Five plants of the family Cucurbitaceae with flavonoid patterns of pollens different from those of corresponding stigmas

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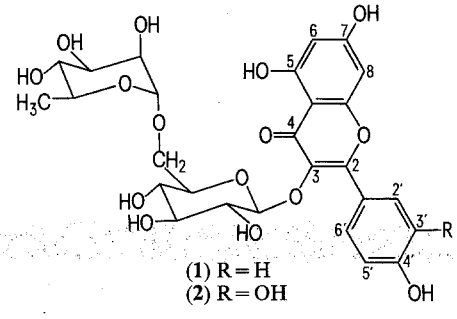
Summary. Flavonoid patterns of pollens of 5 plants of the family Cucurbitaceae are different from those of the corresponding stigmas. The major flavonoid of pollens has been identified as kaempferol 3-O-rutinoside (**1**). Rutin (**2**) has been found in stigmas of 2 species. The other flavonoids of pollens and stigmas of these plants are flavonol 3-O-glycosides.

It is well known that the discovery of the functions of flavones and flavonols in plants is perhaps the most interesting problem in flavonoid field.

The first evidence that theflavonoids of pollens and stigmas may be connected with sex expression in plants was reported by Barber[2] who showed that the anthers and stigmas respectively of male and female flowers of *Cucurbita pepo* contain different glycosides of different quercetin methyl ethers. However, Hartshorne[3] examined anthocyanins from male and female flowers of several plants and could find no significant differences. Recent results[4] have substantially confirmed the report of Barber and have shown that there are differences between the flavonoid patterns of pollens and corresponding stigmas of 3 other species of the genus *Cucurbita (C. maxima, C. moschata* and *C. ficifolia).* Since the above results may be restricted to plants of the genus *Cucurbita,* in the present work the flavonoids of pollens and stigmas of 5 plants (Cucurbitaceae) not belonging to this genus *(Lagenaria vulgaris, Cucumis citrullus, Sechium edule, Ecballium elaterium* and *Bryonia dioica)* have been examined.

Material and methods. For paper chromatography and TLC the solvent mixtures used were: A) 1-butanol-acetic acid-water (4:1:5, upper phase); B) acetic acid-water (5:95); C) 1-butanol-ethanol-water (4:1:2,2); D) phenol saturated with water; E) acetic acid-concentrated HCl-water (30:3:10); F) chloroform-acetic acid (9:1); G) chloroform-methanol-butanone (70:10:6); H) 1-butanol-pyridine-water (6:4:3); I) 1-butanol-acetic acid-ethyl ether-water (9:6:3:1).

Fresh flowers of *Lagenaria vulgaris, Cucumis citrullus, Sechium edule, Ecballium elaterium* and *Bryonia dioica* were collected in Catania. Pollens and homogenized stigmas were extracted 3 times with boiling 95% ethanol; the combined extracts were filtered, concentrated to a small volume in vacuo and re-filtered. Flavonoids were isolated by preparative chromatography on Whatmann 3MM paper in solvent A. Bands were cut off, eluted with 70% ethanol, concentrated and rechromatographed in solvents B and C. Flavonoids (table) were characterized by colour reactions, UV-spectral analysis with usual shift reagents[5], total acid hydrolysis with 2 N HCl (1 h at 100 °C), controlled acid hydrolysis with 10% acetic acid (3.5 h under reflux) and R_f data; identifications of **1** and **2** were confirmed by paper co-chromatography with authentic samples (solvents A, B, C and D). Aglycones (kaempferol or quercetin) obtained by total acid hydrolysis were identified by UV-spectral analysis with shift reagents[5], paper co-chromatography with authentic samples (solvents A, C, D and E), SiO_2 TLC (solvent F) and polyamide TLC (solvent G); some flavonoids were not isolated in sufficient amount for total acid hydrolysis but colour reactions (dark to yellow in $UV+NH_3$), UV-spectral properties and R_f data suggested that they are flavonol 3-O-glycosides. The sugars obtained by total acid hydrolysis of kaempferol 3-O-rutinoside and rutin were identified as rhamnose and glucose by paper co-chromatography (solvents A and H) and SiO_2 TLC (solvent I); controlled acid hydrolysis of these flavonoids gave rhamnose, glucose and rutinose identified as above. The sugars attached to the other flavonoids were not identified but the R_f data of these compounds[6,7] and the chromatographic behaviour of the sugars obtained by controlled acid



(**1**) R = H
(**2**) R = OH

Flavonoids of pollens and stigmas of 5 species of the family Cucurbitaceae*

Species	Organs	Kaempferol glycosides			Quercetin glycosides		Unidentified flavonol glycosides	
		3-Rutinoside	3-Monoglycoside	3-Diglycoside	Rutin	3-Monoglycoside	3-Monoglycoside	3-Diglycoside
Lagenaria vulgaris	Pollen	+++					+	
	Stigma		++		++			
Cucumis citrullus	Pollen	+++						
	Stigma		++					
Sechium edule	Pollen	+++						+
	Stigma							+
Ecballium elaterium	Pollen	+++						+
	Stigma	+++			++			
Bryonia dioica	Pollen	+++					+	
	Stigma			++		++		+

*Partially characterized kaempferol and quercetin 3-O-glycosides as well as unidentified flavonol 3-O-glycosides were all chromatographically distinct compounds. Relative amounts of flavonoids are presented as large (+++), medium (++) and small (+); they were obtained by estimation of the intensity of spots on chromatograms.

hydrolysis suggested that they are mono- or diglycosides (table).

Results and discussion. In earlier studies[8] a flavonoid (7-O-glycosyl-C-glycosylapigenin) was isolated from the leaves of *Bryonia dioica.* The presence of flavonoids in the other 4 plants examined in this work is reported for the first time. The major flavonoid (table) present in pollens of these 5 species is kaempferol 3-O-rutinoside; the minor flavonoids are unidentified flavonol 3-O-glycosides. Kaempferol 3-O-rutinoside is absent in all stigmas but that of *Ecballium elaterium;* the other flavonoids present in stigmas are rutin, kaempferol 3-O-glycosides, quercetin 3-O-glycosides and unidentified flavonol 3-O-glycosides. Hence all flavonoids present in pollens and stigmas of these plants are flavonol 3-O-glycosides. The flavonoid patterns of pollens of 4 species *(Lagenaria vulgaris, Cucumis citrullus, Sechium edule* and *Bryonia dioica)* are completely different from those of the corresponding stigmas and, moreover, some differences have been found between the flavonoids of pollen and stigma of *Ecballium elaterium.*

The above results show that the differences between the flavonoid patterns of pollens and corresponding stigmas are not restricted to species belonging to the genus *Cucurbita*[2,4]. Thus flavonoids of pollens and stigmas may be connected with sex expression in plants belonging to the family Cucurbitaceae. However, since only a small number (9) of species of this family (which contains about 850 species) have been examined, the above suggestion must be confirmed by further studies.

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Sterols of Mediterranean Chlorophyceae[1]

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Summary. The distribution of sterols in 8 Mediterranean green algae has been investigated. C_{29} sterols are the major group in the species examined. 28-Isofucosterol seems to be typical for Ulotrichales, and the rare clerosterol for the genus Codium.

Recently sterols of marine algae have been widely investigated[2,3]. Chlorophyta have been much less extensively studied than Rhodophyta and Phaeophyta, but those species that have been examined show more diversity in their sterol contents. Thus the sterols of marine green algae appear to have value for the systematist as a guide for taxonomy and phylogeny.

In 1976 the most significant works in the field were reviewed by Goad, who also discussed the chemotaxonomic and phylogenetic considerations here briefly summarized[2]. Among the 6 species of Ulotrichales examined, 4 are consistent in containing 28-isofucosterol (**6**) as the main component. The occurrence of this sterol in many vascular plants seems to confirm the possible ancestral role of this order in the evolution of higher plants. The only significant feature revealed by the examination of the sterol fraction of 2 species belonging to the Cladophorales is a high content of cholesterol (**1**), and this suggested that the alkylation reaction of the side chain of the sterols in these 2 species is relatively inefficient. Finally, the 2 Siphonales examined showed a very contrasting sterol composition: in *Halimeda incrassata* clionasterol (**8**) predominates while in *Codium fragile* the major sterol is the unusual clerosterol (**7**) accompanied by minor amounts of codisterol (**5**), its C_{28} analogue.

As a part of a chemical survey on Mediterranean chlorophyta, we examined 8 green seaweeds as reported in the table, and their sterol composition is described in the present paper.

Material and methods. Algae listed in the table were collected from the littoral zone of the east coast of Sicily from Aci Castello to Capo Passero. Each alga (usually 500 g fresh weight) was freeze-dried and extracted with $CHCl_3$ (3×600 ml) at room temperature. Combined extracts were saponified and the non-saponifiable matter was chromatographed on a silica gel column (eluent: C_6H_6-ET_2O, 8:2). The crude sterol fraction, after acetylation with Ac_2O-pyridine 1:1, was further purified by a SiO_2 column using as eluent 40–70° light petroleum-C_6H_6 7:3 and analyzed by GLC-MS (AEI MS 30 instrument connected with a Pye Unicam instrument model 104 gas chromatograph; 1.5 m × 5 mm glass column packed with 2.5% SE 30; N_2 flow 30 ml/min).

When a particular fraction was shown to contain a steryl acetate in a considerable amount, the compound was isolated by PLC on $SiO_2/AgNO_3$ (40–70° light petroleum-C_6H_6 7:3 as eluent) and its identification confirmed by comparison of physical data ($[\alpha]_D$, m.p., IR and PMR) with those of an authentic sample.

Quantitation was performed by GLC of steryl acetates (cholestane as internal standard) using integrated areas of peaks.

The configuration at C-24 of the steryl acetates identified only by GLC-MS was only tentatively assigned as *S,* in view the preponderance of the 24*S*-alkylsterols in the green algae[4].

Results and discussion. Our results, listed in the table, confirmed that 28-isofucosterol is representative of the