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Glandular Hairs and Secreted Material in *Salvia blepharophylla* Brandegee ex Epling Grown in Italy

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Morphological and histological investigations of cuticle and indumentum, as well as identification of the main components of the secreted material, were carried out for *Salvia blepharophylla* Brandegee ex Epling. Besides non-glandular hairs, three types of glandular trichomes (peltate and capitate) are described and compared with trichomes in other species. The histological findings and chemical analysis of the essential oil and leaf surface extracts revealed a complex secretion product. GC-MS analysis of the essential oil showed that eugenol, *cis*-3-hexenyl benzoate, *cis*-jasmone, *trans*-nerolidol, benzyl alcohol and C_{19}-C_{23} *n*-alkanes were the main identifiable components, whereas the flavonoids nuchensin and pedalitin, the neo-clerodane diterpenoid salvianduline D, and the triterpenoids ursolic acid and α-amyrin were isolated from the extract.

Key words: *Salvia blepharophylla* Brandegee ex Epling, glandular hairs, histochemistry, exudate flavonoids, diterpenoids, triterpenoids, essential oil.

INTRODUCTION

The genus *Salvia* comprises many species, some of which are of economic interest (El-Gazzar and Watson, 1970; Werker, Ravid and Putievsky, 1985*a*, *b*). The glandular hairs and essential oil content of various species have been described (Verzár-Petri and Then, 1975; Croteau *et al.*, 1981; Venkatachalam, Kjonaas and Croteau, 1984; Werker *et al.*, 1985*a*, *b*; Werker, 1993; Serrato-Valenti *et al.*, 1997). The aim of this work was to carry out a morphological investigation of the cuticular covering and indumentum of adult leaves of *Salvia blepharophylla*, so as to improve the present knowledge of leaf anatomy, and phytochemistry of the epidermal leaf exudate of *Salvia* spp. *Salvia blepharophylla* is a procumbent ornamental plant, native of Mexico (Epling, 1939), which was introduced to the Hanbury Botanical Gardens, Ventimiglia, Italy in 1978. It has potential as a medicinal plant.

Glandular trichomes are known to be the primary sites of production of bioactive secondary products which may function as plant growth regulators and defend the plant against insects, other pathogens and, possibly, other plants (Croteau, 1977; Bell, 1981; Kelsey, Reynolds and Rodriguez, 1984; Wagner, 1991; Werker, 1993; Duke, 1994). In addition, it has been suggested that, in some desert species, the principal role of glandular trichomes is to secrete large quantities of exudate, which form a continuous layer on the plant surface, increasing light reflectance, thereby reducing leaf temperature (Wagner, 1991; Grayer *et al.*, 1996). In many Lamiaceae species, such glandular exudates consist mainly of mono-, sesqui-, di- and triterpenes, sesquiterpene lactones and flavonoid aglycones (flavones and isoflavones) (e.g. Wollenweber, 1984, 1985; Tomàs-Barberàn and Wollenweber, 1990).

In the present work, light and scanning electron microscopy were used to determine the morphology of glandular hairs; histochemical procedures were used to localize the secreted substances within and on the surfaces of the hairs; and essential oil analysis and preliminary phytochemical investigation of the leaf exudate were carried out to shed light on the chemical nature of the secreted material. The phytochemical work concentrated on the flavonoid and isoprenoid content of the exudate because of their proven antimicrobial, antifungal, insecticidal and insect-deterrent activities (Tomàs-Barberàn, Msonthi and Hostettmann, 1988; Tomàs-Barberàn and Wollenweber, 1990; Wagner, 1991; Grayer and Harborne, 1994; Harborne and Grayer, 1994; Rodríguez-Hahn, Esquivel and Cárdenas, 1994; Dakora, 1995; Grayer *et al.*, 1996).

MATERIALS AND METHODS

Plant material

Small non-flowering branches from established plants of *Salvia blepharophylla* Brandegee ex Epling were obtained from the Hanbury Botanical Gardens of La Mortola, Ventimiglia, Italy. These were identified by Dr P. G.

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Campodonico, and voucher specimens were deposited in the Hanbury Botanical Gardens herbarium. The leaves were used for histochemical, chromatographic and extractive studies.

Microscopical investigation

Light microscopy (*LM*). Both fresh and embedded material was used for bright-field microscopy. Transverse and paradermal hand sections of the lamina were made from the mid-part of fully-grown leaves. Embedded material was prepared as follows: adult leaves were fixed with FAA (formalin 5:acetic acid 5:50% ethanol 90) overnight, then dehydrated in a graded ethanol series and embedded in JB4 resin (Poliscience Inc., Warrington, PA 18976-2590, USA) in BEEM capsules (Brinn and Pickett, 1979). Longitudinal sections were cut at intervals of 5–10 μm using a Reichert OmU2 ultramicrotome equipped with a glass knife. General staining procedures comprised: (1) Toluidine Blue O (TBO) at pH 5·6 as a general stain for tissue structure (Heslop-Harrison and Heslop-Harrison, 1981); (2) Ponceau 2R plus Azure II for a differential staining of storage proteins (red) and cell walls (blue) (Gutmann *et al*., 1996); (3) Toluidine Blue O (TBO) with iodine/potassium iodide (diluted Lugol's stain) poststaining treatment and (4) Safranin O plus Azure II to show phenolic deposits (Gutmann, 1995); (5) Alcian Blue 8GX at pH 2·5 (Pearse, 1985) and (6) Ruthenium Red (Jensen, 1962) for polysaccharides with acidic groups (Amarasinghe, 1990); and (7) Nile Blue for neutral and acid lipids (Jensen, 1962). Specific staining procedures comprised: (8) Nadi reagent for essential oils (David and Carde, 1964); (9) ferric chloride for dihydroxyphenols (catechol-type phenols) (Gahan, 1984); and (10) Vanillin/HCl for flavonoids (Guerin, Delaveau and Paris, 1971).

For fluorescence microscopy, free hand-sectioned fresh material was treated using the following general staining procedures: (1) Nile Red for lipids (Greenspan, Mayer and Fowler, 1985); (2) Fluorol Yellow 088 for lipids (Brundrett, Kendrick and Peterson, 1991); (3) Auramine O for unsaturated, acidic waxes and cutin precursors (Gahan, 1984); and (4) Calcofluor White for cellulose and carboxylated polysaccharides (Hughes and McCully, 1975). For fluorescence and bright-field investigation, a Leitz Dialux fluorescence microscope with a HBO 50W mercury vapour lamp and a filter block H2:BP 390–490 were used.

Scanning electron microscopy (*SEM*). Adult leaf segments were fixed for 24 h in FAA at room temperature and subsequently dehydrated in a graded ethanol series. The specimens underwent critical point drying with liquid CO_2, were gold sputtered and, finally, were examined with a Philips 515 SEM at an acceleration voltage of 20 kV. Photographs were taken using Kodak Technical Pan film.

Identification of secreted material

Extraction and analysis of surface materials. Fresh shoots (2 kg) were immersed in CH_2Cl_2 for 20 sec at room temperature. After filtration, the solvent was removed under reduced pressure, leaving 8 g of extract, from which three major fractions were obtained by column chromatography (Sephadex LH-20 columns; 50 cm × 2·5 cm; $CHCl_3/CH_3OH$ (1:1) eluent; analytical TLC control with Alufolien Kieselgel 60 F_{254} Merck, $CHCl_3/CH_3OH$ 95:5 and $CHCl_3/CH_3OH/HCOOH$ 85:15:0·2; 1 g portions). Two fractions were crystallized from CH_3OH/H_2O. The third was further resolved into its components by column chromatography (silica gel columns; 50 cm × 3 cm; analytical TLC control with Alufolien Kieselgel 60 F_{254} Merck, $CHCl_3$ and $CHCl_3/CH_3OH$ 95:5; 1·2 g portions) with varying mixtures of *n*-hexane and $CHCl_3$, and these were recrystallized from $CHCl_3/CH_3OH$, *n*-hexane/C_2H_5OH and C_2H_5OH. ^{1}H- and ^{13}C-NMR-spectra were performed with a Bruker-DRX 600 spectrometer. EI-MS were obtained with a Hewlett-Packard 5890 gas chromatograph with a 5971A selective mass detector. IR-spectra were obtained with a Perkin-Elmer 1310 spectrophotometer. UV-spectra were performed with a Hewlett-Packard diode array spectrophotometer 8452A. Optical rotation was recorded on a Perkin-Elmer 241 MC polarimeter.

Essential oil extraction and analysis. Fresh aerial parts of non-flowering plants were subjected to steam distillation under reduced pressure to produce an oil yield of 0·07% (w/w). The essential oil was analysed using a Hewlett-Packard 5890 Series II GC-MS apparatus equipped with a capillary column HP-Innowax (30 m long, 0·25 mm internal diameter, 0·25 μm film thickness). The carrier gas was helium with a flow rate of 1 ml min^{-1} and the injection temperature was 250 °C. The column temperature programme was 60 °C for 8 min, followed by an increase of 3 °C min^{-1} to 180 °C, which was then maintained for 5 min, followed by an increase of 40 °C min^{-1} to 250 °C. Identification of the components was achieved by comparison of their mass spectra with WILEY-NBS Library spectra (Wiley 275), and of retention indices with authentic samples. Retention indices were determined experimentally under the same analytical conditions as the oil samples, by the use of standard mixtures of *n*-alkanes (SIGMA Chem. Comp.). They were calculated by linear interpolation between retention times of compound and alkanes, notwithstanding the isothermal periods of analysis, and are acceptable only for internal comparisons within the actual set of chromatograms (Sun *et al*., 1993). The quantification of each component was performed by integration of total ion chromatograms (autointegration method).

RESULTS

Microscopy and histochemistry

The leaves of *Salvia blepharophylla* bore an indumentum of unbranched non-glandular and glandular trichomes on both surfaces. Three types of non-glandular hairs were observed: (1) multicellular, long, thin, simple hairs (Fig. 1A), with distinct articulation between cells, and acute apices, situated on the leaf margins; (2) multicellular, uniseriate, four–five celled short hairs, with swollen basal cells, acute apices and thick warty cell walls (Fig. 1B), abundant on abaxial surfaces, over midribs and major veins; and (3) unicellular papillae associated with a central

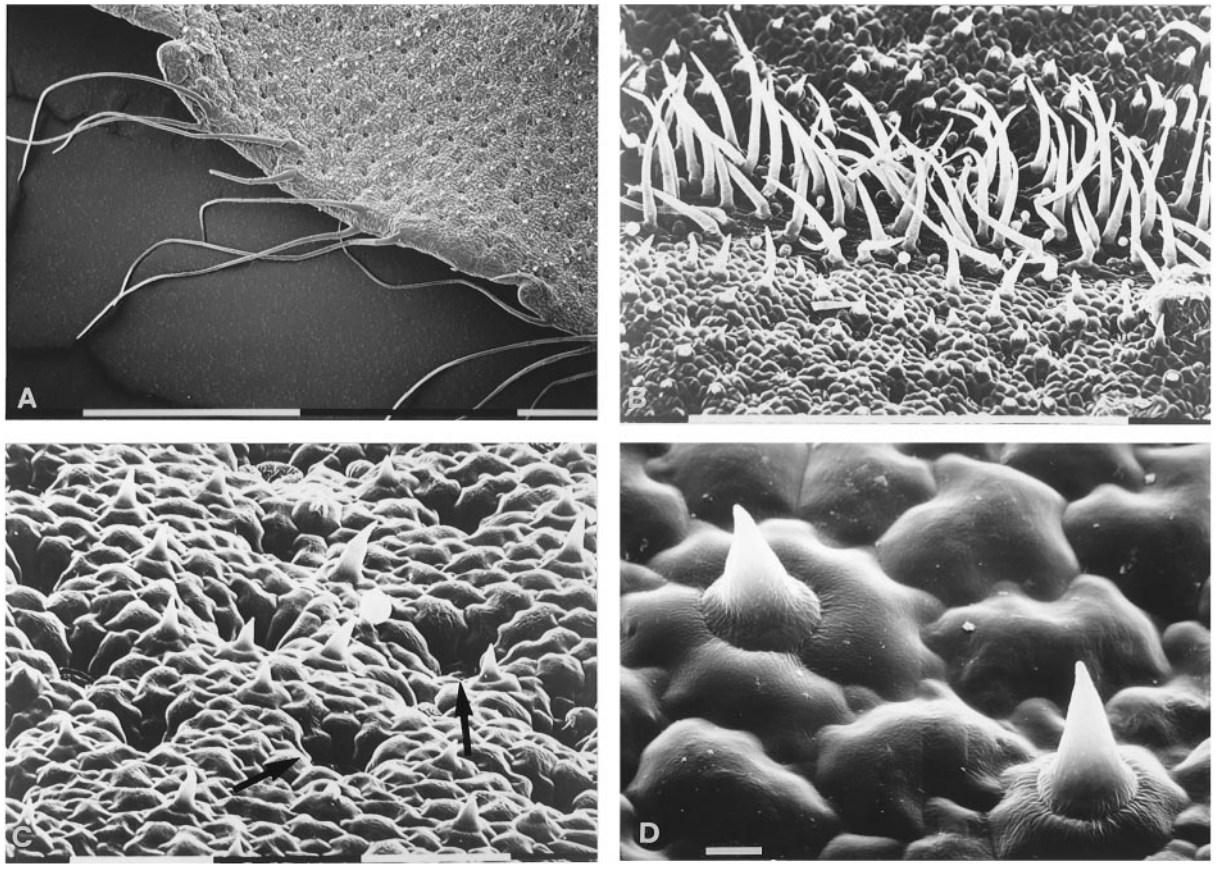


FIG. 1. Scanning electron micrographs of *S. blepharophylla* leaf non-glandular trichomes. A, Multicellular, long simple hairs on the leaf margin. Bar = 1 mm. B, Multicellular uniseriate four–five celled short hairs over midrib. Bar = 1 mm. C, Abaxial leaf surface showing glandular and non-glandular trichomes. Peltate glandular hairs are located in epidermal depressions (arrow). Bar = 1 mm. D, Unicellular papillae. Bar = 10 μm.

circumscribed area of the outer wall of the swollen basal epidermal cell, with enlarged bases and acute apices (Fig. 1D), which were very frequent on the leaf abaxial surface.

The glandular hairs included peltate and capitate types. The peltate trichomes, whose secretion product is released to the outside only when the hair is touched ('long-term glandular hairs', as reported in Werker, 1993), and which, in the adult leaf, were located in epidermal depressions (Fig. 1C), consisted of one basal epidermal cell, a stalk cell (Fig. 5A) and a broad head (Fig. 2B–D) of four secretory cells (very rarely the head consisted of three or five cells). Two types of capitate trichome, whose secretory materials are extruded to the outside soon after their production ('short-term glandular hairs', as reported in Werker, 1993), were present: hairs consisting of one basal epidermis cell, one stalk cell and a round head (Fig. 5B) of two broad cells (capitate type I); and hairs (Fig. 3D) consisting of a stalk cell, a neck cell and a single pear-shaped head cell (capitate type II). The type II capitate hairs were mainly located along the veins, especially on the abaxial side of the leaf.

The surface of the mature peltate glands appeared sutureless because the accumulation of secretions in the subcuticular space distended the cuticle (Fig. 2A). The so-formed subpectic layer-cuticle space increased considerably in volume to constitute a storage pool which engorged with the material produced by the secretory cells (Fig. 5G–I). When touched, the cuticle ruptures, releasing the secretory product over the leaf surface. Two possible mechanisms for release were observed: failure of the cuticle along an equatorial line of weakness (Fig. 2E and F) and the subsequent detachment of the cuticular cap (Fig. 2G), and the passage of volatile components through minute pores observed in the cuticular structure (Fig. 2E).

The apical region of the type I capitate hairs appeared to be either wrinkled or slightly smooth (Fig. 3A) indicating that, initially, the cuticle was closely attached to the secretory cells, emphasizing the cell outlines, but that, subsequently, it became slightly detached from the secretory cell walls. In the small chamber so-formed, the secreted material at first accumulated, but, under pressure, the cuticle soon ruptured (Fig. 3B) releasing the secretion product. Droplets of secreted material, which appeared to have passed through the intact cuticle, were also observed (Fig. 3C).

In type II capitate glandular trichomes, secretory materials were released soon after their production, by the breakage of the cuticle (Fig. 3D) or *via* droplets through the cuticle (Fig. 3E). No appreciable cuticular elevation was observed in this type of capitate hair (Fig. 5E). Both non-glandular and glandular trichomes had a cuticular covering that was thicker than that of the epidermis. Surface warts were

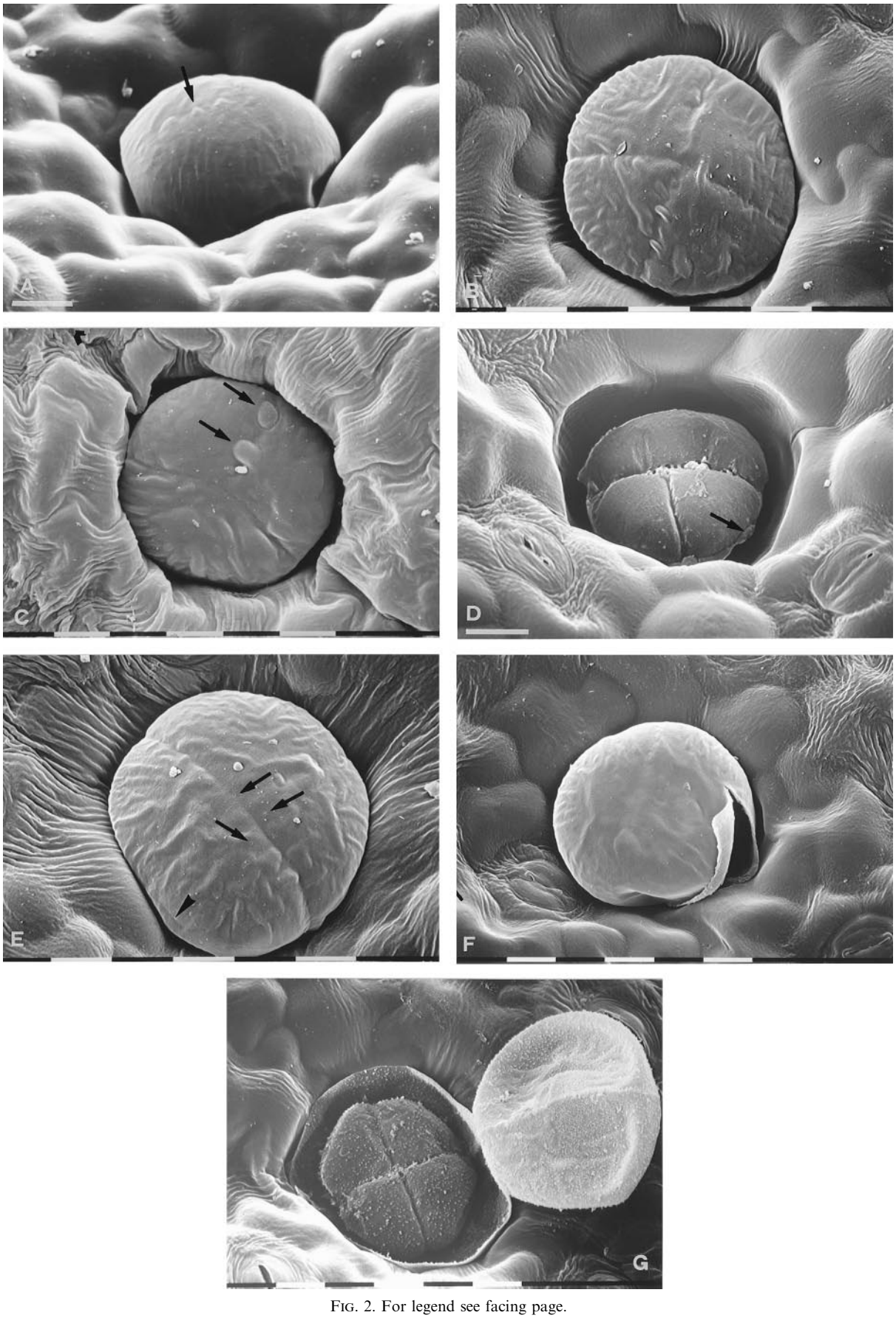


FIG. 2. For legend see facing page.

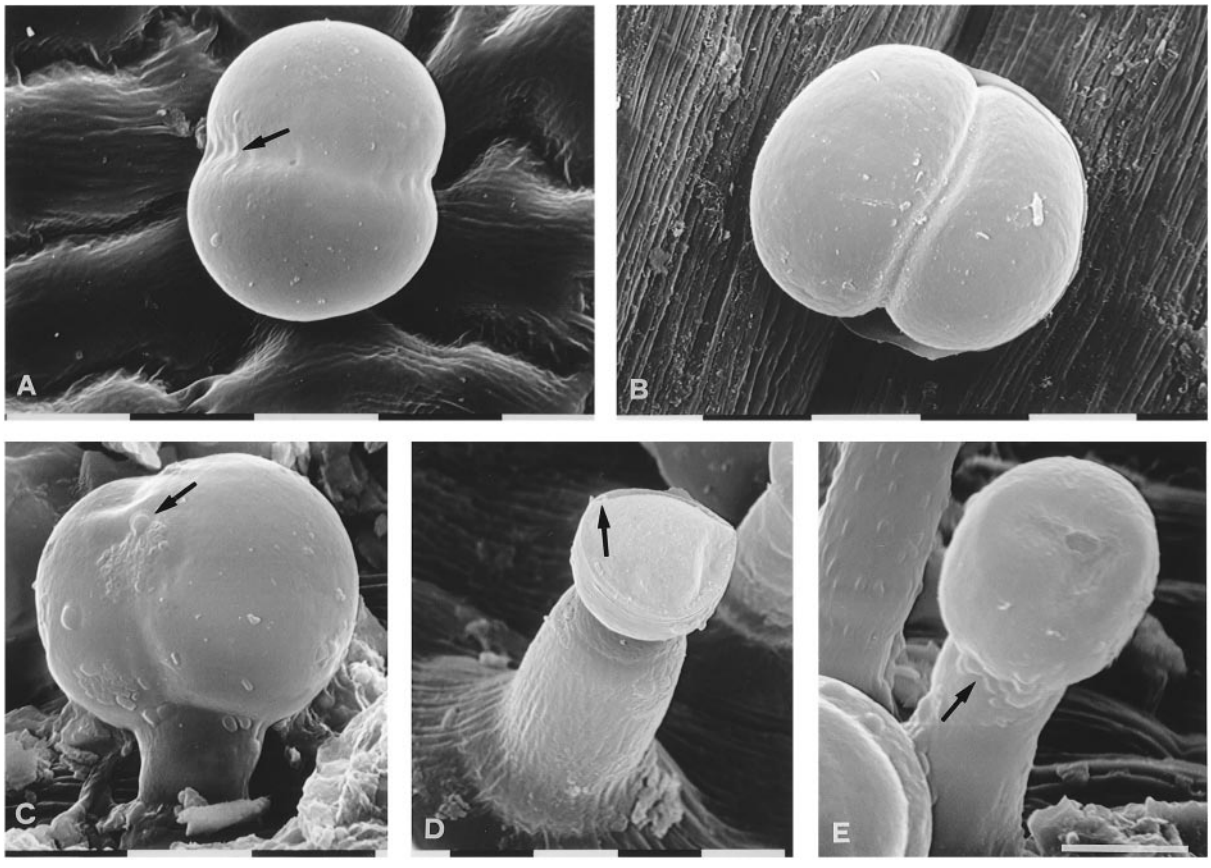


FIG. 3. Scanning electron micrographs of *S. blepharophylla* leaf glandular capitate trichomes. Bars = 10 μm. A–C, Capitate type I. A, The cuticular cap is closely attached to the head cells (arrow). B and C, Possible mechanisms for the release of the secreted material. B, The cuticle has ruptured and the secretion product released. C, Drops of the secreted material are evident on the cuticular surface (arrow). D and E, Capitate type II. Possible mechanisms for the release of the secreted material: by damage of the cuticle (D; arrow) and by passage of droplets through the cuticle (E; arrow).

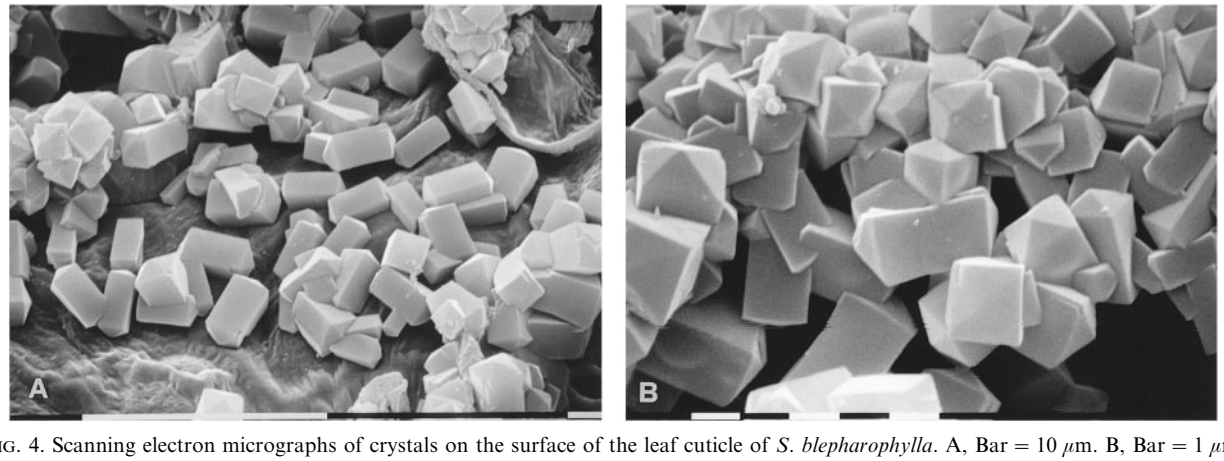


FIG. 4. Scanning electron micrographs of crystals on the surface of the leaf cuticle of *S. blepharophylla*. A, Bar = 10 μm. B, Bar = 1 μm.

FIG. 2. Scanning electron micrographs of *S. blepharophylla* leaf glandular peltate trichomes illustrating different stages of development. Bars = 10 μm. A, Mature peltate gland. Note the drops of secreted material below the cuticular cap (arrow). B, Juvenile trichome. The four secretory head cells are visible. C, Trichome with three secretory head cells. Drops of the secreted material are visible below the cuticle (arrow). D, Trichome with five secretory head cells. The cuticular cap has been ruptured (arrow) and the secretion product released. E–G, Possible mechanisms for the release of the secreted material. E, Cuticular pores (arrows) and equatorial line of weakness (arrowhead) are visible on the cuticular surface. F, The cuticular cap begins to rupture along a line of weakness. G, The cuticular cap has been completely detached.

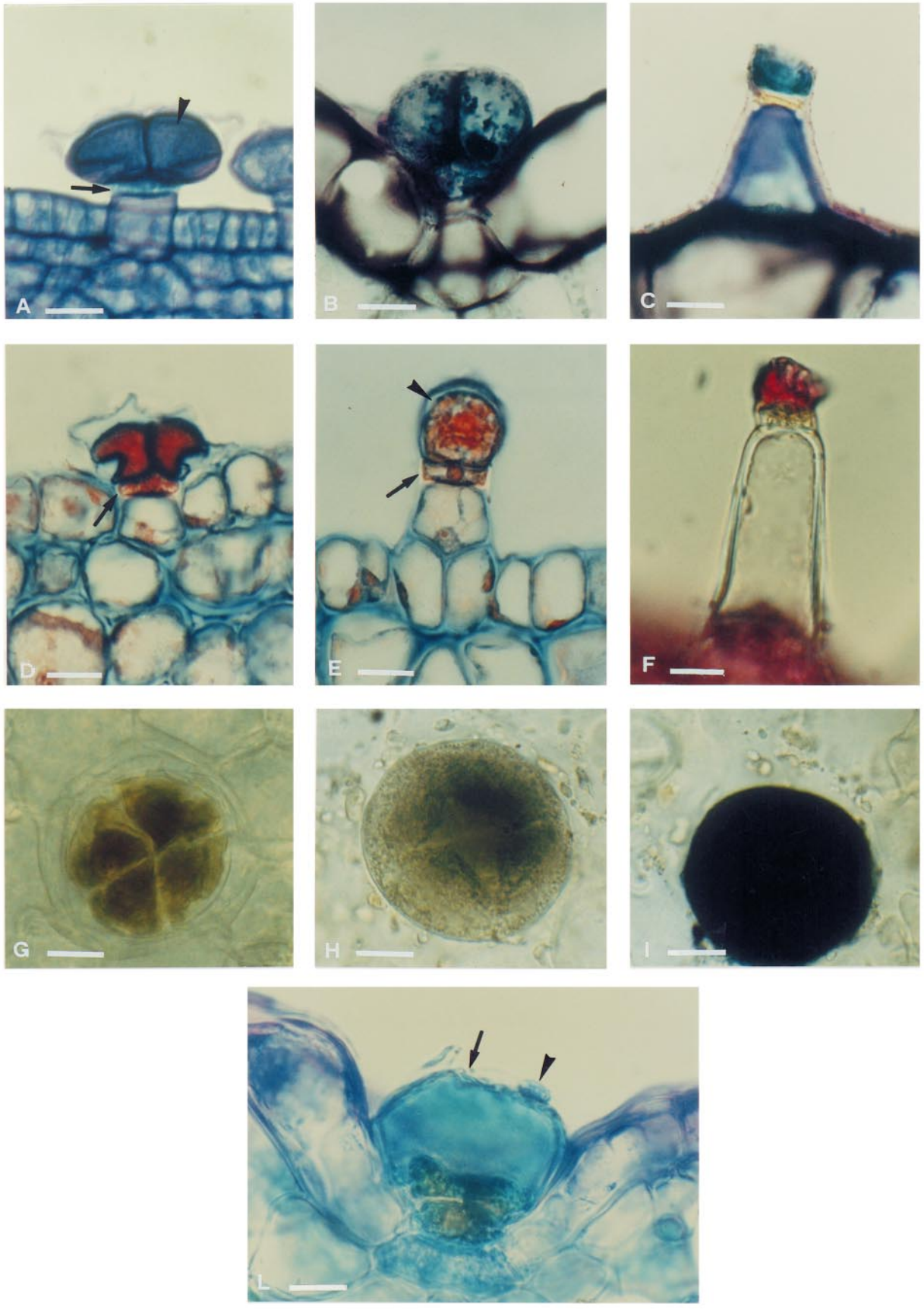


FIG. 5. For legend see facing page.

TABLE 1. *Histochemistry of the secretion products of the glandular trichomes of* Salvia blepharophylla *at the secretory stage*

Staining procedure	Target compounds	Observed colour	Peltate trichomes	Capitate Type-I trichomes	Capitate Type-II trichomes
Nadi	Terpenes	Violet-blue	+++	++	++
Ferric Chloride	O-Dihydroxy phenols	Dark-green	+++	—	—
Vanillin/HCl	Flavonoids	Cherry-red	++	—	—
Auramine O	Unsaturated, acidic waxes and cutin precursors	Bright greenish-yellow fluorescence	+++	++	+++
Fluorol Yellow	Lipids, terpenes, suberin lamellae, hydrophobic structures	Bright yellow fluorescence	+++	+++	+++
Ruthenium Red	Polysaccharides	Red	+++	+++	+++

present in longitudinal rows on non-glandular hairs and some papillae (Fig. 2D).

The histochemistry of the secretion product of the glandular trichomes at the secretory stage is reported in Table 1. In mature peltate trichomes, the elevated cuticle resisted penetration by staining reagents such as Ruthenium Red, Vanillin/HCl and Nile Blue (both hydrophilic and lipophilic substances). The secreted material appeared opaque and emulsified. When the trichome ruptured, the secretion product became stained (Fig. 5L). The cuticle of peltate trichomes stained with Alcian Blue for polysaccharides, Nile Red and Nile Blue for lipids (not shown), but also Calcofluor White for cellulose (Fig. 6C).

In peltate glandular trichomes, the head cells revealed the contemporary presence of phenolic, lipidic and polysaccharidic compounds, being stained with $FeCl_3$, Fluorol Yellow and Ruthenium Red (not shown) even before there was evidence of secretion product in the subcuticular space. In mature organs, both peltate and capitate hairs showed phenolic substances by the dark blue staining of the secreted material with TBO at pH 5·6 (Fig. 5A). Nile Blue (Fig. 5L) and Ruthenium Red (Fig. 5F) procedures also gave positive reactions in all hair types, indicating lipidic and polysaccharidic compounds respectively. In addition, the secretion product in peltate hairs stained positively with Auramine O (Fig. 6E) and Fluorol Yellow (Fig. 6A and B) for lipids, and with $FeCl_3$ (Fig. 5I) and Vanillin/HCl (not shown) for phenolics. Safranin O plus Azure II stained the secreted phenolics only in peltate trichomes, whereas Nile Red revealed the presence of red lipidic droplets in the secretory cell of type II capitate hairs (not shown).

Modified cell walls were present in the stalk and neck cells of both juvenile and mature glandular hairs. The lateral walls of the peltate and capitate type I stalks, as well as the lateral walls of the capitate type II neck cells, showed a strong reaction with the lipid indicators Fluorol Yellow (Fig. 6A and B), Nile Red (not shown) and Auramine O (Fig. 6E), whereas they stained negatively with reagents for polysaccharidic substances such as Calcofluor White (Fig. 6C), Alcian Blue at pH 2·5 (not shown) and Ponceau 2R plus Azure II (Fig. 5D and E).

The stalk cell of peltate and type I capitate hairs, as well as the neck cell of type II capitate hairs, showed positive staining with the lipid reagents Nadi, specific for essential oils, and Nile Blue, both in immature and mature organs (not shown). Furthermore, TBO at pH 5·6 (Fig. 5A) and Ruthenium Red (not shown) stained stalk and neck cells in mature organs metachromatically green and red purple, respectively, indicating the contemporary presence of phenolic and polysaccharidic substances. In peltate hairs, the stalk cell also reacted positively with the phenolic reagents $FeCl_3$ and Vanillin/HCl (not shown).

Phytochemistry

Five compounds were identified following chromatographic separation of the leaf surface extract (Fig. 7). Column chromatography on Sephadex LH-20 gave (in order of elution) a fraction with a mixture of components 3, 4, 5 (3·6 g), a fraction containing 1 and a fraction containing 2. Fractions with 1 and 2 yielded 193 mg and 22 mg of crude nuchensin and pedalitin, respectively (Fig. 7), which were recrystallized from CH_3OH/H_2O. The fraction with 3, 4 and 5 was chromatographed on a silica gel column with mixtures of *n*-hexane/$CHCl_3$ of increasing polarity. Elution with *n*-hexane/$CHCl_3$ (1:1) gave 300 mg of crude 4, which was recrystallized from $CHCl_3/CH_3OH$. Elution with *n*-hexane/$CHCl_3$ (25:75) yielded 35 mg of crude 5, which was recrystallized from *n*-hexane/C_2H_5OH. Elution with $CHCl_3$ gave 250 mg of crude 3, which was recrystallized from

FIG. 5. Bright-field micrographs of *S. blepharophylla* leaf trichomes stained with different reagents. Bars = 5 μm. A, Peltate trichome. Young leaf. TBO pH 5·6. Cross-section showing the metachromatic staining (arrow) of the short stalk cell (green), and the ortochromatic dark blue staining (arrowhead) of the secretory head cells. B and C, TBO with iodine/potassium iodide poststaining treatment. B, Capitate type I. C, Type II capitate trichome. The neck cell has reacted with an ambiguous staining (yellow). D and E, Ponceau 2R plus Azure II. D, Peltate trichome. The stalk cell walls have not reacted (arrow). E, Capitate type II. Note the subcuticular space (arrowhead). The neck walls have not reacted (arrow). F, Ruthenium Red. The secretory cell of a capitate type II trichome is stained positively, indicating the presence of polysaccharides. G–I, $FeCl_3$. Peltate trichome. G, Pre-secretory stage. The head cells are stained. H, Secretory phase. The secreted material appears to be an emulsion. I, Mature trichome. The subcuticular space is filled with phenolic compounds. L, Nile blue test. Peltate trichome. The secreted material has become stained through rupture in the cuticular layer (arrow). A drop of secreted material appears to have been released (arrowhead).

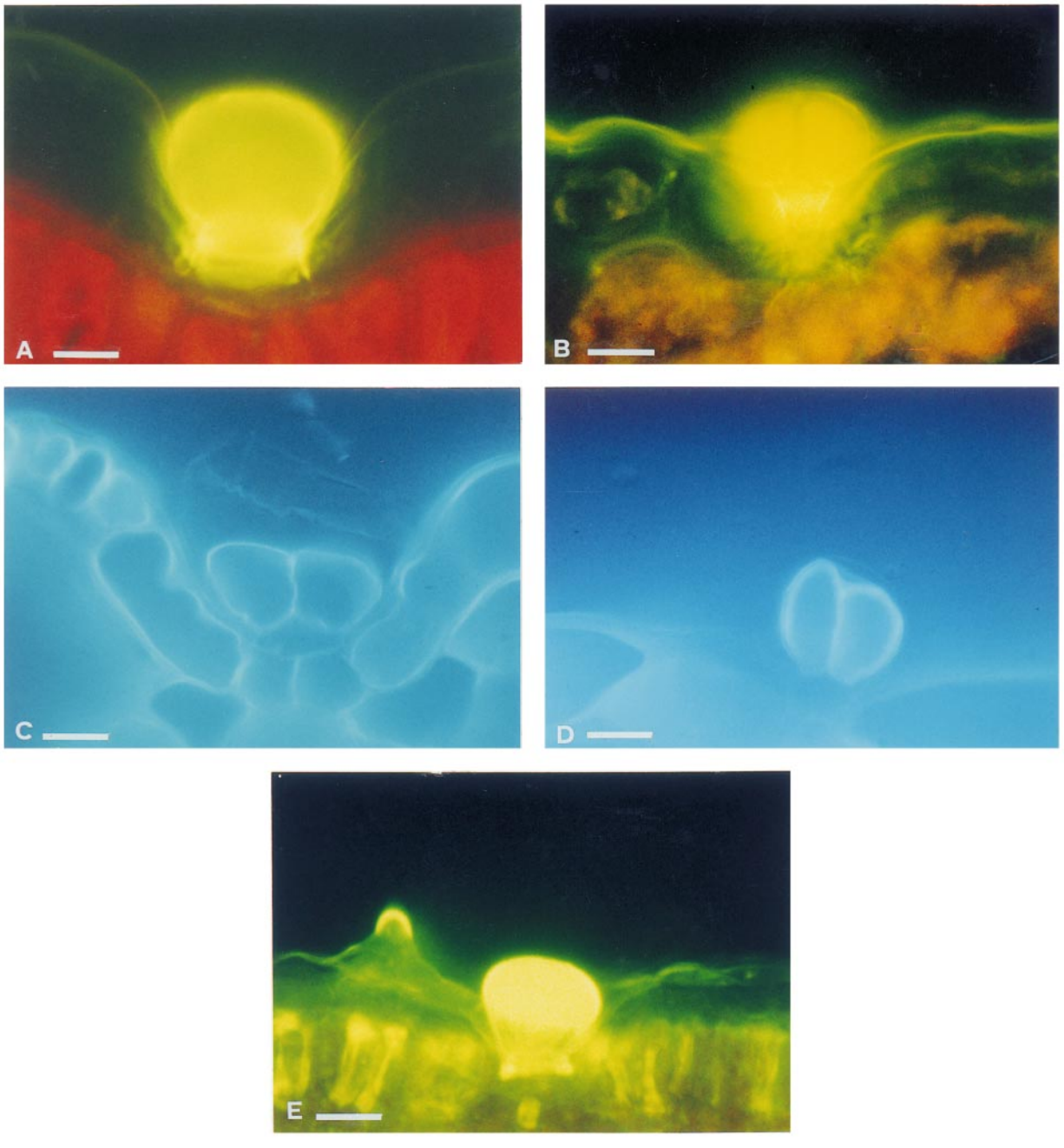
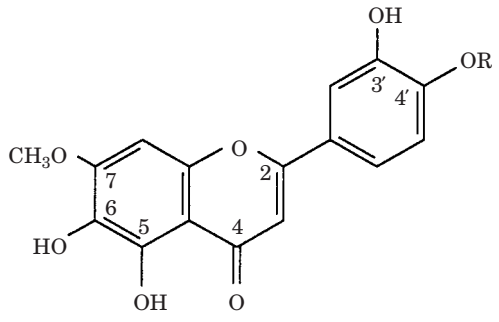


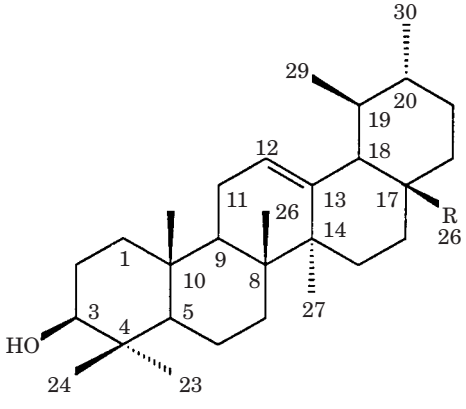
FIG. 6. Fluorescence micrographs of free-hand sections of *S. blepharophylla* leaf trichomes. Bars = 19 μm. A and B, Fluorol Yellow 088. A, Peltate hair. B, Capitate type I showing fluorescence of the secreted material inside the head cells. Note the bright fluorescence of both stalk lateral cell walls. C and D, Calcofluor White. C, Peltate hair. D, Capitate type I. Both stalks lack fluorescence. E, Auramine O staining. The subcuticular space of the peltate hair shows a strong reactivity.

C_2H_5OH. The two flavonoid aglycones, nuchensin (1), and pedalitin (2), and ursolic acid (3) (Fig. 7) were identified by comparison with authentic samples (TLC, IR spectroscopy, UV spectroscopy) previously isolated from the whole extract of *Salvia blepharophylla* (Bisio *et al.*, 1997). Compound 4 was assigned the molecular formula $C_{20}H_{22}O_5$ by EI-mass spectrometry and microanalysis. From its melting point, optical rotation and UV spectra, IR spectra, ^{1}H- and ^{13}C-NMR data, it was identified as salvianduline-D (Maldonado *et al.*, 1994). Compound 5 was identified as α-amirin by its ^{13}C-NMR spectrum (Wehrly and Nishida, 1979).

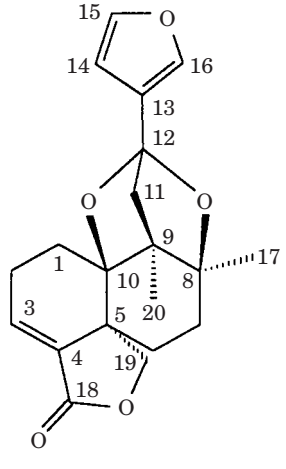
The results of GC-MS analysis of the steam distillate are presented in Table 2. These show the absence of monoterpene and sesquiterpene hydrocarbons; low levels of oxygenated monoterpenes and oxygenated sesquiterpenes (only *trans*-nerolidol identified); and the presence of carotenoid degradation products, non-terpenoid oxygenated substances and linear alkanes.



1: Nuchensin (R = CH_3)
2: Pedalitin (R = H)



3: Ursolic acid (R = COOH)
5: α-Amyrin (R = CH_3)



4: Salvianduline D

FIG. 7. Compounds isolated from the exudate of *Salvia blepharophylla*. 1, Nuchensin (5,6,3′-trihydroxy-7,4′-dimethoxyflavone); 2, pedalitin (5,6,3′,4′-tetrahydroxy-7-methoxyflavone); 3, ursolic acid (3-β-hydroxyurs-12-en-28-oic acid); 4, salvianduline D (8,12;10,12;15,16-triepoxy-*trans*-cleroda-3,13(16),14-triene-19,18-olide); and 5, α-amyrin (urs-12-en-3β-ol).

TABLE 2. *Composition* (*w*/*w*%) *of essential oil of* Salvia blepharophylla

Components	%
Oxygenated monoterpenes	3·9
Linalool	2·1
α-Terpineol + Borneol(?)	1·8
Oxygenated sesquiterpenes	4·0
trans-Nerolidol	4·0
Carotenoid degradation products	8·7
Theaspirane A or B	1·8
β-Damascone	2·6
β-Ionone	2·1
trans-β-Ionone-5,6-epoxide	2·2
Non-terpenoidic oxygenated substances	28·8
Nonanal	1·4
Benzyl alcohol	3·6
cis-Jasmone	4·2
2-Ethylhexanoic acid	2·8
cis-3-Hexenyl benzoate	5·2
Eugenol	10·2
Ethyl palmitate	1·4
Hydrocarbons	24·3
Nonadecane	1·1
Eicosane	2·8
Heneicosane	5·2
Docosane	7·2
Tricosane	8·0
Unidentified	30·3

DISCUSSION

On the basis of external morphology and anatomy, the glandular trichomes of *Salvia blepharophylla* were similar to the two main types occurring in the Lamiaceae: peltate hairs or scales and capitate hairs. In *Salvia blepharophylla*, the peltate hairs were composed of one basal cell, one stalk cell and a broad four-celled head. Very rarely, three or five cells were found. The four-cell feature has also been observed in other *Salvia* species (Gupta and Bhambie, 1980) and other Lamiaceae such as *Teucrium scorodonia* (Antunes and Sevinate-Pinto, 1991), *Teucrium siculum* (Servettaz *et al.*, 1994), *Lamium galeobdolon* (Huphof and Hummel, 1962), *Nepeta racemosa* (Bourett *et al.*, 1994), *Ocimum basilicum* (Werker, 1993), the genera *Ocimum*, *Leucas* and *Ortosiphon* (Bosabalidis and Tsekos, 1984) and the subtribe Hyptidinae (Rudall, 1980). However, the more common arrangement in Lamiaceae consists of a larger secretory head of usually four central cells and six–14 peripheral cells (Werker *et al.*, 1985*b*; Werker, 1993). In the genus *Salvia*, this pattern has been demonstrated for various species such as *S. sclarea* and *S. dominica* (Werker *et al.*, 1985*a*), *S. coccinea* and *S. leucantha* (Gupta and Bhambie, 1980), and *S. officinalis* and *S. fruticosa* (Werker *et al.*, 1985*b*). In *S. aurea*, the head secretory cells are arranged in a single circle but their number varies from six to eight (Serrato-Valenti *et al.*, 1997).

Salvia blepharophylla bore two types of capitate hair, type I and type II, which differed greatly in shape. Type I capitate trichomes consist of two secretory head cells supported by a unicellular stalk, and a basal cell embedded between the ordinary epidermal cells. This kind of capitate hair was also

found in *S. aurea* (Serrato-Valenti *et al.*, 1997), *S. sclarea* (Werker *et al.*, 1985*a*), *S. officinalis* and *S. fruticosa* (Werker *et al.*, 1985*b*) and other *Salvia* species (Schnepf, 1972 cited in Werker *et al.*, 1985*b*). Bourett *et al.* (1994) also reported a similar feature for the small capitate glands in *Nepeta racemosa*, as did Doaigey (1992) for *Lavandula stricta* and *L. coronopifolia*, Antunes and Sevinate-Pinto (1991) for *Teucrium scorodonia* and Bokhari and Hedge (1971) for the genus *Horminum*.

The type II capitate hairs corresponded to those described in *S. sclarea* and *S. dominica*, and named 'small capitate type II hairs' (Werker *et al.*, 1985*a*), and in *Lavandula stricta* (Doaigey, 1992). Moreover, they match the type Ib described for various *Salvia* species (Gupta and Bhambie, 1980) and *Ocimum basilicum*, *O. citriodorum* and *O. sanctum* (Gupta and Bhambie, 1978), and with the two,three-celled stalk and one-celled head hairs described by Bokhari and Hedge (1977) in the *Salvia* species-group centred on *S. aegyptiaca* L.

For *Salvia blepharophylla* there is evidence of only two types of capitate hair. This limited number is in contrast to the large number reported by Werker *et al.* (1985*b*) for *S. sclarea* and *S. dominica*, by Gupta and Bhambie (1980) for eight *Salvia* species, and by Werker *et al.* (1985*b*) for *S. officinalis* and *S. fruticosa*.

The material secreted by the peltate glandular hairs accumulated in the subcuticular space, and sometimes formed a bulge, as in the peltate glands of all the Lamiaceae species that have been examined so far (e.g. Heinrich, 1973; Bruni and Modenesi, 1983; Bosabalidis and Tsekos, 1984; Werker *et al.*, 1985*a*, *b*; Bourett *et al.*, 1994; Serrato-Valenti *et al.*, 1997). The anatomical results in this paper suggest that the secreted material in *S. blepharophylla* can be released by breakage of the cuticle along equatorial lines of weakness, or *via* pores in the cuticular structure. While the first mode is common to the species examined so far (e.g. Antunes and Sevinate-Pinto, 1991; Ascensão, Marques and Pais, 1995; Serrato-Valenti *et al.*, 1997), the second appears to have been described only in capitate trichomes of members of the Lamiaceae (Amelunxen, 1964 cited by Fahn, 1988; Werker *et al.*, 1985*a*, *b*; Werker, 1993). Two potential mechanisms for the release of the secreted material were observed for capitate hairs: (1) the passage of droplets through the intact cuticle; or (2) early rupture of the cuticle, as there was no evidence of accumulation of secretion product in the subcuticular space.

In both peltate and capitate hairs, the secreted material appeared as an emulsion, thus indicating the presence of two chemical phases. An emulsion-like appearance has been described for the secretion products of *S. sclarea* and *S. dominica* (Werker *et al.*, 1985*a*) and *S. aurea* (Bisio, unpubl. res.). Histochemical tests for both hydrophobic and hydrophilic substances in the secretion products of the three types of hair gave positive reactions; for example, Nile Blue indicated fatty acidic substances and Ruthenium Red polysaccharides other than cellulose.

Non-cellulosic polysaccharides have been reported in the secreted material of capitate hairs in various Lamiaceae (Werker *et al.*, 1985*b*; Werker, 1993), as well as in the wall and contents of the four central head cells in peltate hairs. The secreted material of the latter showed only traces of pectic polysaccharidic substances (Werker *et al.*, 1985*b*). The same authors emphasized the correlation between the density of capitate hairs and the location of the vascular tissue. These polysaccharides might act as a lubricant to facilitate leaf expansion (Antunes and Sevinate-Pinto, 1991). The intense deep blue colour developed with TBO could be explained by the additive effects of the purplish-blue metachromasy of the acidic polysaccharides and the greenish-blue metachromasy of phenols (Ling-Lee, Chilvers and Ashford, 1977). Furthermore, in peltate hairs the secretion was stained by phenolic indicators such as ferric chloride and Vanillin/HCl. Chemical complexity of this nature has previously been reported for *Salvia aurea* (Serrato-Valenti *et al.*, 1997), *S. sclarea* and *S. dominica* (Werker, 1985*a*).

Histochemical reactions using Fluorol Yellow, Nile Red and Auramine O indicated the presence of suberin- or cutin-like hydrophobic substances on the side walls of the stalk and neck cells of glandular trichomes. Furthermore, the negative reaction with Calcofluor White was due to the masking of the wall cellulose by lipid substances. This strengthening of the lateral walls of the supporting cells has been observed in both juvenile and fully-developed hairs. Peterson and Vermer (1984) and Fahn (1988) provided evidence that this endodermal feature is common to oil-secreting trichomes, and functions as an effective apoplastic barrier.

In *S. blepharophylla*, the stalk and neck cells of mature glandular hairs were stained by TBO (green metachromasia) showing the presence of phenolic material. Moreover, the presence of pectic substances was demonstrated by positive staining with Ruthenium Red in both stalk and neck cells. Ramalingam and Ravindranath (1970), on the basis of experiments with pure substances, pointed out that the green metachromasia obtained with the reaction of TBO with phenolic materials may also be produced by the presence of diphenol-acid polysaccharide combinations.

A characteristic chemical feature of many Lamiaceae species is the secretion of a lipophilic resin on the leaf surface. These lipophilic substances are secreted by glandular hairs and are known to be composed of a variety of compounds such as terpenes, essential oils, resins, waxes, fats and flavonoid aglycones (Croteau and Johnson, 1984; Wagner, 1991; Duke, 1994). El-Gazzar and Watson (1970) discovered that most of the aromatic genera of the Lamiaceae are in the subfamily Nepetoideae which includes *Salvia*. However, Hegnauer (1966) emphasized that, in *Salvia*, *Hyptis*, *Lycopus* and *Prunella* there are also oil-poor species. On the basis of further data, Cantino and Sanders (1986) noted that one should not expect absolute differences between the two subfamilies of the Lamiaceae in relation to their volatile terpenoid content. In *Salvia blepharophylla*, the low yield of essential oil is probably due to the presence in the glands of compounds with low volatility which can not be recovered by steam distillation (Bicchi *et al.*, 1985).

Flavonoid compounds are considered to be common constituents of materials secreted by glandular hairs (Duke, 1994). According to Wollenweber (1994), flavonoids pass through the cell walls and cuticle, and crystallize on the

surface of secretory glands. Crystals on the epidermis surface were found in *Salvia blepharophylla* (Fig. 4). Flavonoid aglycones are normally detected only on the external surfaces of plants, and are most commonly encountered in plants from xeric and alpine habitats bearing secretory structures (Charrière-Ladreix, 1973; Dell and McComb, 1977; Wollenweber, 1985; Tomás-Barberán and Wollenweber, 1990; Wollenweber, 1994). External flavonoids may play a role in protecting plants from harmful UV radiation, as may external resins of a wax and/or terpenoid nature. This adaptive role of epicuticular layers is comparable to that of pubescence in plants without exudate resins (Tomás-Barberán *et al*., 1988). The occurrence of flavonoid aglycones provides chemical characters for chemotaxonomic studies which are correlated with the adaptation of plants to (semi-) arid habitats (Tomás-Barberán and Wollenweber, 1990). Flavonoid aglycones accumulate on leaf surfaces in association with terpenoid material (Wollenweber, 1984; Tomás-Barberán and Wollenweber, 1990; Wollenweber, 1994). Wollenweber (1984) reports traces of free flavonoids mixed with ursolic acid on leaflets of an *Escallonia* hybrid. External occurrence of lipophilic flavonoid aglycones in the genus *Salvia* has been reported for *Salvia glutinosa* (Wollenweber, 1974) and *S. officinalis*, *S. lavandulaefolia* and *S. triloba* (Tomás-Barberán and Wollenweber, 1990). In a previous study, Bisio *et al*. (1997) isolated various flavonoids and ursolic acid from an ethanolic extract of the aerial parts of *S. blepharophylla*. This work confirms the presence of two flavonoid aglycones, nuchensin and pedalitin, as well as ursolic acid and α-amyrin, common triterpenoids of the waxy coatings of leaves on leaf surfaces. These substances could represent excreted flavonoids and triterpenoids. Salvianduline-D has been found elsewhere only in *Salvia lavanduloides* H.K.B. (Maldonado *et al*., 1994). The presence of this *neo*-clerodane diterpenoid in *S. blepharophylla*, which is of Mexican origin, is consistent with the findings of Rodríguez-Hahn *et al*. (1994), who emphasized that such compounds have been identified only in *Salvia* species of the American Continent.

Work continues on other *Salvia* spp. of Mexican origin, established in the Hanbury Botanical Gardens, in order to characterize the morphology of their glandular trichomes and the chemical composition of their leaf surface extracts.

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