Classification and terminology of plant epicuticular waxes

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Plant cuticles are covered by waxes with considerable ultrastructural and chemical diversity. Many of them are of great systematic significance. Waxes are an essential structural element of the surface and of fundamental functional and ecological importance for the interaction between plants and their environment. An extensive literature has been published since the introduction of scanning electron microscopy (SEM). Hitherto, the area has lacked a complete classification and terminology necessary as a standard for comparative descriptions. A refined classification and terminology of epicuticular waxes is therefore proposed based on high-resolution SEM analysis of at least 13 000 species, representing all major groups of seed plants. In total 23 wax types are classified. Thin wax films appear to be ubiquitous, while thicker layers or crusts are rare. The most prominent structures are local wax projections, which most probably result from self-assembly of wax molecules. These projections are supposed to be mainly of a crystalline nature and are termed crystalloids here. Among these, platelets and tubules are the most prominent types, while platelets arranged in parallel rows and stomatal wax chimneys are the most striking orientation and aggregation patterns. In addition, a comprehensive overview on the correlation between wax ultrastructure and chemical composition is given.

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ADDITIONAL KEY WORDS:—angiosperms – chemistry – crystalloids – cuticle – gymnosperms – systematics – taxonomy – ultrastructure.

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INTRODUCTION

Plant cuticles and epicuticular waxes have been of interest to botanists for more than a century. While in several early publications macroscopically visible effects of cuticles and epicuticular waxes were described, e.g. light reflection ('visible bloom') or wettability (Sprengel, 1793; Darwin, 1886; Kerner von Marilaun, 1913) much of the work at the beginning of this century focused on the fine structure of the cuticle (e.g. Lee & Priestley, 1924; Frey, 1926; Jurasky, 1934; Fritz, 1935).

The first comprehensive study of epicuticular waxes was made by de Bary (1871). In the course of his light microscopic studies, he investigated about 60 species and classified the epicuticular waxes he found into four categories: layers or crusts ('Schichten oder Krusten'), rodlets ('Stäbchen'), granules ('Körnchen') and heaped coverings ('gehäufte Wachsüberzüge'). This publication served as the standard work until the carbon-replica technique was developed to study epicuticular waxes by transmission electron microscopy (Juniper & Bradley, 1958). However only a limited number of species was studied using this technique. With the introduction of the scanning electron microscope (SEM) in botany, which was a quicker and easier technique, although at first of lower magnification, many surfaces were studied. In one of the first investigations, a revised classification of epicuticular waxes based on the work of de Bary was published (Amelunxen, Morgenroth & Picksak, 1967) which was adopted later by several other authors (e.g. Holloway, 1970; Napp-Zinn, 1973; Wilkinson, 1979). In this classification, six types of epicuticular wax structures with 19 different variants were recognized and presented as schematic drawings. Three different variants of granules, five of rodlets, four of layers and crusts, three different heaped wax coverings, two different liquid or amorphous layers, as well as platelets and scales were described. In this classification, platelets were defined as being attached to the surface with their flat side, while scales were defined as being attached to the surface with their narrow side. While German language publications followed this terminology, English language papers used the term platelets instead of scales *sensu* Amelunxen *et al.* (e.g. Baker, 1982; Jeffree, 1986; Baum, Tulloch & Bailey, 1989).

The most recent comprehensive overview of the classification of epicuticular waxes was given by Jeffree (1986). Summarizing most of the published literature, he proposed 14 'wax morphological types' based on the results obtained from the investigations of about 480 taxa (including about 300 *Eucalyptus* species). At least three types can be subsumed within one, because surfaces showing 'amorphous films', 'soft waxes', or being 'essentially wax free' are difficult to distinguish using SEM.

Epicuticular waxes have often been regarded as crystals and the term 'wax crystals' is widespread in the literature. De Bary (1871) expressed this opinion and it was proven for at least some species (Kreger, 1948). The molecular organization of most waxes is not known. Therefore, we shall use the term 'crystalloid' proposed by Barthlott & Wollenweber (1981) which recognizes this uncertainty. Some epicuticular wax crystalloids (e.g. tubules) can recrystallize *in vitro* resulting in tubules of similar shape and dimension as on the plant surface (Jeffree, Baker & Holloway, 1975;

Jetter & Riederer, 1994, 1995). However, recrystallization fails for many other crystalloids. The term 'Biokristall' was proposed by Troll (1948) to describe the different shape of inorganic crystals that crystallize under the influence of the living cell, and which may be of interest in understanding the origin and shape of epicuticular waxes.

Over the last 15 years we have investigated at least 13 000 species of all major groups of angiosperms and all genera of gymnosperms in relation to the systematic significance of epicuticular wax crystalloids. Frölich & Barthlott (1988) began by refining the classification of Amelunxen *et al.* (1967) after having examined the monocotyledons. They accepted 12 different wax types and considered, for the first time, the orientation of wax crystalloids to be important. Secondly, some wax types turned out to be good features in circumscribing major clades of the monocotyledons. The '*Convallaria*-Type' (parallel oriented platelets) was characteristic for liliiflorous families while the '*Strelitzia*-Type' (longitudinally aggregated rodlets) occurred only in farinoseous families and their relatives. Later the '*Aristolochia*-Type' (transversely ridged rodlets) turned out to be characteristic for ancestral woody angiosperms and tubular crystals ('*Berberis*-Type') for ranunculiflorous families and gymnosperms (Hennig *et al.*, 1994). It became necessary, as this work progressed, to find descriptions for the wax crystalloids whose structural diversity was not represented by published classifications. In addition, new results on the relation between wax chemistry and the shape of the crystalloids had to be taken into account. It was shown, for at least some crystalloids, that they represent convergent shapes based on a different chemical composition (Meusel, Leistner & Barthlott, 1994). We present here a refined terminology and classification of epicuticular wax crystalloids, based both on our own investigations and the published literature.

MATERIAL AND METHODS

Material was collected in the field or obtained from different botanic gardens and plant collections throughout the world. Preferably fresh, fully developed and uncontaminated plant material was investigated. If it was not possible to obtain living material, samples were taken from herbarium specimens. Small pieces were cut, affixed to aluminium stubs by double-sided adhesive tape, air dried and sputter coated with gold. The specimens were examined with a Stereoscan 200 (Leica, Bensheim, FRG). The complete list of the investigated species and a detailed description of the methods is in the following publications: Frölich & Barthlott (1988), Hennig *et al.* (1994), Ditsch & Barthlott (1994, 1997), Ditsch, Patha & Barthlott (1995), Engel & Barthlott (1988), Fehrenbach & Barthlott (1988), Theisen & Barthlott (1994), Wilhelmi & Barthlott (1997).

CLASSIFICATION AND TERMINOLOGY

Epicuticular waxes exhibit great micromorphological diversity. Therefore a suitable classification and terminology is necessary to systematize this wealth of different forms. In general, a division can be made between a thin film, which is regarded as the obligatory final layer of the cuticle, and superimposed wax structures. The

latter may be developed as continuous layers, crusts or solitary crystalloids of varying thicknesses. The crystalloids are of differing but characteristic shape and size and may appear in specific arrangements or combinations. Stomatal chimneys are a special feature, which all look very similar, but are derived from either crusts or crystalloids.

Films

Very thin coverings, usually only a few nm thick, representing the obligate outermost border of the cuticle, hardly visible in the SEM and not showing fissures after drying (e.g. *Hydrocotyle bonariensis* Lam., Fig. 1). Films (including 'essentially wax free' surfaces, Jeffree, 1986) represent a very thin covering, probably consisting of few layers of molecules only.

Layers and crusts

Continuous coverings with a considerable thickness (*c.* 0.5–10 µm), sometimes recognizable only after the formation of fissures. It seems reasonable to distinguish between those with ('crusts') or without a surface sculpturing ('smooth layers') and those which may break up into separate blocks/units ('fissured layers').
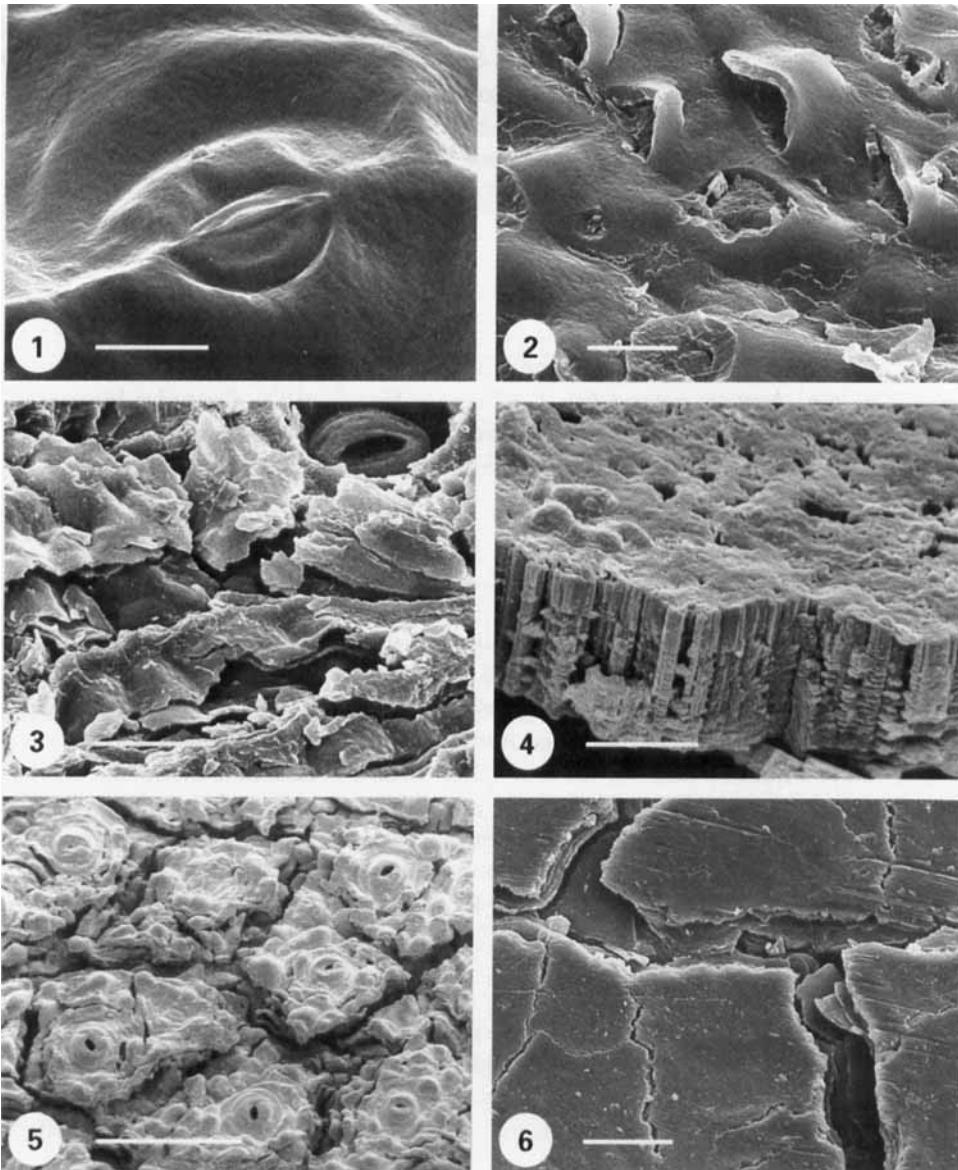
Smooth layers

Continuous coverings usually less than 1 µm thick without a prominent surface sculpturing. Detectable in the SEM mainly due to artificial cracking which separates them from the even thinner films (e.g. *Terminalia* cf. *Seyrigii* (H.Perr) Capuron, Figs 2, 40A). Smooth coverings like 'resinous layers' and 'soft waxes' described by Amelunxen *et al.* (1967) and Jeffree (1986) should be incorporated here.

Crusts

Continuous coverings of considerable thickness (often more than 1 µm) with a more or less prominent surface sculpturing (e.g. *Cynanchum sarcostemma* Lillo, *Copernicia cowellii* Britton & Wilson, Figs 3, 4, 40B). Crusts are massive wax coverings (up to 10 mm in *Copernicia cowellii*) and are characteristic of many succulents (e.g. Asclepiadaceae, Cactaceae). While most crusts are continuous coverings from the beginning, some are the product of the erosion of crystalloids. Sometimes contaminated smooth layers may appear as crusts.

Fissured layers

Usually thick, crusty coverings, fractured by naturally occurring cracks and fissures, often showing a terraced relief (Fig. 40C). The broken, often terraced, appearance is based on the cracking of layers due to the expansion of the epidermal surfaces while the plant grows and wax production proceeds at a different rate. Fissured layers are characteristic for several succulents (e.g. *Copiapoa cinerea* (Phil.) Britton & Rose, *Frerea indica* Dalzell, Figs 5, 6).

Figures 1–6. Films, smooth layers and crusts. Fig. 1. *Hydrocotyle bonariensis* Lam.. (Apiaceae): film. Scale bar = 10 μm. Fig. 2. *Terminalia* cf. *seyrigii* (H. Perrier) Capuron (Combretaceae): smooth layer. Scale bar = 10 μm. Fig. 3. *Cynanchum sarcostemma* Lillo (Asclepiadaceae): crust. Scale bar = 20 μm. Fig. 4. *Copernicia cowellii* Britton & Wilson (Arecaceae): crust. Scale bar = 10 μm. Fig. 5. *Copiapoa cinerea* (Phil.) Britt. & Rose (Cactaceae): fissured layer. Scale bar = 200 μm. Fig. 6. *Frerea indica* Dalzell (Asclepiadaceae): fissured layer. Scale bar = 20 μm.

Crystalloids

Distinct wax projections, usually with a characteristic shape, size and orientation towards the surface, protruding from the ubiquitous wax film. Crystalloids, usually, are characteristic of certain taxa and their shape is fairly constant. In most species

single crystalloids are easily distinguished, but sometimes they cluster very densely. In such cases, they may appear similar to crusts. While crystalloids, in general, show an irregular distribution, they are, in some species, arranged into characteristic orientation and aggregation patterns. If different crystalloids appear upon adjacent cells of one epidermal surface or within one cell we call this 'syntopism'. Where the appearance of the crystalloids depends on varying environmental conditions, we call this 'polymorphism' (cf. section 5).

Granules

Irregular, mostly isodiametric, often rounded crystalloids, which may sometimes be hollow (e.g. *Aegiceras corniculatum* (L.) Blanco, Figs 7, 40D). Granules have rarely been regarded as a typical crystalloid form. It is doubtful whether they are crystals. It is probable that in many cases contaminants were misinterpreted as granules and vice versa.

Platelets and plates

Flat crystalloids, connected to the surface by their narrow side. Platelets are the most common crystalloids and are found in all major plant groups (mosses, ferns, gymnosperms and angiosperms). They may be attached to the surface at different angles, their margin may be entire or irregular and their width/height ratio varies considerably. Platelets without distinct edges that are attached to the surfaces by one edge and orientated perpendicularly are differentiated by their margin ('entire' and 'non entire' platelets). Very thin crystalloids with filamentous extensions are called 'membraneous platelets' while those with distinct edges are called 'plates'. Since platelets and plates are widespread within plants, they are of very little systematic significance unless they are arranged into characteristic orientation patterns.

Entire platelets. Flat crystalloids with entire margin and regular shape, usually 1–3 μm in height and protruding ± perpendicularly from the surface (e.g. *Habropetalum dawei* Hutch. & Dalziel) Airy Shaw, Figs 8, 40E). Entire platelets often are semi-circular and are sometimes arranged into characteristic orientation patterns (cf. section 4.1).

Irregular platelets. Flat crystalloids with irregular margin, usually 1–10 μm in height, protruding perpendicularly from the surface (e.g. *Odosicyos* spec., *Grevillea bipinnatifida* R.Br., Figs 9, 10). Non-entire platelets can be subdivided based on the appearance of the margin which may be gnawed, crenate, sinuate, or laciniate. Their width/height ratio shows great variability and sometimes they possess extensions which may grade into tubules. In many cases they are arranged in characteristic orientation patterns.

Membraneous platelets. Flat, membraneous, usually interconnected crystalloids, protruding from the surfaces at an acute angle, often with threadlike extensions (e.g. *Tilia miqueliana* Maxim., Figs 11, 40F). Membraneous platelets are often found together with other crystalloids and never show specific orientations.
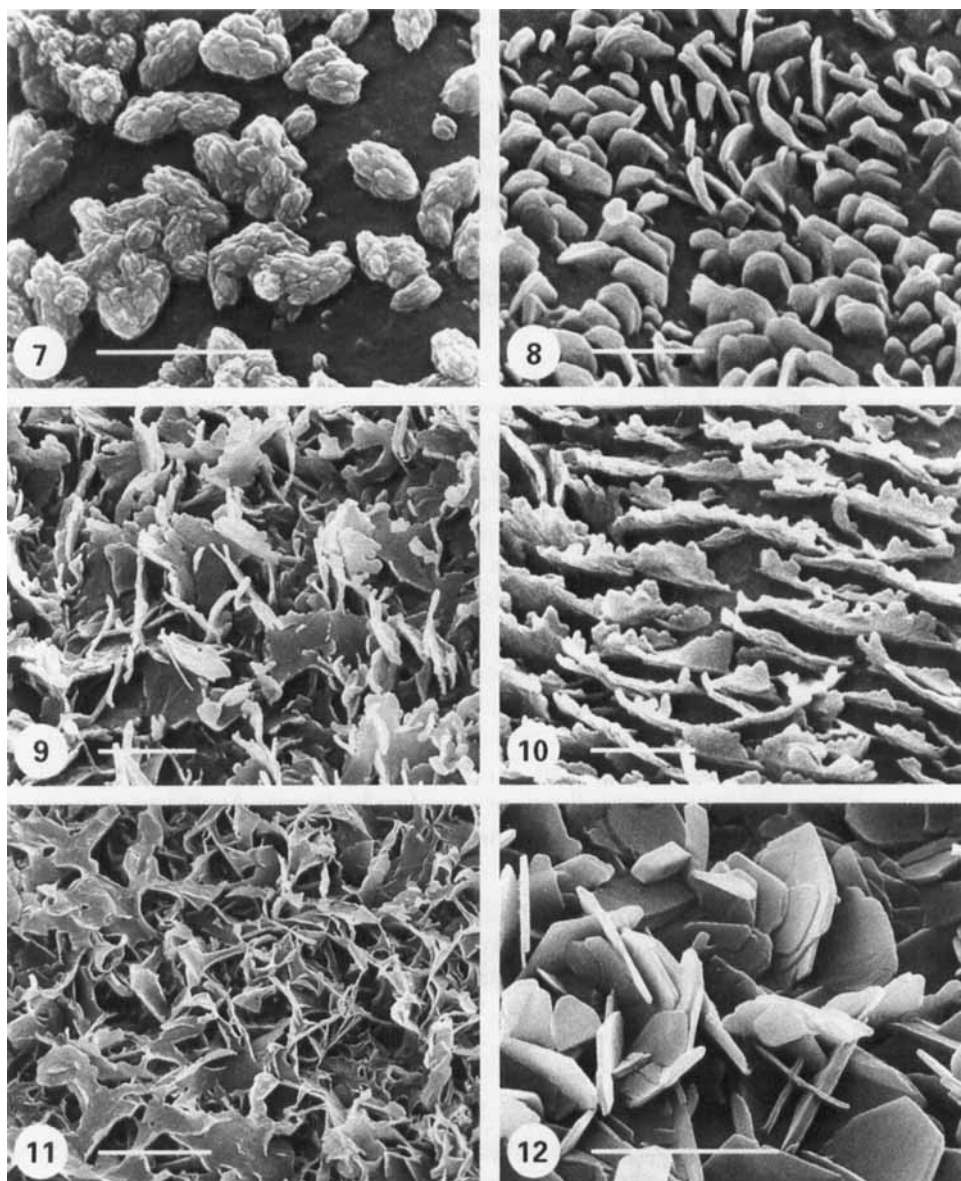
Figures 7–12. Wax crystalloid forms. Fig. 7. *Aegiceras corniculatum* (L.) Blanco (Aegicerataceae): granules. Scale bar = 5 μm. Fig. 8. *Habropetalum dawei* (Hutch. & Dalziell) Airy Shaw (Dioncophyllaceae): entire platelets. Scale bar = 1 μm. Fig. 9. *Odosicyos* spec. (Cucurbitaceae): non entire platelets. Scale bar = 2 μm. Fig. 10. *Grevillea bipinnatifida* R.Br. (Proteaceae): non entire platelets. Scale bar = 1 μm. Fig. 11. *Tilia miqueliana* Maxim. (Tiliaceae): membraneous platelets. Scale bar = 10 μm. Fig. 12. *Lecythis chartacea* Berg (Lecythidaceae): plates. Scale bar = 5 μm.

Plates. Flat, often polygonal crystalloids, with distinct edges (e.g. *Lecythis chartacea* Berg, Figs 12, 41A). Plates show a great variation in size, are attached to the surfaces at varying angles, and are often imbricated like tiles. They are commonly found over veins of leaves and on shoots and are only rarely orientated in a particular direction.

Rodlets

Massive sculptures polygonal or terete in cross section and a distinct longitudinal axis, with a length/width ratio usually not exceeding 50:1. Rodlets can be differentiated by their cross section which may be circular, triangular or polygonal. In addition, rodlets may have a variable diameter along the length of their axis ('transversely ridged rodlets'). 'Coiled rodlets' differ by their small diameter, the narrow windings as well as their cross section which often is kidney shaped. Tubules were misinterpreted as rodlets in various publications, due to the obscured openings or the limited resolution of the SEM. Sometimes it is difficult to distinguish very long rodlets from threads.

Polygonal rodlets. Rodlets with polygonal cross section. Two different types of angular rodlets can be distinguished: (1) *polygonal rodlets* with 4–10 facets, varying in diameter; often arranged into clusters resembling rock-crystals (e.g. *Acsmithia densiflora* (Brongn. & Gris) Hookland, Figs 13, 41C). Usually, polygonal rodlets are not bent and only exceptionally they are very short and compact; (2) *triangular rodlets*, often bent or wound with three distinct facets, ±decumbent or ascending and not arranged in clusters (e.g. *Ledum glandulosum* Nutt., Figs 14, 41B). They were called 'ribbons' in earlier publications (Engel & Barthlott, 1988; Fehrenbach & Barthlott, 1988; Theisen & Barthlott, 1994).

Terete rodlets. Rodlets with ±circular cross section and a diameter of usually more than 0.2 µm (e.g. *Sceletium compactum* L.Bonus, Fig. 15). When coiled the diameter of the coil is more than 1 µm. Usually, terete rodlets are attached perpendicularly to the surface and are often slightly bent. Therefore they were called 'hooked rodlets' in former papers. In many cases, they are found to be longitudinally aggregated or arranged in clusters. Terete rodlets may be confused with tubules if the openings of the latter are obscured.

Coiled rodlets. Rodlets, usually less than 0.2 µm in diameter, coiled throughout their length and often branched. The coils may have a diameter of less than 1 µm (e.g. *Buxus sempervirens* L., Figs 16, 41D). Coiled rodlets may form tubules due to a very narrow radius. Transitional forms between both crystalloids exist and they have a very similar chemical composition. Often, they are furrowed along their length axis, resulting in a kidney-like cross section. Due to branching and webbing, some transitions to platelets may occur.

Transversely ridged rodlets. Rodlets with ridges perpendicular to their longitudinal axis (e.g. *Williamodendron quadrilocellatum.* (van der Werff) Kubitzki & H.G.Richt., Figs 17, 41E). Transversely ridged rodlets are attached perpendicular to the surfaces and are of variable length. Larger crystalloids may be hollow with an irregular cross section. All of them share furrows perpendicular to their long axis alternating with ridges which are sometimes platelet-like or have filamentous extensions (e.g. *Actinidia melanandra* Franch., Fig. 18). The latter merge with branched or longitudinally aggregated rodlets and platelets and may be the expression of 'polymorphism'. They occur convergently within angiosperms. Crystalloids with palmiton (hentriacontan-16-on), an unusual wax component, are characteristic for Magnoliidae *s.s.* Transversely ridged rodlets are often found on shoots.
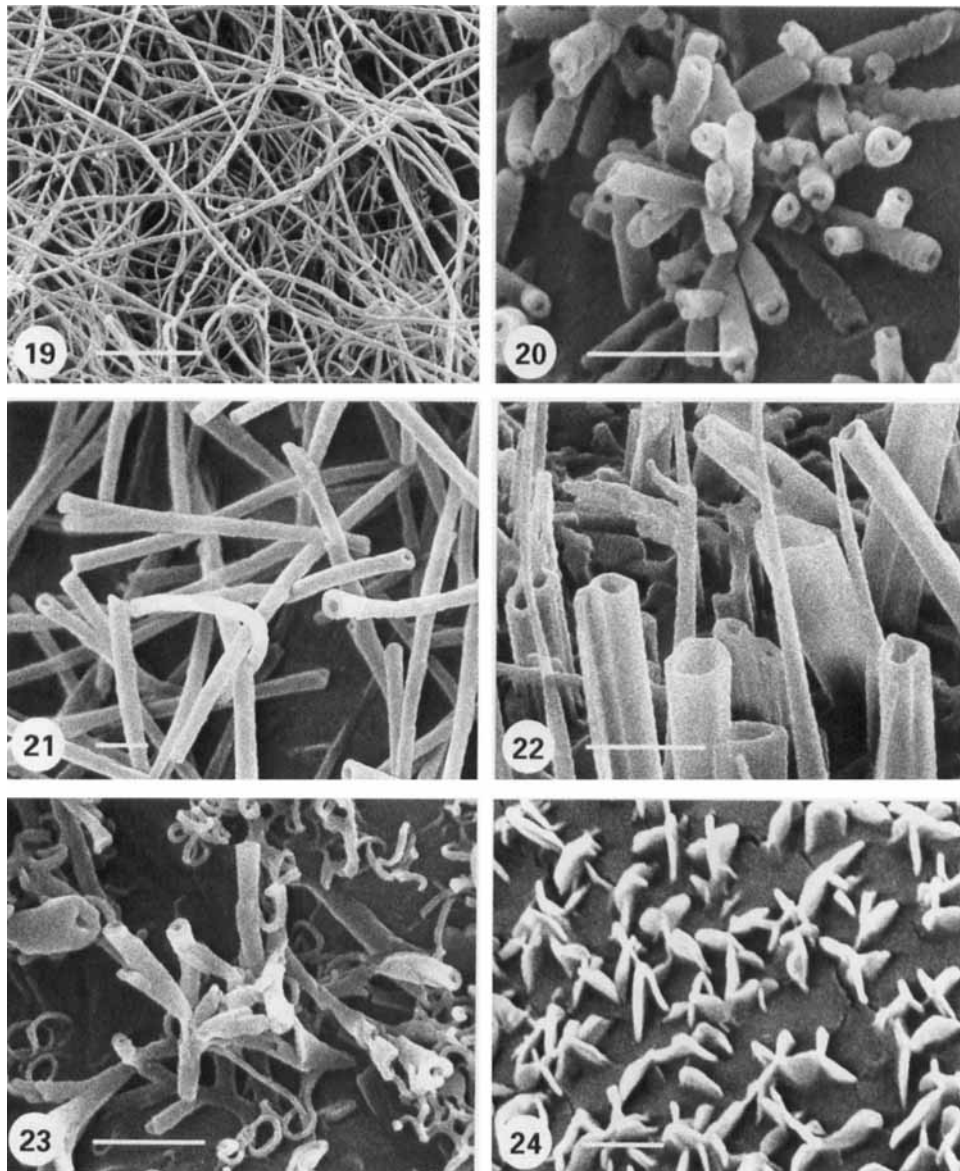
Threads

Massive crystalloids with a ±circular cross section and a length/width ratio exceeding 100:1. Threads are very fine long crystalloids which often form a felt-like
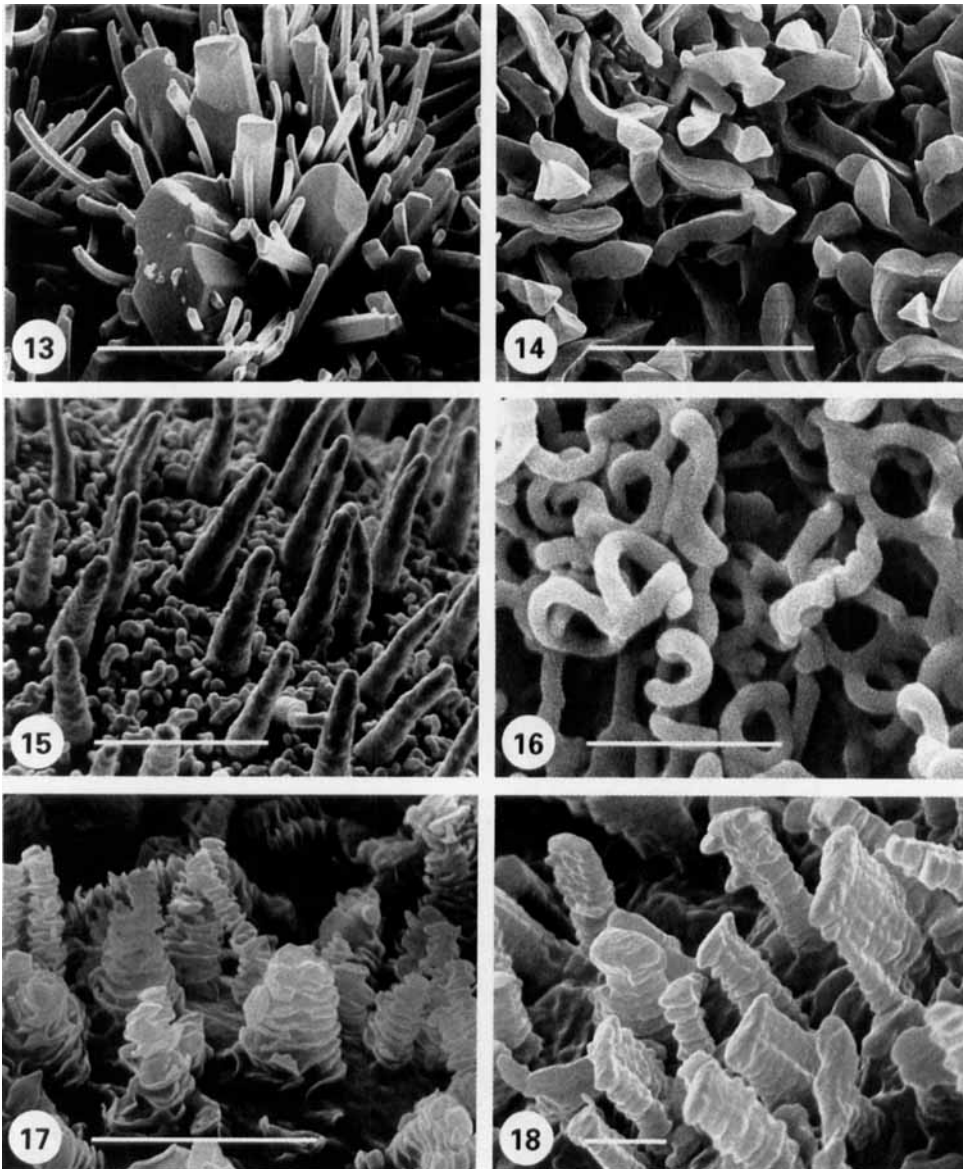
Figures 13–18. Wax crystalloid forms. Fig. 13. *Acsmithia densiflora* (Brongn. & Gris) Hoogland. (Cunoniaceae): polyangular rodlets. Scale bar = 10 µm. Fig. 14. *Ledum glandulosum* Nutt. (Ericaceae): triangular rodlets. Scale bar = 5 µm. Fig. 15. *Sceletium compactum* L. Bolus. (Aizoaceae): terete rodlets. Scale bar = 10 µm. Fig. 16. *Buxus sempervirens* L. (Buxaceae): coiled rodlets. Scale bar = 1 µm. Fig. 17. *Williamodendron quadrilocellatum* (van der Werff) Kubitzki & H.G.Richt. (Lauraceae); transversely ridged rodlets. Scale bar = 5 µm. Fig. 18. *Actinidia melanandra* Franch. (Actinidiaceae): transversely ridged rodlets. Scale bar = 1 µm.

covering (e.g. *Drosera burmanni* Vahl, Figs 19, 41F). Sometimes it is difficult to distinguish between short threads and rodlets. The crystalloids may be flattened, branched or even resemble a bottle-brush. Many threads are produced by glands.

Figures 19–24. Wax crystalloid forms. Fig. 19. *Drosera burmanni* Vahl (Droseraceae): threads. Scale bar = 2 μm. Fig. 20. *Lonicera korolkovii* Stapf (Caprifoliaceae): tubules. Scale bar = 1 μm. Fig. 21. *Columellia oblonga* Ruiz & Pav. ssp. *sericea* (Kunth) Brizicki (Columelliaceae): tubules. Scale bar = 1 μm. Fig. 22. *Amorphophallus maximus* N.E.Br. (Araceae): tubules. Scale bar = 1 μm. Fig. 23. *Buxus sempervirens* L. (Buxaceae): transitional forms of coiled rodlets. Scale bar = 1 μm. Fig. 24. *Calliandra haematoma* (Bert) Benth. (Fabaceae): rosettes. Scale bar = 1 μm.

Tubules

Cylindrical, hollow crystalloids with a terminal opening. Most tubules have very uniform dimensions (0.5–5 μm in length, 0.2–0.3 μm in diameter). They can be divided into two distinct types on the basis of their chemical composition: those characterized by nonacosan-10-ol and those characterized by β-diketones. Both

types are very similar in appearance and hardly distinguishable in the SEM. The most obvious distinguishing feature is the angle of branching. Very often transitional forms between tubules and coiled rodlets are found. Additional kinds of tubules are found which differ in their dimensions from the latter two (e.g. *Amorphophallus maximus* N.E.Br., 3–5 µm long 0.5–1 µm wide, Fig. 22).

Nonacosanol-tubules. Tubules with a ±constant outer diameter of 0.2–0.3 µm, an inner diameter of less than 0.1 µm, and occasional branching at right angles to the main axis (e.g. *Lonicera korolkovii* Stapf, Figs 20, 42A). Epidermal surfaces covered by these tubules are characterized by the dominance of nonacosan-10-ol within the cuticular wax. Often an arrangement into clusters is seen. Sometimes the tubules extend into coiled rodlets which gives an idea of the way in which they originate. These tubules have been described as *Berberis*-Type within angiosperms and are suitable for differentiating between Ranunculidae and Magnoliidae (Hennig *et al.*, 1994). In addition they are characteristic for most gymnosperms (Riederer, 1989).

Diketone-tubules. Tubules with a ±constant outer diameter of 0.2–0.3 µm, an inner diameter of less than 0.1 µm, and occasional branching at acute angles (e.g. *Columellia oblonga* Ruiz. & Pav. ssp. *sericea* (Kunth) Brizicky, Figs 21, 42B). Epidermal surfaces covered by these tubules are characterized by the dominance of β-diketones within the cuticular wax. Branching occurs in ±acute angles and occasionally the ends are funnel-shaped. As in the previous type, transitions to coiled rodlets are not rare, while the formation of clusters is found only exceptionally. Diketone-tubules are found to be a convergent feature among different angiosperms families (e.g. Poaceae, Myrtaceae, Caryophyllaceae, Ericaceae).

Transitional crystalloid forms

Crystalloids sharing features of at least two different crystalloids and which are, therefore, not possible to classify with any degree of clarity. Such crystalloids are found within platelets that show transitions to tubules or coiled rodlets. Many transitions are found between coiled rodlets and tubules, from which they do not differ chemically (e.g. *Buxus sempervirens*, Fig. 23).

Orientation and aggregation patterns of crystalloids

Arrangement of crystalloids into characteristic patterns or assemblies. Apart from the shape of individual crystalloids, their distribution and/or orientation may be of importance to characterize a surface. In most cases there is no special orientation of crystalloids (Fig. 40E) and these are ignored. Characteristic orientation patterns are found preferentially in platelets and they are not restricted to one cell. Aggregation patterns of terete rodlets may be restricted to the anticlinal walls of the epidermal cells (e.g. *Strelitzia*-Type).

Orientation of platelets

Arrangement of platelets into characteristic distribution patterns evenly spread over an epidermal surface. As far as is known, only entire and non-entire platelets are arranged into characteristic distribution patterns. In general, the patterns exceed

one cell in extent. Occasionally locally restricted patterns can be observed around stomata and idioblasts.

Rosettes. Arrangement of platelets into multiple more or less radially assembled clusters, evenly spread over an epidermal surface (e.g. *Calliandra haematoma* (Bert) Benth., Figs 24, 42 C). Rosettes are formed mainly by non-entire platelets. Usually, they consist of 5–15, rarely of only 2–5, or more than 15 single platelets. The size and complexity of the individual rosette seems to be ± taxon specific. This orientation pattern was first recognized as a taxonomic feature for the Fabales, and therefore called 'Fabales-Type' (Ditsch *et al.*, 1995). In addition, this pattern was found in some closely related families such as Connaraceae, Malpighiaceae, Erythroxylaceae (Ditsch & Barthlott, 1997), and within Asteraceae. Massive wax production may build tall rosettes of many platelets and, as a result, appear like longitudinally aggregated rodlets (e.g. *Salix* spp.).

Parallel platelets. Parallel rows of longitudinally aligned platelets. This orientation pattern is formed either by entire or non-entire platelets and always exceeds one cell (e.g. *Convallaria majalis* L., Figs 25, 42D). The mechanism leading to such an orientation is not known. Occasionally orientations are found around idioblasts and stomata. Parallel platelets are characteristic of liliiflorous families within the monocotyledons and were described as '*Convallaria*-Type' (Barthlott & Frölich, 1983).

Parallel grouped platelets. Distinct small groups of platelets orientated in parallel. The angle of the long axis of the individual groups to each other varies. The groups show a great variation in the number of platelets and are usually composed of non-entire platelets (e.g. *Hypericum buckleii* M.A.Curtis, Figs 26, 42E). If the groups comprise only a few platelets, the orientation of the groups to one another is highly variable. In other cases, the platelets are arranged in long rows with a parallel orientation. Between these extremes, all gradations occur. Platelets grouped in parallel were recognized as a typical orientation pattern in the Hypericaceae and, therefore, described as '*Hypericum*-Type' (Ditsch & Barthlott, 1994). In addition, this feature was found in Myrtaceae, Proteaceae, and Haloragaceae (Jeffree, Baker & Holloway, 1976; Ditsch & Barthlott, 1997).

Aggregation patterns of rodlets and tubules

Assemblages of rodlets and tubules evenly distributed over the entire cell surface. Crystalloids may fuse along their longitudinal axis, aggregate to form clusters which may be hemispherical, or sometimes appear like rock crystals. The former and the latter are features of rodlets, while hemispherical clusters are common for tubules.

Clusters of tubules. Assemblage of tubules radiating from a centre, often forming solitary, hemispherical clusters irregularly distributed over the epidermal surface (e.g. *Corydalis cava* Schweigg & Körte, Fig. 27). These clusters are typically composed of nonacosanol-tubules, which are characteristic for Ranunculidae and gymnosperms. Due to the strong branching at ± 90°, the clusters often appear hemispherical and may be up to 5 μm in diameter. They are irregularly distributed over the epidermal surface. If the clusters are very small (0.5–1 μm) and occur closely together, they are often difficult to recognize. In diketones-tubules, clustering is only rarely observed and the clusters appear like fans or brushes. This is caused by the branching which is less dense and at more acute angles.
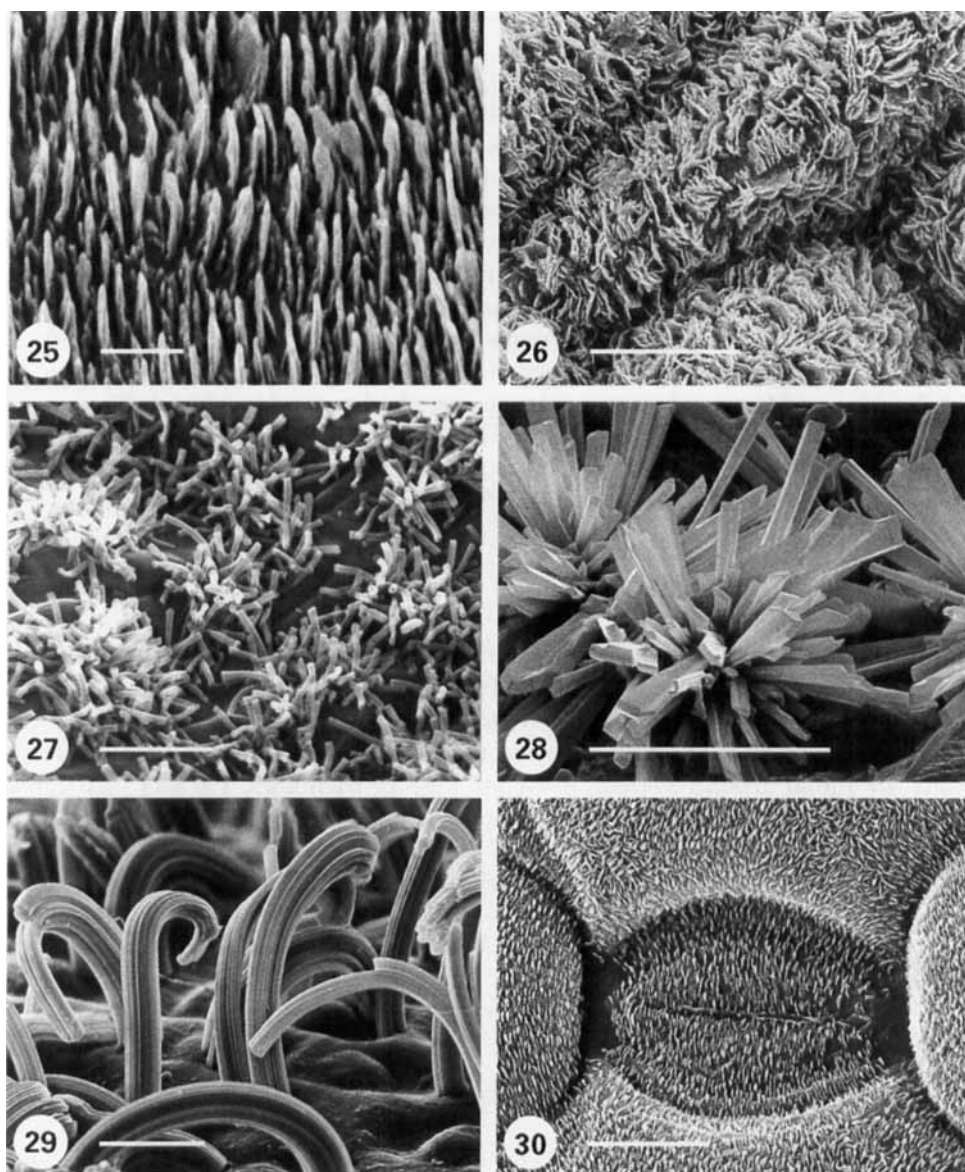
Figures 25–30. Orientation and aggregation patterns of wax crystalloids. Fig. 25. *Convallaria majalis* L. (Convallariaceae): parallel oriented platelets. Scale bar = 1 µm. Fig. 26. *Hypericum buckleii* M.A.Curtis (Hypericaceae): parallel stacked platelets. Scale bar = 10 µm. Fig. 27. *Corydalis cava* Schweigg. & Körte. (Fumariaceae): clusters of tubules. Scale bar = 2 µm. Fig. 28. *Daphne tangutica* Maxim. (Thymelaeaceae): clusters of rodlets. Scale bar = 10 µm. Fig. 29. *Phenacospermum guianense* (Rich.) Endl. & Miq. (Strelitziaceae): longitudinally aggregated rodlets. Scale bar = 10 µm. Fig. 30. *Convallaria majalis* L. (Convallariaceae): locally restricted orientation pattern. Scale bar = 10 µm.

Clusters of rodlets. Assemblage of rodlets radiating from a centre. Generally, these clusters are formed by polygonal rodlets with a straight long axis (e.g. *Tilia* spp.). They strongly resemble groups of rock-crystals, especially if the diameters of the rodlets differ considerably (e.g. *Daphne tangutica* Maxim., Figs 28, 41C). Clusters of

terete rodlets were observed only exceptionally (e.g. 'star scales' in *Jacaranda jasminoides* (Thumb.) Sandwith).

Longitudinally aggregated rodlets. Rodlets fused together along their longitudinal axes. These aggregates are mainly built of terete rodlets and may be ±isodiametric in diameter or appear similar to palisades (e.g. *Phenacospermum guianense* (Rich.) Endl. & Miq., Figs 29, 42F). The latter are in some cases restricted to the anticlinical walls of the epidermal cells. The structures vary in length from 2 to 100 μm. Aggregates which are isodiametric in diameter and relatively long are often bent. This feature is the 'hooked rodlet' described by Amelunxen *et al.* (1967). Longitudinally aggregated rodlets that form chimneys around stomata are very striking. Within monocotyledons, longitudinally aggregated rodlets turned out to be characteristic for Arecidae, Zingiberidae and Commelinidae and were described as '*Strelitzia*-Type' (Barthlott & Frölich, 1983). A similar crystalloid form was developed convergently in some other families, mainly based on elongated rosettes. The massive longitudinally furrowed rodlets in *Wooleya* spp. (Crassulaceae) and *Pinus* spp. (Pinaceae) also must be regarded as convergent.

Locally restricted orientation patterns

Arrangement of crystalloids into characteristic, locally restricted patterns. In contrast to the orientation patterns described in the previous sections that cover the whole epidermal surface, locally restricted patterns are connected to a certain epidermal structure. Some patterns are restricted to the costal areas over leaf veins, others are found around stomata or at the base of trichomes. Parallel platelets or platelets grouped in parallel are common over veins independently of the crystalloid type found on the intercostal parts of the leaf blade. Around stomata, platelets are often arranged in circles or resembling electromagnetic field lines (e.g. *Convallaria majalis*, Fig. 30), while they are oriented in parallel rows on the other epidermal cells. The latter are characteristic for the '*Convallaria*-Type' within liliiflorous mono-cotyledons (Barthlott & Frölich, 1983).

Syntopism

Occurrence of different crystalloids on neighbouring cells or on one cell surface. Within one plant different wax crystalloids may occur on the surface of a particular organ. Here, syntopism means the occurrence of morphologically different crystalloids on adjacent epidermal cells (e.g. *Bupleurum salicifolium* R.Br., Fig. 31) or on one cell surface (e.g. *Benthamia alyxifolia* (F. Muell. ex Benth.) Thiegh., Fig. 32). In most cases, platelets are combined with crystalloids of a different shape.

Up to now little has been known about the chemical composition of individual crystalloids. Therefore it remains unclear whether crystalloids differing in shape are of a different chemical composition. However, it was shown that the shape of wax crystalloids depends strongly on the environmental conditions during crystallization (e.g. Jetter & Riederer, 1994). This is called 'polymorphism'. The best known examples of polymorphism are *Brassica oleracea* L. and *B. napus* L. (Juniper, 1960; Whitecross & Armstrong, 1971; Baker, 1974) as well as *Clarkia elegans* Douglas (Hunt, Holloway & Baker, 1976). Depending on growth conditions, tubules,
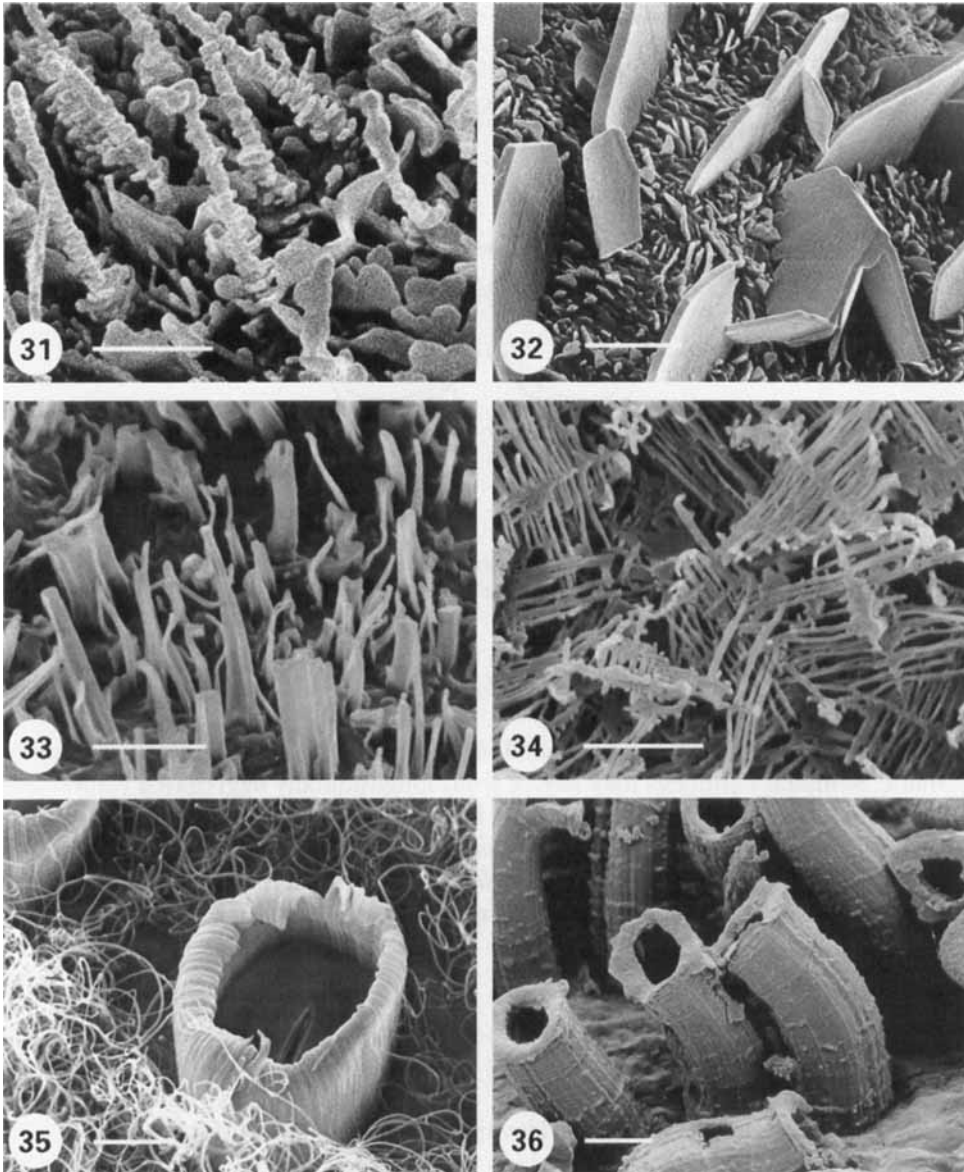
Figures 31–36. Syntopisms and stomatal chimneys. Fig. 31. *Bupleurum salicifolium* R.Br. (Apiaceae): syntopism of platelets and rodlets. Scale bar = 1 µm. Fig. 32. *Benthamia alyxifolia* (F.Muell. ex Benth.) Tiegh. (Loranthaceae): syntopism of plates and platelets. Scale bar = 2 µm. Figs 33, 34. *Brassica oleracea* L. (Brassicaceae): polymorphism. Scale bar = 2 µm. Fig. 35. *Heliconia collinsiana* Griggs (Heliconiaceae): stomatal chimney. Scale bar = 10 µm. Fig. 36. *Colletia cruciata* Gillies & Hook. (Rhamnaceae): stomatal chimney. Scale bar = 20 µm.

platelets, and various rodlets occur adjacent to each other in varying amounts (e.g. *Brassica oleracea*, Figs 33, 34). Therefore, some examples of syntopism may be of a polymorphism that is simply not recognized as such, due to limited knowledge of the wax chemistry.
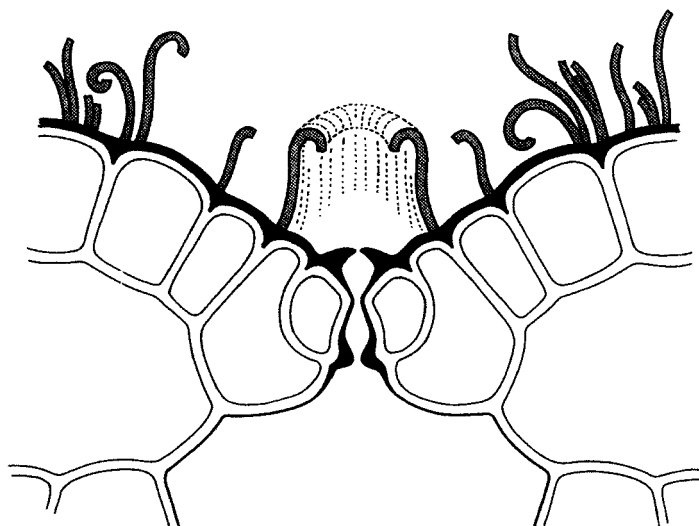
Figure 37. Schematic drawing of the stomatal wax chimneys of *Heliconia collinsiana* Griggs. The wax crystals always protude directly above the anticlinal walls of the stomatal guard cells.

Stomatal chimneys

Crystalloids or aggregations of crystalloids forming hollow cylinders surrounding stomata. Stomatal chimneys may derive from longitudinally aggregated rodlets (e.g. *Heliconia collinsiana* Griggs, Figs 35, 37) or from layers and crusts (e.g. *Colletia cruciata* Gillies & Hook., Fig. 36, *Euphorbia tirucalli* L., Fig. 38). The first are common in monocotyledons characterized by the *Strelitzia*-Type; the latter are characteristic for succulents and many other xerophytic plants.

CORRELATION BETWEEN ULTRASTRUCTURE AND CHEMISTRY

Waxes, according to a strict chemical definition, are esters of long-chain acids and long-chain primary alcohols. However, plant surface lipids, usually called 'epicuticular waxes' are composed of a large mixture of different chemical compounds. They comprise cyclic and long-chain aliphatic components that can be further classified according to their structure, functional groups and by the distribution of their homologues (Fig. 39). Comprehensive reviews of the chemistry of surface waxes are given by Baker (1982), Barthlott & Wollenweber (1981), Bianchi (1995), Martin & Juniper (1970), Tulloch (1976), Walton (1990). Studies of the chemical composition of waxes, mainly the easily obtained alkanes, sometimes serve special chemo-taxonomic purposes (Eglinton *et al.*, 1962; Herbin, 1964; Nishimoto, 1974; Evans *et al.*, 1980; Tulloch, 1981; Wollenweber, 1984; Stevens, 1995).

All information about wax composition was obtained from the isolated cuticular or epicuticular wax, which always more or less includes the obligate wax film, as well as an intracuticular wax component. Nothing is known about the chemical composition of single crystalloids separated from the wax film.

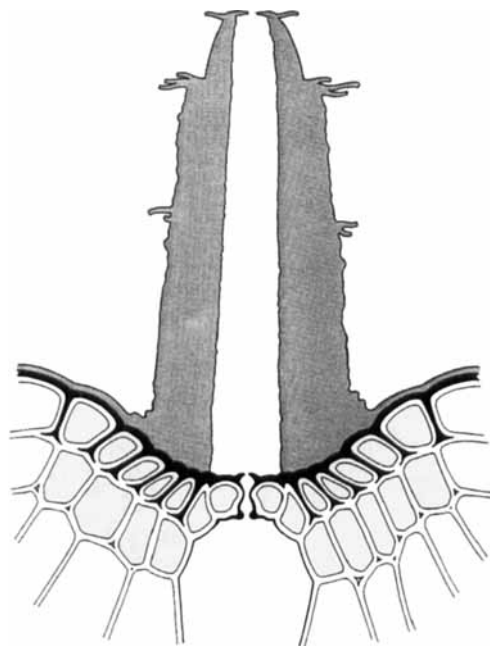Even in the last century, the crystallinity of epicuticular waxes was postulated (de

Figure 38. Schematic drawing of the stomatal wax chimneys of *Euphorbia tirucalli* L.. The wax chimneys derive from a massive crust due to higher wax production of the epidermal cells surrounding the stomata.

Aliphatic compounds
### Most common, but rarely dominant in wax composition

Hydrocarbons	CH_3-$(CH_2)_n$-CH_3	odd	C_{19}-C_{37}
Wax esters	CH_3-$(CH_2)_n$-CO-O-$(CH_2)_m$-CH_3	even	C_{30}-C_{60}
Primary alcohols	CH_3-$(CH_2)_n$-CH_2-OH	even	C_{12}-C_{36}
Fatty acids	CH_3-$(CH_2)_n$-$COOH$	even	C_{12}-C_{36}
Aldehydes	CH_3-$(CH_2)_n$-CHO	even	C_{14}-C_{34}

Less common, but mostly dominant in wax composition

Ketones	CH_3-$(CH_2)_n$-CO-$(CH_2)_m$-CH_3 (e.g. Palmitone)	odd	C_{25}-C_{33}
β-diketones	CH_3-$(CH_2)_n$-CO-CH_2-CO-$(CH_2)_m$-CH_3	odd	C_{27}-C_{35}
Secondary alcohols	CH_3-$(CH_2)_n$-$CHOH$-$(CH_2)_m$-CH_3 (e.g. Nonacosan-10-ol)	odd	C_{21}-C_{33}

Cyclic compounds
e.g. Triterpenoids   e.g. Flavonoids

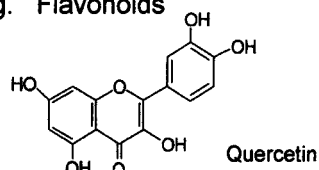HO   β-Amyrin   HO   Quercetin
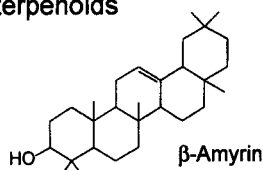
Figure 39. Selection of the main components of epicuticular waxes.

Bary, 1871) and, therefore, a close relationship between their shape and chemical composition was assumed. Nowadays there is common agreement that micromorphology of wax crystalloids depends mainly on their chemical composition
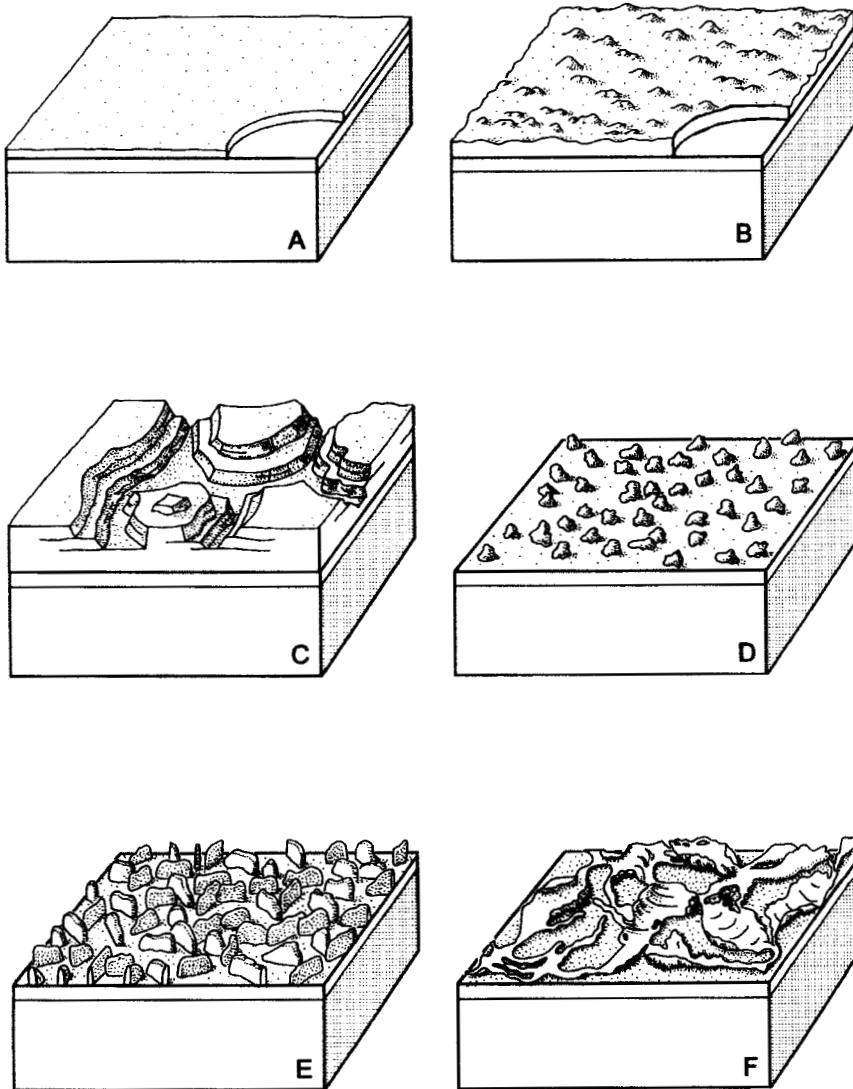
Figure 40. Schematic drawings of the main types of epicuticular wax deposits: A, smooth layers; B, crusts; C, fissured layers; D, granules; E, platelets; F, membraneous platelets.

(surveys in Jeffree *et al.*, 1976; Baker, 1982; Jeffree, 1986). Dominant wax components are usually considered to be responsible for the formation of the crystalloids. Some crystalloid types, especially tubules, demonstrate this by recrystallization of epicuticular waxes and isolated compounds. These *in-vitro* crystals obtained under suitable conditions show the same form and size as the corresponding crystalloids on the plant surface (Hallam, 1970; Jeffree *et al.*, 1975; Jetter & Riederer, 1994, 1995). In these cases, epicuticular waxes are regarded as crystalline. Although Kreger (1948) examined many peculiar plant waxes in terms of crystallinity, this question still remains open for many crystalloid types classified in this paper. Moreover, impressive demonstrations of the relationship between wax micromorphology and chemistry came from wax mutants characterized either by a glaucous or glossy
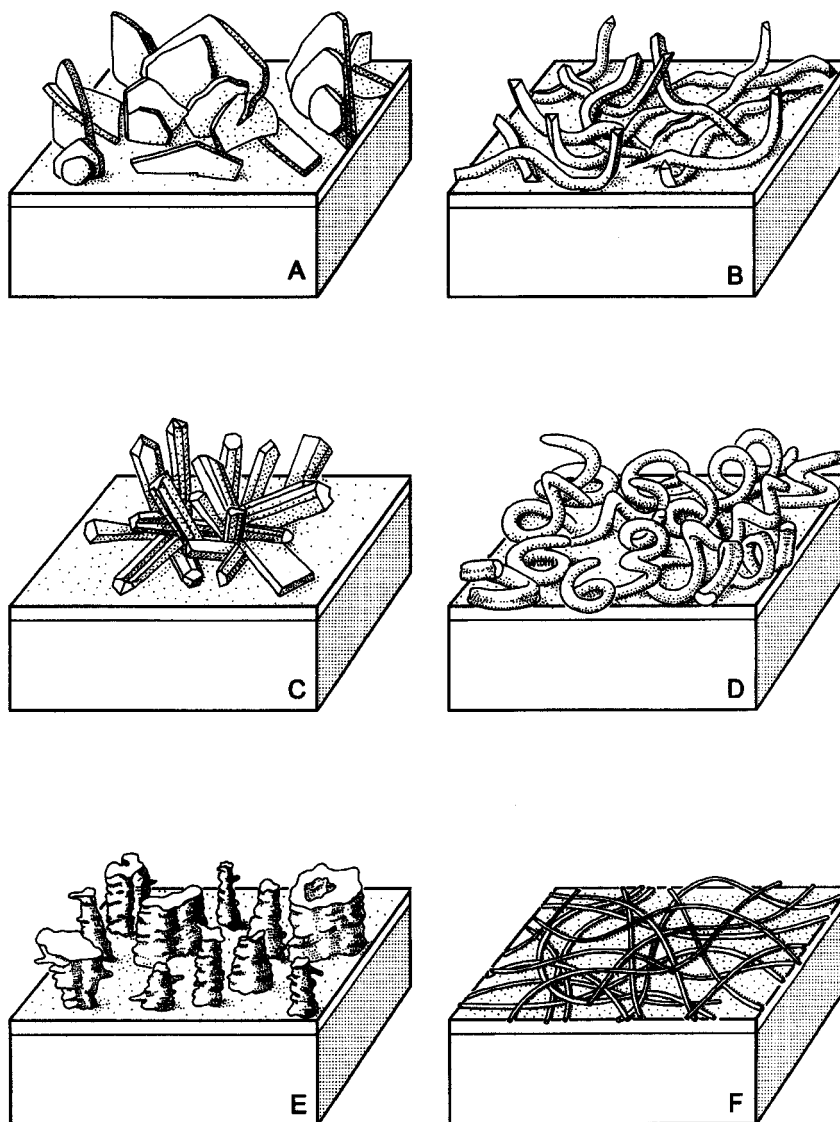
Figure 41. Schematic drawings of the main types of epicuticular wax deposits: A, plates; B, triangular rodlets; C, polygonal rodlets; D, coiled rodlets; E, transversely ridged rodlets; F, threads.

appearance, which are used in studying the biosynthesis of waxes (Wettstein-Knowles, 1974; Jeffree *et al.*, 1976; Koorneef, Hanart & Thiel, 1989; Jenks *et al.*, 1992).

The classification of crystalloid types presented in this paper is almost completely based on micromorphological features. As shown above, it would also be useful, if not necessary, to classify crystalloids by means of their chemical composition. However, in some cases, e.g. tubules, crystalloids can be characterized and differentiated by dominating compounds. In addition chemical composition can be used to define systematically significant wax types, e.g. *Berberis*-Type and *Aristolochia*-Type (Hennig *et al.*, 1994; Barthlott & Theisen, 1995), but this subject of visualized chemotaxonomy is only briefly mentioned here because of the lack of information.
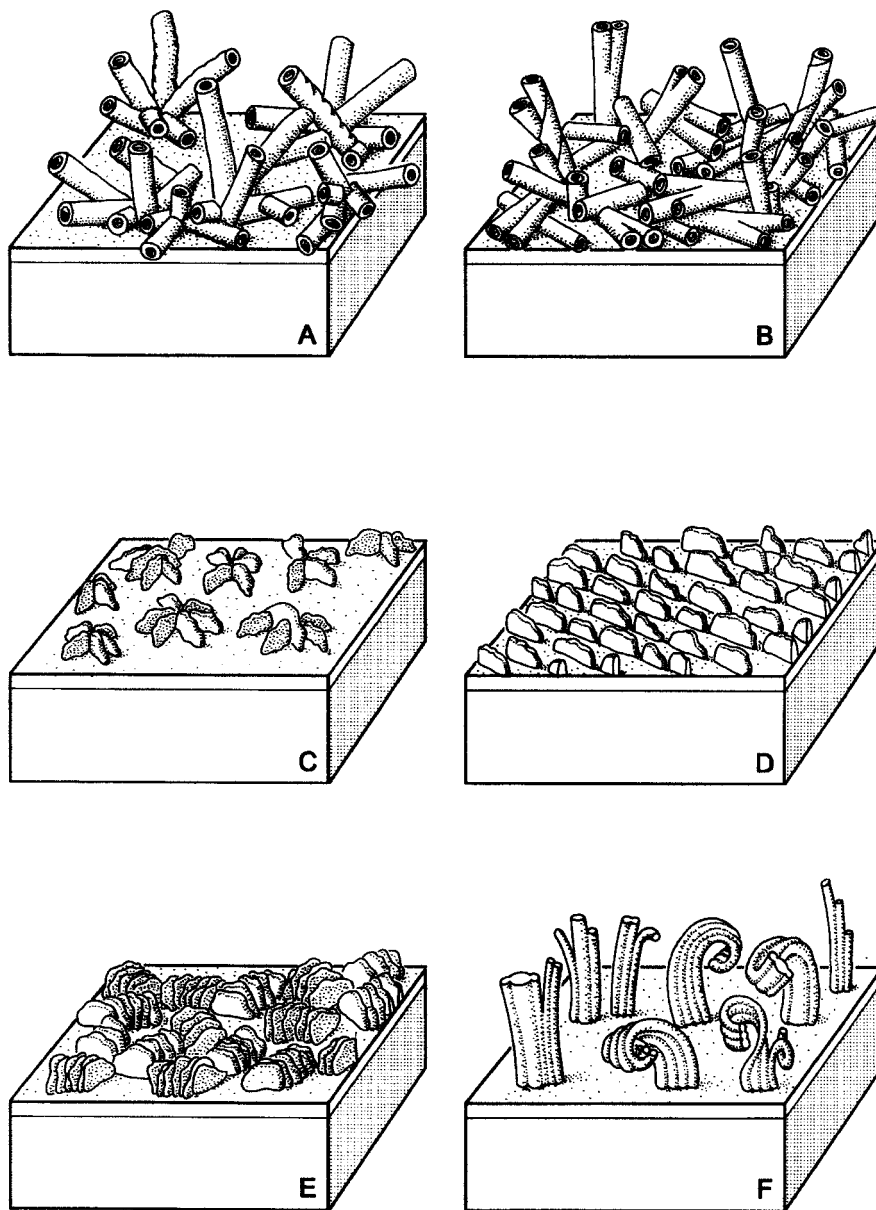
Figure 42. Schematic drawings of the main types of epicuticular wax deposits: A, noncosanol-tubules; B, diketone-tubules; C, rosettes of platelets; D, parallel oriented platelets; E, parallel stacked platelets; F, longitudinally aggregated rodlets.

Chemical composition of major crystalloid types

The following survey of the chemical composition of the main crystalloid types refers only to the dominant components of the bulk wax. No chemical analyses of the pure crystalloids are known.

Platelets are often dominated by primary alcohols. In *Eucalyptus* species, the occurrence of platelets correlates with the dominance of primary alcohols (Hallam & Chambers,

1970). This is also true for many Poaceae and various taxa of different angiosperm families (review in Jeffree, 1986). On the other hand, platelets sometimes are characterized by a high amount of triterpenoids. The leaf wax of the glossy mutant of *Sedum rupestre* L. contains only 0–5% triterpenoids, whereas the triterpenoid content of glaucous leaves covered with platelets ranges from 4 to 62% (Stevens, 1995). Mixtures of triterpenoids from fruits of *Olea europaea* L. and leaves of *Eucalyptus botryoides* Sm. recrystallize *in vitro* as platelets (Baker, 1982).

Polygonal rodlets are associated with the presence of special triterpenoids. In leaf wax from *Tilia tomentosa* Moench, β-amyrine acetate dominates as soon as crystalloids appear on the plant surface (Gülz, Müller & Moog, 1988).

Terete rodlets, which are longitudinally aggregated, show a range of different chemical compositions. The waxes of some members of the Zingiberidae are dominated by wax esters (43–92%), e.g. *Heliconia collinsiana*, *Strelitzia reginae* Banks ex Dryand., (Meusel *et al.*, 1994), *Musa paradisiaca* L., (Baker, 1982) and *Musa balbisiana* Colla, (Freeman & Turner, 1985), as well as the wax of *Copernicia cerifera* Mart. (Vandenburg & Wilder, 1970) and *Typha angustifolia* L. (Meusel *et al.*, 1994). However, the analysis of other species of *Musa* revealed no wax esters. There, alkanes dominate (Freeman & Turner, 1985). If longitudinally aggregated rodlets occur in Poaceae differing compound classes dominate the chemical composition of the wax. Early works on sugar cane wax (*Saccharum officinalis* L.) reveal primary alcohols as the main components (Kreger, 1948). More recent analyses yield high amounts of polymeric aldehydes (Hegenauer, 1963). In contrast to the waxes from the monocotyledonous species mentioned above, the wax from the wax gourd *Benincasa hispida* (Thunb.) Cogn. consists almost completely of triterpenoids (Meusel *et al.*, 1994).

Coiled rodlets are similar in wax chemistry to tubules. Some authors found various β-diketones in the cuticular waxes from *Buxus sempervirens* and some species of *Rhododendron* (Dierickx, 1973; Haas, 1989).

Transversely ridged rodlets appear to be based on different chemical wax compositions. Some authors relate high portions of ketones within the wax to the formation of these crystalloids, e.g. Hall *et al.* (1965) for various species of *Brassica*, Baker (1982) for varieties of *Brassica oleracea*, and Hunt *et al.* (1976) of *Clarkia elegans*. Moreover, Jeffree (1986) considers high amounts of ketones to be responsible for the so-called 'dendritic plates' of several species. For example, in varieties of *Brassica oleracea*, these particular plates are explained as extremely polymorphic crystalloid forms of the transversally ridged rodlets produced by environmental influences on the ultra-structure (Baker, 1982). Of special interest is the occurrence of a certain ketone, hentriacontan-16-one, usually named palmitone. In cuticular waxes it has rarely been found in significant portions, and was recognized for the first time in wax of *Santalum album* L. (Chibnall *et al.*, 1937). The appearance of palmitone in epicuticular wax is predominantly restricted to members of the ancestral Magnoliidae as well as the Paeoniaceae (e.g. Mackie & Ghatge, 1958; Komae & Hayashi, 1971; Gülz *et al.*, 1992; Hennig *et al.*, 1994). Palmitone can be used to define the *Aristolochia*-Type.

Tubules are the best known crystalloids. By means of their main components, two different types can be distinguished. Epicuticular waxes of the so-called *Berberis*-Type always consist of a high percentage of the secondary alcohol nonacosan-10-ol (Holloway, Jeffree & Baker, 1976), often combined with corresponding diols (Jetter

& Riederer, 1995). Sometimes diols prevail in the wax composition, e.g. *Nelumbo nucifera* Gaertn. (Barthlott *et al.*, 1996). The ability of nonacosan-10-ol and its accompanying diols to recrystallize *in-vitro* as tubules has been demonstrated in several investigations (Jeffree *et al.*, 1975; Lister & Thair, 1981; Jetter & Riederer, 1994). On the other hand, epicuticular waxes which appear on plant surfaces as 'diketone tubules' contain predominantly various β-diketones (Wettstein-Knowles, 1974; Jeffree, 1986). In the Poaceae they are often found together with different analogous hydroxy-β-diketones (Tulloch, 1981). Both recrystallize as tubules under suitable *in-vitro* conditions (Hallam, 1970; Jetter, 1993).

Threads as farinose glandular exudates in many species of *Primula* as well as in the gymnogrammoid ferns mostly consist of flavonoids (Wollenweber, 1984). The unsubstituted flavon prevails in the wax of *Primula*. Besides flavonoids, other cyclic compounds chiefly can determine the composition of wax in ferns, rarely chalkones or dihydrochalkones (Wollenweber, 1977), sometimes triterpenoids (Arriaga-Giner, Rumbero & Wollenweber, 1992). The relation to micromorphology has been shown for some components only by recrystallization experiments (Barthlott & Wollenweber, 1981).

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