Water-Soluble Flavonol (= 3-Hydroxy-2-phenyl-4*H*-1-benzopyran-4-one) Derivatives: Chemical Synthesis, Colouring, and Antioxidant Properties

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Water-soluble derivatives of rutin, a very common glycoside of quercetin (= 3,3′,4′,5,7-pentahydroxyflavone = 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one) and a potent plant antioxidant from the flavonol family, were synthesized by simple chemical procedures aimed at introducing carboxy or sulfo groups at the sugar moiety (*Scheme 1*). Such derivatives form stable molecular complexes with malvin, a polyphenolic pigment from the anthocyanin family, and thereby prove to be very effective in the enhancement (hyperchromism) and variation (bathochromism) of natural colours. The H_2O-solubilizing carboxylate and sulfate groups are shown to deeply modify the enthalpy-entropy balance of the pigment-flavonol complexation (copigmentation). A molecular interpretation of the complexation-induced bathochromic shift in the pigment VIS band is proposed. Finally, the H_2O-soluble rutin derivatives are shown to retain the high antioxidant ability of rutin as evidenced by their efficient trapping of the coloured radical DPPH (= 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl).

Introduction. – Flavonols (= 3-hydroxyflavones = 3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-ones) are an important class of flavonoids (polyphenols) abundant in plants and in human diet [1]. Most of their biological activities are attributed to their affinity for a wide range of proteins, including enzymes, and their antioxidant properties [2]. On the other hand, recent epidemiological studies have suggested that flavonoid-rich diets could help prevent the development of cardiovascular diseases, a major cause of mortality in occidental countries [3]. Flavonols also efficiently take part in natural colour expression by forming molecular complexes with polyphenolic plant pigments (anthocyanins), a phenomenon known as copigmentation [4]. Although flavones and flavonols are the most efficient anthocyanin copigments reported so far [5], their efficiency in stabilizing natural colours is limited by their poor solubility (of the order of 10^{-3}M for glycosides and much lower for aglycones) in slightly acidic to neutral aqueous solutions modeling the natural medium. On the other hand, enzymes from the intestinal microflora are known to promote extensive hydrolysis of the glycosidic bonds of flavonol glycosides in the intestine and subsequent degradation of the aglycones into rapidly excreted phenolic acids [6]. However, the quercetin conjugates (glucuronides and sulfates of quercetin and its 3′-methyl ether) found in biologically significant, although relatively low (0.1 – 1 μM), concentrations in the plasma of human volunteers after consumption of a meal rich in quercetin glycosides retain high antioxidant activities [7]. Synthesis of H_2O-soluble flavonol conjugates [8] or chemical modifications of flavonol glycosides may reduce intestinal degradation for a better absorption and a higher plasma concentration.

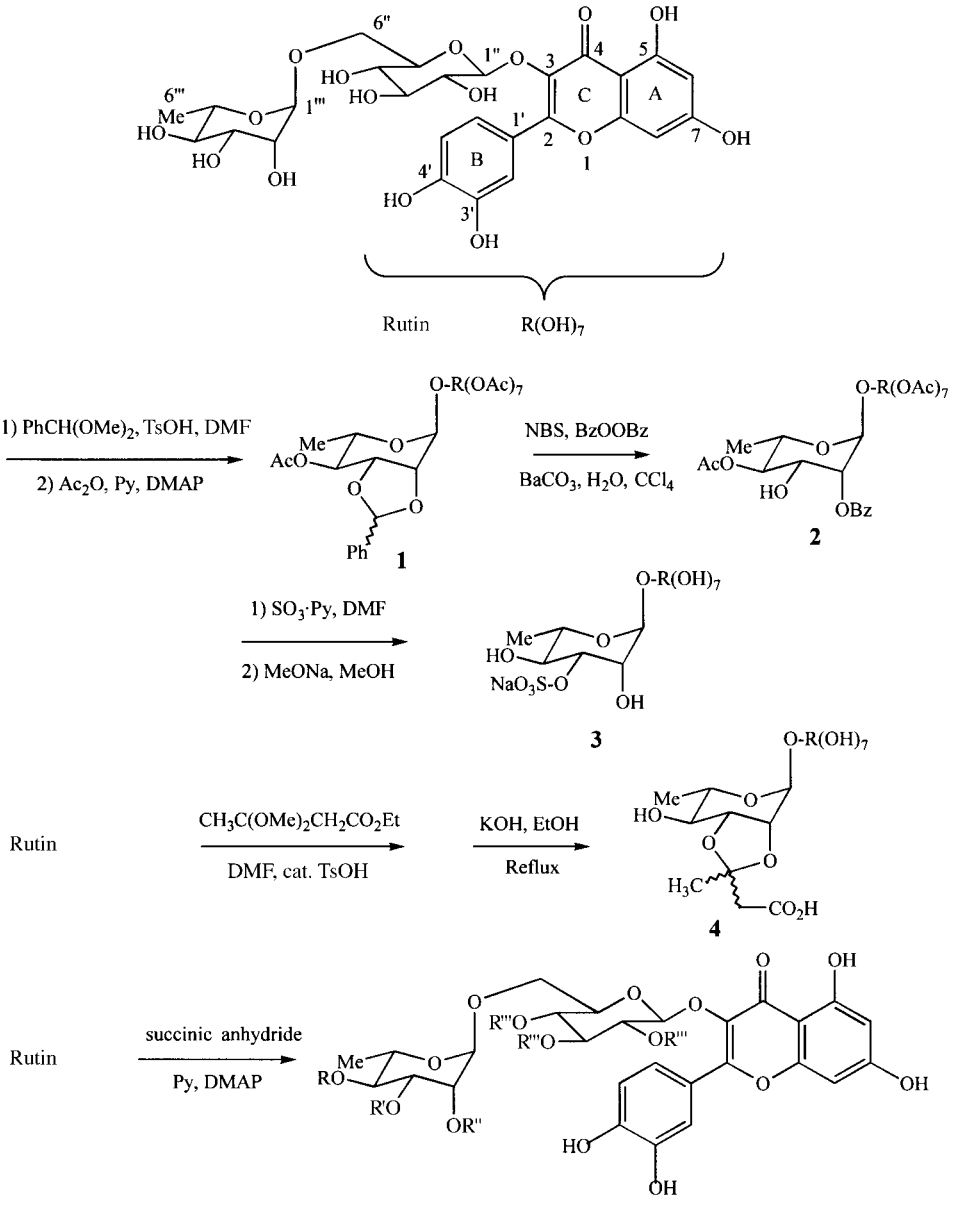
This work describes some simple chemical modifications of rutin (= 3-*O*-(α-L-rhamnopyranosyl-1,6-β-D-glucopyranosyl)quercetin = 3-{[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one; *Scheme 1*) on its sugar moiety (introduction of sulfate and carboxylate groups) aimed at increasing its water solubility and eventually its bioavailability. The influence of the chemical modifications on the colouring and antioxidant properties is discussed. Particular attention will be given to succinylated rutin derivatives since succinyl groups are found in some naturally occurring flavonoid glycosides [1a].

Results and Discussion. – 1. *Synthesis* (see *Scheme 1*). Rutin is one of the most common flavonols in plants. Being commercially available and cheap, it is an ideal material for chemical modifications aimed at modulating its colouring and antioxidant properties. In the first step, the 1,2-*cis*-diol group of the rhamnose moiety is selectively protected by a benzylidene group upon treatment of rutin by benzaldehyde dimethyl acetal in DMF with *p*-toluenesulfonic acid (TsOH) as a catalyst. Subsequent acetylation of the unprotected OH groups (Ac_2O, Py, DMAP = *N*,*N*-dimethylpyridin-4-amine) afforded **1** as a mixture of epimers. Long reaction times were usually required to ensure complete acetylation. In particular, OH–C(5) (δ(H) *ca.* 12.5 ppm) reacted slowly, probably because of the H-bond it forms with the 4-keto group. Upon oxidative ring opening of the benzylidene acetal (NBS (= *N*-bromosuccinimide), benzoyl peroxide, $BaCO_3$, $CHCl_3/H_2O$), **2** was obtained as a single regioisomer in agreement with [9]. Incomplete acetylation of the polyphenolic nucleus invariably resulted in low yields. Treatment of **2** with SO_3·pyridine in DMF and subsequent deacetylation (MeOH, MeONa) gave rutin 3‴-sulfate (sodium salt) (**3**) after elution on a *Dowex* ion-exchange resin (Na^+) and purification on C_{18} silica gel. Alternatively, rutin was treated by the ethyl acetoacetate dimethyl acetal (prepared upon refluxing ethyl acetoacetate with trimethyl orthoformate in MeOH with TsOH as a catalyst) in DMF with catalytic amounts of TsOH. Saponification (KOH, EtOH, reflux) and purification on C_{18} silica gel afforded **4** as a mixture of epimers.

Treatment of rutin with succinic anhydride (6 equiv.) and DMAP (0.5 equiv.) in pyridin at 70° resulted in a quantitative yield of rutin 2″,3″,4″,2‴,3‴,4‴-hexakis(hydrogen succinate) (**5**). No acylation of the phenolic OH groups occurred under these conditions. As expected, **5** is highly soluble in H_2O and aqueous buffers (except in strongly acidic solutions). When the experiment was repeated at 0° with lower concentrations of succinic anhydride (3 equiv.) and DMAP (*ca.* 0.2 equiv.), a mixture **6** of three rutin bis(hydrogen succinates) in a *ca.* 3:3:2 molar ratio was isolated after chromatography on C_{18} silica gel. The ^{1}H-NMR spectrum of **6** suggests that each regioisomer is acylated at O–C(4‴), the second succinyl (= 3-carboxy-1-oxopropyl) group being located either at O–C(3‴) or at one of the glucose O-atoms. Finally, acylation at 0° with 1.5 equiv. of succinic anhydride and *ca.* 0.1 equiv. of DMAP afforded rutin 4‴-(hydrogen succinate) (**7**) in 21% yield. Under mildly acidic conditions (pH 3.5), the H_2O solubility of rutin derivatives **3**–**7** is higher than 10^{-2} M, *i.e.*, at least 10 times higher than that of rutin.

2. *Thermodynamic Investigation of Copigmentation*. Flavonol glycosides such as rutin are abundant in the vacuoles of fruits and flowers where polyphenolic pigments (anthocyanins) are stored. Flavonols and anthocyanins form stable molecular

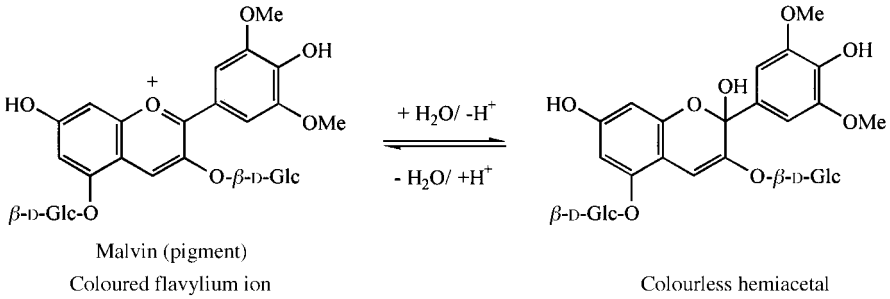
Scheme 1. *Chemical Synthesis of Rutin Derivatives* ***3*** *–* ***5***



5 R = R′ = R″ = R‴ = C(=O)CH_2CH_2COOH
6 R = C(=O)CH_2CH_2COOH, R″ = H, R′,R‴ = 1C(=O)CH_2CH_2COOH, 3 H
7 R = C(=O)CH_2CH_2COOH, R′ = R″ = R‴ = H

complexes upon stacking of their polyphenolic nuclei. Thereby, water addition to the anthocyanin chromophore (*Scheme 2*) and subsequent colour loss are at least partially prevented [4][5a–d].

Scheme 2



Malvin (pigment)
Coloured flavylium ion

Colourless hemiacetal

The efficiency of rutin derivatives **3**–**7** in copigmentation was tested with malvin (= 3,5-bis(β-D-glucopyranosyloxy)-7-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-benzopyrylium chloride; *Scheme 2*), one of the most common naturally occurring anthocyanins. In strongly acidic solution (pH 0.9), where malvin is in a pure flavylium form, copigmentation is manifested by strong bathochromic shifts in the VIS-absorption band of the pigment. For a copigment/pigment molar ratio of 200, the bathochromic shift reaches 40 nm in the case of **4**, **6**, and **7** (*Fig. 1,b*), thus demonstrating the efficiency of H_2O-soluble flavonols in colour variation. Similar bathochromic shifts were previously observed upon addition of quercetin 5′-sulfonate, a H_2O-soluble analog of quercetin, to solutions of anthocyanins and synthetic flavylium ions [10]. Under mildly acidic conditions (pH 3.5), closer to those of natural media, malvin is predominantly in the hemiacetal form (H_2O addition at C(2), see *Scheme 2*), and the solutions are poorly coloured. Addition of **4**, **6**, or **7** (copigment/pigment molar ratio 200) strongly intensifies the colour (*Fig. 1,a*) as the result of a shift in the flavylium-hemiacetal equilibrium induced by the selective stacking of the copigment on the planar flavylium nucleus. Relative hyperchromic shifts ($(A - A_0)/A_0$ at a given wavelength; A_0, A = VIS absorbance in the absence and in the presence of the copigment, resp.) as high as 20 were measured at room temperature. The VIS absorbance gradually decreases when the temperature is raised as a consequence of the partial (endothermic) dissociation of the copigmentation complex. At pH 3.5 where the free flavylium ion is in negligible concentration, the relative hyperchromic shift can be directly related to the binding constant K according to *Eqn. 1* [5a,b] (r = ratio of the molar absorption coefficients of malvin in its bound and free forms; c = total copigment concentration). At the isosbestic point of the free and bound flavylium ions (determined at pH *ca.* 1 where the pigment is in pure flavylium form), r is equal to 1 and *Eqn. 1* can be converted to *Eqn. 2*.

$$\frac{A - A_0}{A_0} = r\,c\,K \tag{1}$$

$$\ln\frac{A - A_0}{c\,A_0} = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{2}$$

Plots of $\ln((A - A_0)/c\,A_0)$ as a function of $1/T$ are linear (correlation coefficients > 0.995, *Fig. 2*) for all copigments in the temperature range investigated (15–45°) and

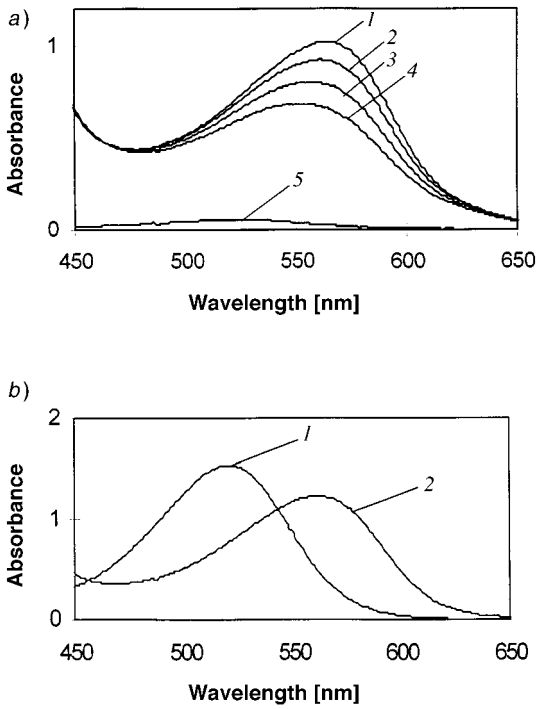


Fig. 1. *Copigmentation of malvin* ($5 \cdot 10^{-5}$ M) *by rutin derivative* **6** (10^{-2} M). a) *At pH 3.5* (0.2M acetate buffer), *VIS spectra of malvin/***6** *at 15.5 (1), 25.0 (2), 35.0 (3) and 44.2° (4); VIS spectrum of malvin alone at 25.3° (5).* b) *At pH 0.9 and 25°, VIS spectrum of malvin alone (2) and of malvin/***6** *(2)*

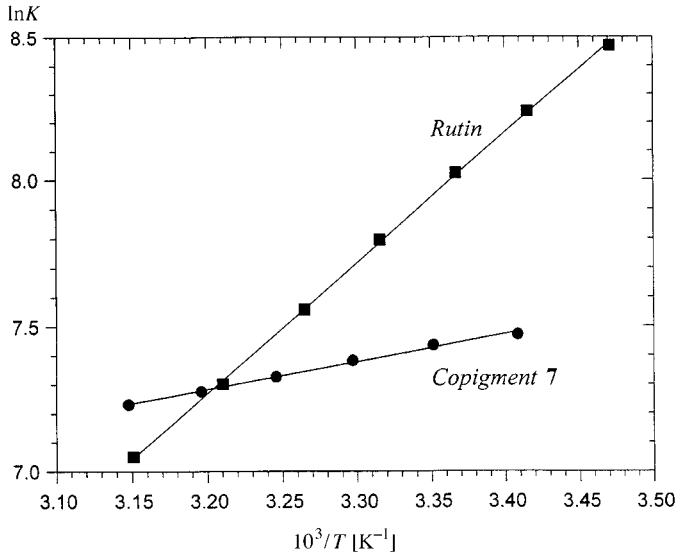


Fig. 2. Van't Hoff *plots for copigmentation of malvin* ($5 \cdot 10^{-5}$ M) *by rutin* (10^{-3} M) *and rutin derivative* **7** (10^{-2} M) *at pH 3.5* (0.2M acetate buffer)

Table 1. *Thermodynamic Parameters for Copigmentation between Malvin and Rutin and Rutin Derivatives* **3**–**7** *Deduced from Temperature Variations in the Range 15–45°* (0.2M acetate buffer, pH 3.5, copigment/pigment molar ratio 200, unless otherwise specified.)

	Rutin	**3**	**4**	**5**	**6**	**7**
ΔH^0 [kJ mol^{-1}]	−37.2 (±0.3)	−33.0 (±0.2)	−16.7 (±0.5)	−17.1 (±0.7)	−13.6 (±0.3)	−7.9 (±0.3)
ΔS^0 [J K^{-1} mol^{-1}]	−59.0 (±0.8)	−62.0 (±0.5)	+8.4 (±1.8)	+0.5 (±2.2)	+16.7 (±1.2)	+35.3 (±0.9)
K [M^{-1}][a])	2750	350	2320	1060	1820	1700
$\Delta\lambda_{max}$ [nm][b])	8	19	41	22	41	40

[a]) Determined at 25° from $\Delta H^0 - T\Delta S^0 = -RT \ln K$. [b]) Determined at pH *ca.* 1 with the following copigment/pigment molar ratios: 200 (**3**, **4**, **6**, **7**), 50 (**5**), and 10 (rutin).

give accurate values for the temperature-independent (*Van't Hoff* behaviour) enthalpy and entropy changes of copigmentation (*Table 1*). The fact that the *Van't Hoff* law is still obeyed with copigments **4** (2 epimers) and **6** (3 regioisomers) suggests that the different isomers display roughly the same affinity for malvin.

The ROESY spectra of rutin and rutin derivatives **3** and **5** (500 MHz, (D_6)DMSO) all display long-range correlations between the aglycone and the rhamnose residue. For instance, H–C(5′) (ring B) is in NOE relationship with H–C(1) (Rha) in rutin, with H–C(3) (Rha) in **3**, and with H–C(6) (Rha) and H–C(2) (Rha) and/or H–C(3) (Rha) in **5**. Moreover, H–C(6′) (ring B) displays NOE connectivities with H–C(1) (Glc), H–C(2) (Glc), and most rhamnose protons in **5**. Hence, rutin and its derivatives display favourable conformations in which the flavonol moiety and the terminal sugar residue (Rha) are in molecular contact. The hydrophobic stacking of the polyphenolic nuclei occurring in copigmentation is thus expected to reduce the solvation of the carboxylate and sulfate groups introduced on the sugar moiety for H_2O-solubility purposes.

The hydration of a neutral group is essentially structure-making ($\Delta S^0 < 0$) because of the ordering of the solvation H_2O molecules to preserve extensive H-bonding between them. On the other hand, the hydration of an ionic group is the sum of a structure-making *neutral* term and of an *electrostatic* term, which is structure-making for the strongly held H_2O molecules of the first solvation shell and structure-breaking ($\Delta S^0 > 0$) for the H_2O molecules of the second solvation shell [11]. The electrostatic term is wholly structure-breaking for large ions. Hence, the partial desolvation of a large ionic group, beside being endothermic, should not strongly contribute to the overall entropy because of compensating structure-making and structure-breaking terms. This is verified in the copigmentation of malvin by **3**: the strong destabilization of the copigmentation complex (in comparison with the malvin · rutin complex) is mainly due to a reduction in exothermicity[1]). Most remarkable is the large influence of the single hydrogen succinate moiety of **7**, which lowers the exothermicity by *ca.* 30 kJ mol^{-1} and turns copigmentation into an entropically favourable process. The

[1]) In spite of its lower affinity toward anthocyanins, **3** remains as efficient as rutin in binding hard metal ions. Hence, from the plots of the absorbance of the aluminum chelates (at the wavelength of the absorption maximum) as a function of the total aluminum concentration and with a simple theoretical treatment developed elsewhere [12], rutin and **3** were shown to form 1:1 complexes with Al^{3+} (0.2M acetate buffer, pH 5.0, 25°). Values for the apparent binding constant (defined as [AlL]/([Al][L], L being the flavonol ligand) were estimated to be 14.5 (±1.5) · 10^3 and 15.9 (±1.7) · 10^3 M^{-1} for rutin and **3**, respectively.

entropic effect may reflect the partial desolvation of the structure-making neutral carboxy group. In their association with malvin, copigments **4**–**6** display thermodynamic behaviours which are intermediate between that of anion **3** and that of **7** (assumed under neutral form). This suggests that the carboxy groups in **4**–**6** are partially dissociated under the conditions of the copigmentation experiments (pH 3.5). Alternatively, the carboxylate groups in **4**–**6** and the sulfate group in **3** may develop favourable electrostatic interactions with the positively charged flavylium nucleus of malvin and thereby maintain a higher exothermicity. Anyway, copigmentation of malvin by the easily available highly H_2O-soluble rutin derivatives **4**–**7** is much less temperature-dependent (and above 40° eventually stronger, see *Fig. 2*) than copigmentation by rutin, a potential advantage for the use of such copigments as colour enhancers in anthocyanin-containing food and beverages.

3. *Investigation of Copigmentation by Fluorescence Spectroscopy.* As evidenced here for the malvin · rutin couple, fluorescence spectroscopy seems a very sensitive method for the quantitative investigation of copigmentation, especially in the case of poorly soluble copigments such as rutin for which large copigment/pigment molar ratios (a condition for accurate investigations by UV/VIS spectroscopy) cannot be achieved. Under strongly acidic conditions (pure flavylium form), malvin displays a broad weak fluoresence emission band around 600 nm (excitation at 540 nm) [13]. Addition of rutin promotes a very efficient quenching of malvin fluorescence at relatively low copigment/pigment molar ratios (2–10). Addition of quercetin 5′-sulfonate to solutions of synthetic flavylium ions also promotes an efficient quenching of flavylium fluorescence, a phenomenon shown to correlate with the ability of the copigment to inhibit the photodegradation of the pigment [14]. *Eqns. 3* and *4* can be easily derived from simple solution chemistry, assuming complete extinction of fluorescence in the copigmentation complex (F, F_0 = fluorescence intensity in the presence and in the absence of copigment, resp.; [L] = free copigment concentration; L_t = total copigment concentration; K = pigment · copigment binding constant; c = total pigment concentration).

The curve-fitting procedure ($r = 0.995$) of the experimental F vs. L_t plot (*Fig. 3*) using *Eqns. 3* and *4* gives a K value of 2560 (± 100) M^{-1}, in good agreement with the value obtained by UV/VIS spectroscopy.

$$F = \frac{F_0}{1 + K[\mathrm{L}]} \tag{3}$$

$$L_t = [\mathrm{L}]\left(1 + \frac{Kc}{1 + K[\mathrm{L}]}\right) \tag{4}$$

4. *Copigmentation and Colour Variation.* Stacking interactions between anthocyanins (flavylium ions) and polyphenolic copigments compete with the nucleophilic addition of H_2O on the chromophore and thereby lower the concentration of the colorless forms at a given pH (*colour intensification*). Simultaneously, the pigment VIS-absorption band is shifted toward longer wavelengths, thus turning the typically red-orange colours of free anthocyanin solutions into deep red and purple colours (*colour variation*) [4][5]. Although still poorly understood, the latter phenomenon plays a key

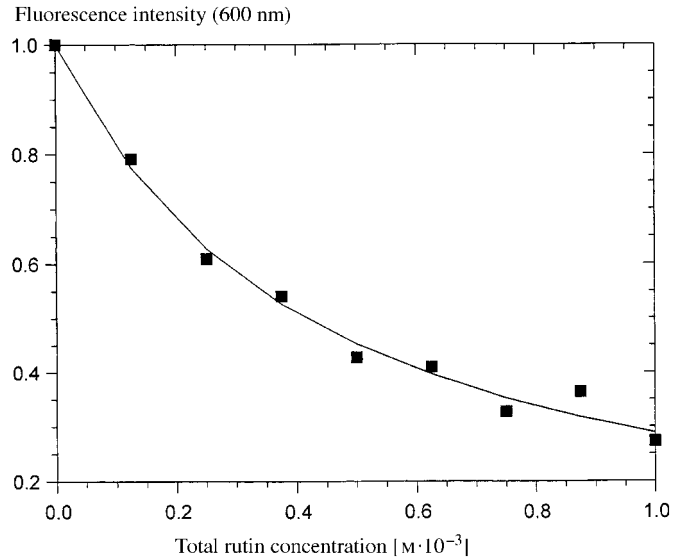


Fig. 3. *Plot of the fluorescence intensity of malvin at 600 nm* (excitation at 540 nm) *as a function of the total rutin concentration* (pH 0.9, 25°). The solid line is the result of the curve-fitting procedure according to *Eqns. 3* and *4*.

role in colour expression by plants. In this work for instance, it is shown that a potent copigment (high binding constant) in large concentrations (high H_2O solubility) actually promotes spectacular colour changes (*Fig. 1*).

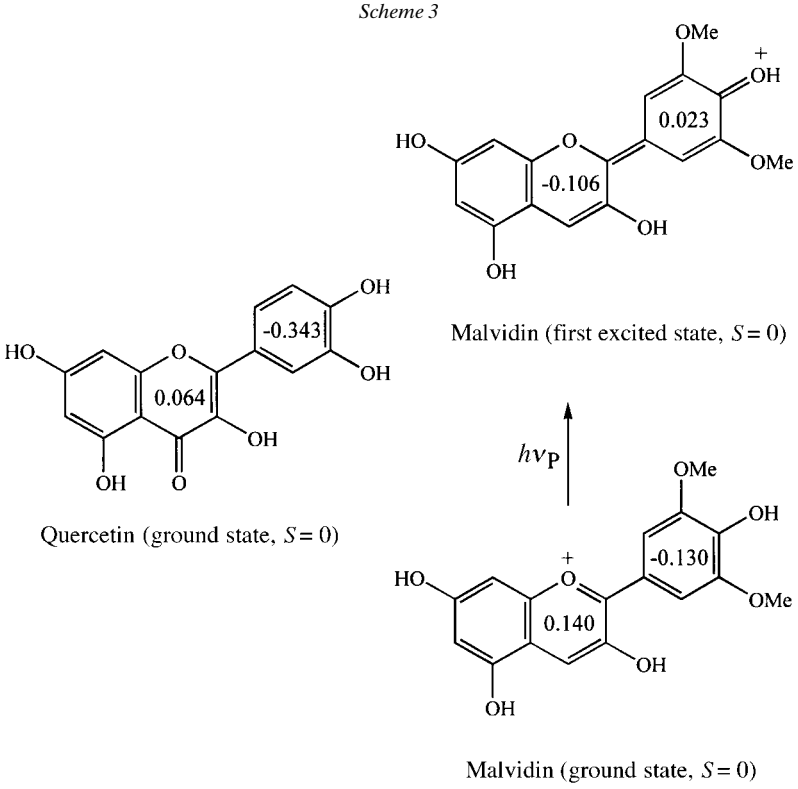
Solvatochromic shifts in UV/VIS spectroscopy are typically interpreted by differences in solvation properties between ground and excited states of solutes. For instance, enhancement in (or weakening of) H-bonding in the excited state explains why an absorption band is bathochromically (or hypsochromically, resp.) shifted when going from a nonprotic to a protic solvent [15]. By analogy, we propose that the copigment-induced bathochromic shift in the pigment VIS band reflects stronger pigment-copigment interactions in the excited state of the pigment. *Van der Waals* and electrostatic solute-solute interactions as well as the hydrophobic effect are the major driving forces in π-stacking complexation in H_2O [16]. Although *van der Waals* interactions and the hydrophobic effects both favour maximal overlap, this is generally not so in the case of electrostatic interactions since repulsion between π electrons is maximized when π electron-rich planar solutes are arranged face-to-face with no offset in the complex. In the case of copigmention by flavones and flavonols (the most potent anthocyanin copigments), such arrangements clearly result in an electrostatic mismatch since both electron-rich rings A, both electron-rich rings B, and both electron-poor rings C are stacked on one another. This unfavourable electrostatic contribution may well be alleviated by a significant offset between the pigment and copigment planes in the complex (at the expense of a small loss in *van der Waals* interactions and hydrophobic-effect contribution).

Semi-empirical quantum-mechanics calculations (AM1 parametrization) in the ground and first excited state (singlet) of malvidin (malvin aglycone) suggest that the electronic transition of lowest energy (VIS band) is accompanied by a strong transfer of

electron density from ring B to ring C, in agreement with the direction of the transition dipole calculated for a series of anthocyanidins (anthocyanin aglycones) in a previous detailed investigation [17]. The electron transfer occurring within the malvidin nucleus upon absorption of a VIS photon is best evidenced by summing the atomic charges borne by the ring-B and -C atoms in the ground and excited states. These ring charges are displayed in *Scheme 3*, together with those for the copigment (quercetin) in the ground state. A quinonoid mesomeric form of the pigment with a positively charged O-atom at C(4′) is chosen to depict the pigment in the excited state since it is more consistent with an electron-poor ring B and an electron-rich ring C. Alternatively, the representation of the probability densities for HOMO and LUMO of ground-state malvidin (*Fig. 4*) confirms that transferring one electron from HOMO to LUMO results in a large increase of π-electron density on ring C with a concomitant decrease on ring B. Hence, in the course of the electronic transition in the VIS range, the π-electron densities on the flavylium rings B and C are changed so as to allow more favourable electrostatic interactions with the corresponding rings in the copigment. Consequently, the pigment-copigment contact must be stronger in the excited state. A simple thermodynamic cycle allows estimation of the increase in the pigment-copigment binding energy ($\Delta\Delta G^0$) when the pigment is promoted from the ground to the first excited state (*Fig. 5*).

From the experimentally determined binding constant of the malvin · rutin complex in the ground state, it can be estimated that more than 95% of the malvin flavylium ion

Scheme 3



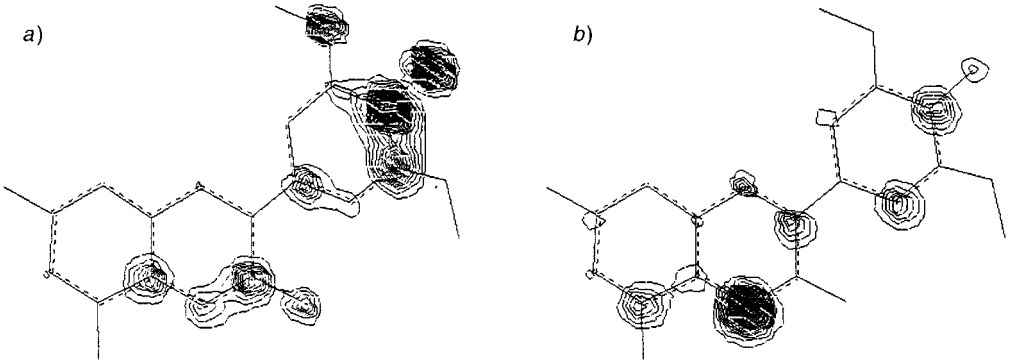


Fig. 4. *Probability densities for* a) *HOMO and* b) *LUMO of ground-state malvidin*. HyperChem program, AM1 parametrization

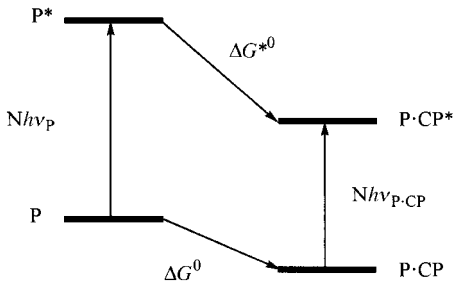


Fig. 5. *Estimation of the increase in the pigment* (P) · *copigment* (CP) *binding energy* ($\Delta\Delta G^0$) *on excitation*

is bound at pH < 1 (no colorless forms) for a copigment/pigment molar ratio of 200. Since such a ratio cannot be reached with rutin because of its poor H_2O solubility, the value for the wavelength of the absorption maximum of the copigmentation complex can be estimated with a highly H_2O-soluble rutin derivative such as **4** or **6** whose affinity for malvin is not significantly depressed by derivatization. The plot of the wavelength of the flavylium absorption maximum as a function of copigment **6** concentration actually shows a clear plateau around 560 nm at large copigment/pigment molar ratios (*Fig. 6*). From the thermodynamic cycle (*Fig. 5*), one gets *Eqn. 5* ($\Delta G^0, \Delta G^{*0}$ = copigmentation free enthalpy change in the ground and first excited states, resp.; K, K^* = pigment · copigment binding constant in the ground and first excited state, resp.; N_A = *Avogadro* constant; h = *Planck* constant; c = speed of light; R = gas constant).

$$\Delta G^0 - \Delta G^{0*} = RT\lambda\nu\frac{K^*}{k} = N_A h(\nu_{\Pi} - \nu_{\Pi X\Pi}) = N_A h c\left(\frac{1}{\lambda_P} - \frac{1}{\lambda_{PCP}}\right) \qquad (5)$$

Energy differences between the vibrational ground states of the electronic ground and first excited states should be considered in the thermodynamic cycle. Hence, a calculation based on wavelengths of absorption maximum for the pigment and its copigmentation complex (λ_P, λ_{PCP}) should be corrected for the fact that vibrational

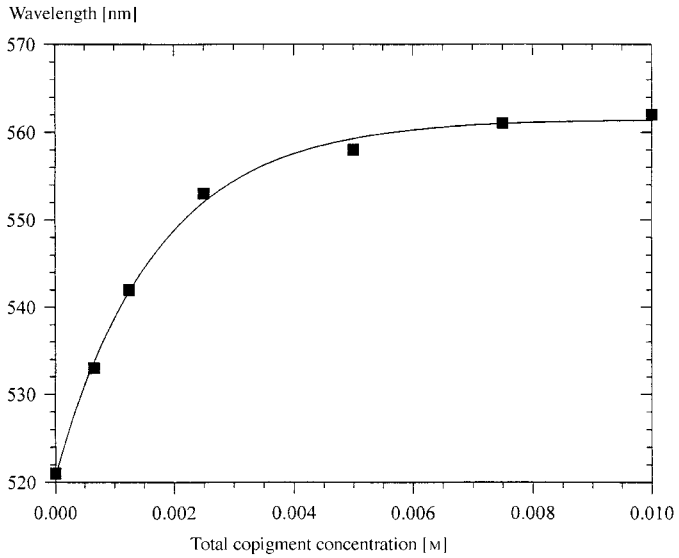


Fig. 6. *Plot of the wavelength of the absorption maximum of malvin* ($5 \cdot 10^{-5}$M) *as a function of the total concentration of rutin derivative* **6** (pH 0.9, 25°)

excited states are actually populated in the electronic excited states (*Franck-Condon* transitions)[2]). However, such corrections must be similar for the flavylium ion in its free and bound forms and are thus expected to cancel out at least partially in *Eqn. 5*. In the case of the malvin · rutin couple, λ_P and λ_{PCP} are equal to 520 and *ca.* 560 nm, respectively, thus giving $\Delta\Delta G^0 = 16.4$ kJ mol^{-1} and $K^*/K = 760$. Hence, this simple model suggests that the binding of potent flavonol copigments to anthocyanins (flavylium ions) may be two to three orders of magnitude stronger when anthocyanins are in their first electronic excited state.

5. *Antioxidant Properties.* Flavonoids can exert their antioxidant properties *via* several mechanisms including quenching of reactive O-species (hydroxyl, alkoxyl, and peroxyl radicals) and inhibition of enzymes involved in their production, chelation of transition-metal ions able to promote radical formation (*Fenton* reaction), and regeneration of membrane-bound antioxidants such as α-tocopherol (vitamin E) [2][7][18].

DPPH (=2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; **8**) is a highly coloured commercially available radical widely used for estimating the ability of antioxidants to trap potentially damaging one-electron oxidants/H-atom-abstracting agents [19]. In particular, antioxidants can be characterized by their stoichiometry *n*, *i.e.* the number of DPPH molecules reduced per molecule of antioxidant. Hence, the antioxidant can be regarded as a source of *n* H-atoms that will convert DPPH to the corresponding hydrazine. When monitoring the H-transfer reactions from a flavonoid antioxidant to

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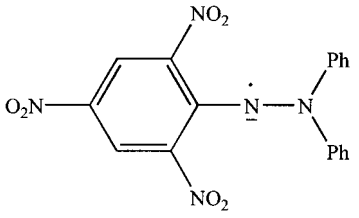
[2]) A better estimate of the energy differences between ground and excited states should be $N_A h(\nu_{abs} + \nu_{em})/2$, ν_{abs} and ν_{em} being the frequencies of absorption and emission maxima. However, such a correction cannot be applied to the bound flavylium ion since copigmentation by rutin leads to a complete quenching of flavylium fluorescence, thereby preventing the determination of ν_{em}.



8

DPPH (**8**) by UV/VIS spectroscopy, two steps can be distinguished: a first step during which the DPPH VIS absorbance (λ_{max} 516 nm in MeOH) quickly decays (typical time interval in MeOH: 60 s) and a second step during which the DPPH VIS absorbance slowly decays over *ca.* 1 h to a final constant value. The fast step essentially refers to abstractions of the most labile H-atoms (OH–C(3), OH–C(3′), OH–C(4′) in the case of quercetin [20]), whereas the slow step reflects the remaining activity in the oxidation-degradation products. A total stoichiometry (n_{tot}) can be determined in a *static* way from the overall amplitude of the kinetic run by *Eqn. 6* (A_f = VIS absorbance corresponding to the plateau at the end of the kinetic run (complete conversion of the antioxidant into inert oxidation-degradation products); A_0 = initial absorbance (just after addition of the antioxidant); c = initial antioxidant concentration; c_0 = initial DPPH concentration).

$$n_{tot} = \frac{c_0}{c}\left(1 - \frac{A_f}{A_0}\right) \quad (6)$$

Of course, the initial DPPH/antioxidant molar ratio c_0/c must be higher than n_{tot} for *Eqn. 6* to apply. A *kinetic* stoichiometry n can also be estimated from the curve-fitting of the kinetic traces featuring the decay of the DPPH band *during the fast step*. To that purpose, an antioxidant of stoichiometry n is simply modeled as n independent sub-units AH (typically, flavonoid OH groups bearing most labile H-atoms), which all transfer a H-atom to DPPH with the same second-order rate constant k. This procedure, already applied to quercetin, rutin [20], and some gallotannins [21], also gives satisfying curve fits ($r > 0.995$) for rutin derivatives **5**–**7** and consistent values for n and k at different DPPH/flavonoid molar ratios (*Table 2*). The k value provides a quantitative estimate of the overall reactivity of the antioxidant toward DPPH (**8**) during the fast step of trapping. Rutin bis(hydrogen succinate) **6** and rutin 4‴-(hydrogen succinate) (**7**) react with DPPH as rapidly as (and eventually, more rapidly than) rutin. However, a higher degree of succinylation significantly slows the rate of trapping. Stoichiometries close to 2 (fast step) are consistent with the formation of quinone intermediates that have been trapped as solvent or benzenesulfinate adducts in the case of quercetin and rutin [20]. To form quinones, aryloxy radicals must further react with DPPH or with themselves (disproportionation). The latter process is more probable since a fast second-order decay of polyphenolic aryloxyl radicals in H_2O ($2k$ in the range 10^6–10^7 M^{-1} s^{-1}) has already been reported in the case of flavonoids from pulse-radiolysis experiments [22] and, in the case of caffeic acid, from electrochemical investigations [23]. Total stoichiometries *ca.* 2–3 times larger than those determined by

Table 2. *H-Abstraction from Rutin and Rutin Derivatives* **5**–**7** *by DPPH* (**8**) *in MeOH at 25°*. For definition of parameters n, n_{tot}, and k, see text.

	DPPH [equiv.]	k [M^{-1} s^{-1}] [a])	n [a])	n_{tot} [b])
5	6	330 (± 7)	1.76 (± 0.01)	–
5	8	276 (± 5)	1.92 (± 0.01)	5.8
6	4	895 (± 9)	2.63 (± 0.01)	–
6	8	736 (± 10)	2.55 (± 0.01)	4.2 (4.3 [c]))
7	4	2220 (± 20)	1.93 (± 0.01)	–
7	6	1850 (± 20)	1.92 (± 0.01)	4.8 (4.7 [d]))
Rutin	8	904 (± 17)	2.23 (± 0.01)	5.9
Rutin	4	932 (± 5)	2.44 (± 0.01)	–

[a]) From the curve fitting of the kinetic traces (typical time intervals: 60 s), see [20]. [b]) From *Eqn. 6*; final DPPH absorbance recorded 1 h after addition of the antioxidant. [c]) DPPH/**6** molar ratio 6. [d]) DPPH/**7** molar ratio 8.

the kinetic method (fast step) are obtained from *Eqn. 6* (*Table 2*), thus showing that quinones are actually intermediates and further react with DPPH. Quinone-DPPH reactions could occur *via* direct H-abstraction from OH–C(7) or, more probably, after intermediate steps of solvent addition [20] and/or dimerization, which allow the regeneration of a benzene-1,2-diol moiety (ring B) liable to transfer its phenolic H-atoms to DPPH. Interestingly, although abstraction of H-atoms from **5** by DPPH is slower than from rutin, the total number of DPPH radicals trapped (n_{tot}) is the same for both compounds. Hence, total or partial succinylation of the sugar moiety of rutin does not seem to significantly alter its antioxidant ability and could eventually prolong it owing to an improved bioavailability.

Experimental Part

General. Malvin (HPLC-pure; *Extrasynthese*, Genay, France), rutin, and DPPH (95%; *Aldrich*) were used as received. UV/VIS Spectra: *Hewlett-Packard-8453* diode-array spectrometer, equipped with a magnetically stirred quartz cell (optical pathlength = 1 cm) thermostated by a water bath; λ_{max} in nm.

Fluorescence spectra: *BioLogic* spectrometer with a thermostated quartz cell.

NMR Spectra: 300-MHz- and 500-MHz-*Bruker* apparatus; at 27°; chemical shifts δ in ppm, coupling constants J in Hz; ^{1}H signals assigned from 1D- and 2D-COSY experiments, ^{13}C signals assigned from DEPT, HMBC, and HSQC experiments (**3**) or from DEPT and comparisons with [24] (**4**, **5**, and **7**). FC = Flash chromatography.

5,7-Bis(acetyloxy)-2-[3,4-bis(acetyloxy)phenyl]-3-{[2,3,4-tri-O-*acetyl-6*-O-*[4*-O-*acetyl-6-deoxy-2,3*-O-*(phenylmethylene)-α*-L-*mannopyranosyl]-β*-D-*glucopyranosyl]oxy}-4*H-*1-benzopyran-4-one* (**1**). A soln. of rutin (5 g, 8.2 mmol) and TsOH (156 mg, 0.82 mmol) in DMF (20 ml) is dried (H_2O traces) upon co-evaporation with toluene (10 ml). Benzaldehyde dimethyl acetal (3.70 ml, 24.6 mmol) and toluene (15 ml) are added, and the mixture is heated at 40° under reduced pressure (*Rotavap*) for the gradual removal of MeOH formed in the course of the reaction (co-evaporation with toluene). Portions of toluene (10 ml) are further added and the co-evaporation repeated until complete disappearence of rutin on TLC. The orange oil thus obtained is poured into CH_2Cl_2 (100 ml). The yellow precipitate (rutin benzylidene acetal) is filtered, washed with Et_2O, and dried. Rutin benzylidene acetal (1.15 g, 1.64 mmol) is then dissolved in pyridin (14 ml), and Ac_2O (7.8 ml, 82 mmol) and DMAP (40 mg, 0.32 mmol) are successively added at 0°. The mixture is stirred at r.t. for 36 h, then at 50° for 24 h. After evaporation of pyridin, the product is precipitated in AcOEt/petroleum ether, washed with petroleum ether, and dried: 82% (from rutin) of **1** (two epimers, *ca.* 1:1 molar ratio). Beige powder. TLC (silica gel, AcOEt/petroleum ether 1:1): R_f 0.62, 0.70. UV/VIS ($CHCl_3$): 302, 261. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.93, 7.91 (2*d*, J = 2.2, 2 H, H–C(2′)); 7.83, 7.77 (2*dd*, J = 2.2, 8.5, 2 H, H–C(6′)); 7.5–7.3 (*m*, 12 H, H–C(5′), Ph); 7.26, 7.18 (2*d*, J = 2.2, 2 H, H–C(8)); 6.84, 6.81 (2*d*, J = 2.2, 2 H, H–C(6)); 6.12, 5.70 (2*s*, 2 H, PhC*H*); 5.65, 5.51 (2*d*, J = 8.1, 2 H, H–C(1″)); 5.27 (*m*, 2 H, H–C(3″)); 5.17 (*m*, 2 H, H–C(2″)); 5.01 (*m*, 2 H, H–C(4″)); 4.89 (*m*,

2 H, H–C(4‴)); 4.82, 4.75 (2*s*, 2 H, H–C(1‴)); 4.38, 4.21 (2*dd*, *J* = 5.2, 8.4, 2 H, H–C(3‴)); 4.01, 3.95 (2*d*, *J* = 5.2, 2 H, H–C(2‴)); 3.6–3.5 (*m*, 8 H, H–C(5″), 2 H–C(6″), H–C(5‴)); 2.44–1.99 (8*s*, 48 H, 8 Ac); 1.01, 0.98 (2*d*, *J* = 6.2, 6 H, Me(6‴)).

5,7-Bis(acetyloxy)-2-[3,4-bis(acetyloxy)phenyl]-3-{[2,3,4-tri-O-*acetyl-6*-O-*(4*-O-*acetyl-2*-O-*benzoyl-6-deoxy-α*-L-*mannopyranosyl-β*-D-*glucopyranosyl]oxy}-4*H-*1-benzopyran-4-one* (**2**). To a soln. of **1** (1 g, 1 mmol) in $CHCl_3$ (100 ml) are successively added *N*-bromosuccinimide (430 mg, 2.5 mmol), $BaCO_3$ (460 mg, 2.4 mmol), H_2O (104 µl, 6 mmol), and benzoyl peroxide (26 mg, 0.1 mmol). The mixture is refluxed under N_2 for 4 h, then cooled, washed with H_2O, dried ($MgSO_4$), and concentrated. Chromatography (silica gel, AcOEt/petroleum ether 7:3) affords **2** (800 mg, 76%). Yellow powder. TLC (silica gel, AcOEt/petroleum ether 1:1): R_f 0.48. UV/VIS ($CHCl_3$): 301. ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.04–7.96 (*m*, H–C(6′), H–C(2′), 2 H_o (Bz)); 7.57 (*d*, *J* = 7.4, H_p (Bz)); 7.44 (*t*, *J* = 7.4, 2 H_m (Bz)); 7.35 (*d*, *J* = 8.5; H–C(5′)); 7.28 (*d*, *J* = 2.2; H–C(8)); 6.83 (*d*, *J* = 2.2; H–C(6)); 5.68 (*d*, *J* = 7.7, H–C(1″)); 5.31 (*t*, *J* = 9.6, H–C(3″)); 5.20 (*dd*, *J* = 9.6, 7.7, H–C(2″)); 5.08 (*d*, *J* = 4.4, H–C(2‴)); 5.03 (*t*, *J* = 9.6, H–C(4″)); 4.90 (*t*, *J* = 9.9, H–C(4‴)); 4.67 (*s*, H–C(1‴)); 3.96 (*dd*, *J* = 9.9, 4.4, H–C(3‴)); 3.65–3.60 (*m*, H–C(5″), H–C(6″), H–C(5‴)); 3.28 (*dd*, *J* = 11.0, 5.1, H–C(6″)); 2.45–2.02 (8*s*, 8 Ac); 1.03 (*d*, *J* = 5.9, Me(6″)).

*3-{[6-*O-*(6-Deoxy-3-*O-*sulfo-α-*L-*mannopyranosyl)-β-*D-*glucopyranosyl]oxy}-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H-*1-benzopyran-4-one Sodium Salt* (**3**). A soln. of **2** (500 mg, 1.8 mmol) and SO_3·pyridin complex (155 mg, 0.9 mmol) in DMF (6 ml) is heated at 50° under N_2 for 12 h. After evaporation, MeOH (5 ml) and 1M MeONa in MeOH (5 ml) are added. The mixture is stirred under N_2 for 12 h in the dark, then neutralized with a *Dowex* resin (H^+ form), filtered, and evaporated. A dil. soln. of the residue in H_2O is passed over a *Dowex* Na^+ ion-exchange resin and then evaporated. FC (C_{18} silica gel, H_2O/MeCN 95:5) affords **3** (100 mg, 30%). Yellow powder. UV/VIS (H_2O): 352, 264. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 7.57 (*dd*, *J* = 8.1, 2.2, H–C(6′)); 7.52 (*d*, *J* = 2.2, H–C(2′)); 6.87 (*d*, *J* = 8.1, H–C(5′)); 6.36 (*d*, *J* = 1.5, H–C(8)); 6.17 (*d*, *J* = 1.5, H–C(6)); 5.17 (*d*, *J* = 7.4, H–C(1″)); 4.38 (br. *s*, H–C(1‴)); 4.05 (*dd*, *J* = 8.8, 3.7, H–C(3‴)); 3.67 (br. *s*, H–C(2‴)); 3.36–2.99 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), 2 H–C(6″), H–C(4‴), H–C(5‴)); 0.95 (*d*, *J* = 5.9, Me(6‴)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 178.1 (C(4)); 165.0 (C(7)); 161.7 (C(5)); 157.8 (C(8a)); 157.4 (C(2)); 149.1 (C(4′)); 145.4 (C(3′)); 134.4 (C(3)); 122.7 (C(6′)); 122.0 (C(1′)); 116.9 (C(2′)); 116.1 (C(5′)); 104.8 (C(4a)); 102.7 (C(1′)); 102.0 (C(1‴)); 99.5 (C(6)); 94.6 (C(8)); 77.7 (C(3‴)); 77.1 (C(5″)); 76.4 (C(2″)); 74.8 (C(4‴)); 71.1 (C(2‴)); 70.7 (C(3″)); 69.6 (C(4″)); 69.1 (C(5‴)); 68.7 (C(6″)); 18.3 (C(6‴)). ES-MS (neg. mode): 711.1 ([*M* – H]$^+$), 689.1 ([*M* – Na]$^+$).

*3-{[6-*O-*[2,3-*O-*(2-Carboxy-1-methylethylidene)-6-deoxy-α-*L-*mannopyranosyl]-β-*D-*glucopyranosyl}oxy}-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H-*1-benzopyran-4-one* (**4**). As described for **1**, with rutin (0.5 g, 0.8 mmol), TsOH (16 mg, 0.08 mmol), DMF (10 ml), and toluene (10 ml), then ethyl acetoacetate dimethyl acetal (1.2 g, 7 mmol) and toluene (10 ml), and finally portions of toluene (10 ml). After evaporation, 1M KOH in EtOH (10 ml) is added. The mixture is heated at 60° under N_2 for 1.5 h, then acidified with a *Dowex* resin (H^+ form) to pH *ca.* 1, filtered, and evaporated. Chromatography (C_{18} silica gel, H_2O/MeCN 4:1) affords **4** (2 epimers, *ca.* 1:1 molar ratio; 472 mg, 85%). UV/VIS (H_2O): 351, 255. TLC (silica gel, AcOEt/butanone/HCO_2H/H_2O 14:2:1:1): R_f 0.5. ^{1}H-NMR (CD_3OD, 500 MHz): 7.71 (*d*, *J* = 2.3, 2 H, H–C(2′)); 7.64 (*dd*, *J* = 8.6, 2.3, 2 H, H–C(6′)); 6.89 (*d*, *J* = 8.6, 2 H, H–C(5′)); 6.45 (*s*, 2 H, H–C(8)); 6.25 (*s*, 2 H, H–C(6)); 5.21, 5.17 (2*d*, *J* = 7.4, 2 H, H–C(1″)); 4.82, 4.80 (2*s*, 2 H, H–C(1‴); 4.01–3.80 (*m*, 6 H, H–C(6″), H–C(2‴), H–C(3‴)); 3.53–3.14 (*m*, 14 H, H–C(2″), H–C(3″), H–C(4″), H–C(5″), H–C(6″), H–C(4‴), H–C(5‴)); 2.76, 2.68 (2*d*, *J* = 14.3, 2 H, C(Me)CH_2); 2.59, 2.53 (2*d*, *J* = 13.9, 2 H, C(Me)CH_2); 1.62, 1.40 (2*s*, 6 H, C(*Me*)CH$_2$); 1.11, 1.10 (2*d*, *J* = 6.6, 6 H, Me(6‴)). ^{13}C-NMR (CD_3OD, 300 MHz): 178.3 (C(4)); 172.5 (CO_2H); 164.9 (C(7)); 161.9 (C(5)); 158.2 (C(8a)); 157.4 (C(2)); 148.8 (C(4′)); 144.8 (C(3′)); 134.5 (C(3)); 122.4 (C(6′)); 122.0 (C(1′)); 116.7 (C(2′)); 115.0 (C(5′)); 108.2 (*C*(Me)CH$_2$); 104.6 (C(4a)); 103.3 (C(1″)); 98.9 (C(1‴)); 97.7 (C(6)); 93.9 (C(8)); 78.8 (C(3″)); 77.1 (C(5″)); 76.4 (C(2″)); 76.4 (C(4‴)); 76.0 (C(2‴)); 74.7 (C(3‴)); 74.4 (C(4″)); 70.5 (C(5″)); 66.9 (C(6″)); 65.7 (?); 47.2, 46.6 (C(Me)*C*H$_2$); 23.8, 23.2 (C(*Me*)CH$_2$); 16.5 (Me(6‴)). ES-MS (neg. mode): 693.1 ([*M* – H]$^+$).

*2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-{[2,3,4-tris-*O-*(3-carboxy-1-oxopropyl)-6-*O-*[2,3,4-tris-*O-*(3-carboxy-1-oxopropyl)-6-deoxy-α-*L-*mannopyranosyl]-β-*D-*glucopyranosyl}oxy}-4*H-*1-benzopyran-4-one* (**5**). A soln. of rutin (500 mg, 0.82 mmol), succinic anhydride (821 mg, 8.2 mmol), and DMAP (50 mg, 0.41 mmol) in pyridine (20 ml) is heated at 70° under N_2 for 24 h. After removal of pyridine and addition of H_2O (20 ml), the mixture is acidified with *Dowex* (H^+ form) to pH 2, filtered, washed with $CHCl_3$ (3 × 30 ml), and lyophilized. FC (C_{18} silica gel, $H_2O \rightarrow H_2O$/MeCN 4:1) affords **5** (934 mg, 95%). Yellow powder. UV/VIS (MeOH): 356, 257. TLC (silica gel, AcOEt/butanone/HCO_2H/H_2O 20:2:1:1): R_f 0.53. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.57 (1*s*, OH–C(5)); 7.65 (*d*, *J* = 2.2, H–C(2′)); 7.44 (*dd*, *J* = 2.2, 8.8, H–C(6′)); 6.83 (*d*, *J* = 8.8, H–C(5′)); 6.41

(*d*, *J* = 1.5, H–C(8)); 6.19 (*d*, *J* = 1.5, H–C(6)); 5.64 (*d*, *J* = 8.1, H–C(1″)); 5.38 (*t*, *J* = 9.6, H–C(3″)); 5.15 (*t*, *J* = 9.6, H–C(2″)); 4.97 (*t*, *J* = 9.6, H–C(4″)); 4.90 (*d*, *J* = 2.9, H–C(2‴)); 4.87 (*dd*, *J* = 10.3, 2.9, H–C(3‴)); 4.74 (*t*, *J* = 10.3, H–C(4‴)); 4.44 (*s*, H–C(1‴)); 3.7–3.5 (*m*, H–C(5″), 2 H–C(6″), H–C(5‴)); 2.41 (*s*, 6 CH_2CH_2COOH); 0.94 (*d*, *J* = 5.9, Me(6‴)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 177.6 (C(4)); 174.5–174.0 (CO_2H); 172.4–171.8 (COCH$_2$CH$_2$COOH); 165.1 (C(7)); 162.0 (C(5)); 157.3 (C(8a)); 157.1 (C(2)); 149.7 (C(4′)); 145.6 (C(3′)); 133.8 (C(3)); 121.8 (C(6′)); 121.2 (C(1′)); 117.2 (C(2′)); 116.2 (C(5′)); 104.7 (C(4a)); 99.8 (C(1″)); 99.5 (C(1‴)); 98.3 (C(6)); 94.5 (C(8)); 73.2 (C(3‴)); 73.0 (C(5″)); 72.3 (C(2″)); 71.1 (C(4‴)); 70.8 (C(3‴)); 69.4 (C(2‴)); 69.1 (C(4″)); 67.4 (C(6″)); 66.7 (C(5‴)); 29.7–29.3 (CH$_2$CH$_2$COOH); 17.8 (Me(6‴)). FAB-MS (pos. mode): 1211.4 ([*M* + H]$^+$).

*3-{[6-*O*-[3,4-Bis-*O*-(3-carboxy-1-oxopropyl)-6-deoxy-α-*L*-mannopyranosyl]-β-*D*-glucopyranosyl}oxy}- and 3-{[2- or 3- or 4-*O*-(3-Carboxy-1-oxopropyl)-6-*O*-[4-*O*-(3-carboxy-1-oxopropyl)-6-deoxy-α-*L*-mannopyranosyl]-β-*D*-glucopyranosyl}oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one* (**6**; isomer ratio *ca.* 3 : 2 : 2). Succinic anhydride (98 mg, 0.98 mmol) is added to a soln. of rutin (200 mg, 0.33 mmol) and DMAP (8.6 mg, 0.07 mmol) in pyridine (10 ml) at 0° under N_2. After stirring for 6 h at 0°, pyridine is evaporated and MeOH (10 ml) added. The mixture is acidified with *Dowex* and filtered, the filtrate evaporated and the residue subjected to chromatography (C_{18} silica gel, $H_2O \rightarrow H_2O$/MeOH 65 : 35): **6** (3 regioisomers; 75 mg, 28%). Beige solid. UV/VIS (MeOH): 358, 257. TLC (silica gel, AcOEt/butanone/HCO_2H/H_2O 20 : 2 : 1 : 1): R_f 0.25. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.58, 12.56, 12.53 (3*s*, 3 OH–C(5)); 7.53–7.49 (*m*, 3 H–C(2′), 3 H–C(6′)); 6.85 (*d*, *J* = 8.1, 3 H–C(5′)); 6.39 (*s*, 3 H–C(8)); 6.20 (*s*, 3 H–C(6)); 5.52, 5.46 (2*d*, *J* = 8.1, 2 H–C(1″)); 5.44–5.35 (*m*, 1 H–C(1″), 1 H(Glc)); 4.83 (*m*, 3 H–C(4‴)); 4.65 (*t*, *J* = 9.5, 1 H(Glc)); 4.59 (*dd*, *J* = 9.5, 3.7, 1 H–C(3‴)); 4.44, 4.41, 4.39 (3*s*, 3 H–C(1‴)); 3.75–3.05 (other H signals); 2.50 (*m*, CH_2CH_2COOH); 1.02, 0.99, 0.74 (3*d*, *J* = 5.9, 3 Me(6‴)). FAB-MS (pos. mode): 811.6 ([*M* + H]$^+$).

*3-{[6-*O*-[4-*O*-(3-Carboxy-1-oxopropyl)-6-deoxy-α-*L*-mannopyranosyl]-β-*D*-glucopyranosyl}oxy}-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one* (**7**). Succinic anhydride (49 mg, 0.49 mmol) is added to a soln. of rutin (200 mg, 0.33 mmol) and DMAP (4 mg, 0.03 mmol) in pyridine (10 ml) at 0° under N_2. After stirring for 7 h at 0°, pyridine is evaporated and MeOH (10 ml) added. The mixture is acidified with *Dowex* and filtered, the filtrate evaporated, and the residue subjected to chromatography (C_{18} silica gel, $H_2O \rightarrow H_2O$/MeCN 70 : 30): **7** (50 mg, 21%). Beige solid. UV/VIS (MeOH): 359, 257. TLC (silica gel, AcOEt/butanone/HCO_2H/H_2O 20 : 2 : 1 : 1): R_f 0.22. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 7.55–7.46 (*m*, H–C(2′), H–C(6′)); 6.84 (*d*, *J* = 8.1, H–C(5′)); 6.37 (*s*, H–C(8)); 6.17 (*s*, H–C(6)); 5.42 (*d*, *J* = 7.4, H–C(1″)); 4.81 (*t*, *J* = 8.8, H–C(4‴)); 4.39 (*s*, H–C(1‴)); 3.72–3.05 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), 2 H–C(6″), H–C(2‴), H–C(3‴), H–C(5‴)); 2.41 (*s*, CH_2CH_2COOH); 1.00 (*d*, *J* = 5.9, Me(6‴)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 178.0 (C(4)); 175.3 (CO_2H); 172.9 (COCH$_2$CH$_2$COOH); 165.9 (C(7)); 162.0 (C(5)); 157.3 (C(8a), C(2)); 149.7 (C(4′)); 145.9 (C(3′)); 134.0 (C(3)); 122.0 (C(6′)); 121.7 (C(1′)); 117.1 (C(2′)); 116.2 (C(5′)); 104.4 (C(4a)); 101.6 (C(1″), C(1‴)); 99.8 (C(6)); 94.6 (C(8)); 78.7 (C(3″)); 76.3 (C(5″)); 72.7 (C(2″), C–C(4‴)); 71.4 (C(3‴)); 71.2 (C(2‴)); 69.2 (C(4″)); 68.5 (C(6″)); 67.4 (C(5‴)); 31.3 (CH_2 (Suc)); 30.6 (CH_2 (Suc)); 18.6 (Me(6‴)). FAB-MS (pos. mode): 711.2 ([*M* + H]$^+$).

Semiempirical Quantum-Mechanics Calculations. Run *in vacuo* on a *Pentium-90* PC with the HyperChem program (*Autodesk*, Sausalito, California, USA) and the AM1 parametrization. Atomic charges in the ground state and probability densities for HOMO and LUMO were obtained after previous geometry optimization. Atomic charges in the excited state were the result of so-called 'single-point calculations' (without geometry optimization).

Data Analysis. The curve fittings were carried out on a *Pentium-120* PC (Scientist program, *MicroMath*, Salt Lake City, Utah, USA) through least-square regression. Optimized values for the parameters and the corresponding standard deviations are reported.

Copigmentation Experiments by UV/VIS Spectroscopy. A given copigment is added to a $5 \cdot 10^{-5}$ M soln. of malvin in a 0.2M acetate buffer at pH 3.5 (copigment/pigment molar ratio 20–200, depending on the copigment solubility). An aliquot of the soln. is placed in the spectrometer cell, and UV/VIS spectra are recorded every 5° in the temp. range 15–45°. The soln. is kept *ca.* 30 min at the selected temp. before each measurement to allow the structural transformations of the pigment (H_2O addition) to reach complete equilibrium. The experiment is repeated with the soln. of malvin without copigment. Both solns. are then acidified to pH 0.9–1.0 with conc. HCl (no significant dilution) for the determination of the isosbestic point of malvin (flavylium form) and its copigmentation complex.

Copigmentation Experiments by Fluorescence Spectroscopy. Aliquots (10 µl) of a $2.5 \cdot 10^{-2}$ M soln. of rutin in MeOH are added to 2 ml of a $5 \cdot 10^{-5}$ M soln. of malvin at pH 0.9–1.0 (acetate buffer acidified with conc. HCl soln.). Emission spectra are recorded after each addition (excitation at 540 nm).

H-Abstraction by DPPH (**8**). To 2 ml of freshly prepared $2 \cdot 10^{-4}$ M **8** in MeOH placed in the spectrometer cell, freshly prepared 10^{-3} M flavonol in MeOH (50–100 µl) is added. Spectra are recorded every 0.5 s over 30–90 s for the determination of parameters n and k. Kinetic runs over 1 h are used for the determination of n_{tot}.

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