Organ Specific Composition of Epicuticular Waxes of *Cistus albidus* L., Cistaceae

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Cistus albidus, Epicuticular Wax Composition, Organ Specific Patterns

Epicuticular waxes from various organs of *Cistus albidus* L. showed always an organ specific composition.

Leaf wax contained homologous series of very long chained and saturated wax components, such as hydrocarbons, wax esters, fatty acids and alcohols. Additionally in this wax extract triterpenoids and great amounts of resin were found. Sepal wax had nearly the same wax composition like that of leaves with a trend to shorter chain lengths and not so great amounts of resin.

Petal wax contained the common wax lipids, too, but the homologous series showed a shift to shorter chain lengths, and great amounts of unsaturated lipids were found in hydrocarbons, wax esters and fatty acids. In addition triterpenol esters were found but no resin.

Stamen wax was similar to that of petals. The shift to shorter chain lengths and the presence of great amounts of unsaturated lipids showed a characteristic distribution for stamens.

Seed wax contained only saturated wax lipids. In contrast to leaf waxes, the short chain compounds were dominating, and triterpenoids and resin were missing.

The chemical wax composition of the various organs of *C. albidus* was correlated to the surface structures of these organs by SEM pictures.

Introduction

All aerial organs of higher plants are covered with a thin continuous wax layer. Often these wax layers are of fluid consistancy, for example on petals and seeds. But on leaves this wax layer is most superimposed by crystalline structures of very different geometrical forms [1–3]. The chemical composition of these epicuticular waxes is species specific [4, 5] and in addition several hints in literature show also organ specific wax patterns [6–16]. *C. albidus* L. [17] is a perennial evergreen scrub and a characteristic element of the macchias and garigues of the Mediterranean region. This plant was used for many studies concerning epicuticular waxes [15, 16, 18–21] and flavonoids [22–24]. Therefore, this *Cistus* species was selected to compare the wax composition of five different aerial organs such as leaves, sepals, petals, stamens and seed coats from the same plant in one vegetation period under reproducible conditions.

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Materials and Methods

Plant materials

Plants of *C. albidus* were grown from seeds and cultivated under equal environmental conditions in the fields of the Botanical Institute of the University of Cologne. Three plants of different origin were used for parallel studies. Quantitative and qualitative compositions of the epicuticular waxes were analyzed from leaves, sepals, petals, stamens, and seed coats.

Methods

Epicuticular waxes were extracted from leaves, sepals, petals, and stamens with chloroform. They were dipped consecutively into three beakers of chloroform for a total of 3 min. Seeds were treated with hexane in the same manner. The wax extraction was completely, as shown by SEM (see Fig. 9). The raw wax was redissolved in pentane and the soluble parts were chromatographed on a silica gel column (Merck 60, Darmstadt) with the following solvents of increasing polarity: 1. pentane to elute hydrocar-

bons, 2. 2-chloropropane to elute wax esters and 3. methanol to elute alcohols and fatty acids.

These fractions were checked by TLC, GC and chemical reactions as described previously [25–27]. For TLC, precoated silica gel plates (Merck 60, Darmstadt) were used. The solvent system was toluene for R_{f1}, dichloromethane:ethyl acetate (24:1) for R_{f2}. Plates, impregnated with silver nitrate in acetonitrile, were run in dichloromethane:ethyl acetate (24:1) for R_{f3}. As spray reagents were taken bromothymol blue and carbazole for triterpenoids and sterols [28].

For gas chromatography a Hewlett Packard model 5710 A with FID and integrator 3380 S was used for most substances. A 25 m glass capillary column DUHT-OV 101 was programmed from 160–340 °C with 4 °C/min advance. A 10 m glass capillary column FFAP was used for fatty acid methyl esters, temp. 180–280 °C, rate 4 °C/min.

The surface of leaves, sepals, petals, stamens and seed coats were studied with a scanning electron microscope, type Philips PSME 500. The fresh plant material was sputtered with gold. Pictures were taken with a polaroid camera.

Results

Epicuticular waxes were extracted from the various organs of *C. albidus* in very different amounts. Leaves resulted in 3.6% epicuticular extract/dry weight, sepals in similar amounts, but petals contained only 0.6% wax/dry weight, stamens 1.0% and seeds only 0.1%. Fig. 1 demonstrates these correlations and shows also the relation of wax layer to the surface of these organs. Seeds and petals have the thinnest wax layers, whereas sepals and especially leaves show multiple values, because their extracts contained not only the common epicuticular wax components but also great amounts of resin (about 50–60%). This is a characteristic observation of most *Cistus* leaves [24] and demonstrated in Fig. 1. Leaves had an extractable epicuticular layer of about 200 $\mu g \times cm^{-2}$, petals of 5 $\mu g \times cm^{-2}$, and seeds of 13 $\mu g \times cm^{-2}$. Plants of arid regions showed often similar leaf wax layers including great amounts of resin. Thick deposits of 60–300 $\mu g \times cm^{-2}$ have been found on leaves of the Mediterranean plants *Ceratonia siliqua, Pistacia lentiscus*, and *Olea europaea* [5].

All waxes of the *C. albidus* organs studied contained the following components: hydrocarbons, wax esters, fatty acids, alcohols and, with the exception of seeds, triterpenoids. Leaves and sepals showed in addition great amounts of resin, see Table I. The waxes from all organs of *C. albidus* showed a composition of long chained lipids with characteristic and specific patterns for each organ.

Hydrocarbons

Hydrocarbons were found in all organ waxes in form of homologous series with prevailing odd carbon numbers. The composition of these hydrocarbons was different in their chain length maxima and the occurrence of unsaturated compounds, see Fig. 2. Leaves contained alkanes, with chain lengths ranging from C_{19} to C_{37} with one dominating compound C_{29} of more than 50% [15]. Sepals had nearly the same alkane composition, but their distribution pattern is not so steep as from leaves, and unsaturated hydrocarbons were found in amounts of 3%. Petals showed a quite different distribution pattern.

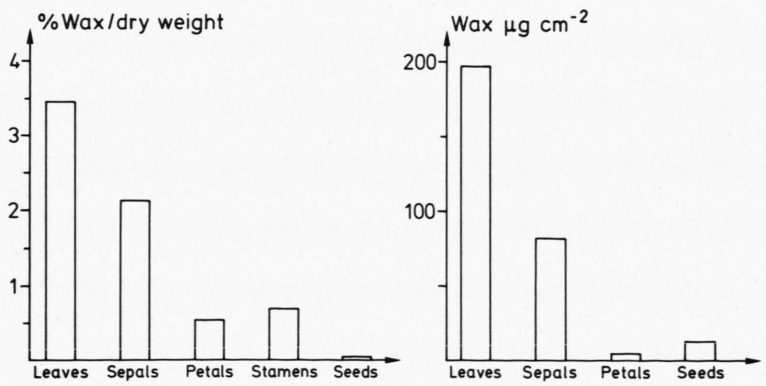


Fig. 1. Yield of epicuticular waxes from various organs of *C. albidus:* A) in relation to the dry weight (% dry wt.) and B) in relation to the surface ($\mu g \times cm^{-2}$).

Table I. Composition and yield of epicuticular waxes from various organs of *C. albidus*.

	Leaves		Sepals		Petals		Stamens		Seeds	
	mg wax	% wax	mg wax	% wax	mg wax	% wax	mg wax	% wax	mg wax	% wax
Hydrocarbons	282	9.4	51	9.1	103	31.2	17	30.9	9	18.0
Wax esters	488	16.3	141	22.0	86	26.1	7	12.2	17	34.0
Triterpenol esters	–	–	–	–	15	4.6	2	4.1	–	–
Unidentified	54	1.8	9	1.3	3	0.6	9	16.4	5	9.2
Fatty acids	144	4.8	30	4.5	15	4.6	3	5.4	15	30.0
Alcohols	16	0.5	3	0.4	4	1.1	2	3.9	1	2.6
Resin + triterpenoids	1731	57.7	327	51.1	71	21.6	1	1.8	–	–
Lost on column	285	9.5	79	12.3	33	10.0	14	25.5	3	6.0
Raw wax	3000	100.0	640	100.7	330	99.8	55	100.2	50	99.8

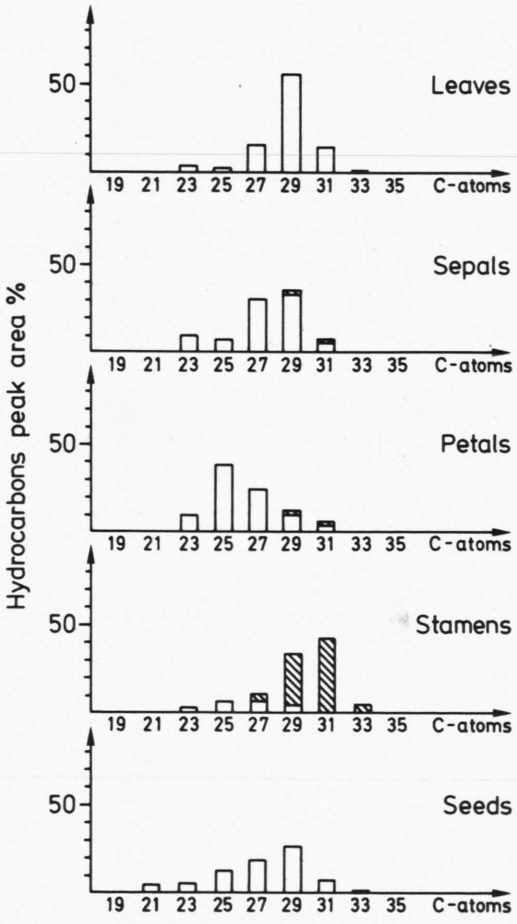


Fig. 2. Distribution patterns of hydrocarbons from waxes of various organs of *C. albidus*. □ Saturated, ▨ unsaturated.

The hydrocarbon maximum shifted to C_{25} and alkenes were found of about 8% [15, 20]. Stamen hydrocarbons contained alkenes of about 80%, and the dominating alkene had the chain length C_{31}. Seed waxes again contained most saturated alkanes, but their distribution pattern was very flat and the main component C_{29} was present with less than 30%. Alkenes were found only in traces [16]. Wax hydrocarbons of *C. albidus* showed always organ specific compositions and distribution patterns with distinct maxima.

Wax esters

Wax esters with chain lengths ranging from C_{32} to C_{54} were present in all waxes. But the homologous series of prevailing even numbered C-atoms differed in chain length maxima and the presence of unsaturated esters, see Fig. 3. Leaf waxes contained only saturated wax esters. The distribution pattern had a maximum of C_{44} with about 34% [15]. The saturated alcohols and fatty acids of these esters were checked by ethanolysis. Sepal wax had nearly the same pattern with identical maximum of C_{44} (30%). Petals showed a quite different composition of wax esters. Ethanolysis of these esters resulted in the presence of many unsaturated fatty acids [15]. The esters with chain length C_{36} (about 27%) were predominant and contained also unsaturated alcohols. In addition to the wax esters triterpenol esters were found with about 17%. Stamen wax esters showed similar results

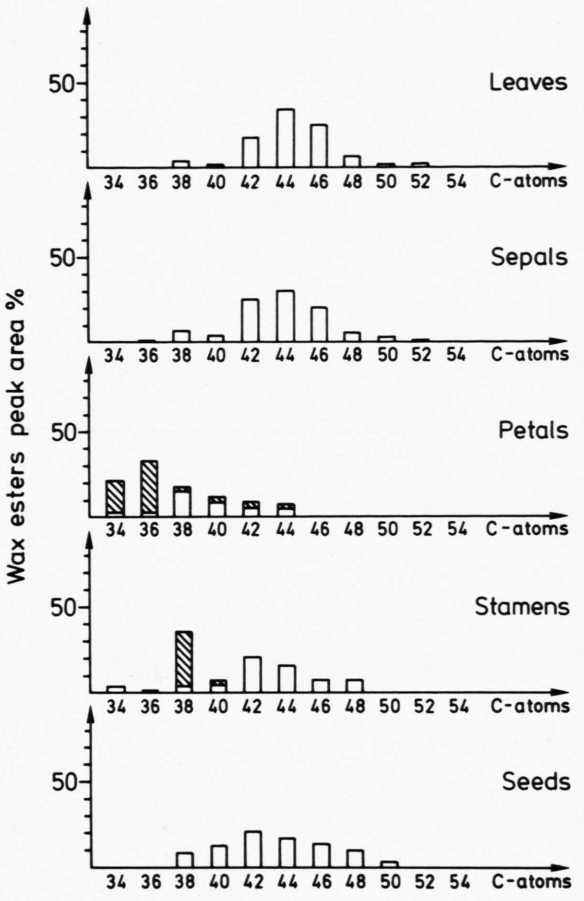


Fig. 3. Distribution patterns of wax esters from waxes of various organs of *C. albidus*. □ Saturated, ▨ unsaturated.

to those of petals. They also had great amounts of unsaturated fatty acids. The maximum of the wax esters distribution pattern was found with C_{38} in this organ. Triterpenol esters were also detected in amounts of about 13%. Seed waxes contained homologous series of saturated wax esters in a flat distribution pattern. Triterpenol esters could not be detected [16]. And again wax esters of *C. albidus* showed organ specific compositions and distribution patterns with distinct and in this case flat maxima.

Fatty acids

Free fatty acids were found in all *C. albidus* waxes in homologous series with chain lengths from C_{14} to C_{30}, see Fig. 4. In leaf waxes free fatty acids with the maxima of C_{22} (50%) and C_{24} (38%) were found only saturated. Sepal waxes contained nearly the same fatty acid composition, but oleic acid was present in 2%. In petal waxes most unsaturated fatty acids could be detected with one dominating chain length of C_{18}. Oleic acid, linoleic acid and also linolenic acid were found in these waxes. Stamen waxes showed similar compositions of fatty acids as petals. Only the proportions of the unsaturated acids were different. The seed waxes contained only saturated free fatty acids. But in this case the short chain fatty acid C_{16} (90%) was dominating.

Alcohols

In all waxes of the various organs of *C. albidus* primary alcohols with chain lengths from C_{16} to C_{30} were found. The chain lengths C_{20}, C_{22}, C_{24}, and C_{26}

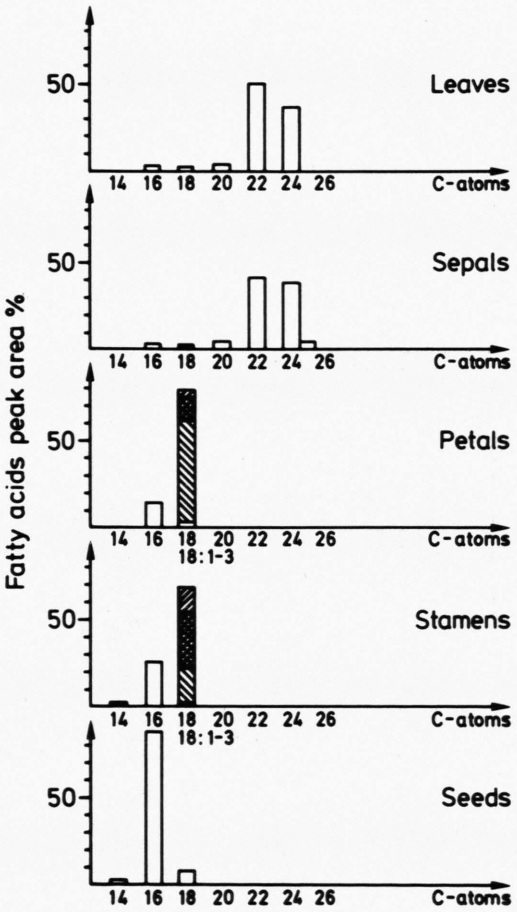


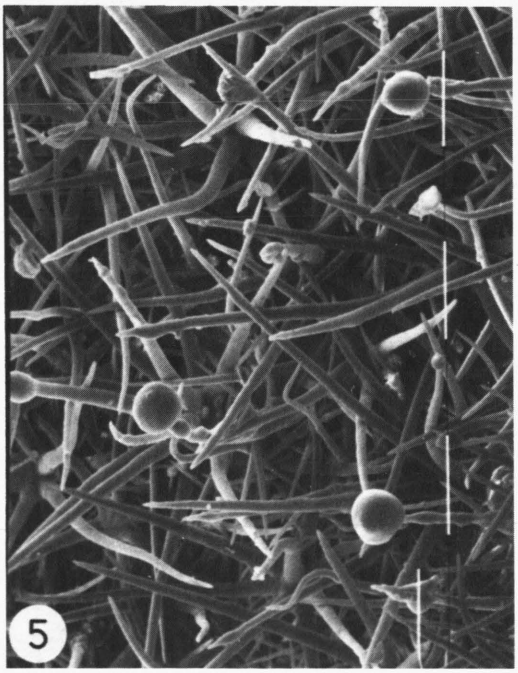
Fig. 4. Distribution patterns of fatty acids from waxes of various organs of *C. albidus*. □ Saturated, ▨ unsaturated.

were predominant. The alcohols were saturated and showed no organ specific compositions. In addition triterpenoids could be identified in the waxes of leaves, sepals, petals and stamens but not in seeds. These triterpenoids consisted of a very complex mixture of triterpenols which could not be identified in detail up to date.

Discussion

The epicuticular waxes of the various organs of *C. albidus* showed characteristic organ specific compositions. Leaf wax consisted of homologous series of very long chained and saturated lipids with distinct maxima. In addition to the common wax components the leaf extract included great amounts of resinous terpenes. Sepal wax had nearly the same wax compositions like those of leaves. But the maxima of the patterns were not so strongly marked, unsaturated compounds could be detected in small amounts and the proportion of resin was essentially lower. Petal wax was quite different from that of leaves or sepals. The distribution patterns showed a shift to shorter chain lengths and especially unsaturated lipids could be found in the hydrocarbons, wax esters and fatty acids series. In addition triterpenol esters were found in this wax, but no resin. Stamen wax was similar to that of petals concerning the shorter chain lengths and the presence of unsaturated compounds. But this wax showed an originally organ specific composition. Seed coat wax showed a special lipid composition. All common wax lipids were found to be saturated. The distribution patterns of alkanes and wax esters showed a flat figure and short chain compounds were dominating in contrast to leaf wax.

These epicuticular wax compositions of various organs of *C. albidus* should be now correlated to the surface structures of these organs with pictures produced by the scanning electron microscopy. The SEM pictures of a leaf surface (Fig. 5) show a very dense cover of hairs and glandular trichomes. So it was very difficult to have a look at the bottom of the leaf surface in order to see the structure of the surface wax. Only with a magnification of more than 3200× we were able to have a look into the underground of the trichomes and could see a waxy or liquid wax layer with no crystalline structures, see Fig. 6. The very long chained and saturated lipid composition of the leaf wax may suggest a crystalline structure according to other wax studies [3, 29].



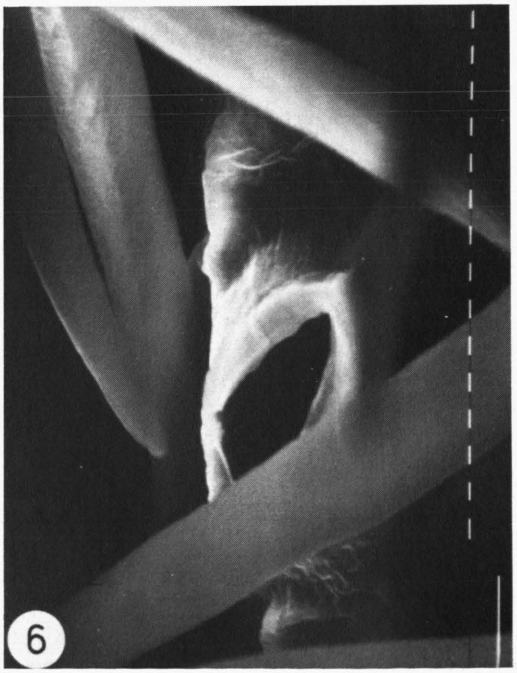


Fig. 5 + 6. SEM from the surface of a *C. albidus* leaf. Fig. 5: Bar = 100 μm; Fig. 6: Bar = 1 μm.

But, because of the great amounts of resin on these leaves, the wax lipids may be redissolved in these resinous substances. SEM pictures of the surface of a sepal show identical waxy layer and therefore are not documented here. The SEM pictures of the surface of a petal (Fig. 7) demonstrate a thin continuous wax layer of fluid consistancy. The predominance of short chained and unsaturated lipids in this wax corroborated these results. The surface of the petals in addition shows parallel series of papillae. They were found in all *Cistus* petals in similar arrangements. The seed coat surface appears in the SEM picture (Fig. 8) as a folded continuous wax layer of fluid consistency. The wax composition of seed coats has only saturated lipids. But in contrast to the leaf wax, these lipids show a shift to shorter chain lengths and in addition no dominating main component was observed. This wax could be extracted completely with hexane as demonstrated in Fig. 9, according to the composition of wax lipids most with short chain length components. In this picture the strongly folded epidermal cell walls of the seed coat are now visible.

The organ specific wax compositions and surface structures may probably be correlated to their organ

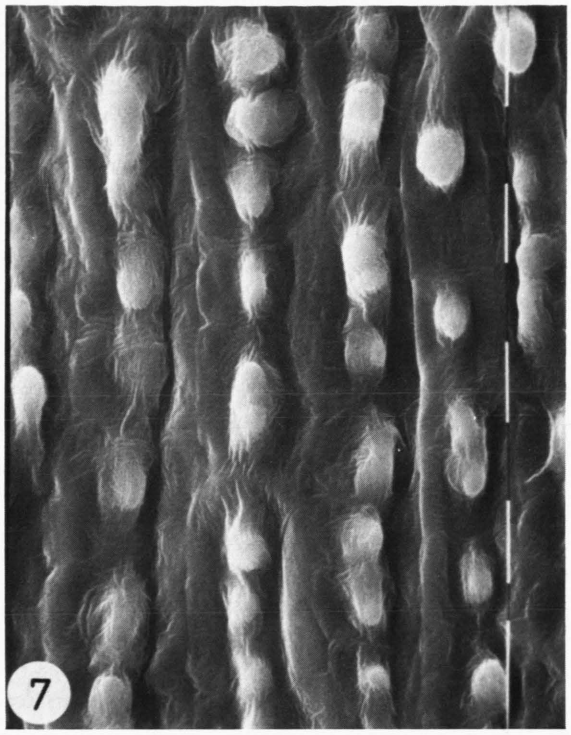


Fig. 7. SEM from the surface of a *C. albidus* petal. Bar = 10 μm.

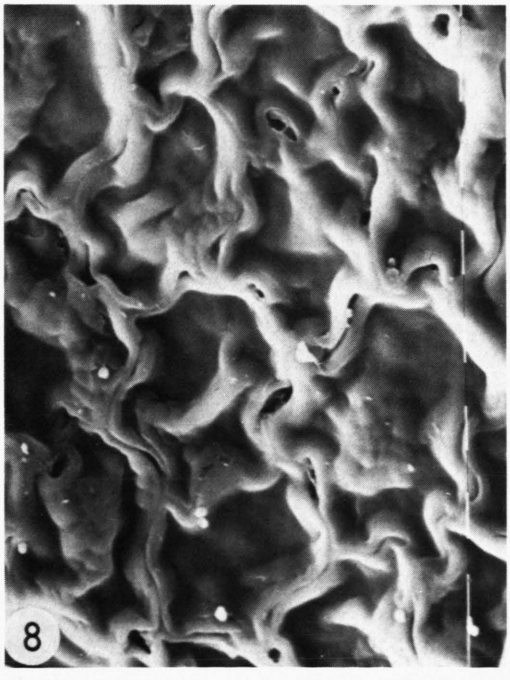


Fig. 8 + 9. SEM from the surface of a *C. albidus* seed. Fig. 8: Bar = 10 μm; Fig. 9: Waxes were extracted with hexane. Bar = 1 μm.

specific physiological functions. Leaves and sepals of *C. albidus* are very long living organs, leaves about one year. Their surface had to adapt themselves to the demands of long living organs in a *semi* arid climate. They should be steadfast against environmental factors such as wind, rain, sun or gaseous, liquid and solid particals. The SEM pictures 5 and 6 demonstrate that the leaf surface of *C. albidus* is covered with dense standing trichomes and a wax layer of fluid consistency. The chemical analysis of this wax extract shows in addition to the common saturated and long chained lipids great amounts of resin, 5 times of the wax. The secretion of resin is observed often in xeromorphic plants of arid and *semi* arid regions and found in nearly all *Cistus* species [24]. Thus, the high amounts of resin may be the reason for the fluid or waxy surface layer.

In contrast to leaves and sepals, the petals and stamens of *C. albidus* are very short living organs of only one day. They must develope themselves in a few minutes out of their buds. Therefore, the surface wax layer must follow these movements and has to be of elastic properties. The waxes of both organs are characterized by high amounts of unsaturated and short chain compounds. The SEM pictures (Fig. 7) in agreement to these results show a fluid wax layer on the surface of petals and also stamens.

The surface of the seed coats had to adapt itself to the process of shrinking and of swelling by germination. According to the dominance of short chain but saturated lipids, the surface of seed coats has a fluid wax layer, too, as shown in Fig. 8.

The differences in the chemical composition of epicuticular waxes from the various organs of *C. albidus* may be primarily conditioned by enzymes, regulating the chain length and the desaturation of the lipids. Each organ has enzymes specific for the biosynthesis of distinct chain lengths for different lipids, and desaturation enzymes, which are activated in petals and stamens, but not in leaves and seed coats, or they are missing in the last organs.

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[1] C. A. Jeffree, E. A. Baker, and P. J. Holloway, in: Microbiology of Aerial Plant Surfaces (C. H. Dickinson and T. F. Preese, eds.), pp. 119–158, Academic Press, New York 1976.
[2] W. Barthlott and E. Wollenweber, Tropische und subtropische Pflanzenwelt **32,** 33–97, Akad. Wiss. Lit. Mainz, F. Steiner, Wiesbaden 1981.
[3] P.-G. Gülz, Ber. dtsch. Bot. Ges. **99,** 89–97 (1986).
[4] A. P. Tulloch, in: Chemistry and Biochemistry of Natural Waxes, (P. E. Kolattukudy, ed.), pp. 235–287, Elsevier, Amsterdam 1976.
[5] E. A. Baker, in: The Plant Cuticle (D. F. Cutler, K. L. Alvin, and G. E. Price, eds.), Academic Press, New York 1982.
[6] V. Wollrab, Collect. Czech. Chem. Commun. **32,** 1304–1308 (1967).
[7] K. Stransky, M. Streibl, and V. Herout, Collect. Czech. Chem. Commun. **32,** 3213–3220 (1967).
[8] V. Wollrab, Collect. Czech. Chem. Commun. **33,** 1584–1600 (1968).
[9] V. Wollrab, M. Streibl, and F. Sorm, Collect. Czech. Chem. Commun. **33,** 2257–2265 (1968).
[10] K. Stransky and M. Streibl, Collect. Czech. Chem. Commun. **34,** 103–117 (1969).
[11] G. A. Herbin and P. A. Robins, Phytochemistry **8,** 1985–1998 (1969).
[12] P. J. Holloway and E. A. Baker, Ann. appl. Biol. **66,** 145–154 (1970).
[13] E. A. Baker, J. Procopiou, and G. M. Hunt, J. Sci. Fd. Agric. **26,** 1093–1101 (1975).
[14] S. Nagy, H. E. Nordby, and J. C. Lastinger, Phytochemistry **14,** 2443–2445 (1975).
[15] P.-G. Gülz, P. Proksch, and D. Schwarz, Z. Pflanzenphysiol. **92,** 341–347 (1979).
[16] P. Krollmann, C. Eich, and P.-G. Gülz, Z. Naturforsch. **39c,** 521–524 (1984).
[17] E. F. Warburg, *Cistus* L., in: Flora Europaea, **Vol. 2** (T. G. Tutin *et al.*, eds.), p. 282, Cambridge University Press, Cambridge, Mass. 1968.
[18] P.-G. Gülz, E. Latza, L. Köhlen, and R. Schütz, Z. Pflanzenphysiol. **89,** 59–67 (1978).
[19] P. Proksch, R. Engel, and P.-G. Gülz, Z. Pflanzenphysiol. **89,** 227–237 (1978).
[20] P.-G. Gülz, E. Amigoni, B. Schmitz, and H. Egge, Z. Pflanzenphysiol. **94,** 35–43 (1979).
[21] P.-G. Gülz and G. Dielmann, Z. Pflanzenphysiol. **100,** 175–178 (1980).
[22] J. De Pascual Teresa, J. G. Urones, P. Basabe, and J. A. Pinto Del Rey, An. Quim. **74,** 345–350 (1978).
[23] E. Wollenweber and K. Mann, Z. Naturforsch. **39c,** 303–306 (1984).
[24] T. Vogt, P. Proksch, and P.-G. Gülz, J. Plant Physiol. **131,** 25–36 (1987).
[25] P.-G. Gülz, H. Hemmers, J. Bodden, and F.-J. Marner, Z. Naturforsch. **42c,** 191–196 (1987).
[26] P.-G. Gülz, R. W. Scora, E. Müller, and F.-J. Marner, J. Agric. Food Chem. **35,** 716–720 (1987).
[27] P.-G. Gülz, J. Bodden, E. Müller, and F.-J. Marner, Z. Naturforsch. **43c,** 19–23 (1988).
[28] P. Gosh and S. Thakur, J. Chromatogr. **240,** 515–517 (1982).
[29] H. Hemmers, P.-G. Gülz, and K. Hangst, Z. Naturforsch. **41c,** 521–525 (1986).