Acylated Flavone Glucosides: Synthesis, Conformational Investigation, and Complexation Properties

by **Bertrand Alluis** and **Olivier Dangles***

Laboratoire des Polyphénols, Bât. 303, Université Claude Bernard-Lyon I, UPRES A-CNRS 5078, 43, bld du 11 Novembre 1918, F-69622 Villeurbanne (Phone: (33)(0) 472431989, Fax: (33)(0) 472431323, E-mail: dangles@univ-lyon1.fr)

Acylated flavonoid glycosides are ubiquitous in plants; however, the possible influence of the acyl groups on the typical properties of flavonoids is rather poorly documented. In this work, a series of chrysin (= 5,7-dihydroxyflavone) glucosides acylated with aromatic and aliphatic acid residues has been synthesized in a simple three-step procedure. Aromatic acyl groups are shown to impose folded conformations on the chrysin glucosides owing to their ability to stack on the polyphenolic nucleus. The acyl groups may also cause significant changes in the ability of the flavonoid glucosides to bind hard metal ions and proteins, as demonstrated in this work in the case of Al^{3+} and bovine serum albumin.

Introduction. – Flavonoids are a broad class of polyphenolic secondary metabolites abundant in plants and in human diet [1]. Beside their important biological roles in nitrogen fixation and chemical defense, flavonoids possess a broad range of pharmacological properties including anti-cancer, anti-viral, and anti-inflammatory properties [2], which may be the consequence of their antioxidant properties and their affinity for proteins, including enzymes. Flavonoids also protect plants from damaging UV-B radiation [3] and efficiently contribute to natural color expression by forming stable molecular complexes with polyphenolic pigments (anthocyanins), a phenomenon known as copigmentation [4].

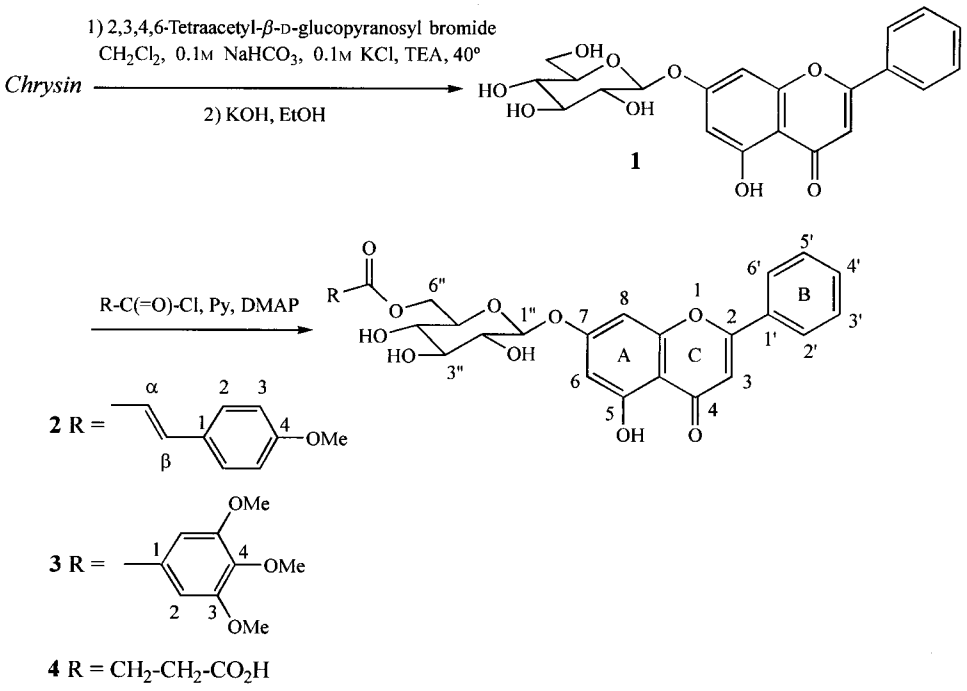
Most flavonoids occur in nature as glycosides in which at least one of the OH groups (most frequently, HO–C(3), HO–C(5), and HO–C(7)) of the chromophore (aglycone) is glycosylated by mono- to tetrasaccharides involving neutral sugars such as D-glucose, D-galactose, L-rhamnose, and D-xylose. Acylation of sugar OH groups by a variety of aliphatic (acetic, malonic, succinic *etc.*) and aromatic (*p*-hydroxybenzoic, *p*-coumaric, caffeic *etc.*) acids brings additional structural diversity to the flavonoid family [1]. Although the possible influence of such acyl groups on the binding and antioxidant properties of flavonoids is still poorly documented, there is evidence that they play an important role in protection against UV-B radiation [3] and in pigmentation [4]. For instance, anthocyanins bearing aromatic-acid moieties typically display much more stable colors than their deacylated counterparts. Indeed, in a process known as *intramolecular copigmentation*, the aromatic acyl groups stack on the anthocyanin chromophore (2-phenyl-1-benzopyrylium nucleus) and thereby protect the pyrylium ring from the nucleophilic attack of water molecules on C(2), which yields colorless hemiacetals and chalcones.

To systematically investigate the possible influence of acyl groups on the properties of flavonoids, the chemical synthesis of acylated flavonoids is an attractive alternative to extraction from plants.

In this work, a series of chrysin (= 5,7-dihydroxyflavone = 5,7-dihydroxy-2-phenyl-4*H*-1-benzopyran-4-one) derivatives has been synthesized upon regioselective glycosidation of chrysin at *O*–C(7), followed by acylation of the sugar moiety by a variety of aliphatic or aromatic acids. The properties of the chrysin derivatives (preferred conformations, metal complexation, protein complexation) have been investigated by UV/VIS, fluorescence, and NMR spectroscopy, and by molecular modeling.

Results and Discussion. – 1. *Synthesis. Scheme 1* summarizes the main steps of the chemical synthesis. Chrysin was regioselectively glycosylated at *O*–C(7) under phase-transfer conditions according to a procedure previously used in the synthesis of anthocyanin models (sat. K_2CO_3/CH_2Cl_2, tris[2-(2-methoxyethoxy)ethyl]amine, 40°) [5]. Similar conditions were also used in the glycosidation of isoflavones [6]. The HO–C(5) group, which is strongly H-bonded to the C(4)=O group (δ 12–13 ppm in (D_6)DMSO), does not react under our conditions. Replacing the saturated K_2CO_3 solution with 0.1M $NaHCO_3$/0.1M KCl slows the competitive hydrolysis of the glycosylbromide and results in higher yields. After deacetylation (KOH, EtOH), 7-*O*-(β-D-glucopyranosyl)chrysin (**1**) was obtained. Its characteristics are in agreement with those reported in the literature on a sample isolated from cell cultures of 'old man'

Scheme 1. *Synthesis of Chrysin Derivatives* **1**–**4**



cactus (*Cephalocereus senilis*) [7]. The glycosidation in the galactose series is significantly faster than in the glucose series and gives higher yields. In the mannose series, the main product is the orthoester resulting from the nucleophilic attack of the phenolate anion on the AcO–C(2) group with subsequent elimination of Br^- ion in accord with the literature [8]. Attempts to prepare glucuronides under these conditions were unsuccessful.

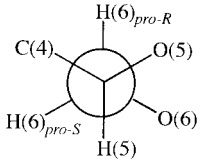
Acylation of **1** by electron-rich aromatic acids (*p*-methoxybenzoic acid, 3,4,5-trimethoxybenzoic acid, *p*-methoxycinnamic acid) was attempted by different procedures. No reaction occurred when **1** was directly treated with the aromatic acid by the *N*,*N*′-dicyclohexylcarbodiimide(DCC)/4-(dimethylamino)pyridine (DMAP) procedure. Under *Mitsunobu* conditions (diisopropyl azodicarboxylate (DIAD)/Ph_3P), the slow formation of acylated products could be evidenced by TLC. Acylation of **1** by *p*-methoxycinnamyl or 3,4,5-trimethoxybenzoyl chloride in pyridine with DMAP as a catalyst (0.2–0.5 equiv.) was much faster and afforded moderate yields (*ca.* 30%) of 6″-*O*-acylflavones **2** and **3**, respectively. Although relatively low yielding, the acylation step was highly regioselective and resulted in a mixture of only **1** and the acylated product, which were easily separated on silica gel. Hence, the regioselective acylation of unprotected flavonoid glucosides by the DMAP/acyl chloride method is an efficient and very simple alternative to acylation of flavonoid derivatives bearing partially protected D-glucose moieties, which has already been reported [9]. Heating or replacing pyridine by pyridine/CH_2Cl_2 1:1 and pyridine/DMF 4:1 mixtures did not significantly improve the yields. In pure DMF, the reaction of **1** with 3,4,5-trimethoxybenzoic acid chloride gave 7-*O*-(6″-*O*-formyl-β-D-glucopyranosyl)chrysin as the main product. TLC Analysis showed the acylation of **1** by *p*-methoxybenzoyl chloride in pyridine to be less regioselective. Only the 3″-*O*-acyl derivative could be isolated in significant yield (11%).

Acylation of 7-*O*-(β-D-galactopyranosyl)chrysin was much slower than that of **1**. The corresponding acylated products were not isolated in this case.

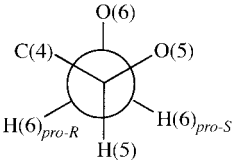
When **1** was treated with succinic anhydride and DMAP in pyridine, 7-*O*-(6″-*O*-succinyl-β-D-glucopyranosyl)chrysin (**4**) could be isolated after chromatography on silica gel (along with minor amounts of the 3″-regioisomer). On the other hand, addition of an excess of succinic anhydride (5 equiv.) and heating at 70° afforded water-soluble 7-*O*-(2″,3″,4″,6″-tetra-*O*-succinyl-β-D-glucopyranosyl)chrysin in quantitative yield. The 6″-malonyl analog of **4** has been isolated from *Pueraria lobata* cells fed with chrysin [10].

2. *Conformational Investigation*. ROESY Analysis (500 MHz) of 7-*O*-(6″-*O*-*p*-methoxycinnamyl-β-D-glucopyranosyl)chrysin (**2**) and 7-*O*-(6″-*O*-(3,4,5-trimethoxybenzoyl)-β-D-glucopyranosyl)chrysin (**3**) revealed long-range correlations between the chrysin and acyl moieties. In **3** ($CDCl_3$/(D_6)DMSO), the correlated protons are H–C(2) and H–C(6) of the 3,4,5-trimethoxyphenyl moiety on the one hand and H–C(6) of the chrysin moiety on the other hand. In **2** (CD_3OD, 5°), the correlated protons are H–C(α) of the cinnamyl moiety and H–C(6) of the chrysin moiety. Hence, 7-*O*-β-D-glucopyranosylflavones acylated on their primary sugar OH group by benzoic- or cinnamic-acid derivatives seem to adopt folded conformations in which both aromatic moieties are in *van der Waals* contact. This is reminiscent of the intramolecular copigmentation process in acylated anthocyanins (pigments) [4][11], *i.e.*, stacking interactions between the 2-phenyl-1-benzopyrylium chromophore and the

aromatic acyl groups which efficiently take part in color stabilization and variation. As evidenced in this work, aryl-aryl interactions are also found in the flavone family. Although such interactions are expected to be stronger in aqueous media because of the hydrophobic effect, they are easily detectable in organic solvents. Thus, the relatively rigid sugar moiety seems to drive together aromatic nuclei located at C(1″) and C(6″). Favorable electrostatic and *van der Waals* interactions between the aryl groups must compensate the loss of free rotation about the sugar C(5″)–C(6″) bond. Such conformational restrictions are manifested by differences in the value of the coupling constant between H–C(5″) and $H_{pro\text{-}R}$–C(6″) in the series **1**–**3**. Since the *tg* conformation in glucose units is strongly destabilized by 1,3-*syn* periplanar repulsions between C(4″)–O and C(6″)–O bonds, $H_{pro\text{-}R}$–C(6″) can be identified as the proton at C(6″) displaying the larger *J*(5,6) value, an assignment confirmed by selective deuteration experiments [12]. The *gt* conformation actually places H–C(5″) and $H_{pro\text{-}R}$–C(6) in an antiparallel arrangement, allowing a strong spin-spin coupling, and also orients the acyl moiety toward the flavone nucleus, thus favoring aryl-aryl interactions. The $J(5,6_{pro\text{-}R})$ value in (D_6)DMSO is 7.4 Hz in **2** and **3**, whereas it is only 3.7 Hz in **1**. Such differences point to a higher fraction of *gt* conformation in **2** and **3** with respect to the *gg* conformation. From a modified *Karplus* equation relating $J(5,6_{pro\text{-}R})$ to the fractions of *gg* and *gt* conformers [6], and assuming no *tg* conformer in agreement with a non-measurable coupling constant between H–C(5″) and $H_{pro\text{-}S}$–C(6″), 77% of *gg* conformer can be estimated for **1**, whereas 60% of **2** and **3** would adopt the *gt* conformation.



gt conformation



gg conformation

It is also noteworthy that $H_{pro\text{-}S}$–C(6) is strongly deshielded with respect of $H_{pro\text{-}R}$–C(6) in the acylated flavones as a consequence of the magnetic anisotropy effect of the C=O bond. Thus, $\Delta\delta$ is 0.60 ppm in **3** and 0.34 ppm in **2**, whereas it is only 0.17 in non-acylated **1**. This suggests that $H_{pro\text{-}R}$–C(6) lies above the plane of the ester group, whereas $H_{pro\text{-}S}$–C(6) would lie approximately in the plane of the ester group.

All ^{1}H-NMR signals of the chrysin moiety in **2** are shielded with respect to the corresponding signals in **1**, especially H–C(2′), H–C(6′) (– 0.26 ppm), and H–C(3) (– 0.13 ppm). Similar diamagnetic shifts are observed when the *p*-methoxycinnamyl moiety of **2** is compared with the methyl *p*-methoxycinnamate, especially in the case of the aromatic H-atoms (*ca.* – 0.2 ppm). Such observations suggest that the interacting aromatic nuclei lie approximately parallel to each other (*face-to-face* arrangement).

The above-mentioned structural information can be reasonably justified by molecular modeling on **2** and **3** by means of the classical MM+ force field. The charge distributions on both aromatic nuclei (which are of crucial importance for determining their relative positions in the stacked conformation [13]) were previously deduced from semi-empirical quantum-mechanical calculations on 7-*O*-methylchrysin,

methyl *p*-methoxycinnamate, and methyl 3,4,5-trimethoxybenzoate. The calculations were repeated with different initial values for the torsion angles about C(5″)–C(6″), C(6″)–O, C(1″)–O (glycosidic bond), and C(7)–O. In the case of **2**, two energy-minimized conformations close in stability were obtained, the main characteristics of which are shown in *Fig. 1* (upper part): the C(5″)–C(6″) bond adopts a *gt* conformation. C(2″) and C(7) are held in an antiparallel arrangement in agreement with the *exo*-anomeric effect. The atom C(1″) is approximately in the plane of the flavone nucleus in agreement with an sp^2-hybridized O-atom at C(7). The C(5″)–C(6″) bond is approximately perpendicular to the plane of the ester group. Consequently, the $H_{pro\text{-}S}$–C(6″) and $H_{pro\text{-}R}$–C(6″) bonds lie 13–17 and 131–134°, respectively, out of the plane of the ester group, in agreement with the δ values of $H_{pro\text{-}S}$–C(6″) and $H_{pro\text{-}R}$–C(6″). The only difference between the two energy-minimized conformations is the configuration of the α,β-unsatured ester group which is '*cisoid*' in one and '*transoid*' in the other. Both conformations allow *face-to-face* molecular contacts between the acyl group and the B ring of the flavone nucleus, which are in agreement with the observed ROESY correlations. A similar optimized conformation was obtained for **3** (*Fig. 1*, lower part).

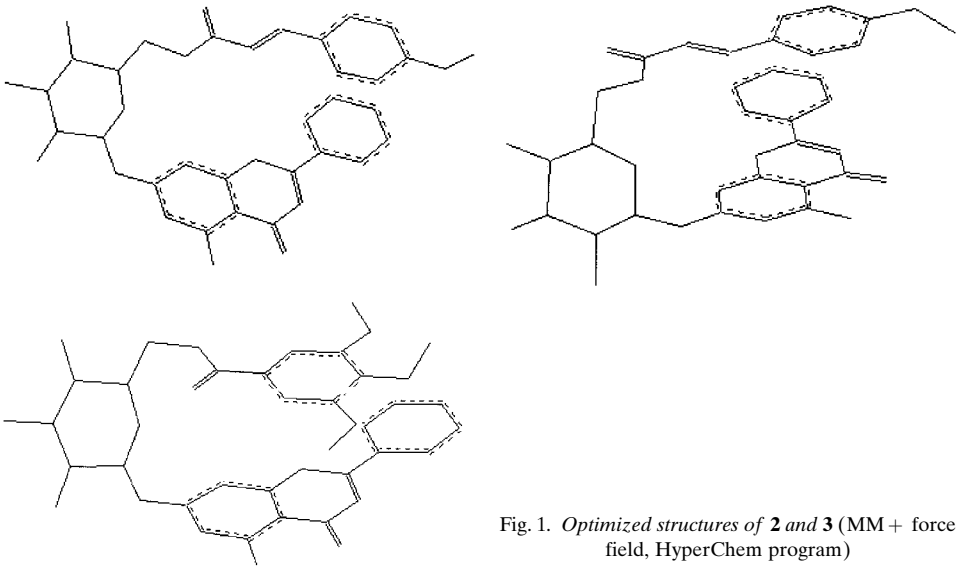


Fig. 1. *Optimized structures of* **2** *and* **3** (MM+ force field, HyperChem program)

3. *Metal Complexation*. 5-Hydroxyflavones are able to chelate hard metal ions through their 5-hydroxy-4-oxo group [14]. To probe the possible influence of acyl moieties on the stability of the metal chelates, complexation by Al^{3+} was investigated with chrysin and flavones **1**, **2**, and **4**. To that purpose, UV/VIS spectra of each flavone were recorded for different Al^{3+} concentrations in MeOH/0.2M acetate buffer 1:1 at pH 5.3. Bathochromic shifts typical of metal complexation were observed (*Fig. 2*). The complexation reaction may be written as:

$$M + LH \rightarrow ML + H^+$$

LH, M, and ML stand for the flavone (neutral form), metal ion, and 1:1 chelate, respectively. The H^+ corresponds to *HO*–C(5), which is removed upon chelation of the metal ion. The equilibrium constant is $K = ([ML][H^+])/([M][LH])$. Since the pH is constant in the complexation experiment, the affinity of the flavone for M can also be expressed by $K' = K/[H^+] = [ML]/([M][LH])$.

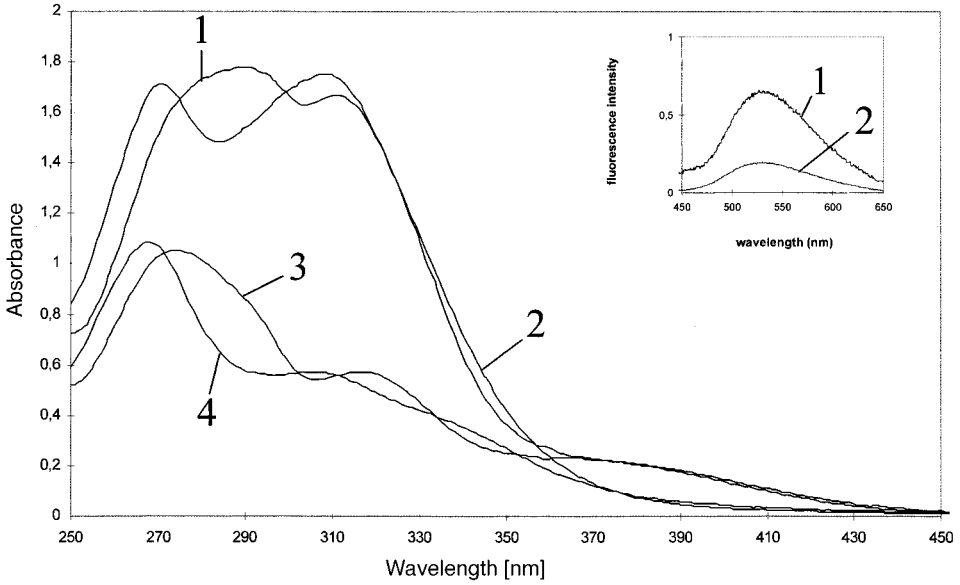


Fig. 2. *UV/VIS Spectra of* **1** *and* **2**, *and their* Al^{3+}*-complexes in MeOH/0.2*M *acetate buffer 1:1* (pH 5.3, 22°). *Spectrum 1*: **2** + $AlCl_3$ (50 equiv.); *spectrum 2*: **2**; *spectrum 3*: **1** + $AlCl_3$ (50 equiv.); *spectrum 4*: **1**. *Inset*: fluorescence spectra of the Al^{3+} complexes of **1** and **2** in MeOH. *Spectrum* 1: **1** + $AlCl_3$ (10 equiv., excitation wavelength: 405 nm): *spectrum 2*: **2** + $AlCl_3$ (10 equiv., excitation wavelength: 415 nm).

The plots of the absorbance (at a fixed wavelength of the chelate absorption band) as a function of the Al^{3+} concentration can be fitted to *Eqns. 1* and *2* with the sole assumption of 1:1 complexation (*A:* absorbance, A_0: absorbance in the absence of metal ion, [M]: free metal concentration, M_t: total metal concentration, *c:* total flavone concentration). The constant *r* stands for the following ratio of molar absorption coefficients, $\varepsilon(ML)/\varepsilon(L)$.

$$A = A_0(1 + rK'[M])/(1 + K'[M]) \quad (1)$$

$$M_t = [M](1 + cK' + K'[M])/(1 + K'[M]) \quad (2)$$

Values for parameters *r* and *K'* are reported in *Table 1*. The *K'* value of **1** is lower than that of chrysin as expected from the electron-withdrawing effect of the D-glucopyranosyl group. Interestingly, introduction of a succinyl group at *O*–C(6″) (flavone **4**) raises the *K'* back to the level of its value for chrysin, thus suggesting participation of the carboxylate group in complexation.

Table 1. *Aluminium Complexation of Chrysin and Chrysin Derivatives in MeOH/0.2*M *Acetate Buffer 1:1* (pH 5.3, 22°). For definition of *r* and *K'*, see text.

Flavone	K' [M^{-1}]	r (385 nm)
Chrysin	3240 ± 290	3.45 ± 0.04
1	1710 ± 220	3.63 ± 0.08
2	2330 ± 210	4.02 ± 0.06
4	3340 ± 430	4.41 ± 0.09

When $AlCl_3$ (10 equiv.) is added to a solution of **1** in CD_3OD, a strong deshielding of the H–C(3) NMR signal ($\Delta\delta$ 0.34 ppm) is observed, whereas the other signals are only weakly affected. This suggests that ring A in the complex tends to adopt an aromatic pyrylium structure (*Scheme 2*). Moreover, in agreement with the literature [15], addition of $AlCl_3$ (10 equiv.) to a solution of flavone (chrysin, **1**, and **2**) in MeOH converts the essentially non-fluorescent flavone nucleus to a complex that displays a fluorescence emission band with λ_{max} in the range 520–540 nm (excitation wavelengths in the range 400–440 nm), depending on the flavone structure (*Table 2*). Such emission bands are typical of pyrylium-like chromophores [16]. Interestingly, whereas both **1** and **2** display emission bands with almost identical λ_{max}, the fluorescence intensity in **1** is 3–4 times as strong as in **2** (*Fig. 2*). Thus, the *p*-methoxycinnamyl moiety in **2** promotes an efficient quenching of the Al^{3+}-bound flavone fluorescence.

Scheme 2. *Structure of the Flavone-Aluminium Complexes*

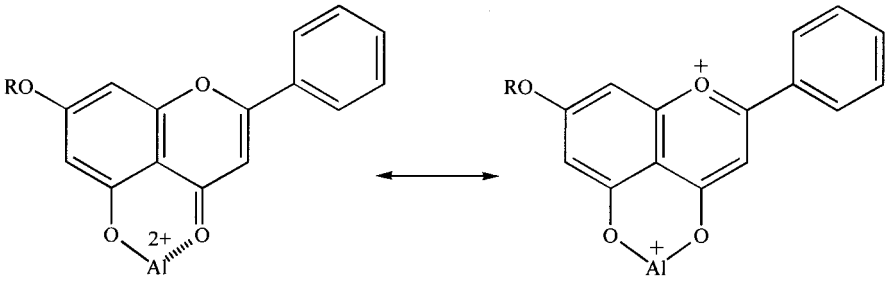


Table 2. *Spectroscopic Parameters of Flavone-Aluminium Complexes in MeOH*. Aluminium/flavone molar ratio: 10. Values in brackets are excitation wavelengths.

	λ_{max} [nm]	
Flavone	Absorption	Fluorescence emission (exc.)
Chrysin	281, 330, 324	523 (440)
1	277, 323, 384	534 (405)
2	289, 315, 383	532 (415)

4. *Protein Complexation*. Human serum albumin (HSA), the well-known carrier of fatty acids in blood, has been shown to bind quercetin (3,3′,4′,5,7-pentahydroxyflavone) and structurally related flavonoids, as well as quercetin conjugates detected in the plasma of rats and humans fed a quercetin-rich diet, *in vitro* [17]. Recently, binding of quercetin to bovine serum albumin (BSA) was quantitatively investigated by fluorescence spectroscopy [18]. At pH 7.4, BSA promotes a strong saturable enhance-

ment of quercetin fluorescence emission around 530 nm (excitation at 450 nm), which points to quercetin adopting a pyrylium character in the complex. From *Scatchard* analysis, the quercetin-BSA complex was shown to display a 1:1 stoichiometry and a stability constant K equal to 103 ($\pm$ 16) $\times 10^3$ M^{-1} at 25°. Re-treating the data using a simple 1:1 binding model gives: $K = 125$ ($\pm$ 12) $\times 10^3$ M^{-1}.

BSA-Promoted fluorescence enhancements are strongly dependent on the substitution of the flavonoid nucleus and, for instance, are absent in the case of chrysin and its derivatives. Hence, chrysin and its derivatives fluoresce neither in their free form nor in their BSA-bound form. To investigate their binding to BSA, competitive experiments were thus carried out. Increasing concentrations of chrysin, **1**, **2**, and methyl *p*-methoxycinnamate were added to a solution of BSA (7.52×10^{-5}M) and quercetin (10^{-4}M). In *Fig. 3* are plotted the corresponding changes in fluorescence intensity. In the case of chrysin, the gradual quenching of quercetin fluorescence at 520 nm continues until complete extinction and reflects the replacement of quercetin by chrysin within the BSA binding site. The spectral changes can be quantitatively accounted for from a simple competitive model (*Eqns. 3* and *4*, *F:* fluorescence intensity, F_1: molar fluorescence intensity of the bound indicator (quercetin), I_t: total indicator concentration, K_I: indicator-BSA binding constant, [P]: free protein concentration, P_t: total protein concentration, *K:* flavone-BSA binding constant, L_t: total flavone concentration).

$$F = F_I I_t K_I [P]/(1 + K_I[P]) \quad (3)$$

$$P_t = [P](1 + K_I I_t/(1 + K_I[P]) + K L_t/(1 + K[P])) \quad (4)$$

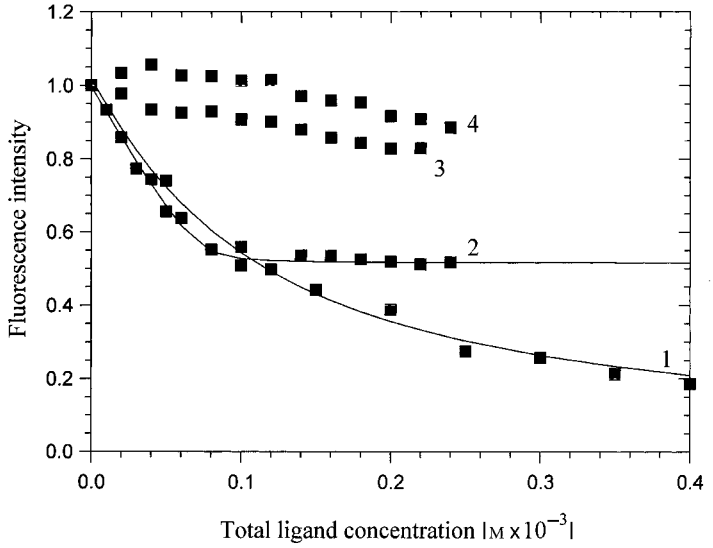


Fig. 3. *Changes in the fluorescence intensity at 530 nm* (excitation wavelength: 455 nm) *as a function of the total ligand concentration in a 0.02*M phosphate *buffer containing BSA* (7.52×10^{-5}M) *and quercetin* (10^{-4}M) (25°, pH 7.4, ionic strength: 0.2M). Ligands: chrysin (*1*), **2** (*2*), **1** (*3*), and methyl *p*-methoxycinnamate (*4*). In the case of chrysin, the solid line is the result of the curve-fitting procedure.

The curve-fitting procedure ($r = 0.997$) of the experimental *F vs.* L_t plot gives a *K* value of 155 (± 11) $\times 10^3$ M^{-1}, thus showing that chrysin roughly displays the same affinity for BSA as quercetin.

Surprisingly, upon addition of **2**, the fluorescence intensity decreases until it reaches a clear-cut plateau for a 1:1 flavone **2**/quercetin molar ratio (*Fig. 3*). The plateau fluorescence intensity is *ca.* half the initial value (without **2**). Hence, a large and constant residual quercetin fluorescence is still observed at large flavone **2**/quercetin molar ratios. Such a phenomenon is absent or much less pronounced in the case of flavone **1** and methyl *p*-methoxycinnamate. Hence, **2** seems to bind BSA in a noncompetitive way, thus resulting in the formation of a flavone **2**-quercetin-BSA ternary complex, which, owing to the quercetin ligand, is still fluorescent although less strongly than the quercetin-BSA binary complex. The apparently quantitative formation of the ternary complex for a 1:1 quercetin/flavone **2** molar ratio suggests that **2** binds to the quercetin-BSA complex with a very high affinity. The flavone nucleus and the aromatic acid moiety of **2** both take part in the formation of the ternary complex since **1** and methyl *p*-methoxycinnamate by themselves cause much weaker spectral changes in the fluorescence of bound quercetin. Hence, flavonoid glycosides bearing aromatic acyl groups may act as potent bidentate ligands for serum albumin.

Similarly, polyphenols from the gallotannin family bind to BSA and gelatin with an affinity that dramatically increases with the number of galloyl groups on the D-glucose core [19].

In conclusion, acyl groups frequently encountered in the structure of flavonoid glycosides may markedly affect the binding and conformational properties of flavonoids. Succinyl and malonyl groups may increase the stability of metal-flavonoid complexes through participation of the free carboxylate group in binding hard metal ions. Aromatic acyl groups favor folded conformations through stacking interactions with the flavonoid nucleus. Such interactions may significantly influence the antioxidant properties of flavonoids and their binding to proteins, as briefly evidenced in this work in the case of serum albumin.

Experimental Part

General. Chrysin, quercetin, $AlCl_3 \cdot 6H_2O$, and BSA (fraction V, Mol. wt. 66500 g mol^{-1}) were from *Sigma-Aldrich.* HPLC: *Hypersil 5* silica column (250 × 4.6 mm), flow rate 1 ml/min, *Jasco 880 PU* pump equipped with a *Shimadzu SPD-6A* UV/VIS detector. TLC: silica plates (Kieselgel F_{254}, *Merck*), UV visualization. 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. ^{1}H- and ^{13}C-NMR Spectra: 300-MHz and 500-MHz Bruker apparati at 27°; chemical shifts (δ) in ppm, coupling constants (J) in Hz. ^{13}C-NMR signals tentatively assigned from DEPT and comparisons with the literature [7][9][10][20].

*5-Hydroxy-2-phenyl-7-(tetra-*O*-acetyl-β-*D*glucopyranosyloxy)-4*H*-1-benzopyran-4-one.* A soln. of tetra-*O*-acetyl-α-D-glucopyranosylbromide (1.62 g, 3.93 mmol) in CH_2Cl_2 (25 ml) was slowly added to a soln. of chrysin (1 g, 3.93 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (1.26 ml, 3.93 mmol) in 25 ml of 1M $NaHCO_3$ 1M KCl 1:1. The mixture was heated at 40° for 72 h. During this period, additional tetra-*O*-acetyl-α-D-glucopyranosylbromide was added (2 × 0.81 g, 2 × 1.95 mmol) to complete the reaction. After addition of H_2O (100 ml) and extraction with CH_2Cl_2 (3 × 100 ml), the org. phases were successively washed with 1M HCl (100 ml) and H_2O (100 ml). After drying ($MgSO_4$), the mixture was concentrated and purified by FC (silica gel; AcOEt/petroleum ether 3:7). Yield 63% (1.44 g, yellow powder). UV/VIS (CH_2Cl_2): 313. TLC (AcOEt/petroleum ether (2:3)): R_f 0.47. HPLC (AcOEt/petroleum ether 1:1): t_R 5.9 min. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 12.87

(*s*, HO–C(5)); 8.09 (*d*, *J* = 6.6, H–C(2′), H–C(6′)); 7.64–7.57 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.09 (*s*, H–C(3)); 6.85 (*d*, *J* = 1.5, H–C(8)); 6.48 (*d*, *J* = 1.5, H–C(6)); 5.78 (*d*, *J* = 8.1, H–C(1″)); 5.41 (*t*, *J* = 9.5, H–C(4″)); 5.12 (*dd*, *J* = 8.1, 9.5, H–C(2″)); 5.03 (*t*, *J* = 9.5, H–C(3″)); 4.35 (*m*, H–C(5″)); 4.20 (*dd*, *J* = 11.8, 5.9, $H_{pro\text{-}R}$–C(6″)); 4.12 (*d*, *J* = 11.8, $H_{pro\text{-}S}$–C(6″)); 2.03 (*s*, 3 Ac); 1.99 (*s*, Ac). ^{13}C-NMR ($CDCl_3$/(D_6)DMSO 9 : 1, 50 MHz): 182.5 (C(4)); 170.6 (CO of Ac); 170.1 (CO of Ac); 169.4 (CO of Ac); 169.2 (CO of Ac); 164.45 (C(2)); 162.3 (C(7)); 162.1 (C(5)); 157.4 (C(9)); 132.1 (C(4′)); 131.1 (C(1′)); 129.1 (C(3′), C(5′)); 126.3 (C(2′), C(6′)); 107.0 (C(3)); 106.0 (C(10)); 99.9 (C(1″)); 98.1 (C(6)); 95.6 (C(8)); 72.6 (C(5″)); 72.4 (C(3″)); 71.0 (C(2″)); 68.3 (C(4″)); 62.0 (C(6″)); 20.6 (Me).

*7-(β-*D*-Glucopyranosyloxy)-5-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one* (**1**). A soln. of the compound described above (1.44 g, 2.46 mmol) in 1M KOH/EtOH (40 ml) was refluxed for 3 h under N_2. After cooling, addition of *Dowex 50* (H^+ form) to pH 3 (wet pH paper), filtration, and drying under vacuum, **1** (1.025 g, 99%) was obtained. Yellow powder. UV/VIS (MeOH): 268, 306. TLC (AcOEt/butan-2-one/HCOOH/H_2O 30 : 2 : 1 : 1): R_f 0.32. ^{1}H-NMR ((D_6)DMSO, 300 MHz): 12.81 (*s*, HO–C(5)); 8.11 (*d*, *J* = 6.6, H–C(2′), H–C(6′)); 7.62–7.59 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.07 (*s*, H–C(3)); 6.88 (*d*, *J* = 1.5, H–C(8)); 6.48 (*d*, *J* = 1.5, H–C(6)); 5.09 (*d*, *J* = 6.6, H–C(1″)); 3.71 (*d*, *J* = 10.3, $H_{pro\text{-}S}$–C(6″)); 3.50–3.20 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″)); $H_{pro\text{-}R}$–C(6″)). ^{13}C-NMR ($CDCl_3$/(D_6)DMSO 9 : 1, 75 MHz): 183.0 (C(4)); 164.6 (C(2)); 164.0 (C(7)); 162.0 (C(5)); 158.0 (C(9)); 133.1 (C(4′)); 131.4 (C(1′)); 130.1 (C(3′), C(5′)); 127.4 (C(2′), C(6′)); 106.4 (C(3)); 106.3 (C(10)); 100.7 (C(1″)); 100.5 (C(6)); 95.8 (C(8)); 78.0 (C(5″)); 77.3 (C(3″)); 73.9 (C(2″)); 70.4 (C(4″)); 61.4 (C(6″)). FAB-MS (pos. mode): 417.1.

*5-Hydroxy-2-phenyl-7-(tetra-*O*-acetyl-β-*D*-galactopyranosyloxy)-4*H*-1-benzopyran-4-one.* Synthesized according to the procedure described above for *5-hydroxy-2-phenyl-7-(tetra-*O*-acetyl-β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one* with tetra-*O*-acetyl-α-D-galactopyranosyl bromide as the glycosyl donor. Yield 81% (orange powder). TLC (AcOEt/petroleum ether 2 : 3): R_f 0.54. HPLC (AcOEt/petroleum ether 1 : 1): t_R 6.2 min. ^{1}H-NMR ($CDCl_3$/300 MHz): 12.73 (*s*, HO–C(5)); 7.89 (*d*, *J* = 5.9, H–C(2′), H–C(6′)); 7.58–7.51 (*m*, H–C(3′), H–C(4′), H–C(5′)); 6.70 (*s*, H–C(3)); 6.63 (*d*, *J* = 2.1, H–C(8)); 6.48 (*d*, *J* = 2.1, H–C(6)); 5.56–5.49 (*m*, H–C(4″), H–C(3″)); 5.16 (*d*, *J* = 7.4, H–C(1″)); 5.14 (H–C(2″), partially masked by H–C(1″)); 4.24 (*dd*, *J* = 11.0, 5.2, H_a–C(6″)); 4.17–4.13 (*m*, H–C(5″), H_b–C(6″)); 2.20 (*s*, Ac); 2.10 (*s*, 2 Ac); 2.03 (*s*, Ac). ^{13}C-NMR ($CDCl_3$, 75 MHz): 183.0 (C(4)); 170.9 (CO of Ac); 170.6 (CO of Ac); 170.5 (CO of Ac); 169.7 (CO of Ac); 164.8 (C(2)); 162.8 (C(7)); 162.6 (C(5)); 157.8 (C(9)); 132.5 (C(4′)); 131.5 (C(1′)); 129.6 (C(3′), C(5′)); 126.7 (C(2′), C(6′)); 107.4 (C(3)); 106.4 (C(10)); 100.3 (C(1″)); 99.1 (C(6)); 95.9 (C(8)); 71.9 (C(5″)); 71.1 (C(3″)); 68.7 (C(2″)); 67.4 (C(4″)); 61.9 (C(6″)); 21.1 (Me).

*7-(β-*D*-Galactopyranosyloxy)-5-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one.* Synthesized according to the procedure described for **1**. Yield 97% (orange powder). TLC (AcOEt/butanone/HCOOH/H_2O 10 : 2 : 1 : 1): R_f 0.62. ^{1}H-NMR ((D_6)DMSO, 300 MHz): 8.09 (*d*, *J* = 5.9, H–C(2′), H–C(6′)); 7.64–7.57 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.06 (*s*, H–C(3)); 6.87 (*d*, *J* = 2.2, H–C(8)); 6.48 (*d*, *J* = 2.2, H–C(6)); 5.04 (*d*, *J* = 7.4, H–C(1″)); 3.70–3.40 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), 2 H–C(6″)). ^{13}C-NMR ($CDCl_3$ (D_6)DMSO 9 : 1, 75 MHz): 183.0 (C(4)); 164.5 (C(2)); 164.2 (C(7)); 162.0 (C(5)); 158.0 (C(9)); 133.1 (C(4′)); 131.5 (C(1′)); 130.0 (C(3′), C(5′)); 127.4 (C(2′), C(6′)); 106.4 (C(3)); 106.3 (C(10)); 101.3 (C(1″)); 100.5 (C(6)); 95.8 (C(8)); 76.6 (C(5″)); 74.0 (C(3″)); 70.9 (C(2″)); 68.9 (C(4″)); 61.1 (C(6″)). FAB-MS (pos. mode): 417.1.

*5-Hydroxy-7-[6-*O*-(*p*-methoxycinnamyl)-β-*D*-glucopyranosyloxy]-2-phenyl-4*H*-1-benzopyran-4-one* (**2**). *p*-Methoxycinnamyl chloride (94 mg, 0.48 mmol) was added in several portions to a soln. of **1** (100 mg, 0.24 mmol) and DMAP (15 mg, 0.12 mmol) in anh. pyridine (20 ml) under N_2. After stirring for 96 h at r.t., pyridine was removed under vacuum. Addition of acetone (20 ml), neutralization by *Dowex* (H^+ form), filtration, removal of the solvent under vacuum, and FC (silica gel; AcOEt/petroleum ether 7 : 3 to pure AcOEt), **2** (43 mg, 31%) was obtained as a white powder, as well as unreacted **1** (25 mg). UV/VIS (MeOH): 271, 309. TLC (AcOEt): R_f 0.33. HPLC (AcOEt/petroleum ether 7 : 3): t_R 15.1 min. ^{1}H-NMR ((D_6)DMSO, 300 MHz): 12.82 (*s*, HO–C(5)); 8.07 (*d*, *J* = 7.4, H–C(2′), H–C(6′)); 7.65–7.54 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.53 (*d*, *J* = 16.2, H–C(β)); 7.46 (*d*, *J* = 8.8, H–C(2), H–C(6), of acyl); 7.03 (*s*, H–C(3)); 6.87 (*d*, *J* = 2.2, H–C(8)); 6.78 (*d*, *J* = 8.8, H–C(3), H–C(5) of acyl); 6.49 (*d*, *J* = 2.2, H–C(6)); 6.41 (*d*, *J* = 16.2, H–C(α)); 5.19 (*d*, *J* = 6.6, H–C(1″)); 4.85 (*d*, *J* = 4.4, HO–C(2″)); 4.64–4.60 (*m*, HO–C(3″), HO–C(4″)); 4.49 (*d*, *J* = 11.8, $H_{pro\text{-}S}$–C(6″)); 4.16 (*dd*, *J* = 11.8, 7.4, $H_{pro\text{-}R}$–C(6″)); 3.76 (*s*, MeO); 3.40–3.70 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″)). ^{13}C-NMR ((D_6)DMSO, 75 MHz): 183.0 (C(4)); 167.1 (CO of acyl); 164.4 (C(2)); 163.8 (C(7)); 161.9 (C(5)); 157.9 (C(9)); 145.3 (C(β)); 133.0 (C(4′)); 131.3 (C(1′)); 130.8 (C(4) of acyl); 130.7 (C(2), C(6) of acyl); 129.9 (C(3′), C(5′)); 127.2 (C(2′), C(6′)); 115.8 (C(α)); 115.2 (C(1) of acyl); 115.0 (C(3), C(5) of acyl); 106.5 (C(3));

106.2 (C(10)); 100.6 (C(1″)); 100.2 (C(6)); 95.6 (C(8)); 77.1 (C(5″)); 14.7 (C(3″)); 73.8 (C(2″)); 70.8 (C(4″)); 64.3 (C(6″)); 56.1 (MeO). ESI-MS (pos. mode): 577.1.

*5-Hydroxy-2-phenyl-7-[6-*O*-(3,4,5-trimethoxybenzoyl)-β-*D*-glucopyranosyloxy]-4*H*-1-benzopyran-4-one* (**3**). From **1** as described for **2** with 3,4,5-trimethoxybenzoyl chloride as the acylating reagent. Yield 30%. White powder. UV (MeOH): 266, 303. TLC (AcOEt/butan-2-one/HCOOH/H_2O 20:2:1:1): R_f 0.60. HPLC (AcOEt/petroleum ether 1:1): t_R 21.3 min. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 7.87 (*d*, *J* = 7.4, H–C(2′), H–C(6′)); 7.61–7.47 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.11 (*s*, H–C(2), H–C(6) of acyl); 6.96 (*s*, H–C(3)); 6.80 (*d*, *J* = 2.2, H–C(8)); 6.45 (*d*, *J* = 2.2, H–C(6)); 5.19 (*d*, *J* = 6.6, H–C(1″)); 4.77 (*d*, *J* = 11.8, $H_{pro\text{-}S}$–C(6)); 4.17 (*dd*, *J* = 11.8, 8.1, $H_{pro\text{-}R}$–C(6)); 3.93 (*t*, *J* = 8.1, H–C(5″)); 3.60 (*s*, 2 *m*-MeO); 3.50 (*s*, *p*-MeO); 3.40–3.20 (*m*, H–C(2″), H–C(3″), H–C(4″)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 182.7 (C(4)); 166.0 (CO of acyl); 164.4 (C(2)); 163.6 (C(7)); 161.6 (C(5)); 157.9 (C(9)); 153.4 (C(3), C(5) of acyl); 142.4 (C(4) of acyl); 132.5 (C(4′)); 131.1 (C(1′)); 129.9 (C(3′), C(5′)); 127.1 (C(2′), C(6′)); 125.5 (C(1) of acyl); 107.1 (C(2), C(6) of acyl); 106.3 (C(3)); 106.1 (C(10)); 100.4 (C(1″)); 99.7 (C(6)); 95.5 (C(8)); 76.7 (C(5″)); 74.5 (C(3″)); 73.5 (C(2″)); 71.6 (C(4″)); 65.7 (C(6″)); 60.6 (*p*-MeO); 56.5 (2 *m*-MeO). ESI-MS (pos. mode): 611.1.

*5-Hydroxy-2-phenyl-7-(6-*O*-succinyl-β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one* (**4**). From **1** as described for **2** with succinic anhydride (2 equiv.) as the acylating reagent. After chromatography (silica gel; AcOEt/petroleum ether 9:1 to AcOEt/MeOH 9:1), the 6″-*O*-acyl (**4**, yield 16%) and the 3″-O-acyl (yield 5%) regioisomers were obtained.

Data of **4**. TLC (AcOEt/butan-2-one/HCOOH/H_2O 20:2:1:1): R_f 0.50. HPLC (AcOEt/petroleum ether 1:1): t_R 15.3 min. UV/VIS (MeOH): 268, 305. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 8.06 (*d*, *J* = 5.9, H–C(2′), H–C(6′)); 7.60–7.57 (*m*, H–C(3′), H–C(4′), H–C(5′)); 6.98 (*s*, H–C(3)); 6.85 (*d*, *J* = 2.2, H–C(8)); 6.46 (*d*, *J* = 2.2, H–C(6)); 5.08 (*d*, *J* = 7.4, H–C(1″)); 4.38 (*d*, *J* = 10.3, $H_{pro\text{-}S}$–C(6″)); 3.60–3.10 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), $H_{pro\text{-}R}$–C(6″)); 2.50 (br. *s*, 2 CH_2). MS (positive mode): 517.1.

*5-Hydroxy-2-phenyl-7-(3-*O*-succinyl-β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one.* TLC (AcOEt/butan-2-one/HCOOH/H_2O 20:2:1:1): R_f 0.78. ^{1}H-NMR ($CDCl_3$/(D$_6$)DMSO 9:1, 300 MHz): 12.55 (*s*, HO–C(5)); 7.75 (*d*, *J* = 5.9, H–C(2′), H–C(6′)); 7.42–7.37 (*m*, H–C(3′), H–C(4′), H–C(5′)); 6.53 (*s*, H–C(3), H–C(8)); 6.34 (*s*, H–C(6)); 5.00–4.94 (*m*, H–C(1″), H–C(3″)); 3.75–3.45 (*m*, H–C(2″), H–C(4″), H–C(5″), 2 H–C(6″)); 2.42 (br. *s*, 2 CH_2).

*5-Hydroxy-2-phenyl-7-(2,3,4,6-tetra-*O*-succinyl-β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one.* From **1** as described for **2** with succinic anhydride (4 equiv.) as the acylating reagent in pyridine at 70°. Yield 90%. Yellow powder. TLC (AcOEt/butan-2-one/HCOOH/H_2O 20:2:1:1): R_f 0.87. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 8.07–8.04 (*d*, *J* = 6.6, H–C(2′), H–C(6′)); 7.62–7.55 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.01 (*s*, H–C(3)); 6.84 (*d*, *J* = 2.2, H–C(8)); 6.46 (*d*, *J* = 2.2, H–C(6)); 5.70 (*d*, *J* = 7.4, H–C(1″)); 5.44 (*t*, *J* = 9.5, H–C(3″)); 5.15 (*dd*, *J* = 7.4, 9.5, H–C(2″)); 5.04 (*t*, *J* = 9.5, H–C(4″)); 4.33 (*dd*, *J* = 10.3, 5.1, $H_{pro\text{-}R}$–C(6″)); 4.20–4.10 (*m*, H–C(5″), $H_{pro\text{-}S}$–C(6″)); 2.52–2.43 (*m*, 8 CH_2). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 183.0 (C(4)); 173.6 (2 CO_2H); 173.1 (2 CO_2H); 172.6 (CO of acyl); 172.3 (CO of acyl); 172.2 (CO of acyl); 171.9 (CO of acyl); 164.8 (C(2)); 162.7 (C(7)); 161.8 (C(5)); 157.9 (C(9)); 133.2 (C(4′)); 131.3 (C(1′)); 130.0 (C(3′), C(5′)); 127.3 (C(2′), C(6′)); 107.0 (C(3)); 106.4 (C(10)); 100.5 (C(1″)); 97.4 (C(6)); 96.2 (C(8)); 72.2 (C(5″)); 72.1 (C(3″)); 71.1 (C(2″)); 68.8 (C(4″)); 62.9 (C(6″)); 29.5–29.2 (CH_2). FAB-MS (pos. mode): 816.9.

Flavone-Aluminium Complexation. In a typical experiment, a freshly prepared 10^{-4}M soln. of flavone in MeOH was diluted twice into a 0.2M acetate buffer (final pH 5.3). In one part of this soln. $AlCl_3 \cdot 6H_2O$ was added to a final concentration of 5×10^{-3}M. If necessary, the pH was brought back to 5.3 upon addition of conc. NaOH (without significant dilution). The flavone and flavone-aluminium solns. were then mixed in different ratios in order to prepare samples having intermediate Al^{3+} concentrations for spectral measurements.

Flavone-BSA Complexation. Quercetin soln. (250 µl, 10^{-2}M) in MeOH was added to 25 ml of a 7.52×10^{-5}M soln. of BSA (5 mg/ml) in 0.02M Na_2HPO_4 phosphate buffer (pH 7.4, *I* adjusted to 0.2M by NaCl addition). Aliquots (10 µl) of a 10^{-2}M soln. of chrysin or of a 4×10^{-3}M soln. of **1**, **2**, or the methyl of *p*-methoxycinnamate in DMSO/MeOH 1:1 were added to 2 ml of the quercetin/BSA soln. Emission spectra (excitation at 450 nm) were recorded after each addition.

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.

Calculations. Semi-empirical quantum-mechanics calculations (AM1) and molecular-mechanics calculations (MM+) were run at 0 K in vacuum on a *Pentium 90* PC with the HyperChem program (Autodesk, Sausalito, California, USA). In a first step, AM1 calculations were run on 5-*O*-methylchrysin, methyl *p*-methoxycinnamate, and methyl 3,4,5-trimethoxybenzoate for a reliable estimation of charge distributions.

The optimized chrysin and aromatic acid moieties were incorporated into the structures of flavones **2** and **3**, the energies of which were then minimized by the MM+ force field. The energy-minimization procedure was repeated with different sets of input data files for the torsion angles about the C(1″)–O bond (glycosidic bond), the C(5″)–C(6″) and C(6″)–O bonds (D-glucose moiety) and the C(7)–O bond (chrysin moiety).

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