dioxyaniline; their physicochemical data are not reported since they were prepared only from diastereomeric mixtures of homoleucine and not from pure isomers.

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Flavonoids from pollens and stigmas of male and female flowers of four species of the genus *Cucurbita*

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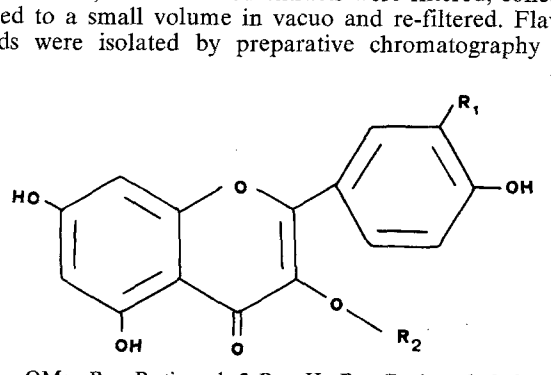
Summary. Differences have been found between the flavonoid patterns of pollens and corresponding stigmas of *Cucurbita pepo, C. maxima, C. moschata* and *C. ficifolia*. The major flavonoids have been identified as isorhamnetin-3-O-rutinoside (**1**), kaempferol-3-O-rutinoside (**2**), kaempferol-3-O-robinobioside (**3**) and rutin (**4**). A flavonol glycoside previously isolated from stigmas of *C. pepo* is absent in this material.

There is no evidence that the flavonoids of pollens and stigmas are connected with sex expression of plants. Hartshorne[2] examined flavonoids from both male and female flowers of several plants and could find no relationship between anthocyanidin type and sex. Barber[3] reported, however, that the anthers and stigmas respectively of male and female flowers of *Cucurbita pepo* contain different glycosides of different quercetin methyl ethers.

In view of the great interest in biological function and physiological properties of flavonoid compounds, in the present work the flavonoids of pollens and stigmas of *Cucurbita maxima, C. moschata* and *C. ficifolia* have been studied and the flavonoids of pollen and stigmas of *C. pepo* have been re-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, 1-butanol-ethanol-water (4:1:2,2); C, acetic acid-water (5:95); D, acetic acid-conc. HCl-water (30:3:10); E, phenol saturated with water F, 1-butanol-pyridine-water (6:4:3); G, ethyl acetate-butanone-formic acid-water (5:3:1:1); H, 1-butanol-acetic acid-ethyl ether-water (9:6:3:1:1); I, chloroform-ethyl acetate (1:1); L, chloroform-acetic acid (9:1).

Fresh flowers of *Cucurbita pepo, C. maxima, C. moschata* and *C. ficifolia* 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 C and B. When complete separation was not achieved, further purification was obtained by preparative SiO_2 TLC in solvent G.



1 R_1 = OMe; R_2 = Rutinosyl, **2** R_1 = H; R_2 = Rutinosyl, **3** R_1 = H; R_2 = Robinobiosyl, **4** R_1 = OH; R_2 = Rutinosyl.

Flavonoids were identified by UV-spectral analysis with usual shift reagents[4], total acid hydrolysis with 2 N HCl (1 h at 100 °C), controlled acid hydrolysis with 10% acetic acid (3.5 h under reflux), methylation (Me_2SO_4-K_2CO_3-Me_2CO) followed by acid hydrolysis and R_f data; identifications of **2** and **4** were confirmed by paper co-chromatography with authentic samples (solvents A, B, C, E). Aglycones obtained by total acid hydrolysis of **1**, **2** and **4** were identified respectively as isorhamnetin, kaempferol and quercetin by UV-spectral analysis with shift reagents[4], paper co-chromatography with authentic samples (solvents A, B, D and E) and SiO_2 TLC (solvent L); the sugars obtained by total acid hydrolysis of **1**, **2** and **4** were identified as glucose and rhamnose by paper co-chromatography (solvents A and F), SiO_2 TLC (solvent H) and GLC of their TMS ethers[5]. Controlled acid hydrolysis of **1**, **2** and **4** gave rutinose, glucose and rhamnose identified as above. Methylation followed by acid hydrolysis of **1** and **4** gave 5,7,3′,4′-tetra-O-methylquercetin, 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose; 5,7,4′-tri-O-methylkaempferol and the above methylated sugars were obtained from **2**. The partially methylated aglycones were identified by UV-spectral analysis[4], MS and paper co-chromatography with authentic samples (solvents A and C); 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose were identified by paper chromatography according to Petek[6] and SiO_2 TLC (solvent I)[7]. 3 minor flavonoids (**5**–**7**) were isolated from pollens of *C. maxima* and *C. ficifolia* but were not present in sufficient amount for analysis; UV-spectra, R_f data and colours (dark to yellow in UV + NH_3) suggest that they may be flavonol-3-O-monoglycosides.

Results and discussion. The major flavonoids (table) have been identified as isorhamnetin-3-O-rutinoside (**1**), kaempferol-3-O-rutinoside (**2**), kaempferol-3-O-robinobioside (**3**) and rutin (**4**); the minor flavonoids (**5**–**7**) may be flavonol-3-O-monoglycosides. Re-examination of pollen and stigmas of *C. pepo* confirms the report of Barber[3] that flavonoids of these materials are different. Barber reported[3],

however, the presence of one flavonoid in anthers (a glucose rhamnoside of isorhamnetin) and one in corresponding stigmas of the above plant (possessing an aglycone similar with isorhamnetin and the unusual combination, glucose and fructose). Kaempferol-3-O-rutinoside and rutin have now been isolated from stigmas of *C. pepo* but the flavonoid containing fructose has not been found in this material; these results may be related to phytogeographical factors[8]. From the physiological view point, it is of interest that differences have been found between the flavonoid patterns of pollens and corresponding stigmas of the 4 species (table).

Flavonoids of pollens and stigmas of 4 species of the genus *Cucurbita**

Species	Pollen	Stigma
Cucurbita pepo	**1**	**2,4**
C. maxima	**1,2,3,5,6**	**1,2,3**
C. moschata	**1,2,3**	**2**
C. ficifolia	**1,4,7**	**1,2**

* Flavonoids 5-7 were not present in sufficient amount for analysis; preliminary investigations suggest that they may be flavonol-3-O-monoglycosides.

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A possible pathway for the biosynthesis of adenochromines

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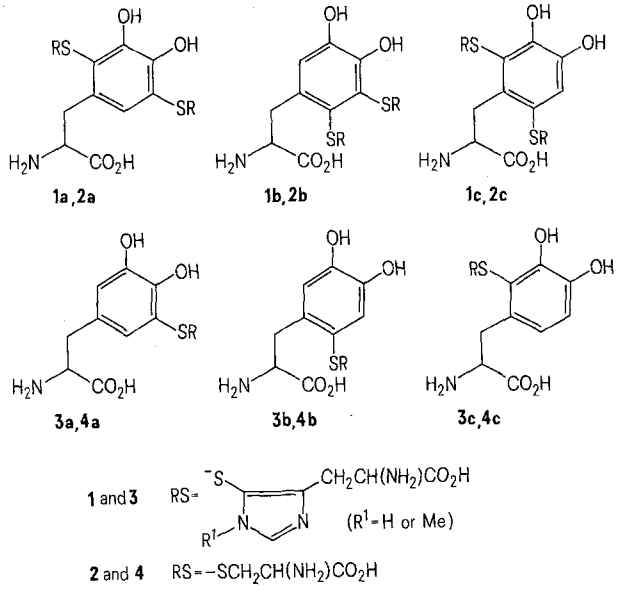
Summary. 5-Thiolhistidine reacted with dopaquinone produced in vitro by tyrosinase oxidation of dopa to give high yields of secoadenochromines (**3**) along with a small amount of adenochromines (**1**) which are the amino-acids responsible for the iron-(III)-binding properties of adenochromes.

Adenochromines (**1a**, **1b**, **1c**) are component amino-acids of a unique group of iron-(III)-binding peptides, adenochromes, which occur in the branchial heart of *Octopus vulgaris*[3].

Suggestions for the biosynthesis of the adenochromines followed from their close structural similarity with the cysteinyldopas[4], especially 2,5-S,S-dicysteinyldopa[5] (**2**), which are formed by addition of cysteine to dopaquinone produced by tyrosinase oxidation of dopa[6]. When examined in vitro this reaction leads mainly to the formation of the monoadducts i.e. 5-S-(**4a**), 2-S-(**4c**) and 6-S-(**4b**) cysteinyldopas (74%, 14% and 1%) along with a small amount (5%) of the diadduct 2,5-S,S-dicysteinyldopa (**2**). Therefore a similar reaction between 5-thiolhistidine and dopaquinone could account for the biosynthesis of adenochromines by way of the monoadducts **3a**, **3b**, **3c**, known as secoadenochromines.



To test this hypothesis we have studied the enzymic oxidation of dopa and secoadenochromines A, B and C in the presence of 5-thiolhistidine.

A) *Tyrosinase oxidation of dopa and 5-thiolhistidine.* A solution of L-dopa (12.5 mg; 0.063 mmoles) and 5-thiol-L-histidine dihydrochloride[7] (35.2 mg; 0.125 mmoles) in 0.05 M sodium phosphate buffer, pH 6.8, was vigorously stirred at 24 °C (oxygen not bubbled into the solution) in the presence of mushroom tyrosinase (4.4 mg; 2750 units/mg; from Sigma Chem. Co.) and the course of the reaction was followed by monitoring the UV spectrum (in 0.1 N HCl) of aliquots taken at suitable intervals. Since after 45 min the formation of secoadenochromines (**3**) became evident (increase of absorbance at 293 nm), the oxidation was stopped by acidification to pH 1 with 6 N HCl and the reaction mixture was passed through a column (0.9×26 cm) of Dowex 50 W-X2 (200-400 mesh, H^+ form). After washing with 1 N HCl (10 ml) the column was eluted with 3 N HCl at a flow rate of 9 ml/h and fractions of 5 ml were collected and monitored spectrophotometrically. Fractions 12-15 gave 13.3 mg of 5-thiolhistidine disulphide; 16-20, 13.3 mg of a mixture of **3b** and **3c** contaminated with some (~ 10%) disulphide; 22-29, 19.8 mg (about 55%) of **3a** containing a trace of **1b**, and 32-38, 1.6 mg (3%) of **1a** and **1c** which were not separable from each other.