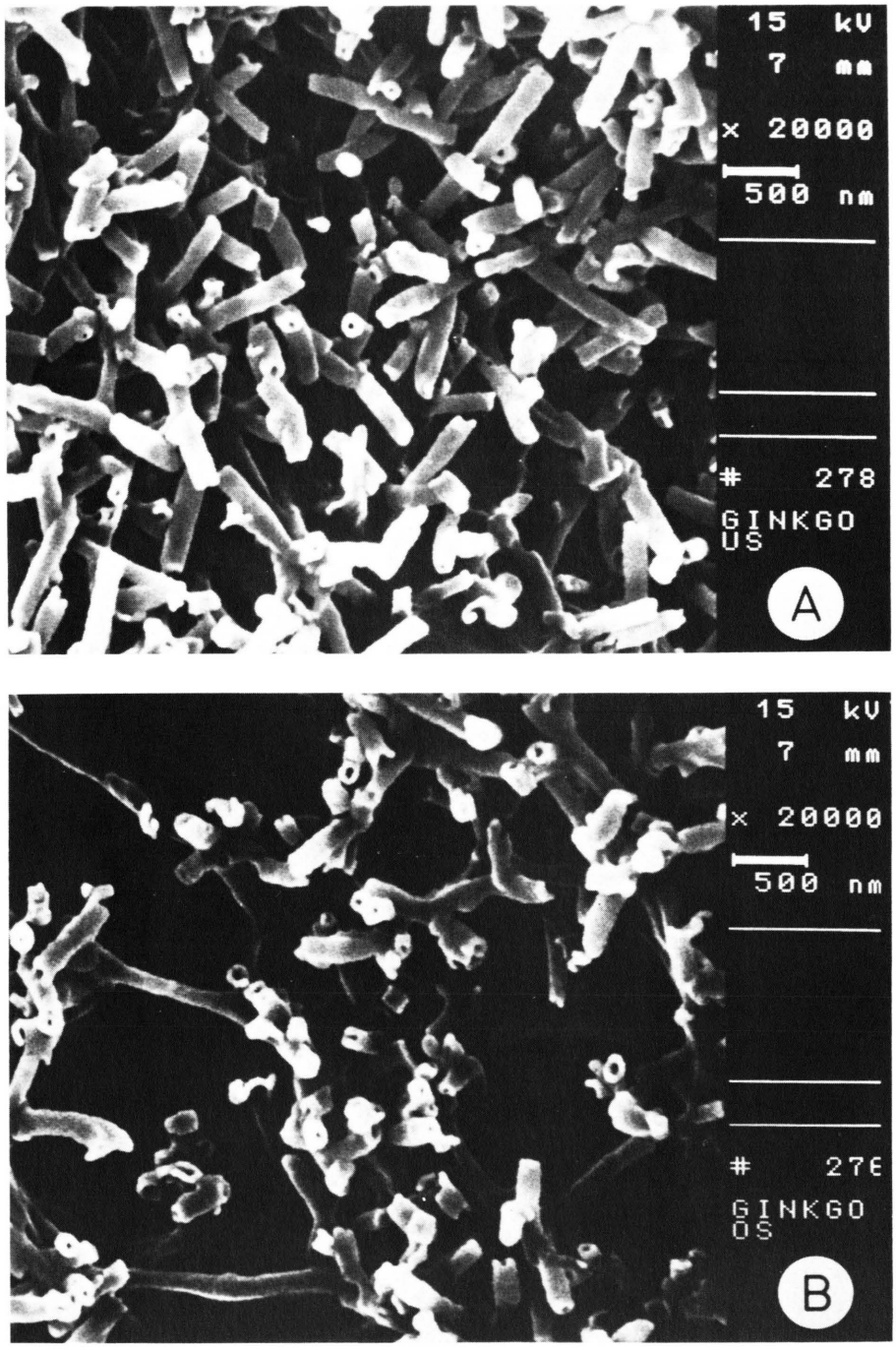

Fig. 2. *Ginkgo biloba* surface structures of a mature leaf at a magnification of 20,000.
A. Abaxial leaf surface with a dense arrangement of small tubules, the openings of the tubules are clearly visible. Bar = 500 nm.
B. Adaxial epidermis with a comparable arrangement of small tubules. Bar = 500 nm.

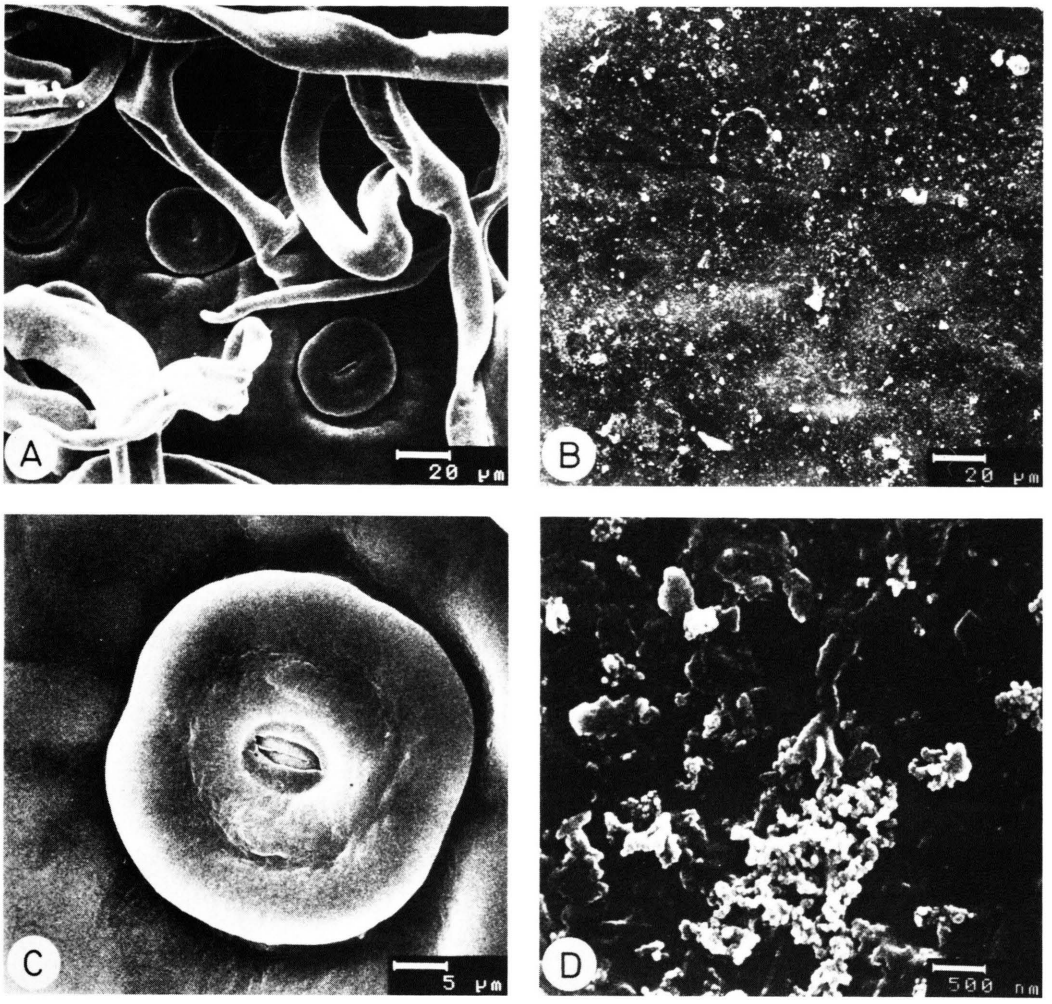

Fig. 3. *Magnolia grandiflora* surface structures of a mature leaf.
A. Abaxial epidermis with a continuous wax layer, singular trichomes and stomata. Bar = 20 µm.
B. Adaxial epidermis with a continuous wax layer and irregular sculptures. Bar = 20 µm.
C. Abaxial leaf surface around a stoma without any wax sculptures or crystalloids. Bar = 5 µm.
D. Adaxial leaf surface with irregular wax sculptures in granular shapes. Bar = 500 nm.

lets, which consist of several piled up layers or may be described as cross rippled rodlets (Fig. 4C). The rodlets have a length of 500–1000 nm and a width of 200 nm (outer diameter). The upper (adaxial) leaf side did not show structures as the abaxial epidermis. The adaxial epidermal cells are rather plain but are also covered with a continuous wax layer and a dense arrangement of wax crystalloids. These crystalloids do not have the same definite ultrastructure as those of the abaxial surface. In contrast to the single sculptured rodlets of the abaxial surface, the adaxial crystalloids usually appeared melted up or melted together without any clearly visible structure, or were found as granular sculptures (Fig. 4D).

Discussion

Wax lipids and taxonomic aspects

G. biloba leaf wax contained the usual very long chained wax lipids of deciduous broadleaf trees in form of homologous series of hydrocarbons, wax

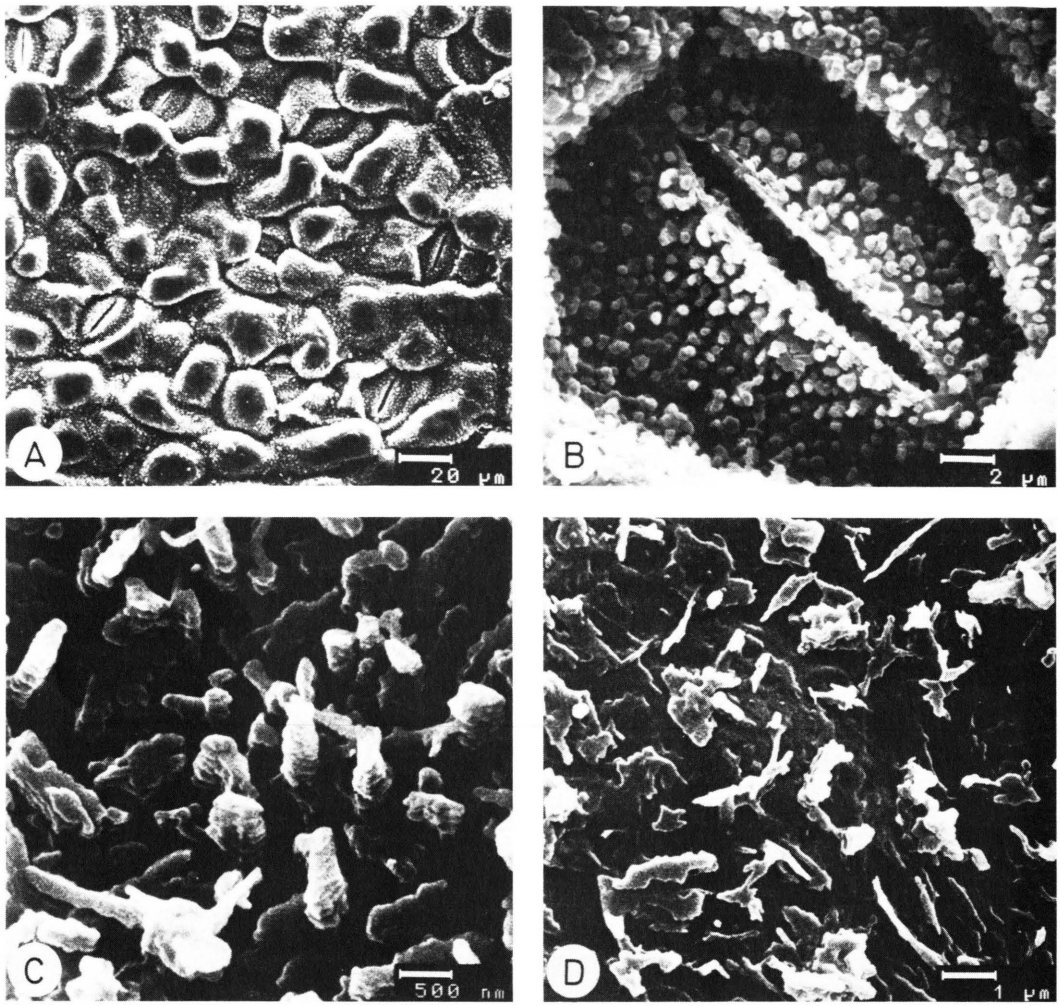

Fig. 4. *Liriodendron tulipifera* surface structures from a mature leaf.
A. Abaxial epidermis with many cuticular papillae, stomata and covered with a continuous wax layer superimposed with wax crystalloids. Bar = 20 µm.
B. Dense and regular arrangement of wax crystalloids around a stoma. Bar = 2 µm.
C. Wax crystalloids in shape of angular rodlets consisting of piled up layers on the abaxial leaf surface. Bar = 500 nm.
D. Wax crystalloids on the adaxial leaf surface in shape of irregular and melted rodlets. Bar = 1 µm.

esters, benzyl acyl esters, aldehydes, acetates, primary alcohols and fatty acids. None of these lipid classes revealed a dominating main component. Therefore, none of these lipids is responsible for the observed wax crystalloids. In addition to these lipids, about 15% nonacosan-10-ol was present in *Ginkgo* leaf wax. This secondary alcohol, with the hydroxy group in position Δ 10, is a characteristic component of waxes of all conifer needles analyzed so far [22].

It was found in many species of *Abies* [23, 24], *Picea* [5, 10, 23, 25, 26], *Pinus* [25, 27], *Chamaecyparis* [4, 5] and *Juniperus* [4, 28]. Secondary alcohols, mostly with hydroxy groups in positions other than Δ 10, were also reported for Papaveraceae, Ranunculaceae, Liliaceae, Rosaceae and Compositae [5, 29, 30]. The presence of nonacosan-10-ol in *Ginkgo* leaf wax demonstrates the close phylogenetic relationship of this plant with the Gymnosperms. Nonacosan-10-ol and homologues fatty

acids are the only wax lipids which are in common with these plant families. The composition of coniferyl waxes is quite different from that of *Ginkgo* and deciduous trees. Coniferyl waxes contain high amounts of ω-hydroxy fatty acids, α,ω-diols, and estolites in addition to nonacosan-10-ol and usual fatty acids in species specific compositions [4, 5, 10, 22–30].

Magnolia and *Liriodendron* are systematically closely related plant genera. However, they show rather different wax compositions. Both waxes contain the usual long chain wax lipids, namely hydrocarbons, wax esters, benzyl acyl esters, aldehydes, primary alcohols and fatty acids in form of homologous series with no main component dominating. They differ, however, in the presence of other wax compounds. Thus, the wax of *M. grandiflora* consists only of the above listed lipid classes, while *L. tulipifera* wax, additionally contains hentriacontan-16-one and several triterpenols. β-Amyrin, α-amyrin and lupeol are found very often in epicuticular waxes [8, 9, 37, 38], while friedelanol and friedelanone are present very rarely in leaf waxes [9, 38]. In addition, four triterpenols yet unidentified were also found in this wax. Hentriacontan-16-one (palmitone) was detected already in 1934 by Chibnal [13] and found in several *Brassica* species [13–15] and plants of the genera *Annona, Aristolochia* and *Umbellaria* [20].

Nonacosan-10-ol forms tubules

Both surfaces (abaxial and adaxial) of *G. biloba* leaves are densely covered with wax crystalloids as shown by SEM. These wax crystalloids have the shape of round tubules (Fig. 2, A and B). The openings of these tubules could clearly be seen in the SEM on both leaf surfaces at the rather high magnification of 20,000. Similar tubules as on *Ginkgo* leaves [31] were also found on surface of fruits of *G. biloba* [32]. The same tubules as on *Ginkgo* leaves were found on all young conifer needles as shown by SEM for Pinaceae, Cupressaceae and Taxaceae [33–36]. The genera of the Ranunculales and Papaverales, studied so far, have also these characteristic wax tubules, which are formed by the secondary alcohol nonacosan-10-ol [20]. In all conifer plants and also in *Ginkgo* a clear correlation between the presence of nonacosan-10-ol and the occurrence of small crystalline wax tubules on the leaf surfaces was observed [5].

Hentriacontan-16-one forms angular rodlets

Even distribution patterns of wax lipids result in continuous wax layers without any wax crystalloids as found on the surface of *M. grandiflora.* Usually wax crystalloids can be observed, if one lipid class is present in concentrations of about 40% of the total wax and one main component dominating with more than 80%. For example, in *Tilia* wax [39, 40] β-amyrenyl acetate was found amounting to 44% of the wax, which formed quadrangular rodlets. In *Quercus* wax [41], alcohols were present of about 40% and tetracosanol is dominating with more than 90%, forming a dense arrangement of platelets. Triterpenols are not very suitable for crystallization from wax layers, since usually mixtures of these substances are observed. Thus, in wax of *L. tulipifera* β-amyrin, α-amyrin and lupeol are found in almost equal amounts and in addition other triterpenols are also present. Similar mixtures of triterpenols are found in waxes of *Castanea sativa* and *Aesculus hippocastanum* [38], too. Hentriacontan-16-one (palmitone) also tends to crystallize and forms crystalloids in shape of most angular rodlets, consisting of several piled up layers thus leading to a cross rippled surface structure (Fig. 4). In *L. tulipifera* wax these crystalloids are found corresponding to a concentration of 23% of hentriacontan-16-one. This type of wax crystalloids in shape of transversally ridged rodlets is called "Aristolochia Type" by Barthlott [42] and is found primarily in numerous genera of the Magnoliidae according to Dahlgren's system of classification of Angiosperms [43, 44]. Even Magnoliales have variable surface structures as shown in this paper for *M. grandiflora* and *L. tulipifera.* The wax crystalloids described above were found within the Magnoliaceae in the species *M. kobus* var. *stellata, M.* × *wattsonia* and in the genus *Liriodendron* but not in *M. grandiflora.* Within the Annonaceae these rodlets were found in all species of the genus *Annona,* within the Lauraceae in all genera and within the Aristolochiaceae especially in the genus *Apoma* and *Aristolochia* [20, 42].

Conclusion

Some compounds are much more suitable for crystallization than others. Thus, nonacosan-10-ol forms crystalloids in shape of tubules, already in a

concentration of 15% of the total wax in *G. biloba* and in all conifer needles. The presence of hentriacontan-16-one in leaf waxes in a concentration of 20% and more is always correlated with the same wax ultrastructure in shape of angular rodlets consisting of several piled up layers. Possibly, these compounds are not very soluble in the mixture of other wax lipids. These two lipids may be examples of a close correlation between special compounds or classes of compounds and the specific micromorphology of wax crystalloids they form.

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Note added in proof: "Components of *Ginkgo biloba* leaf wax" were also studied by H. L. Casal and P. Moyna, Phytochemistry **18,** 1738–1739 (1979).

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