Quercetin (= 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one) Glycosides and Sulfates: Chemical Synthesis, Complexation, and Antioxidant Properties

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Glycosides, acylated glycosides, and sulfates of quercetin (= 3,3′,4′,5,7-pentahydroxyflavone; **1**), which is, together with its derivatives, among the most common polyphenols found in plants and in the human diet, were prepared and quantitatively investigated for their ability to bind metal ions (Al^{III}, Fe^{II}, Fe^{III}), enhance and vary natural colours (anthocyanin copigmentation), and trap potentially damaging radicals (antioxidant activity).

Introduction. – Flavonoids and other polyphenols are ubiquitous secondary metabolites in plants [1] which, as food components, enjoy increasing interest from researchers, food manufacturers, and consumers. Indeed, polyphenol-rich diets (*e.g.*, the regular consumption of fruits, vegetables, red wine, and tea) have been repeatedly correlated with a low risk of developing cardiovascular diseases and cancers [2], the two major causes of mortality in occidental countries. Because of their antioxidant properties and their ability to bind to a wide range of proteins including enzymes [1][3], polyphenols may be partially responsible for these protective effects.

Besides phenolic acids (benzoic and cinnamic acid derivatives), flavonols (3-hydroxyflavones) are one of the main classes of dietary polyphenols [1]. Their antioxidant properties essentially stem from their ability to trap reactive O-species (ROS) and bind transition-metal ions, which, if not suitably complexed, could enter redox cycles that produce ROS [3]. This work focuses on quercetin (3,3′,4′,5,7-pentahydroxyflavone; **1**), the most representative dietary flavonol (abundant in onions, apples, and tea) and a potent antioxidant. In most naturally occurring flavonols, at least one of the OH groups of the polyphenolic nucleus (aglycone) is glycosylated by mono- and oligosaccharides involving neutral sugars such as D-glucose, D-galactose, L-rhamnose, and D-xylose. Quercetin β-D-glucopyranosides are particularly interesting because their biodisposability, irrespective of the location of the sugar moiety on the polyphenolic nucleus, is better than that of quercetin and other quercetin glycosides, possibly as a consequence of a facilitated intestinal absorption *via* glucose transporters [4]. Moreover, quercetin sulfates deserve particular attention, not only because of their natural occurrence [1][5], but also because the quercetin conjugates found in the plasma of human volunteers after consumption of a meal rich in plant products are actually glucuronides and sulfates [6].

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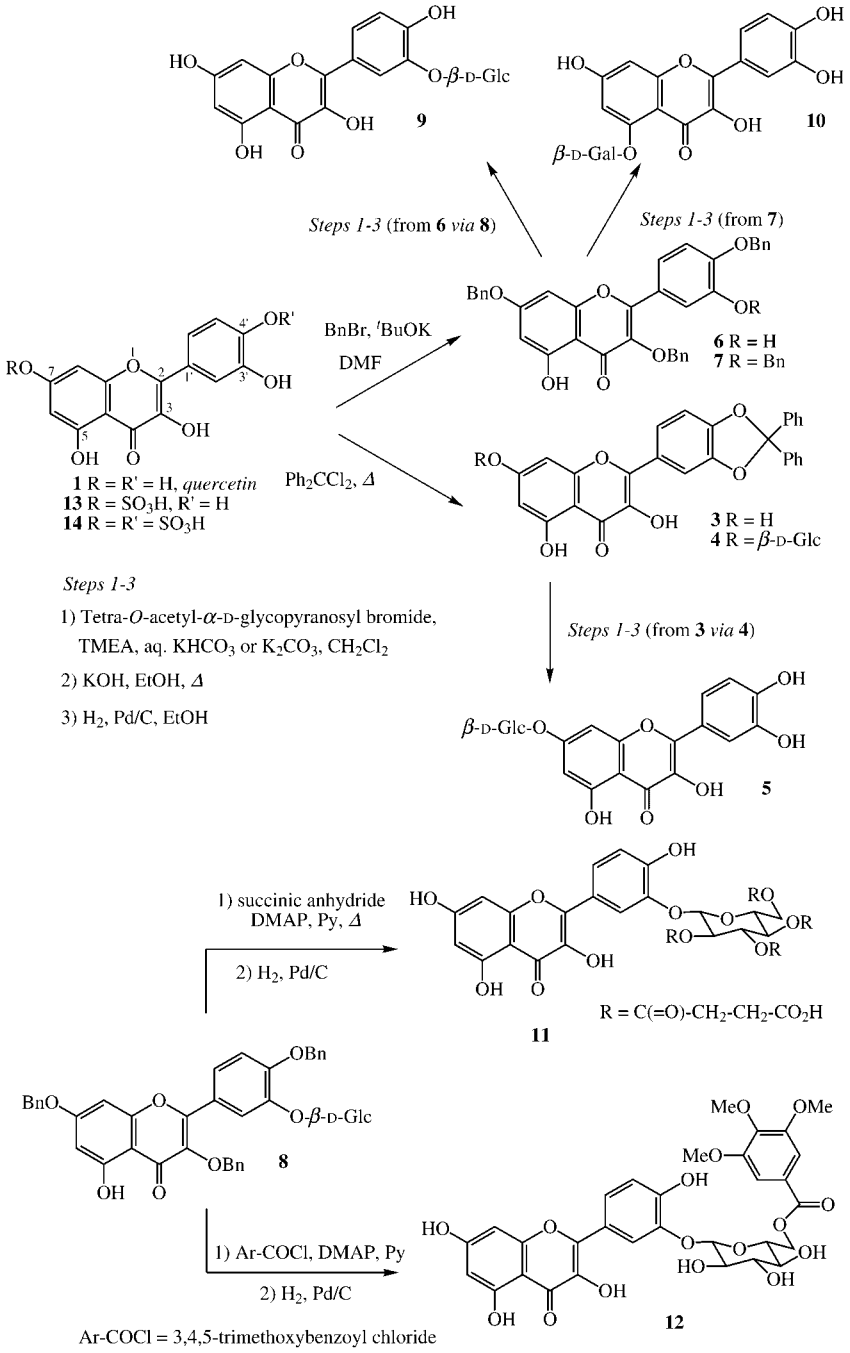
In this work, simple methods for the preparation of quercetin glycosides, *via* a phase-transfer glycosidation step, are described, quercetin sulfates being prepared following already published procedures [7]. The quercetin derivatives are then tested for their ability to bind metal ions (Al^{3+}, Fe^{3+}, Fe^{2+}) and trap DPPH (diphenylpicrylhydrazyl), a coloured free radical commonly used for assessing the antioxidant (H-atom-donating) activity. In addition, the highly H_2O-soluble sulfates are also demonstrated to form exceptionally stable molecular complexes with polyphenolic plant pigments (anthocyanins), thereby allowing natural colours to be deeply intensified and varied (copigmentation). Quite frequently in plants, some sugar OH groups in flavonoid glycosides are acylated by a variety of aromatic and aliphatic acids [1] including succinic acid (= butanedioic acid). Some easily available and highly H_2O-soluble succinylated quercetin glycosides are also investigated for their ability to bind metal ions with the possible participation of their free carboxy groups.

Results and Discussion. – 1. *Synthesis* (see *Scheme 1*). The direct glycosidation of quercetin (**1**) by 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**) under mildly alkaline to neutral phase-transfer conditions that have proved suitable for the glycosidation of flavones and other acidic phenols [8] did not allow us to isolate any glycoside. Since the most acidic OH groups (OH–C(7), OH–C(4′)) are dissociated even in neutral aqueous solutions [9], one may infer that the corresponding polyanions are much too polar to be transferred into the organic phase to further react with the glycosyl donor. To facilitate the phase transfer of quercetin anions and also to better control the regioselectivity, quercetin (**1**) was partially protected. After a failed attempt to prepare the methylene acetal, **1** was converted to its diphenylmethylene acetal **3** in 33% yield by directly heating it with Ph_2CCl_2 (4 equiv.) at 170° (*Scheme 1*). The reaction in refluxing pyridine (5 equiv. of Ph_2CCl_2) gave slightly lower yields (25%). Glycosidation of **3** by **2** under phase-transfer conditions (CH_2Cl_2, sat. $KHCO_3$ solution, tris[2-(2-methoxyethoxy)ethyl]amine (TMEA), 40°) resulted in the regioselective formation of the 7-*O*-β-D-glucopyranoside **4** in moderate yields (*ca.* 30% after saponification of the acetate groups). The regioselectivity is in agreement with the preferential dissociation of OH–C(7) in the aqueous phase. Subsequent hydrogenolysis of the diphenylmethylene acetal (yield 60%) led to 7-*O*-β-D-glucopyranosylquercetin (**5**) or quercimeritrin, whose spectral characteristics are in agreement with those of a sample extracted from plants [10].

The strategy of partial benzylation and subsequent glycosidation has already been described as a means to prepare quercetin glucosides [11]. When a solution of **1** in DMF was treated with benzyl bromide (10 equiv.) and ^{t}BuOK (5 equiv.) at room temperature, a mixture of 3,4′,7-tri-*O*-benzyl- and 3,3′,4′,7-tetra-*O*-benzylquercetin (resp., **6** and **7**) was obtained. Flash chromatography (silica gel) of the mixture yielded **6** and **7** in 40 and 14% yields, respectively. Glycosidation of **6** by **2** under the usual conditions led to the 3′-*O*-β-D-glucoside **8** in 48% yield after saponification of the acetate groups. The choice of a sat. $KHCO_3$ solution for the aqueous phase seems the best compromise since it allowed the dissociation of the weakly acidic OH–C(3′) and at the same time minimized the hydrolysis of **2**. With 1M $KHCO_3$ or sat. K_2CO_3 solution, the yields were significantly lower (*ca.* 30%). The absence of reactivity of OH–C(5) is attributable to the strong intramolecular H-bond it forms with C(4)=O (δ 12.67 in

Scheme 1. *Chemical Synthesis of Quercetin Derivatives*



$(D)_6$DMSO for OH–C(5) in **6**). Subsequent hydrogenolysis of the benzyl groups of **8** led to 3′-*O*-*β*-D-glucopyranosylquercetin (**9**). Alternatively, **7** could be glycosylated by 2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide under more alkaline conditions (sat. K_2CO_3 solution) to deprotonate the weakly acidic OH–C(5) proton (yield 35%, after saponification of the acetate groups); after debenzylation, 5-*O*-*β*-D-galactopyranosylquercetin (**10**) was obtained. Our method is a significant improvement over the previously reported synthesis of 3′- and 5-*O*-glucosides **9** and **10** of quercetin (yields 30 and 8%, respectively, for the glycosidation step under *Koenigs-Knorr* conditions) [11a].

The partial or total succinylation of flavonoid sugar moieties has been shown to be a very efficient way to increase the water solubility of flavonoids for application as colour enhancers [12]. The reaction is regioselective for the sugar OH groups and does not even require the preliminary protection of the aglycone OH groups. Thus, when **8** was treated with succinic anhydride (6 equiv.) in pyridine in the presence of *N*,*N*-dimethylpyridin-4-amine (DMAP; 1 equiv.) at 70°, the corresponding 2″,3″,4″,6″-tetrakis(hydrogen succinate) was isolated in quantitative yield; subsequent hydrogenolysis led to **11** (*Scheme 1*).

Acylation of the chrysin (5,7-dihydroxyflavone) 7-*O*-glucoside by aromatic acyl chlorides has been shown to occur regioselectively (yield *ca.* 30%) at the sugar primary OH group [8a]. Similarly, treatment of **8** by 3,4,5-trimethoxybenzoyl chloride (2 equiv. added in several portions within 2 h) in pyridine in the presence of DMAP (0.5 equiv.) afforded the 6-*O*-acyl derivative as the only product (*Scheme 1*). The low yield (16%) suggests that the steric hindrance exerted by the aglycone on the sugar moiety is stronger with 3′-*O*-*β*-D-glucosides than with 7-*O*-*β*-D-glucosides. Subsequent debenzylation gave 3′-*O*-[6-*O*-(3,4,5-trimethoxybenzoyl)-*β*-D-glucopyranosyl]quercetin (**12**). The signals of some aglycone protons in **12** are significantly shielded with respect to the corresponding signals in non-acylated **9** (−0.11 and −0.32 ppm for H–C(6) and H–C(8), resp.). Similarly, the signal of the equivalent H_{ortho} protons of the acyl group are shielded by −0.30 ppm with respect to the corresponding signals in methyl 3,4,5-trimethoxybenzoate. These observations suggest intramolecular stacking interactions between both aromatic moieties in **12**, as already observed in the acylated 7-*O*-*β*-D-glucosides of chrysin [8a].

Quercetin sulfates **13** and **14** were prepared upon treatment by tetrabutylammonium hydrogen sulfate ($(Bu_4N)HSO_4$) and dicyclohexylcarbodiimide (DCC) in pyridine according to an already published procedure [7]. Their spectral characteristics are in agreement with the literature.

2. *Metal Complexation*. Metal complexation by polyphenols is a phenomenon with many biological implications. Formation of stable H_2O-soluble polyphenol complexes with Al^{III} may, *e.g.*, contribute to general mechanisms of Al^{III} uptake in plants and Al^{III} toxicity in humans in relation to neurological and bone disorders [13]. Al^{III} Complexation by polyphenols may also inhibit the antioxidant activity of dietary polyphenols circulating in plasma [14] and participate in colour expression in plants [15]. Moreover, polyphenols can take part in reductive mechanisms of iron uptake from ferritin or soil oxides [16]. They can, however, decrease the absorption of iron from the diet [17]. Finally, polyphenols, especially flavonoids, can prevent ROS generation (*e.g.*, the *Fenton* reaction) by sequestering iron ions under inactive complexes [18] although pro-oxidant effects have been evidenced, depending on the flavonoid structure and iron concentration.

Quercetin is able to chelate metal ions *via* the 3-hydroxy-4-oxo, 5-hydroxy-4-oxo, and 3′,4′-dihydroxy (catechol) groups [9][19]. In quercetin derivatives, one of the binding sites may be cancelled because of derivatization. The complexation reaction may be written as:

$$M + LH_n \rightarrow [ML] + nH^+$$

LH_n, M, and [ML] stand for the flavonol (neutral form), metal ion, and 1:1 chelate, respectively. Upon complexation, the metal ion displaces n protons from the flavonol nucleus. Except in the case of succinylated flavonol glycosides, for which n may be higher than 2 because of the participation of free carboxy groups in the binding, n is equal to 1 when chelation occurs at the 3-hydroxy-4-oxo or 5-hydroxy-4-oxo group (deprotonation of OH−C(3) or OH−C(5)), and to 2 when chelation occurs at the catechol group (deprotonation of OH−C(3′) and OH−C(4′)). The equilibrium constant is $K = ([[ML]] \cdot [H^+]^n)/([M] \cdot [LH_n])$. Since the pH is fixed in the complexation experiment, the apparent affinity of the flavonol for M can also be quantified by $K' = K/[H^+]^n = [[ML]]/([M] \cdot [LH_n])$.

The plots of the absorbance (at a fixed wavelength of the chelate absorption band) as a function of the total metal concentration can be fitted against *Eqns. 1* and *2* with the sole assumption of 1:1 complexation (A, A_0 = absorbances in the presence and absence of metal ion, respectively; [M] = free metal concentration; M_t = total metal concentration; *c:* total flavonol concentration). Therein, r stands for the ratio of molar absorption coefficients $\varepsilon([ML])/\varepsilon(LH_n)$.

$$A = A_0 \frac{1 + rK'[M]}{1 + K'[M]} \tag{1}$$

$$M_t = [M]\left(1 + \frac{K'c}{1 + K'[M]}\right) \tag{2}$$

Complexation of Al^{3+} by quercetin derivatives was investigated in MeOH/0.2M acetate buffer 1:1 (pH 5.0, 25°) to allow comparisons with the poorly H_2O-soluble quercetin. Upon addition of Al^{3+}, new absorption bands in the range 420–430 nm appear that correspond to the (flavonolato)aluminium complexes (*Fig. 1*). Values for parameters r and K' deduced from the curve-fitting procedure are reported in *Table 1*. If Al^{3+} complexation primarily occurred with the catechol group, one would expect a sharp decrease in the K' value upon derivatization of the catechol group (glycosidation, sulfation). This is not the case. For instance, the K' value is slightly higher for 3′-β-D-glucopyranosylquercetin (**9**) than for quercetin itself (**1**). In addition, the 3-hydroxy-4-oxo group must be a better chelating site for Al^{3+} than the 5-hydroxy-4-oxo group since complexation on the latter site is accompanied by the removal of the OH−C(5) proton, which is strongly stabilized by H-bonding with C(4)=O. Consistently, the affinity of quercetin for Al^{3+} is higher than that of chrysin (5,7-dihydroxyflavone) under the same conditions [8a]. Hence, binding is very likely to occur *via* the 3-hydroxy-4-oxo group in this series, in agreement with previous investigations of Al^{III} complexation by flavonols in MeOH [19]. Al^{3+} Complexation is strongest for **11**, a result suggesting that one or two carboxylate groups take part in the binding. Although remote from the binding site,

the 7-*O*-sulfate group of **13** also exerts a favourable influence on the complexation (*Table 1*), possibly by long-range electrostatic interactions. The observation that the second sulfate group of **14** does not further increase the apparent binding constant may be explained by conflicting electronic effects. Indeed, sulfation of both OH–C(7) and OH–C(4′) is expected to lower the electron-donating properties of both groups and thus the electron-density at C(4)=O (*via* conjugation) and, to a lesser degree, at OH–C(3).

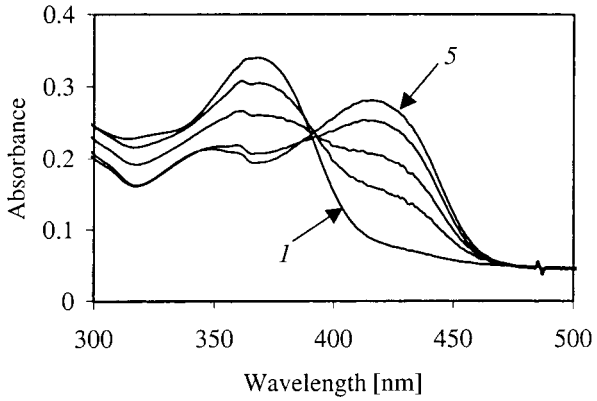


Fig. 1. *UV/VIS Spectra of* **11** ($5 \cdot 10^{-5}$ M) *and of its* Al^{3+} *complex at pH 5.0 and 25°* (MeOH/0.2M acetate buffer 1:1). Al^{3+}/**11** molar ratio 0 (*1*), 0.25 (*2*), 1 (*3*), 2.5 (*4*), and 5 (*5*)

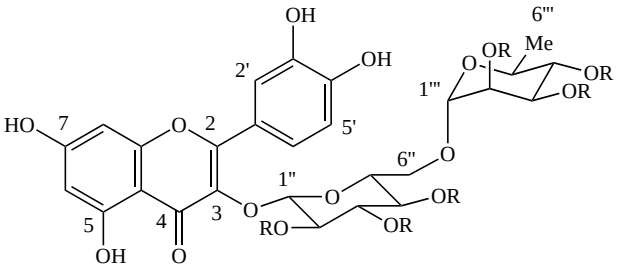
Table 1. *Complexation of* Al^{3+} *by Quercetin Derivatives* (pH 5.0, 25°). For the definition of *r* and *K*′, see text.

	$K' \cdot 10^{-3}$ [M^{-1}]	*r*(420 nm)
quercetin [a])	4.6 (± 0.2)	12.2 (± 0.1)
9[a])	7.0 (± 0.8)	10.7 (± 0.2)
11[a])	39.7 (± 8.1)	7.9 (± 0.2)
13[a])	18.8 (± 3.8)	21.0 (± 0.7)
14[a])	13.4 (± 3.0)	23.4 (± 1.0)
rutin[b])	12.0 (± 1.2)	36.9 (± 0.6)
15[b])	5.1 (± 0.5)	5.1 (± 0.1)

[a]) MeOH/0.2M Acetate buffer 1:1. [b]) MeOH/0.2M Acetate buffer 5:95.

Aluminium complexation was also investigated with rutin (= 3-*O*-(α-L-rhamnopyranosyl-1 → 6-β-D-glucopyranosyl)quercetin), a commercially available and ubiquitous quercetin glycoside, and its hexakis(hydrogen succinate) **15** [12]. Rutin is a better Al^{III} ligand than **15** (*Table 1*). Hence, polysuccinylation of a sugar moiety may increase or decrease the affinity of the aglycone for Al^{3+} depending on the location of the glycosyloxy group at the polyphenolic nucleus.

The well-known affinity of polycarboxylate ligands for iron drove us to test the flavonoid poly(hydrogen succinates) **11** and **15** as iron ligands. However, the general propensity of iron complexes with polyphenols to precipitate required adaptation of the experimental procedure. The complexation kinetics was monitored after addition of a small volume of concentrated metal solution in the spectrometer cell. The



rutin R = H

15 R = C(=O)$CH_2CH_2CO_2H$

absorbance values used in the estimation of K' correspond to the plateau typically reached in a few minutes (*Fig. 2*). This method allowed us to ensure that the complexation equilibrium was reached and that precipitation did not begin yet, two conditions to be met for a rigorous analysis.

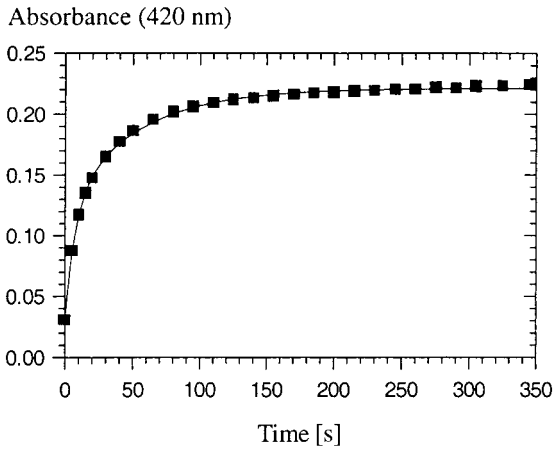


Fig. 2. *Time dependence of the absorbance at 420 nm of* **15** ($5 \cdot 10^{-5}$ M) *after addition of* Fe^{2+} (0.5 equiv.) *at pH 5 and 25°* (MeOH/0.2M acetate buffer 5:95)

Complexation of Fe^{2+} and Fe^{3+} by quercetin, rutin, and their succinylated derivatives **11** and **15** (*Figs. 3* and *4*) could be satisfactorily investigated by means of the 1:1 binding model (*Eqns. 1* and *2*) from which values for parameters r and K' were estimated (*Tables 2* and *3*). For each metal ion, the affinities of quercetin and **11** are of the same order of magnitude. Hence, the possible participation of the carboxylate groups of **11** may compensate for the introduction of a bulky, electron-withdrawing sugar moiety at the 3′ position, which, in addition, suppresses the ability of the B ring to complex metal ions. The affinity of **15** for both Fe^{2+} and Fe^{3+} is very significantly higher than that of rutin, an observation clearly pointing to the efficient participation of the free carboxylate groups of **15**. Remarkably, the affinity of **15** for Fe^{3+} is so high that the pH must be lowered to 1 for an accurate estimation of the corresponding K' value.

Under such acidic conditions, the binding of Fe^{3+} by rutin is not even detectable. In a pH 1 solution of Fe^{III} and **15** in equal concentrations (10^{-3} M), it can thus be estimated that *ca.* 80% of Fe^{III} is bound, whereas Fe^{III} remains in its free form in the presence of rutin under the same conditions. Since the ROESY spectrum of **15** displays several long-range correlations between the B ring and the rhamnose moiety [12], it may be assumed that the ligand can adopt a folded conformation in which the catechol group and two succinate groups (probably at Rha) are in close proximity, thus providing a very efficient multidentate binding site for Fe^{3+}.

3. *Anthocyanin Complexation*. Anthocyanins are polyphenolic pigments mainly responsible for the red, purple, and blue colours displayed by flowers and fruits [1]. In

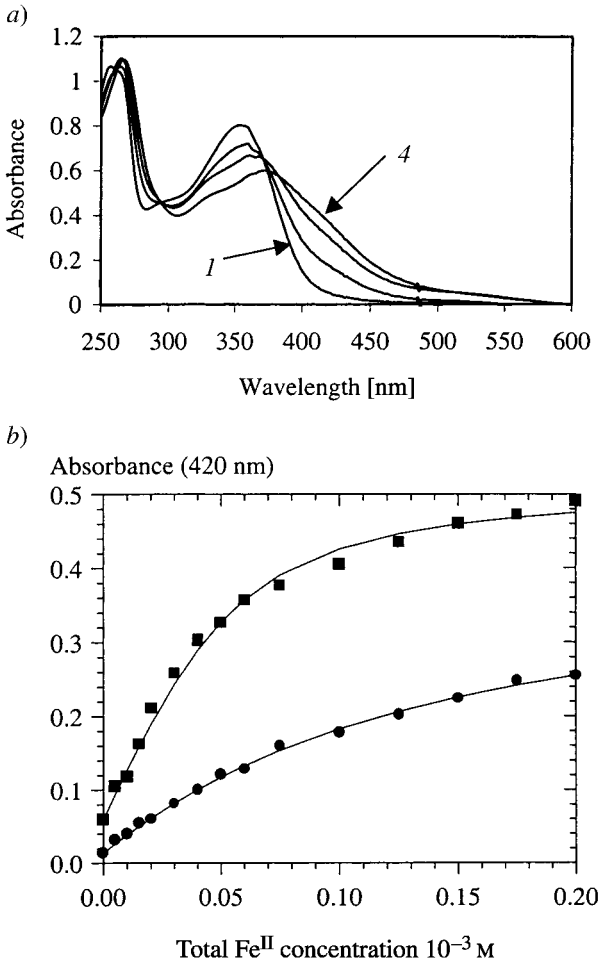


Fig. 3. a) *UV/VIS Spectra of* **15** ($5 \cdot 10^{-5}$ M) *and its* Fe^{2+} *complex at pH 5.0 and 25°* (MeOH/0.2M acetate buffer 5:95; Fe^{2+}/**15** molar ratio 0 (*1*), 0.4 (*2*), 1 (*3*), and 2 (*4*); b) *Plot of the absorbance at 420 nm as a function of the total metal concentration for rutin* (●) *and* **15** (■). The solid lines are the results of the curve-fitting procedures according to *Eqns. 1* and *2*.

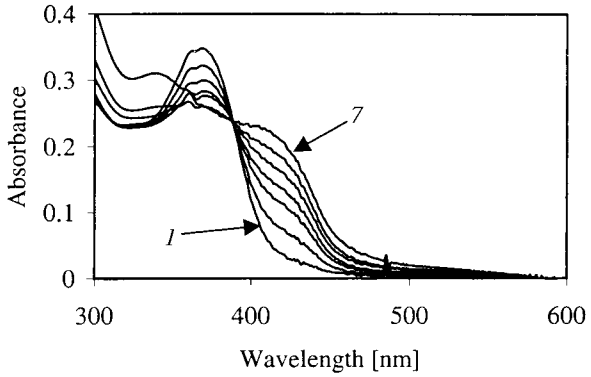


Fig. 4. *UV/VIS Spectra of* **11** ($5 \cdot 10^{-5}$ M) *and of its* Fe^{3+} *complex at pH 5.0 and 25°* (MeOH/0.2M acetate buffer 1:1; Fe^{3+}/**11** molar ratio 0 (*1*), 0.1 (*2*), 0.2 (*3*), 0.3 (*4*), 0.5 (*5*), 1 (*6*), and 2 (*7*)).

Table 2. *Complexation of* Fe^{2+} *by Quercetin Derivatives* (pH 5, 25°). For the definition of *r* and *K′*, see text.

	$K' \cdot 10^{-3}$ [M^{-1}]	*r*(420 nm)
quercetin [a])	151 (± 48) [c])	4.8 (± 0.2)
11 [a])	183 (± 97) [c])	2.0 (± 0.1)
rutin [b])	10.2 (± 0.9)	28.9 (± 1.1)
15 [b])	71 (± 13)	8.7 (± 0.2)

[a]) MeOH/0.2M acetate buffer 1:1. [b]) MeOH/0.2M acetate buffer 5:95. [c]) Large standard deviations because of quasi-irreversible binding.

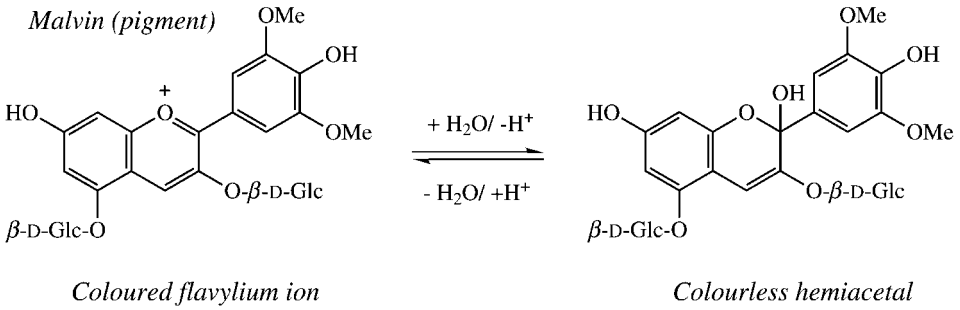
Table 3. *Complexation of* Fe^{3+} *by Quercetin Derivatives* (25°). For the definition of *r* and *K′*, see text.

	$K' \cdot 10^{-3}$[M^{-1}]	*r*(420 nm)
quercetin [a])	25.8 (± 3.4)	6.3 (± 0.2)
11 [a])	59 (± 14)	2.7 (± 0.1)
rutin [b])	22.5 (± 1.9)	5.2 (± 0.1)
15 [c])	24.0 (± 2.1)	6.0 (± 0.2)

[a]) MeOH/0.2M acetate buffer 1:1, pH 5. [b]) MeOH/0.2M acetate buffer 5:95, pH 4. No detectable binding in MeOH/0.1M HCl 5:95. [c]) MeOH/0.1M HCl 5:95.

the vacuoles where anthocyanins are stored, the mildly acidic aqueous conditions favour the reversible conversion of the colored forms (flavylium ions) to colorless hemiacetals *via* water addition at C(2) (*Scheme 2*). Fortunately, phenolic acids and flavonoid glycosides, which are also abundant in vacuoles, form stable stacking molecular complexes with the large planar polyphenolic nuclei of flavylium ions [20]. This phenomenon, known as copigmentation, efficiently competes with H_2O addition on the pyrylium moiety and thus helps maintain anthocyanins in their colored forms. Since conversion of anthocyanins into colorless forms may be the first step toward pigment degradation [21], copigmentation may be viewed as an efficient color-

Scheme 2. *Water Addition to Malvin*



Coloured flavylium ion *Colourless hemiacetal*

stabilizing process, and there is great interest in plant extracts rich in both anthocyanins and potent water-soluble copigments for use as food colourings [22].

The efficiency of water-soluble quercetin derivatives **11**, **13**, and **14** in copigmentation was tested with malvin (*Scheme 2*), one of the most common naturally occurring anthocyanins. In strongly acidic solution (pH 1), where malvin is in a pure flavylium form, copigmentation is manifested by bathochromic shifts in the VIS absorption band of the pigment (*Fig. 5*) that may reflect a stronger interaction of the copigment with the pigment in its first excited state than with the ground-state pigment [12]. In mildly acidic solutions (pH 3.5), closer to those of natural media, copigmentation of malvin was investigated by monitoring the loss in VIS absorbance in malvin/copigment solutions (copigment/pigment molar ratios 200 for **11**, 20 for **13** and **14**) induced by increasing the temperature as a consequence of the partial (endothermic) dissociation of the copigmentation complexes (*Fig. 5*) [12][23]. Under such conditions, the relative hyperchromic shift at the isosbestic point of the free and bound flavylium ions (determined at pH *ca.* 1 where the pigment is in the pure flavylium form) can be very simply related to the binding constant K according to *Eqn. 3* (A, $A_0 =$ values for the VIS absorbance in the presence and absence of copigment, respectively; $c =$ total copigment concentration) [23].

$$\frac{A - A_0}{A_0} = cK \tag{3}$$

From the linear plots of $\ln[(A - A_0)/A_0]$ as a function of $1/T$ (*Van't Hoff* behaviour), values for the temperature-independent enthalpy and entropy changes of copigmentation could be estimated (*Table 4*). Whereas **11** binds to malvin with an affinity close to that measured with analogous succinylated rutin derivatives [12], quercetin sulfates **13** and **14** were found to be exceptionally potent copigments. Hence, at pH 1, bathochromic effects of *ca.* 40 nm are reached with a copigment/pigment molar ratio of 20, *i.e.*, 10 times lower than with the most efficient rutin derivatives. Moreover, the values for the copigmentation binding constant K (on the order of 10^4 M^{-1} at 25°) are by far the highest recorded until now. At first sight, the strong affinity of sulfates **13** and **14** for malvin could stem from an additional electrostatic attraction between the negatively charged sulfate group(s) and the positively charged flavylium nucleus of

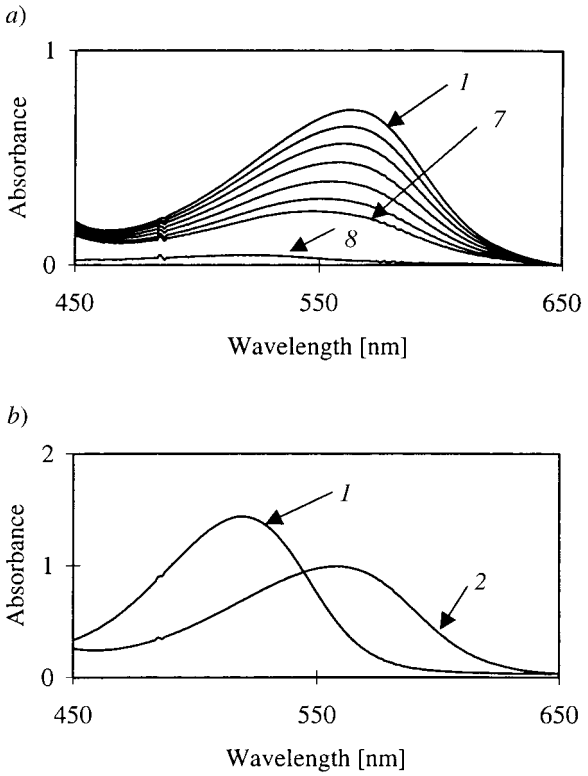


Fig. 5. *Copigmentation of malvin* ($5 \cdot 10^{-5}$ M) *by* **13** (10^{-3} M): a) *At pH 3.5* (0.2M acetate buffer), *VIS spectra of malvin/***13** *at 15.4° (1), 20.5° (2), 25.1° (3), 29.7° (4), 34.9° (5), 39.8° (6) and 44.8° (7); VIS spectrum of malvin alone at 25.1° (8).* b) *At pH 0.9 and 25°, VIS spectra of malvin alone (1) and of malvin/***13** *(2).*

Table 4. *Thermodynamic Parameters for Copigmentation of Malvin by Quercetin Derivatives Deduced from Temperature Variations in the Range 15–45°* (0.2M acetate buffer, pH 3.5).

	11	**13**	**14**
ΔH°[kJ mol^{-1}]	−23.6 (±0.1)	−37.3 (±0.6)	−34.7 (±0.3)
ΔS°[J K^{-1} mol^{-1}]	−18.0 (±0.4)	−45.6 (±1.5)	−40.0 (±0.8)
K [M^{-1}] [a])	1560	14470	8940
$\Delta\lambda_{max}$ [nm] [b])	27	39	36

[a]) Determined at 25° from $\Delta H^\circ - T\Delta S^\circ = RT\ln K$. [b]) Determined at pH *ca.* 1 and 25° with the copigment/pigment molar ratios 100 (**11**) and 20 (**13**, **14**).

malvin. However, since disulfate **14** binds to malvin less strongly than monosulfate **13** mainly for enthalpic reasons, it is not clear that favourable electrostatic interactions make an important contribution to the stability of copigmentation complexes. Comparisons with quercetin (virtually insoluble in weakly acidic buffers) being impossible, we just point out that the malvin-**13** complexation is as exothermic as the malvin-rutin complexation [12]. The more favourable entropy term in the case of the malvin-**13** complexation may indicate that, being quite rigid, **13** binds to malvin with

relatively little reorganization. Although less potent than the quercetin sulfates, **11** is still a good copigment. Its binding to malvin, although weakly exothermic, displays a relatively favourable entropy term. Similar thermodynamic behaviors were already observed with the succinylated rutin derivatives and were analyzed in terms of compensating *structure-breaking* and *structure-making* contributions that accompany the desolvation of carboxy/carboxylate groups upon binding [12].

From the ^{1}H-NMR spectra of malvin, copigment **14**, and a solution of malvin and **14** in equimolar concentrations (*Fig. 6, Table 5*), it can be seen that copigmentation induces very large shieldings of all protons at the interacting polyphenolic nuclei. Hence, each partner is located inside the cone of diamagnetic anisotropy of the other, in agreement with a face-to-face arrangement in the complex (stacking).

Table 5. *Complexation-Induced Shieldings* $\Delta\delta$ ($= \delta$(complex) $- \delta$(free form)) [ppm] *of the Proton Signals of Malvin and* **14** *in* $D_2O/CD_3OD/CF_3CO_2D$ *90 : 9 : 1.* Copigment/pigment molar ratio 1, 25°.

	$\Delta = \delta$(complex) $- \delta$(free form)[ppm]					
	H–C(4)	H–C(6)	H–C(8)	H–C(2′)	H–C(5′)	H–C(6′)
14	–	−0.48	−0.57	−0.81	−0.45	−0.59
malvin	−0.51	−0.56	−0.26	−0.62	–	−0.62

The influence of the solvant on the pigment-copigment interaction was studied with the malvin-**13** couple. A solution of malvin ($5 \cdot 10^{-5}$ M) and **13** ($5 \cdot 10^{-4}$ M) in 0.1M aqueous HCl and the same solution in MeOH acidified by a concentrated HCl solution to a final concentration of 0.1M, were mixed in different proportions. The VIS spectra of the resulting solutions were recorded and the wavelength of VIS absorption maximum plotted as a function of the percentage of MeOH (*Fig. 7*). The experiment was repeated with the pigment malvin alone. In the absence of copigment, the λ_{max} value raises monotonously with the percentage of MeOH, as expected from the negative solvatochromism of flavylium ions (first excited state less polar than ground state). In the presence of the copigment, the λ_{max} value first sharply decreases and then slowly increases when the amount of MeOH increases. Such variations reflect the combination of two factors when the percentage of MeOH increases: the dissociation of the copigmentation complex which tends to lower the λ_{max} value (dominant factor in the range 0–40%) and the negative solvatochromism of malvin, which tends to elevate the λ_{max} value (dominant factor in the range 50–100%). Both plots are superimposed beyond a percentage of MeOH of 40–50%, thus suggesting that, despite its stability in H_2O, the copigmentation complex is completely dissociated in MeOH/H_2O mixtures in which MeOH is the main component. Hence, although stronger in MeOH ($\varepsilon_r = 32.6$) than in H_2O ($\varepsilon_r = 78.5$), electrostatic interactions do not allow maintenance of the pigment-copigment association under such conditions. The hydrophobic effect requires a highly organized network of H-bonded H_2O molecules around the nonpolar parts of organic solutes and must be severely weakened when MeOH molecules gradually displace H_2O molecules from the solvation shells. Hence, unlike electrostatic interactions, the hydrophobic effect, which is maximal in pure H_2O, appears as a crucial contribution to the copigmentation driving force, in agreement with previous investigations [24].

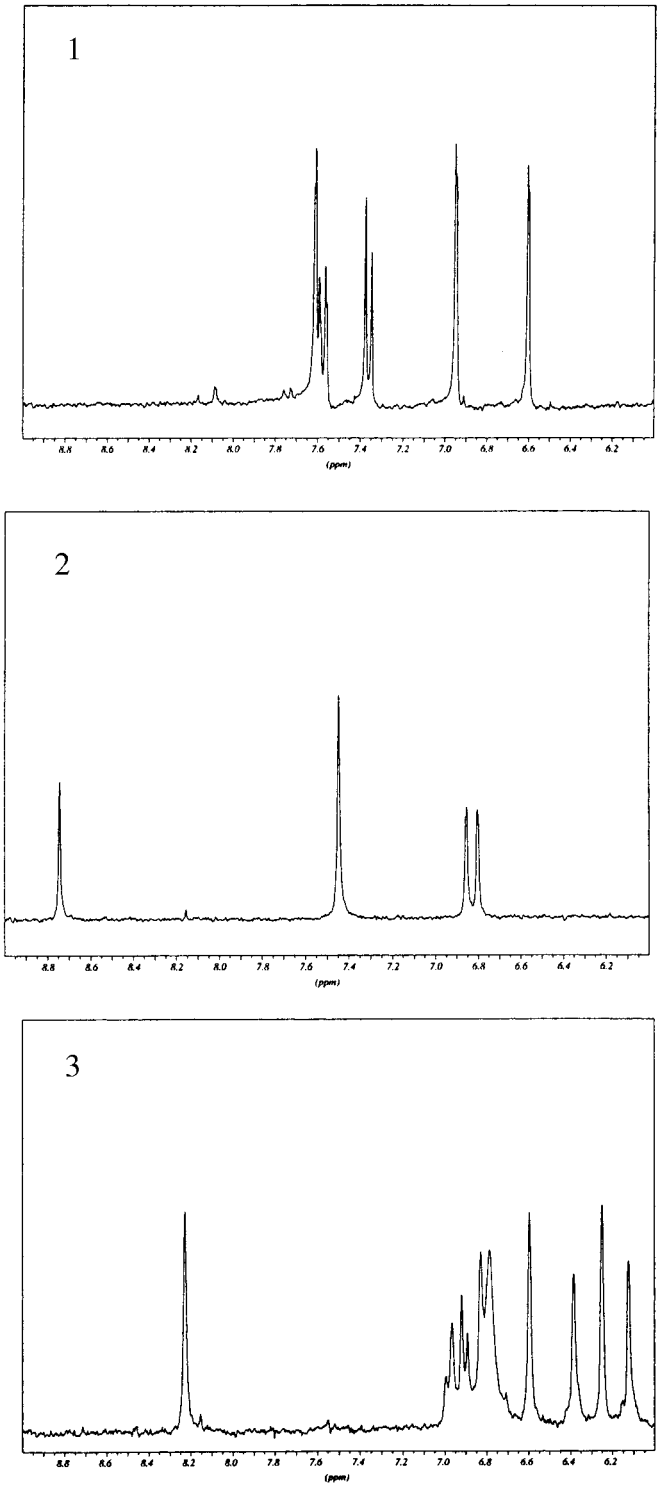


Fig. 6. *^{1}H-NMR Spectra* (300 MHz) *of* **14** (*1*), *malvin* (*2*) *and malvin/***14** (*3*; copigment/pigment molar ratio 1) *at* 27° *in* $D_2O/CD_3OD/CF_3CO_2D$ 90 : 9 : 1).

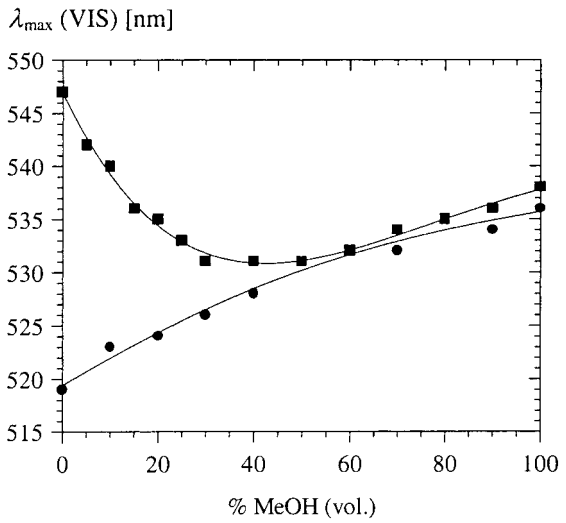
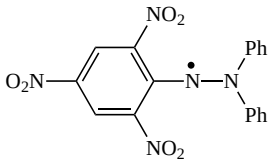


Fig. 7. *Plot of the wavelength of the VIS absorption maximum of malvin* ($5 \cdot 10^{-5}$ M) *as a function of the percentage of MeOH in MeOH/H_2O mixtures.* ● = malvin alone; ■ = malvin + **13** (10 equiv.). The acidity was kept constant (0.1M HCl), 25°.

4. *Antioxidant Properties.* One of the main mechanisms by which flavonoids exert their antioxidant activity consists in transferring electrons and/or H-atoms to biological ROS (superoxide, hydroxyl, lipid alkoxyl, and peroxyl radicals) [3][25]. Investigating the reaction of antioxidants with DPPH (diphenylpicrylhydrazyl), a highly coloured commercially available radical, provides a simple way to compare antioxidants for their H-atom-donating ability [12][26]. In this work, the decay of the DPPH VIS absorbance (λ_{max} 515 nm in MeOH) following the addition of the antioxidant was monitored over one minute and quantitatively analysed (see *below*). The kinetic runs were repeated with different DPPH/antioxidant molar ratios. In most cases, the solutions were kept in the spectrometer cell for 1 h to ensure the complete conversion of the antioxidant to inert oxidation-degradation products. From the overall amplitude of VIS absorbance, the stoichiometry (n) of the antioxidant (number of DPPH radicals trapped per molecule of antioxidant) was estimated by *Eqn. 4* (A_f = absorbance 1 hour after addition of the antioxidant to the DPPH solution, A_0 = initial absorbance, c = initial antioxidant concentration, c_0 = initial DPPH concentration). Of course, the initial DPPH/antioxidant molar ratio c_0/c must be higher than n for *Eqn. 4* to apply.

$$n = \frac{c_0}{c}\left(1 - \frac{A_f}{A_0}\right) \tag{4}$$

Flavonols displaying a free 1,2-dihydroxybenzene (catechol) moiety such as quercetin (**1**) may be assumed to react with DPPH according to the following mechanism (*Scheme 3*) [27]: *i*) H-Abstraction from the flavonol (FH_2) to DPPH occurs with simultaneous formation of aryloxyl radicals *FH* (rate constant k_1). *ii*) Fast disproportionation of *FH* into the parent flavonol and its quinone follows. The latter



DPPH

subsequently undergoes fast solvent addition to yield F[2]). Like FH_2, F also displays a catechol group with labile H-atoms and can further react with DPPH. *iii*) H-Abstraction from F to DPPH occurs with simultaneous formation of aryloxyl radicals (rate constant k_2), which subsequently disproportionate ($f = 1/2$).

This mechanism, which requires free OH groups at positions 3, 3′, and 4′, is assumed to hold for quercetin derivatives **5**, **10**, and **13**. It is consistent with the evidence of quinone intermediates, the structural identification of the quinone-solvent adduct in the case of quercetin, the high stoichiometries ($n \geq 4$), usually measured with flavonols having a 1,2-dihydroxybenzene moiety [27], and investigations of polyphenolic radicals by pulse radiolysis [29][3]). Assuming a quasi-stationary state for the radicals, *Eqns. 5–7* were derived and used in the curve-fitting of the kinetic traces to give optimized k_1 and k_2 values in reasonable agreement for different DPPH/antioxidant molar ratios (*Table 6*).

$$-\frac{d}{dt}[\text{DPPH}] = k_1[FH_2][\text{DPPH}] + k_2[F][\text{DPPH}] \quad (5)$$

$$-\frac{d}{dt}[F\text{H}_2] = \frac{k_1}{2}[FH_2][\text{DPPH}] \quad (6)$$

$$\frac{d}{dt}[F] = \frac{k_1}{2}[FH_2][\text{DPPH}] - fk_2[F][\text{DPPH}] \quad (7)$$

In the case of rutin, the glycosidation of OH–C(3′) cancels the *o*-quinone/*p*-quinonoid tautomerism so that the rutin quinone (F) is much less prone to solvent addition and is actually detectable by ^{1}H-NMR [27]. Its residual antioxidant activity is simply accounted for by postulating a H-atom transfer to DPPH with rate constant k_2 ($f = 1$ in *Eqn. 7*).

Glycosidation of OH–C(5) (**10**) and OH–C(7) (**5**) does not significantly lower the antioxidant stoichiometry, in agreement with the above-mentioned mechanism, in which neither OH–C(5) nor OH–C(7) plays a role. However, both glycosides react with DPPH more slowly than the aglycone (quercetin). The steric hindrance of the glycosyl groups as well as their probable electron-withdrawing effect on the polyphenolic nucleus may account for this observation. In contrast, glycosidation of OH–C(3′) (**9**) and sulfation of OH–C(4′) (**14**) both result in a dramatic decrease in the antioxidant stoichiometry and the apparent rate of H-atom abstraction. The

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[2]) F is represented as a mixture of pyran- and furan-containing forms (in equilibrium *via* a non-detected triketone form), since the latter structure has been proposed for the product isolated after electrochemical oxidation of quercetin [28].

[3]) The fast second-order decay of quercetin radicals in aqueous solution ($2k = 3.4 \cdot 10^6$ M^{-1} s^{-1}) [29] suggests that their recombination *via* disproportionation may be faster than their reaction with a second DPPH radical.

Scheme 3. *Mechanism for the Reaction of Quercetin with DPPH*

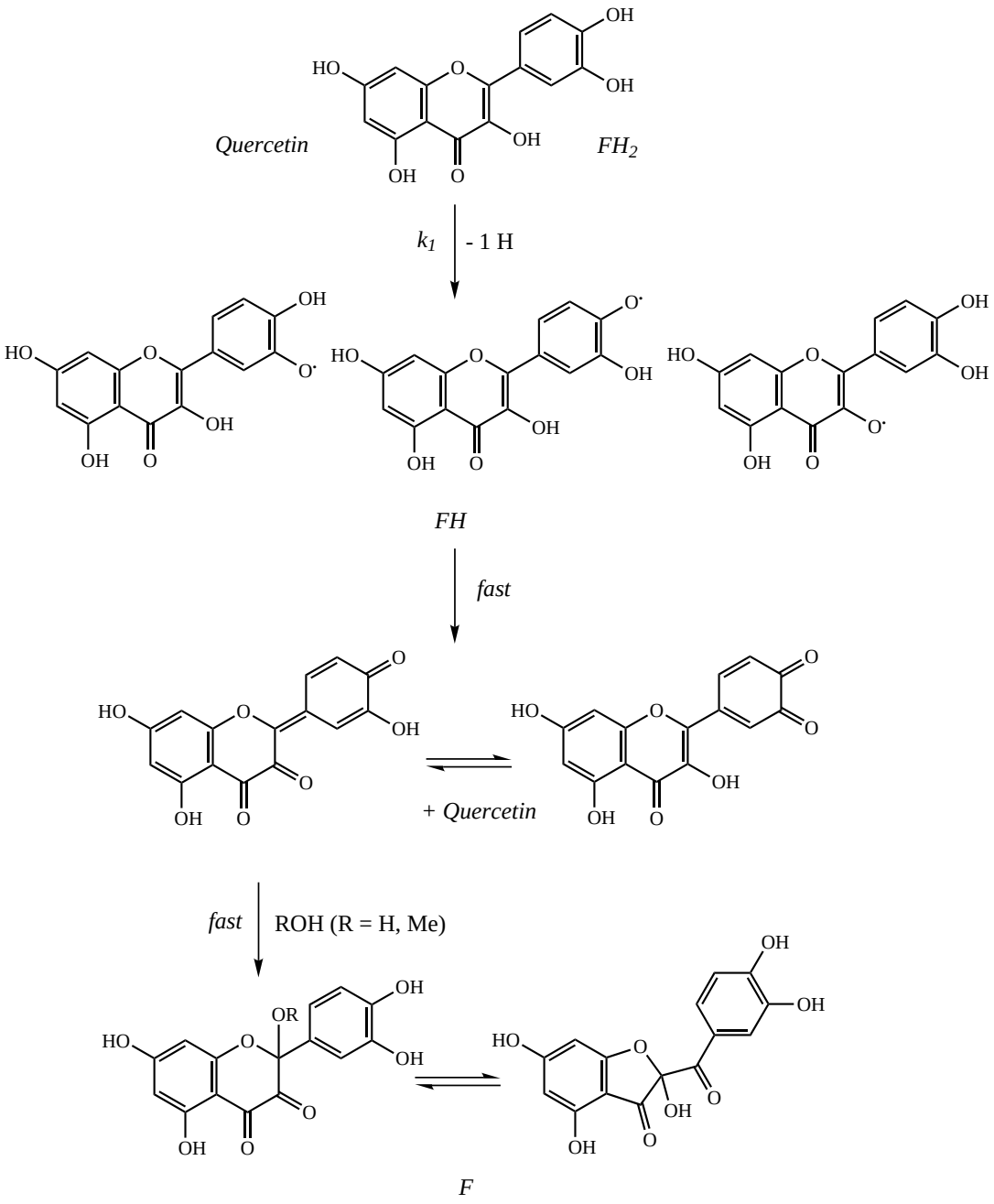


Table 6. *H-Abstraction from Quercetin Derivatives by DPPH* (MeOH, 25°). For the definition of parameters k_1, k_2 and n, see text.

	DPPH [equiv.]	k_1 [M^{-1} s^{-1}][a])	k_2 [M^{-1} s^{-1}][a])	n[b])
quercetin	4	2519 (± 77)	617 (± 18)	–
	8	2800 (± 71)	776 (± 14)	6.8
rutin	4	1481 (± 11)	43 (± 2)	–
	8	1840 (± 17)	50 (± 1)	5.9
5	6.4	639 (± 6)	213 (± 4)	–
	9.4	728 (± 9)	250 (± 5)	*ca.* 7
9	4–8	[c])	–	*ca.* 1
10	6.2	437 (± 4)	364 (± 9)	–
	9	505 (± 11)	433 (± 22)	6.3
13[d])	4	6190 (± 180)	126 (± 3)	–
	6	5610 (± 150)	91 (± 1)	4.6
14	4–8	[c])	–	*ca.* 0.4

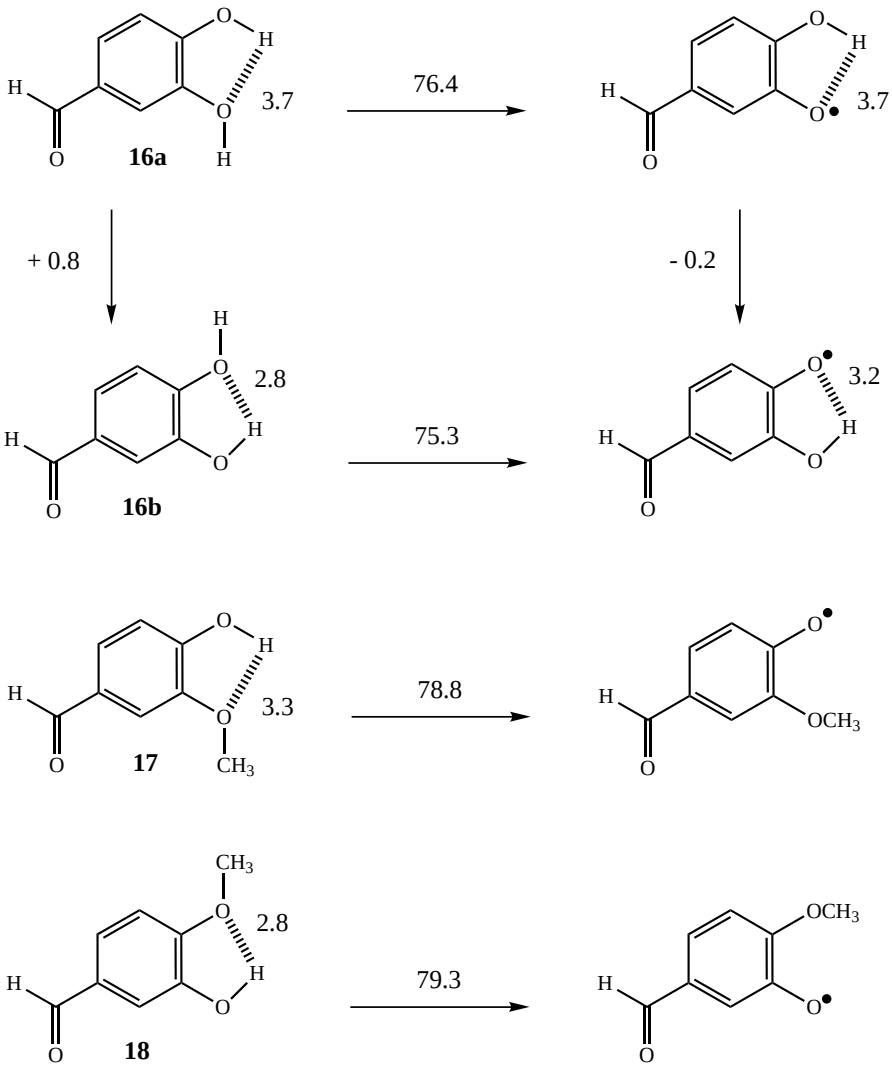
[a]) From the curve-fitting of the kinetic traces (time interval: 60 s) according to *Eqns. 5–7*. [b]) From *Eqn. 4*; final absorbance A_f recorded 1 h after addition of the antioxidant. [c]) Slow decay, no valid kinetic model. [d]) A transient absorption of the quinone intermediate at the wavelength of detection (ε at 515 nm *ca.* 8000 M^{-1} cm^{-1}) must be assumed for a correct curve-fitting.

radicals derived from **9** and **14** are probably much less stable than those of quercetin and may quickly recombine to form inert dimers[4]). These results confirm that the 1,2-dihydroxy substitution at the B ring is essential to the antioxidant activity. It may thus be assumed that the OH–C(4′) and OH–C(3′) are primarily involved in H-atom transfer reactions to DPPH and/or that each OH group strongly facilitates H-abstraction from the other by stabilizing the corresponding radical by a combination of electronic and H-bonding effects. The favourable role of the catechol group in the antioxidant (H-atom-donating) activity can be rationalized from *in vacuo* semi-empirical quantum-mechanics calculations (AM1 parametrization) with simple phenols such as 3,4-dihydroxybenzaldehyde (**16**), 4-hydroxy-3-methoxy-benzaldehyde (**17**), and 3-hydroxy-4-methoxybenzaldehyde (**18**), in which the aldehyde moiety models the electron-withdrawing enone moiety of flavones and flavonols (*Scheme 4*). The rotamer of **16** in which the most acidic OH group (OH–C(4)) acts as the H-bond donor (**16a**) is more stable by 0.8 kcal mol^{-1} than the one in which OH–C(3) is the donor (**16b**), in agreement with a more stable intramolecular H-bond (3.7 *vs.* 2.8 kcal mol^{-1}). Although dissociation of the OH group at C(3) is favoured in **16a** since it preserves the most stable H-bond, dissociation of the OH group at C(4) in **16b** is less endothermic by 1.1 kcal mol^{-1}, probably because of an extended electron-delocalization in the radical with respect to the parent phenol, but also because the intramolecular H-bond involving the OH–C(3) donor is stronger by 0.4 kcal mol^{-1} in the radical than in the parent phenol. Despite a probable gain in electron-delocalization upon dissociation of the OH group at C(4) in **17**, the corresponding energy is higher by 3.5 kcal mol^{-1} than that of **16b**, a gap roughly matching the inevitable loss in H-bonding upon H-abstraction from **17**. Although accompanied by the loss of a weaker H-bond,

4) The second-order decay of flavonoid radicals in aqueous solution is very significantly faster with flavonoids devoid of a 1,2-dihydroxy substitution at the B-ring [29].

dissociation of the OH group at C(3) in **18** is slightly more endothermic than dissociation of the OH group at C(4) in **17**, suggesting that electron-delocalization in the radicals is the prevailing factor.

Scheme 4. *Semi-Empirical Quantum-Mechanics Calculations with Simple Phenols* (see text)[a])



[a]) Energy values in kcal mol^{-1}.

The quercetin conjugates detected in the plasma of human volunteers after a meal rich in plant products are sulfoglucuronides of quercetin and 3′-*O*-methylquercetin [6], the exact structures of which are still unknown. Models of quercetin conjugates (quercetin 3-*O*-sulfate, and enzymatically prepared glucuronides of quercetin and 3′-*O*-

methylquercetin), although less potent than quercetin, still significantly retard lipid peroxidation in plasma low-density lipoproteins (LDL), probably *via* regeneration of the endogenous amphiphilic antioxidant α-tocopherol at the LDL surface (H-atom transfer from the flavonols to the α-tocopheryl radical). In addition, the plasma of rats intragastrically administered with quercetin contains the same type of conjugates as those found in man and is more resistant to peroxidation than control plasma [30]. Finally, glucosides and methyl ethers of quercetin have been demonstrated to inhibit lipid peroxidation in LDL and liposomes, 7-*O*-methylquercetin and the 3- and 7-*O*-β-D-glucosides being much more potent than 3′-*O*-methylquercetin and the 4′-*O*-β-D-glucoside [31]. The present work dealing with quercetin sulfates and glycosides (as models of glucuronides) is consistent with the latter investigations. When glycosidation or sulfation does not take place at the catechol moiety (B ring), which displays the most labile H-atoms, it, however, induces a slowing down of the H-atom transfer reactions (with the possible exception of quercetin 7-*O*-sulfate). When glycosidation or sulfation takes place at the catechol moiety, it results in the suppression of one of the most labile H-atoms and makes the residual H-atom (OH–C(3′) or OH–C(4′)) less reactive. It also cancels out the formation of *o*-quinones that provide an efficient way for subsequent degradations (high stoichiometries). Consequently, the antioxidant activity is drastically reduced. Hence, one may speculate that the residual antioxidant activity of the circulating conjugates is due to a fraction in which the B ring is spared.

Experimental Part

General. Malvin (*Extrasynthèse*, Genay, France), and quercetin (**1**) and DPPH (*Aldrich*) were used as received. Quercetin 7-*O*-sulfate (**13**), quercetin 4′,7-di-*O*-sulfate (**14**), and rutin 2″,3″,4″,2‴,3‴,4‴-hexakis(hydrogen succinate) (**15**) were synthesized according to already published procedures [7][12]. UV/VIS Spectra: *Hewlett-Packard-8453* diode-array spectrometer, equipped with a magnetically stirred quartz cell (optical path length 1 cm), with a thermostated water bath; λ_{max} in nm. NMR Spectra: 300-MHz- and 500-MHz-*Bruker* apparatus at 27°; chemical shifts λ in ppm, coupling constants J in Hz; ^{1}H signals assigned from 1D and 2D-COSY spectra; ^{13}C signals assigned from DEPT and comparisons with [32].

*2-(2,2-Diphenyl-1,3-benzodioxol-5-yl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one* (**3**). A mixture of quercetin (**1**; 2 g, 5.91 mmol) and Ph_2CCl_2 (4.54 ml, 23.65 mmol) under N_2 is heated at 170° for 15 min under vigorous stirring. After cooling in an ice bath and dilution in a few ml of CH_2Cl_2, purification by FC (silica gel, AcOEt/light petroleum 1:9 → 1:4) affords **3** (920 mg, 33%). Beige solid. TLC (silica gel, AcOEt/light petroleum ether 1:4): R_f 0.16. UV/VIS (MeOH): 251, 359. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 11.80 (*s*, OH–C(5)); 7.79–7.76 (*m*, H–C(4′), H–C(6′)); 7.62–7.58 (*m*, H$_m$ of Ph); 7.40–7.39 (*m*, H$_o$ and H$_p$ of Ph); 7.00 (*d*, J = 8.1, H–C(7′)); 6.42 (*s*, H–C(8)); 6.30 (*s*, H–C(6)). FAB-MS (neg. mode): 465.0 ($[M-H]^-$).

*2-(2,2-Diphenyl-1,3-benzodioxol-5-yl)-3,5-dihydroxy-7-[(tetra-*O*-acetyl-β-D-glucopyranosyl)oxy]-4*H*-1-benzopyran-4-one.* A soln. of 2,2,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**; 530 mg, 1.29 mmol) in CH_2Cl_2 (20 ml) is added to a soln. of **3** (500 mg, 1.07 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TMEA; 343 µl, 1.07 mmol) in sat. $KHCO_3$ soln. (20 ml) under N_2. After heating at 40° for 24 h, a second portion of **2** (530 mg) is added and the mixture heated for 24 more h. After cooling and addition of H_2O (100 ml), the mixture is extracted with CH_2Cl_2 (3 × 100 ml) and washed with 0.1M HCl (100 ml) and H_2O (2 × 100 ml). After drying ($MgSO_4$), evaporation, and purification by FC (silica gel, light petroleum ether/AcOEt 4:1 → 3:2), the protected glucoside (222 mg, 26%) is obtained as a yellow oil. TLC (silica gel, AcOEt/light petroleum ether 2:3): R_f 0.21. UV/VIS (MeOH): 301, 362. ^{1}H-NMR ($CDCl_3$, 300 MHz): 11.74 (*s*, OH–C(5)); 7.79 (*dd*, J = 8.8, 1.5, H–C(6′)); 7.76 (*d*, J = 1.5, H–C(4′)); 7.61–7.54 (*m*, H$_m$ of Ph); 7.40–7.38 (*m*, H$_o$ and H$_p$ of Ph); 7.01 (*d*, J = 8.8, H–C(7′)); 6.58 (*d*, J = 2.2, H–C(8)); 6.44 (*d*, J = 2.2, H–C(6)); 5.39–5.28 (*m*, H–C(2″), H–C(4″)); 5.19 (*d*, J = 7.4, H–C(1″)); 5.15 (*t*, J = 9.5, H–C(3″)); 4.29 (*dd*, J = 11.7, 5.1, 1 H–C(6″)); 4.21 (*dd*, J = 11.7, 2.9, 1 H–C(6″)); 3.95 (*ddd*, J = 9.5, 5.1, 2.9, H–C(5″)); 2.11–2.05 (4 *s*, 4 Ac). ^{13}C-NMR ($CDCl_3$, 75 MHz): 175.7 (C(4)); 171.6 (Me*C*O); 171.0 (Me*C*O); 170.6 (Me*C*O); 169.7 (Me*C*O); 162.6 (C(7)); 161.4 (C(5)); 156.6

(C(8a)); 149.5 (C(3′a)); 148.1 (C(2)); 146.4 (C(7′a)); 140.1 (C(3)); 136.4 (C_{ipso} Ph); 129.8 (C_p (Ph)); 128.9 (C_m (Ph)); 126.6 (C_o (Ph)); 124.8 (Ph_2C); 123.5 (C(7′)); 118.3 (C(5′)); 109.1 (C(6′)); 108.3 (C(4′)); 105.6 (C(4a)); 99.7 (C(1″)); 98.6 (C(6)); 95.7 (C(8)); 73.0 (C(5″)); 72.8 (C(3″)); 71.3 (C(2″)); 68.6 (C(4″)); 62.4 (C(6″)); 21.5–20.6 (Me (Ac)).

*2-(2,2-Diphenyl-1,3-benzodioxol-5-yl)-3,5-dihydroxy-7-(β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one* (**4**). A soln. of the protected glucoside (20 mg, 0.024 mmol) and KOH (27 mg, 0.48 mmol) in EtOH (5 ml) is refluxed for 4 h, then cooled, and neutralized with *Dowex* (H^+ form). After filtration and concentration, **4** (16 mg, 99%) is obtained as a white solid. TLC (silica gel, AcOEt/butan-2-one/HCO_2H/H_2O 20:2:1:1): R_f 0.67. UV/VIS (MeOH) = 256, 371. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 7.85 (*s*, H–C(4′)); 7.74 (*d*, *J* = 8.8, H–C(6′)); 7.59–7.53 (*m*, H_m (Ph)); 7.50–7.45 (*m*, H_o and H_p (Ph)); 7.25 (*d*, *J* = 8.8, H–C(7′)); 6.85 (*d*, *J* = 1.5, H–C(8)); 6.43 (*d*, *J* = 1.5, H–C(6)); 5.08 (*d*, *J* = 7.4, H–C(1″)); 3.71 (*d*, *J* = 10.3, 1 H–C(6″)); 3.51–3.16 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), H–C(6″)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 177.1 (C(4)); 163.7 (C(7)); 161.2 (C(5)); 156.7 (C(8a)); 148.8 (C(3′a)); 147.6 (C(2)); 147.1 (C(7′a)); 140.2 (C(3)); 137.8 (C_{ipso} (Ph)); 130.4 (C_p (Ph)); 129.5 (C_m (Ph)); 126.6 (C_o (Ph)); 124.8 (Ph_2C); 124.1 (C(7′)); 118.0 (C(5′)); 109.8 (C(6′)); 108.7 (C(4′)); 105.6 (C(4a)); 100.7 (C(1″)); 99.8 (C(6)); 95.3 (C(8)); 78.0 (C(5″)); 77.3 (C(3″)); 73.9 (C(2″)); 70.4 (C(4″)); 61.4 (C6″)). FAB-MS (neg. mode): 627.0 ($[M-H]^-$).

*3,5-Dihydroxy-2-(3,4-dihydroxyphenyl)-7-(β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one* (**5**). A mixture of **4** (60 mg, 0.10 mmol) and 10% Pd/C (102 mg, 0.10 mmol) in THF/EtOH 1:1 (4 ml) is vigorously stirred under H_2 (1 bar) for 24 h at r. t., then purged with N_2, filtered on *Celite*, and washed with EtOH. Evaporation and purification by FC (silica gel, AcOEt/light petroleum ether 4:1 → AcOEt/MeOH 9:1), gave **5** (27 mg, 60%). Yellow solid. TLC (silica gel, AcOEt/butan-2-one/HCO_2H/H_2O 20:2:1:1): R_f 0.31. UV/VIS (MeOH): 256, 367. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.51 (*s*, OH–C(5)); 9.66 (*s*, OH–C(4′)); 9.51 (*s*, OH–C(3′)); 9.31 (*s*, OH–C(3)); 7.72 (*d*, *J* = 2.2, H–C(2′)); 7.55 (*dd*, *J* = 8.8, 2.2, H–C(6′)); 6.90 (*d*, *J* = 8.8, H–C(5′)); 6.76 (*d*, *J* = 2.2, H–C(8)); 6.42 (*d*, *J* = 2.2, H–C(6)); 5.07 (*d*, *J* = 6.6, H–C(1″)); 3.66 (*d*, *J* = 10.3, 1 H–C(6″)); 3.52–3.16 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), C(6″)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 175.7 (C(4)); 162.3 (C(7)); 160.0 (C(5)); 157.4 (C(8a)); 147.6 (C(4′)); 147.4 (C(2)); 144.8 (C(3′)); 136.0 (C(3)); 121.8 (C(1′)); 119.9 (C(6′)); 115.7 (C(5′)); 115.3 (C(2′)); 104.2 (C(4a)); 99.8 (C(1″)); 98.7 (C(6)); 94.1 (C(8)); 77.2 (C(5″)); 76.4 (C(3″); 73.5 (C(2″)); 69.6 (C(4″)); 60.7 (C(6″)). FAB-MS (neg. mode): 463.0 ($[M-H]^-$).

*3,7-Bis(benzyloxy)-2-[4-(benzyloxy)-3-hydroxyphenyl]-5-hydroxy-4*H*-1-benzopyran-4-one* (**6**) *and 3,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]-5-hydroxy-4*H*-1-benzopyran-4-one* (**7**). ^{t}BuOK (3.32 g, 29.6 mmol) is added to a soln. of **1** (2.0 g, 5.9 mmol) in anh. DMF (30 ml) under N_2. After stirring for 2 h at r.t., a first portion of benzyl bromide (3.52 ml, 29.6 mmol) is added and a second one (3.52 ml) after 12 h. After 24 h, H_2O (200 ml) and 1M HCl (20 ml) are added, and the mixture is extracted with AcOEt (3 × 100 ml), washed with H_2O (2 × 100 ml), dried ($MgSO_4$) and evaporated. Purification by FC (silica gel, light petroleum ether → light petroleum ether/AcOEt 85:15) affords **6** (1.33 g, 40%) and **7** (577 mg, 14%).

Data of **6**: Yellow solid. TLC (silica gel, AcOEt/light petroleum ether 1:4): R_f 0.33. UV/VIS (MeOH): 354. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.67 (*s*, OH–C(5)); 9.47 (*s*, OH–C(3′)); 7.56 (*d*, *J* = 1.5, H–C(2′)); 7.52–7.29 (*m*, H–C(6′), 3 Ph); 7.13 (*d*, *J* = 8.8, H–C(5′)); 6.80 (*d*, *J* = 1.5, H–C(8)); 6.47 (*d*, *J* = 1.5, H–C(6)); 5.23–5.22 (br. *s*, PhCH_2); 5.02 (*s*, 1 PhCH_2). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 179.0 (C(4)); 165.0 (C(7)); 161.9 (C(5)); 157.2 (C(8a)); 150.1 (C(3′)); 147.5 (C(2)); 147.4 (C(4′)); 137.6 (C_{ipso} (Bn)); 137.5 (C_{ipso} (Bn)); 137.4 (C_{ipso} (Bn)); 136.9 (C(3)); 129.4–128.4 (CH (Bn)); 123.3 (C(1′)); 121.4 (C(5′)); 116.5 (C(6′)); 114.1 (C(2′)); 106.2 (C(4a)); 99.3 (C(6)); 94.0 (C(8)); 74.2 (PhCH_2); 70.8 (PhCH_2); 70.6 (PhCH_2).

Data of **7**: Yellow solid. TLC (silica gel, AcOEt/light petroleum ether 1:4): R_f 0.52. UV/VIS (MeOH): 352. FAB-MS (pos. mode): 663.2 ($[M+H]^+$). ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.63 (*s*, OH–C(5)); 7.75 (*d*, *J* = 1.5, H–C(2′)); 7.65 (*dd*, *J* = 8.8, 1.5, H–C(6′)); 7.49–7.27 (*m*, 4 Ph); 7.22 (*d*, *J* = 8.8, H–C(5′)); 6.85 (*d*, *J* = 2.2, H–C(8)); 6.48 (*d*, *J* = 2.2, H–C(6)); 5.24 (br. *s*, 2 PhCH_2); 5.06 (*s*, 1 PhCH_2); 4.97 (*s*, 1 PhCH_2). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 179.0 (C(4)); 165.0 (C(7)); 161.8 (C(5)); 157.0 (C(8a)); 156.5 (C(4′)); 151.5 (C(3′)); 148.4 (C(2)); 137.8 (C_{ipso} (Ph)); 137.7 (C_{ipso} (Bn)); 137.6 (C_{ipso} (Bn)); 137.4 (C_{ipso} (Bn)); 136.9 (C(3)); 129.4–128.4 (CH (Bn)); 123.2 (C(1′)); 123.1 (C(5′)); 115.0 (C(6′)); 114.3 (C(2′)); 106.2 (C(4a)); 99.3 (C(6)); 94.4 (C(8)); 74.3 (PhCH_2); 71.0 (PhCH_2); 70.9 (PhCH_2); 70.7 (PhCH_2). FAB-MS (neg. mode): 571.3 ($[M-H]^-$).

*3,7-Bis(benzyloxy) 2-[4-(benzyloxy)-5-hydroxy-3-[(tetra-*O*-acetyl-β-*D*-glucopyranosyl)oxy]phenyl]-4*H*-1-benzopyran-4-one.* A soln. of **2** (99 mg, 0.24 mmol) in CH_2Cl_2 (10 ml) is added to a soln. of **6** (90 mg, 0.16 mmol) and TMEA (50 µl, 0.16 mmol) in sat. $KHCO_3$ soln. (10 ml). The mixture is heated at 40° for 96 h, 3 portions of **2** (66 mg, 0.16 mmol) being added each 24 h to complete the reaction. After cooling and addition of H_2O (20 ml), the mixture is extracted with CH_2Cl_2 (3 × 20 ml) and washed with 1M HCl (30 ml) and H_2O (50 ml). After drying ($MgSO_4$), evaporation, and purification by FC (silica gel, light petroleum ether/AcOEt

4:1 → 3:2), the protected glucoside (70 mg, 48%) is obtained as a yellow solid. TLC (silica gel, AcOEt/light petroleum ether 2:3): R_f 0.46. ^{1}H-NMR ($CDCl_3$, 300 MHz): 12.66 (*s*, OH–C(5)); 7.95 (*d*, *J* = 2.2, H–C(2′)); 7.71 (*dd*, *J* = 8.8, 2.2, H–C(6′)); 7.43–7.36 (*m*, 3 Ph); 7.02 (*d*, *J* = 8.8, H–C(5′)); 6.54 (*d*, *J* = 1.5, H–C(8)); 6.45 (*d*, *J* = 1.5, H–C(6)); 5.33–5.24 (*m*, H–C(2″), H–C(4″)); 5.16–5.14 (3 *s*, 3 PhCH_2); 5.03 (*d*, *J* = 9.5, H–C(3″)); 4.77 (*d*, *J* = 10.3, 1 H–C(6″)); 4.70 (*d*, *J* = 7.4, H–C(1″)); 4.05 (*m*, H–C(6″)); 3.06 (*m*, H–C(5″)); 2.07–1.98 (4 *s*, 4 Ac). ^{13}C-NMR ($CDCl_3$, 75 MHz): 179.1 (C(4)); 171.0 (Me*C*O); 170.7 (Me*C*O); 169.8 (Me*C*O); 169.7 (Me*C*O); 165.0 (C(7)); 162.5 (C(5)); 157.1 (C(8a)); 156.2 (C(3′)); 151.6 (C(2)); 146.6 (C(4′)); 138.2 (C(3)); 137.1 (C_{ipso} (Bn)); 136.6 (C_{ipso} (Bn)); 136.2 (C_{ipso} (Bn)); 129.6–127.6 (CH (Bn)); 124.2 (C(5′)); 123.6 (C(1′)); 119.3 (C(6′)); 114.8 (C(2′)); 106.6 (C(4a)); 100.2 (C(1″)); 99.1 (C(6)); 93.5 (C(8)); 74.9 (Ph*C*H$_2$); 72.8 (C(5″)); 72.2 (C(3″)); 71.2 (C(2″)); 71.1 (Ph*C*H$_2$); 70.9 (Ph*C*H$_2$); 68.4 (C(4″)); 62.0 (C(6″)); 21.5–20.8 (4 *Me*CO).

*3,7-Bis(benzyloxy)-2-[4-(benzyloxy)-3-(β-*D*-glucopyranosyloxy)phenyl]-5-hydroxy-4*H*-1-benzopyran-4-one* (**8**). Saponification of the acetate groups of the protected glucoside gives **8** (48 mg, 99%). White solid. TLC (silica gel, AcOEt): R_f 0.17. UV/VIS (MeOH): 348. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.63 (*s*, OH–C(5)); 7.79 (*d*, *J* = 1.5, H–C(2′)); 7.70 (*dd*, *J* = 8.8, 1.5, H–C(6′)); 7.52–7.29 (*m*, 3 Ph); 7.19 (*d*, *J* = 8.8, H–C(5′)); 6.90 (*d*, *J* = 1.5, H–C(8)); 6.48 (*d*, *J* = 1.5, H–C(6)); 5.27 (*s*, 1 PhCH_2); 5.23 (*s*, 1 PhCH_2); 5.05 (*s*, 1 PhCH_2); 4.89 (*d*, *J* = 8.1, H–C(1″)); 3.69 (*d*, *J* = 11.8, 1 H–C(6″)); 3.46 (*dd*, *J* = 11.8, 5.2, 1 H–C(6″)); 3.35–3.13 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 179.0 (C(4)); 165.0 (C(7)); 161.8 (C(5)); 157.1 (C(8a)); 156.8 (C(3′)); 151.3 (C(2′)); 147.6 (C(4′)); 137.7 (C_{ipso} (Bn)); 137.6 (C_{ipso} (Bn)); 137.2 (C_{ipso} (Bn)); 136.9 (C(3)); 129.4–128.3 (CH (Bn)); 124.4 (C(5′)); 123.4 (C(1′)); 116.9 (C(6′)); 115.0 (C(2′)); 106.2 (C(4a)); 101.7 (C(1″)); 99.4 (C(6)); 94.3 (C(8)); 78.1 (C(5″)); 77.6 (C(3″)); 74.1 (C(2″)); 74.0 (Ph*C*H$_2$); 71.2 (Ph*C*H$_2$); 71.1 (Ph*C*H$_2$); 70.5 (C(4″)); 60.7 (C(6″)). FAB-MS (pos. mode): 735.5 ([M + H]$^+$).

*2-[3-(β-*D*-Glucopyranosyloxy)-4-hydroxyphenyl]-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one* (**9**). Hydrogenolysis of the benzyl groups of **8** and purification by FC (silica gel, AcOEt/light petroleum ether 9:1 → AcOEt/MeOH 9:1) give **9** (11 mg, 37%). Yellow solid. TLC (silica gel, AcOEt/butan-2-one/HCO_2H/H_2O 20:2:1:1): R_f 0.35. UV/VIS (MeOH): 269, 366. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.45 (*s*, OH–C(5)); 10.78 (*s*, OH–C(7)); 9.45 (*s*, OH–C(4′)); 9.32 (*s*, OH–C(3)); 7.96 (*d*, *J* = 2.2, H–C(2′)); 7.84 (*dd*, *J* = 8.8, 2.2, H–C(6′)); 6.97 (*d*, *J* = 8.8, H–C(5′)); 6.49 (*d*, *J* = 1.5, H–C(8)); 6.19 (*d*, *J* = 1.5, H–C(6)); 4.78 (*d*, *J* = 6.6, H–C(1″)); 3.77 (*d*, *J* = 11.0, 1 H–C(6″)); 3.57 (*dd*, *J* = 11.0, 5.1, 1 H–C(6″)); 3.37–3.15 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 179.0 (C(4)); 161.5 (C(7)); 161.4 (C(5)); 157.0 (C(8a)); 156.8 (C(3′)); 151.1 (C(2)); 146.3 (C(4′)); 136.6 (C(3)); 124.2 (C(5′)); 123.0 (C(1′)); 117.0 (C(6′)); 116.6 (C(2′)); 106.2 (C(4a)); 103.5 (C(1″)); 99.3 (C(6)); 94.6 (C(8)); 78.1 (C(5″)); 76.8 (C(3″)); 74.1 (C(2″)); 70.4 (C(4″)); 61.5 (C(6″)). FAB-MS (neg. mode): 463.1 ([*M* − H]$^-$).

*3,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]-5-[(tetra-*O*-acetyl-β-*D*-galactopyranosyl)oxy]-4*H*-1-benzopyran-4-one.* A soln. of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (372 mg, 0.91 mmol) in CH_2Cl_2 (15 ml) is added to a soln. of **7** (400 mg, 0.6 mmol) and TMEA (193 µl, 0.6 mmol) in sat. K_2CO_3 soln. (15 ml). After heating at 40° for 24 h, a second portion of glycosyl donor (250 mg, 0.6 mmol) is added and the mixture further heated for 24 h. After the usual treatment and purification by FC (silica gel, light petroleum ether/AcOEt 4:1 → 3:2), the protected glucoside (209 mg, 35%) is obtained as a yellow oil. TLC (silica gel, AcOEt/light petroleum ether 2:3): R_f 0.45. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.73 (*s*, H–C(2′)); 7.55 (*d*, *J* = 8.8, H–C(6′)); 7.45–7.16 (*m*, 4 Ph); 6.94 (*d*, *J* = 8.8, H–C(5′)); 6.92 (*d*, *J* = 2.2, H–C(8)); 6.72 (*d*, *J* = 2.2, H–C(6)); 5.20 (*s*, 2 PhCH_2); 5.11 (*s*, 1 PhCH_2); 5.12–4.99 (*m*, H–C(3″), H–C(4″)); 4.93 (*t*, *J* = 8.1, H–C(2″)); 4.91 (*s*, 1 PhCH_2); 4.74 (*d*, *J* = 8.1, H–C(1″)); 4.29–4.16 (*m*, H–C(5″), 2 H–C(6″)); 2.15 (*s*, Ac); 2.09 (*s*, Ac); 2.02 (*s*, Ac); 1.98 (*s*, Ac).

*3,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]-5-(β-*D*-galactopyranosyloxy)-4*H*-1-benzopyran-4-one.* Saponification of the acetate groups of the protected galactoside gives a white solid (99 mg, 100%). TLC (silica gel, AcOEt/butan-2-one/HCOOH/H_2O 20:2:1:1): R_f 0.57. UV/VIS (MeOH): 253, 352. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 7.76 (*d*, *J* = 1.5, H–C(2′)); 7.65 (*dd*, *J* = 8.8, 1.5, H–C(6′)); 7.52–7.28 (*m*, 4 Ph); 7.22 (*d*, *J* = 8.8, H–C(5′)); 7.08 (*d*, *J* = 2.2, H–C(8)); 6.92 (*d*, *J* = 2.2, H–C(6)); 5.25 (*s*, 2 PhCH_2); 4.96 (*s*, 2 PhCH_2); 4.81 (*d*, *J* = 8.1, H–C(1″)); 3.77–3.45 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), 2 H–C(6″)). FAB-MS (neg. mode): 823.1 ([*M* − H]$^-$).

*3,7-Dihydroxy-2-(3,4-dihydroxyphenyl)-5-(β-*D*-galactopyranosyloxy)-4*H*-1-benzopyran-4-one* (**10**). Hydrogenolysis of the benzyl groups of the partially protected galactoside and purification by FC (silica gel, AcOEt/light petroleum ether 4:1 → AcOEt/MeOH 4:1) give **10** (25 mg, 49%). Yellow solid. TLC (silica gel, AcOEt/butan-2-one/HCO_2H/H_2O 20:2:1:1): R_f 0.11. UV/VIS (MeOH): 368. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 11.11 (*s*, OH–C(7)); 9.61 (*s*, OH–C(4′)); 9.34 (*s*, OH–C(3′)); 8.90 (*s*, OH–C(3); 7.66 (*d*, *J* = 2.2,

H–C(2′)); 7.52 (*dd*, *J* = 8.8, 2.2, H–C(6′)); 6.90 (*d*, *J* = 8.8, H–C(5′)); 6.80 (*d*, *J* = 2.2, H–C(8)); 6.71 (*d*, *J* = 2.2, H–C(6)); 4.70 (*d*, *J* = 7.4, H–C(1″)); 3.65–3.24 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), 2 H–C(6″)). FAB-MS (neg. mode): 463.0 ($[M-H]^-$).

3,7-Bis(benzyloxy)-2-{4-(benzyloxy)-3-{[2,3,4,6-tetra-O-*(3-carboxy-1-oxopropyl)-β-*D-*glucopyranosyl]-oxy}phenyl}-5-hydroxy-4*H-*1-benzopyran-4-one.* A soln. of **8** (200 mg, 0.27 mmol), DMAP (33 mg, 0.27 mmol), and succinic anhydride (163 mg, 1.63 mmol) in anh. pyridine (10 ml) is heated at 70° under N_2 for 16 h. After evaporation of pyridine under vacuum and dilution with acetone (10 ml), the mixture is neutralized with *Dowex* (H^+ form) to pH 2–3 (wet pH paper), filtered, and evaporated. Yellow solid (300 mg, 98%). TLC (silica gel, AcOEt/butan-2-one/HCO_2H/H_2O 20:2:1:1): R_f 0.88. ^{1}H-NMR ((D_6)DMSO, 300 MHz): 12.61 (*s*, OH–C(5)); 12.16 (*s*, CO_2H); 7.83 (*d*, *J* = 2.2, H–C(2′)); 7.78 (*dd*, *J* = 8.8, 2.2, H–C(6′)); 7.46–7.31 (*m*, 3 Ph); 7.27 (*d*, *J* = 8.8, H–C(5′)); 6.84 (*d*, *J* = 2.2, H–C(8)); 6.49 (*d*, *J* = 2.2, H–C(6)); 5.37 (*t*, *J* = 9.5, H–C(3″)); 5.24–5.21 (*m*, 3 PhCH_2); 5.20 (*d*, *J* = 8.1, H–C(1″)); 5.13 (*dd*, *J* = 8.1, 9.5, H–C(2″)); 5.00 (*t*, *J* = 9.6, H–C(4″)); 4.97 (*d*, *J* = 11.0, 1 H–C(6″)); 4.11–4.01 (*m*, 1 H–C(6″), H–C(5″)); 2.42 (*s*, 8 CH_2 (suc)). FAB-MS (neg. mode): 1133.0 ($[M-H]^-$).

3,5,7-Trihydroxy-2-{4-hydroxy-3-{[2,3,4,6-tetra-O-*(3-carboxy-1-oxopropyl)-β-*D-*glucopyranosyl]oxy}phenyl}-4*H-*1-benzopyran-4-one* (**11**). Hydrogenolysis of the benzyl groups of the preceding compound gives a crude mixture, which is diluted with H_2O (50 ml), washed with $CHCl_3$ (3 × 50 ml) and lyophilized: **11** (210 mg, 90%). Yellow-green solid. TLC (silica gel, AcOEt/butan-2-one/HCO_2H/H_2O 20:2:1:1): R_f 0.82. UV/VIS (MeOH): 368. ^{1}H-NMR ((D_6)DMSO, 300 MHz): 7.92 (*d*, *J* = 1.5, H–C(2′)); 7.83 (*dd*, *J* = 8.1, 1.5, H–C(6′)); 7.00 (*d*, *J* = 8.1, H–C(5′)); 6.47 (*d*, *J* = 1.5, H–C(8)); 6.17 (*d*, *J* = 1.5, H–C(6)); 5.48 (*t*, *J* = 9.5, H–C(4″)); 5.42 (*d*, *J* = 8.1, H–C(1″)); 5.10 (*dd*, *J* = 9.5, 8.1, H–C(2″)); 5.06 (*t*, *J* = 9.5, H–C(3″)); 4.23–4.05 (*m*, H–C(5″), 2 H–C(6″)); 2.38 (*s*, 8 CH_2 (suc)). ^{13}C-NMR ((D_6)DMSO, 75 MHz): 176.6 (C(4)); 174.8–174.7 (4 CO_2H); 173.1–172.1 (4 CO_2R); 161.3 (C(7)); 157.5 (C(5)); 157.1 (C(8a)); 151.2 (C(2)); 146.2 (C(4′)); 145.4 (C(3′)); 136.8 (C(3)); 125.7 (C(5′)); 122.8 (C(1′)); 117.0 (C(6′)); 116.0 (C(2′)); 103.0 (C(4a)); 100.4 (C(1″)); 99.7 (C(6)); 94.6 (C(8)); 72.2 (C(5″)); 71.7 (C(3″)); 71.5 (C(2″)); 68.8 (C(4″)); 62.7 (C(6″)); 31.5–30.1 (8 CH_2 (suc)). FAB-MS (neg. mode): 863.1 ($[M-H]^-$).

3,7-Bis(benzyloxy)2-{4-(benzyloxy)-3-{[6-O-*(3,4,5-trimethoxybenzoyl)-β-*D-*glucopyranosyl]oxy}phenyl}-5-hydroxy-4*H-*1-benzopyran-4-one.* In small portions, 3,4,5-trimethoxybenzoyl chloride (95 mg, 0.41 mmol) is added to a soln. of **8** (100 mg, 0.14 mmol) and DMAP (9 mg, 0.07 mmol) in anh. pyridine under N_2. After stirring for 72 h at r.t., evaporation under vacuum, and dilution in AcOEt (50 ml), the mixture is washed with 0.1M HCl (50 ml), sat. $NaHCO_3$ (50 ml), and sat. NaCl soln. (2 × 50 ml), dried, and evaporated and the residue purified by FC (silica gel, AcOEt/light petroleum ether 3:2 → 4:1): Yellow solid (21 mg, 16%). TLC (silica gel, AcOEt): R_f 0.33. UV/VIS (MeOH): 268, 345. ^{1}H-NMR ((D_6)DMSO, 300 MHz): 12.49 (*s*, OH–C(5)); 7.83 (*dd*, *J* = 8.8, 1.5, H–C(6′)); 7.77 (*d*, *J* = 1.5, H–C(2′)); 7.53–7.30 (*m*, 3 Ph); 7.18 (*d*, *J* = 8.8, H–C(5′)); 6.86 (*s*, H–C(2‴), H–C(6‴)); 6.58 (*d*, *J* = 2.2, H–C(8)); 6.40 (*d*, *J* = 2.2, H–C(6)); 5.26–5.22 (*m*, 3 PhCH_2); 5.04 (*d*, *J* = 11.0, 1 H–C(6′)); 4.97 (*d*, *J* = 7.4, H–C(1″)); 4.92 (br. *d*, *J* = 11.0, 1 H–C(6″)); 3.55 (*s*, 1 MeO); 3.44 (*s*, 2 MeO); 3.39–3.20 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″)). FAB-MS (pos. mode): 929.7 ($[M+H]^+$).

3,5,7-Trihydroxy-2-{4-hydroxy-3-{[6-O-*(3,4,5-trimethoxybenzoyl)-β-*D-*glucopyranosyl]oxy}phenyl}-4*H-*1-benzopyran-4-one* (**12**). Hydrogenolysis of the benzyl groups of the preceding compound and purification by FC (*C18* silica gel, $H_2O \rightarrow H_2O$/MeOH 1:1) gave **12** (4 mg, 64%). Yellow solid. TLC (silica gel, AcOEt/butan-2-one/HCO_2H/H_2O 20:2:1:1): R_f 0.72. UV/VIS (MeOH): 265, 371. FAB-MS (neg. mode): 657.5 ($[M-H]^-$). ^{1}H-NMR ((D_6)DMSO, 300 MHz): 7.89 (*d*, *J* = 8.8, H–C(6′)); 7.84 (*s*, H–C(2′)); 6.96 (*d*, *J* = 8.8, H–C(5′)); 6.88 (*s*, H–C(2‴), H–C(6‴)); 6.16 (*s*, H–C(8)); 6.08 (*s*, H–C(6)); 4.95 (*d*, *J* = 7.4, H–C(1″)); 4.89 (*d*, *J* = 10.3, 1 H–C(6″)); 4.18 (*dd*, *J* = 10.3, 6.6, H–C(6″)); 3.56 (*s*, 1 MeO); 3.55 (*s*, 2 MeO); 3.42–3.28 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″)). ^{13}C-NMR ((D_6)DMSO, 125 MHz): 166.1 (C(4)); 166.0 (CO (acyl)); 165.1 (C(7)); 156.6 (C(5)); 153.6 (C(8a)); 153.1 (C(3‴), C(5‴)); 146.1 (C(3′)); 145.7 (C(2)); 142.8 (C(4′)); 142.2 (C(4‴)); 125.6 (C(1′)); 125.5 (C(1‴)); 124.3 (C(5′)); 117.1 (C(6′)); 115.8 (C(2′)); 107.5 (C(4a)); 107.0 (C(2‴), C(6‴)); 103.2 (C(1″)); 102.2 (C(6)); 94.6 (C(8)); 76.5 (C(5″)); 75.0 (C(3″)); 73.8 (C(2″)); 71.3 (C(4″)); 65.0 (C(6″)); 60.8 (1 MeO); 56.8 (2 MeO).

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

Copigmentation of Malvin. 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 for **13** and **14** and 200 for **11**). 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 by conc. HCl soln. (no significant dilution) for the determination of the isosbestic point of malvin (flavylium form) and its copigmentation complex.

H-Abstraction by DPPH. To 2 ml of freshly prepared $2 \cdot 10^{-4}$ M DPPH in MeOH placed in the spectrometer cell, 50–100 µl of a freshly prepared 10^{-3} M soln. of flavonol in MeOH were added. UV/VIS spectra were recorded every 0.5 s over 1 min for the determination of the rate constants of H-atom abstraction. Runs over 1 h were used for the determination of the total antioxidant stoichiometry.

Aluminium Complexation by Flavonols. In a typical experiment, freshly prepared 10^{-4} M flavonol in MeOH was diluted twice with a 0.2M acetate buffer (final pH 5.0). In one part of this soln., $AlCl_3 \cdot 6\,H_2O$ was added to a final concentration of $5 \cdot 10^{-3}$ M. If necessary, the pH was brought back to 5.0 by addition of conc. NaOH soln. (without significant dilution). The flavonol and flavonol/aluminium solns. were then mixed in different ratios to prepare samples having intermediate Al^{3+} concentrations for spectral measurements.

Iron Complexation by Flavonols. In a typical experiment, a few µl of $3 \cdot 10^{-3}$ M $FeSO_4 \cdot 7\,H_2O$ or $Fe(NO_3)_3 \cdot 9\,H_2O$ in MeOH was added at time zero to a sample (2 ml) of flavonol ($5 \cdot 10^{-5}$ M) in a given buffer placed in the thermostated cell of the UV/VIS spectrometer. Spectra were recorded over a few min until equilibrium was reached. Absorbance values measured at equilibrium were used for the determination of the apparent iron-flavonol binding constants. No precipitation occurred under such conditions.

Semi-Empirical Quantum-Mechanics Calculations. Energy optimizations were run *in vacuo* on a *Pentium-90* PC with the HyperChem program (*Autodesk*, Sausalito, California, USA) and the AM1 parametrization. The unrestricted *Hartree-Fock* method was selected for both the open-shell (aryloxyl radicals) and close-shell species (phenols). The bond-dissociation energies were identified to the energy differences between the aryloxyl radicals and the corresponding phenols. The H-bond dissociation energies in phenols and aryloxyl radicals were identified to the energy differences between non-H-bonded and H-bonded rotamers (180° rotation about the C–OH bond).

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