Semisynthesis of Linarin, Acacetin, and 6-Iodoapigenin Derivatives from Diosmin

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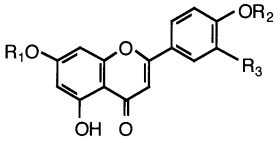
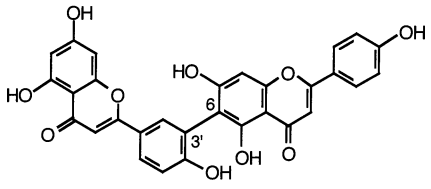
Semisynthesis of linarin and acacetin from the *Citrus* flavonoid diosmin was performed via, as first intermediate, the 3′-*O*-phenyltetrazolyl ether of diosmin. This paper relates also a semisynthetic access to 6-iodoapigenin derivatives, which are key compounds in the synthesis of some biflavonoids such as robustaflavone.

Diosmin (diosmetin 7-*O*-rutinoside) (**1**) is a natural flavone glycoside therapeutically used to improve the symptoms of venous and lymphatic vessel insufficiency. Diosmin is readily obtained by dehydrogenation of the corresponding flavanone glycoside, hesperidin, that is abundant in the pericarp of various *Citrus*.[1] The readily availability of **1** or its aglycone diosmetin (**2**) from the low-priced hesperidin makes these flavones excellent starting materials for semisynthetic modifications. In previous papers, we described the preparation of original flavones endowed with new biological activities compared with **1** and **2** (modulation of multidrug resistance, inhibition of phosphodiesterases IV).[2,3] The purpose of this present study is twofold and consists of the semisynthetic access to linarin (**3**) on one hand and to 6-iodoapigenin derivatives on the other hand. Linarin is the main active compound of some medicinal plants such as *Buddleia cordata* from Mexico[4] and *Chrysanthemum zawadskii* var. *latilobum* from Korea.[5] This flavonoid was shown to possess antipyretic, analgesic, and anti-inflammatory activities, which could account for some indications of these plants in folk medicine.[4,5] More recently, linarin was identified in the well-known species in herbal medicine *Valeriana officinalis*, and its sedative and sleep-enhancing properties were described for the first time.[6] 6-Iodoapigenin derivatives are known to be key intermediates for access to some biflavonoids with two apigenin (**4**) units connected via at least one 6-position such as robustaflavone (**5**), an inhibitor of hepatitis B virus (HBV).[7]

Synthesis of linarin (**3**) or 6-iodoapigenin derivatives from diosmin (**1**) requires removal of the 3′-hydroxyl group in a two-step deoxygenation sequence. Two distinct methods were conceivable through reduction of either a sulfonate ester[8] or an electron-withdrawing ether such as phenyltetrazolyl ether.[9] The unlikely regiospecific sulfonation of the 3′-hydroxy group in the presence of the rutinosyl moiety prompted consideration of 3′-OH ether formation (Scheme 1). Treatment of diosmin (**1**) with 5-chloro-1-phenyl-1*H*-tetrazole (DMF, $KHCO_3$, 80 °C, 2 h) gave the tetrazolyl ether (**6**) as the main compound. **6** could be isolated and identified as a pure compound after crystallization from methanol (ESIMS *m/z* 775 $[M + Na]^+$; mp 175−177 °C; 40% yield). However, according to TLC (silica gel, CH_2Cl_2−MeOH, 85:15), the crude, dried residue of **6** could be submitted to the next step without further purification. Hydrogenolysis of the tetrazolyl ether (**6**) was investigated under various conditions (H_2/Pd−C in a Parr apparatus, transfer hydrogenation with hydrazine, formic acid, or ammonium formate).[9,10] The best results were observed with 10% Pd−C and ammonium formate in refluxing methanol, which cleaved the C3′−O bond within 4.5 h. Workup of the reaction allowed isolation of pure linarin (**3**); however, the majority of this compound remained either adsorbed on or crystallized within the charcoal after filtration, which was then selectively eluted with DMF. The identification of **3** was done by comparison (physical and spectroscopic data) with an authentic sample. The overall yield of the conversion of diosmin to linarin without isolation of the intermediate tetrazolyl ether was 53%.



	R_1	R_2	R_3
1	β-rutinosyl	Me	OH
2	H	Me	OH
3	β-rutinosyl	Me	H
4	H	H	H
9	H	Me	H



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With access to linarin, the second aim of this study was the synthesis of 6-iodoapigenin derivatives. The regioselective 6-iodination was accomplished with benzyltrimethylammonium dichloroiodate (BTMA·ICl_2)[11] according to a method recently developed in our laboratory.[12] Linarin was first converted to 5-hydroxyhexaacetyllinarin (**7**) in two steps: (a) Ac_2O−pyridine, rt, 48 h; (b) TFA, rt, 6 h;[13] 88%). Reaction of **7** with 1 equiv of BTMA·ICl_2 (CH_2Cl_2−MeOH, $CaCO_3$, rt, 24 h) led to formation of the amorphous compound **8** in 98% yield. **8** was homogeneous on TLC (silica gel, CH_2Cl_2−MeOH, 98:2), but its ^1H NMR spectrum

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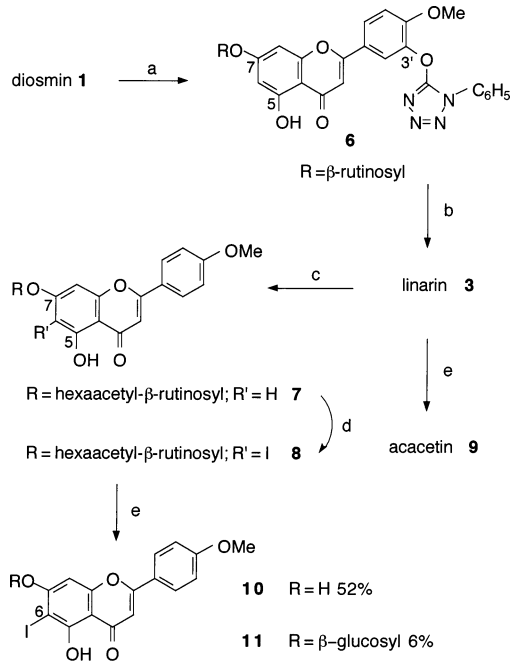
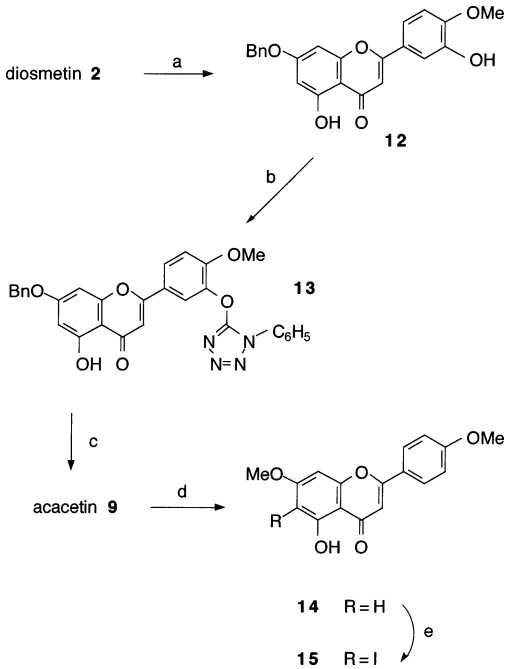
Scheme 1[a]



[a] Reagents: (a) $KHCO_3$, 5-chloro-1-phenyl-1*H*-tetrazole in DMF; (b) Pd−C, $HCOONH_4$ in MeOH; (c) Ac_3O in pyridine; TFA; (d) BTMA·ICl_2, $CaCO_3$ in CH_2Cl_2−MeOH; (e) NaOH−THF; HCl until pH 4; removal of THF; naringinase; (e) HCl 11 N.

Scheme 2[a]



[a] Reagents: (a) $KHCO_3$, benzyl chloride in DMF; (b) $KHCO_3$, 5-chloro-1-phenyl-1*H*-tetrazole in DMF; (c) Pd−C, $HCOONH_4$ in MeOH; (c) K_2CO_3 MeI in DMF; (e) BTMA,ICl_2, $CacO_3$ in CH_2Cl_2−MeOH.

displayed a mixture of two compounds in a 92:8 ratio [easily inferred from the ^{1}H NMR spectrum by comparing integration of signals of the hydrogen-bonded phenolic proton at 13.90 (major) and 14.05 ppm (minor)]. The iodo substitution at C-6 for the major compound was proven by comparison of HMQC and HMBC NMR experiments on **7** and **8**, which indicated a strong shielding by the iodine of C-6 at δ 71.0 ppm for **8** (99.5 ppm for **7**), Finally, the observation in the ESI mass spectrum of **8** of a single peak at *m*/*z* 993 [M + Na]$^+$ confirmed **8** to be a mixture of two derivatives, monoiodinated at C-6 for the major product and, by analogy with our previous results,[12] at C-8 for the minor component. The last step of the sequence was the hydrolysis of the glycosidic bond, which was not expected to pose problems. However, the various acid hydrolytic conditions mainly gave rise to acacetin (**9**), resulting from a protodehalogenation mechanism.[14] This unwanted result prompted us to turn to enzymatic hydrolysis. Compound **8** was submitted sequentially in a one-pot procedure to saponification (THF−1 N NaOH, rt, 3 h), then, after removal of THF and adjustment of the medium to pH 4, to the action of naringinase for 120 h at 40 °C. Successive extractions of the reaction with CH_2Cl_2 and EtOAc afforded 6-iodoacacetin (**10**) (EIMS *m*/*z* 410 M$^+$; mp 254−256 °C; 52% yield) and 6-iodoacacetin 7-*O*-glucoside (**11**) (ESIMS *m*/*z* 595 (M + Na)$^+$; mp 239−240 °C; 6% yield) resulting from a partial hydrolysis. A detailed NMR study (HMQC, HMBC, NOESY) of **10** unambiguously confirmed the 6-iodo substitution. The protodehalogenation encountered during the acid hydrolysis indicated that it is more judicious to carry out the 6-iodination step with an aglycone rather than a glycoside. Therefore, acacetin (**9**) was easily prepared by acid hydrolysis of linarin (HCl 11 N, 50 °C, 1.5 h, 70%).

An alternative pathway to acacetin (**9**) from the readily available diosmetin (**2**) was also performed via the isolated intermediate compounds 7-benzyldiosmetin (**12**) and 7-benzyl-3′-phenyltetrazolyldiosmetin (**13**) in 30% overall yield (Scheme 2). Last, methylation of **9** at the 7-position (K_2CO_3, MeI, DMF, rt) afforded 7,4′-dimethylapigenin (**14**) (95%), which was iodinated to 7,3′-dimethyl-6-iodoapigenin (**15**) (73%).[12]

To the best of our knowledge, this work describes the first semisynthesis of linarin and acacetin from an easily available citroflavonoid and allows access to key intermediates in the synthesis of some biflavonoids.

Experimental Section

General Experimental Procedures. Melting points were determined with a micro-Koffler and are uncorrected. ^{1}H NMR spectra, NOESY experiments, and ^{1}H−^{1}H (COSY) and ^{1}H−^{13}C (HMQC and HMBC) were performed with a Bruker AM-400. EIMS were registered on an Automass Thermoquest with EI source (70 eV) and ESIMS on a Navigator Aqa thermoquest with an ES source (MeOH, flow rate 5 μL/min) (70 eV). TLC data were obtained with Merck 60 F 254 silica precoated on aluminum sheets. Samples of linarin and acacetin were from Extrasynthese.

Linarin (3) from Diosmin (1). To a suspension of **1** (4.864 g, 8 mmol) and $KHCO_3$ (1.6 g, 16 mmol) in DMF (100 mL) was added 5-chloro-1-phenyl-1*H*-tetrazole (2.98 g, 16 mmol) and the mixture stirred under nitrogen for 2 h at 80 °C. The reaction mixture was cooled, filtered, and evaporated to dryness. One-hundredth of the dried residue (78 mg) was crystallized from MeOH and provided **6** (24 mg, 40%).

3′-*O*-(1-Phenyltetrazol-5-yl)diosmin (6): pale-yellow crystals; mp 175−177 °C; ^{1}H NMR (DMSO-d_6) δ [aglycone moiety] 3.88 (3H, s, OCH_3-4′), 6.47 (1H, d, J = 2 Hz, H-6), 6.81 (1H, d, J = 2 Hz, H-8), 7.00 (1H, s, H-3), 7.48 (1H, d, J = 8.8 Hz, H-5′), 7.62 (1H, t, J = 8.8 Hz, H-4 phenyltetrazole), 7.70 (2H, t, J = 8.8 Hz, H-3 and H-5 phenyltetrazole), 7.87 (2H, d, J = 8.8 Hz, H-2 and H-6 phenyltetrazole), 8.12 (1H, dd, J = 8.8 and 2 Hz, H-6′), 8.34 (1H, d, J = 2 Hz, H-2′), 12.8 (1H, s, OH-5); [sugar moiety] inner glucose 3.18 (1H, H-4″), 3.29 (2H, H-2″ and H-3″), 3.47 (1H, H-6″), 3.58 (1H, H-5″), 3.86 (1H, H-6″), 5.06 (1H, d, J = 7.2 Hz, H-1″); terminal rhamnose 1.07 (3H, d, J = 6.4 Hz, H-6‴), 3.18 (1H, H-4‴), 3.40 (1H, H-5‴), 3.47 (1H, H-3‴), 3.68 (H-2‴), 4.55 (1H, s, H-1‴); 4.44, 4.59, 4.68, 5.18, 5.20, 5.43 (6H, sugar hydroxyls); ^{13}C NMR (DMSO-d_6) δ [aglycone moiety] 56.0 (CH_3, OCH_3-4′), 94.5 (CH, C-8), 99.2 (CH, C-6), 104.4 (CH, C-3), 105.4 (C, C-10), 113.8 (CH, C-5′), 120.0 (CH, C-2′), 122.8 (CH, C-2 and C-6 phenyltetrazole),

122.9 (C, C-1′), 126.2 (CH, C-6′), 129.9 (CH, C-4 phenyltetrazole), 130.1 (CH, C-3 and C-5 phenyltetrazole), 142.0 (C, C-3′), 153.1 (C, C-4′), 156.8 (C, C-9), 162.4 (C, C-2), 163.0 (C, C-7), 181.8 (C, C-4); [sugar moiety] inner glucose 66.1 (CH_2, C-6″), 69.5[a] (CH, C-4″), 72.8 (CH, C-2″), 75.6 (CH, C-5″), 76.1 (CH, C-3″), 99.8 (CH, C-1″); terminal rhamnose 17.6 (CH_3, C-6‴), 68.1 (CH, C-5‴), 70.1[a] (CH, C-2‴), 70.2[a] (CH, C-3‴), 72.1 (CH, C-4‴), 100.5 (CH, C-1‴), [a]interchangeable; ESIMS *m/z* 775 $[M + Na]^+$; HRESIMS *m/z* 775.2120 (calcd for $C_{35}H_{36}N_4O_{15}Na$, 775.2075).

A solution of the remaining dried residue in MeOH (500 mL) was added to ammonium formate (2.52 g, 40 mmol) and 10% Pd−C (1.75 g) and the mixture stirred at reflux under nitrogen for 5 h. The reaction mixture was cooled, and the catalyst was separated and washed with MeOH (twice 50 mL) then DMF until the filtrate was colorless. Evaporation to dryness of DMF afforded pure linarin (**3**) (2.485 g, 53%) as an off-white powder.

Linarin (3): mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ [aglycone moiety] 3.86 (3H, s, OCH_3-4′), 6.49 (1H, d, J = 2 Hz, H-6), 6.78 (1H, d, J = 2 Hz, H-8), 6.92 (1H, s, H-3), 7.14 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 8.04 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 12.9 (1H, s, OH-5); [sugar moiety] 1.10 (3H, d, J = 6.4 Hz, H-6‴), 3.1−3.9 (10 sugar protons) 4.52 (1H, s, H-1‴), 5.04 (1H, d, J = 7.2 Hz, H-1″), 4.40, 4.52, 4.64, 5.12, 5.17, 5.40 (6H, sugar hydroxyls); ^{13}C NMR (DMSO-d_6) δ [aglycone moiety] 55.7 (CH_3, OCH_3-4′), 95.0 (CH, C-8), 99.9 (CH, C-6), 104.0 (CH, C-3), 105.7 (C, C-10), 114.9 (CH, C-3′ and C-5′), 122.8 (C, C-1′), 128.6 (CH, C-2′ and C-6′), 157.1 (C, C-9), 161.3 (C, C-5), 162.6 (C, C-4′), 163.1 (C, C-7), 164.1 (C, C-2), 182.2 (C, C-4); [sugar moiety] inner glucose 66.3 (CH_2, C-6″), 69.8[a] (CH, C-4″), 73.3 (CH, C-2″), 75.9 (CH, C-5″), 76.5 (CH, C-3″), 100.2 (CH, C-1″); terminal rhamnose 18.0 (CH_3, C-6‴), 68.5 (CH, C-5‴), 70.6[a] (CH, C-2‴), 71.2[a] (CH, C-3‴), 72.3 (CH, C-4‴), 100.7 (CH, C-1‴), [a]interchangeable; ESIMS *m/z* 615 $[M + Na]^+$.

6-Iodoacacetin (10) from Linarin (3). A suspension of linarin (**3**) (0.850 g, 1.44 mmol) in a mixture of pyridine−Ac_2O (6:4) (10 mL) was stirred at 80 °C until dissolution. The reaction was left for 48 h at room temperature. A standard workup of the mixture provided a dry residue of pure linarin heptaacetate [TLC on silica gel (CH_2Cl_2−MeOH, 98:2): homogeneous colorless spot with $FeCl_3$] (1.193 g, 94%). The residue was dissolved in TFA (10 mL), and the mixture was left at room temperature for 6 h. The medium was diluted with iced water and carefully adjusted to pH 6 with 30% aqueous NaOH. Extraction with CH_2Cl_2 afforded 5-hydroxyhexaacetyllinarin (**7**) [TLC on silica gel (CH_2Cl_2−MeOH, 98:2): homogeneous blue spot with $FeCl_3$] (1.068 g, 94%).

5-Hydroxyhexaacetyllinarin (7): yellowish amorphous powder; ^{1}H NMR ($CDCl_3$) δ [aglycone moiety] 3.90 (3H, s, OCH_3-4′), 6.42 (1H, d, J = 2 Hz, H-6), 6.56 (1H, d, J = 2 Hz, H-8), 6.59 (1H, s, H-3), 7.03 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.84 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 12.85 (1H, s, OH-5); [sugar moiety] inner glucose 3.67 (1H, H-6″), 3.82 (1H, H-6″), 3.92 (1H, H-5″), 5.18 (1H, H-4″), 5.23 (1H, H-1″), 5.25[a] (1H, H-2″), 5.35 (1H, H-3″); terminal rhamnose 1.15 (3H, d, J = 6.4 Hz, H-6‴), 3.88 (1H, H-5‴), 4.73 (1H, s, H-1‴), 5.02 (1H, H-4‴), 5.23[a] (1H, H-2‴), 5.26[a] (H-3‴); 1.93−2.12 (18H, 6s, 6 sugar acetyles), [a]interchangeable; ^{13}C NMR ($CDCl_3$) δ [aglycone moiety] 55.0 (CH_3, OCH_3-4′), 95.0 (CH, C-8), 99.5 (CH, C-6), 104.1 (CH, C-3), 106.6 (C, C-10), 114.2 (CH, C-3′ and C-5′), 123.0 (CH, C-2′), 128.0 (CH, C-2′ and C-6′), 157.0 (C, C-9), 162.0 (C, C-7), 162.0 (C, C-5), 162.4 (C, C-4′), 164.6 (C, C-2), 182.1 (C, C-4); [sugar moiety] inner glucose 66.4 (CH_2, C-6″), 68.3 (CH, C-4″), 70.0 (CH, C-3″), 71.5[a] (CH, C-2″), 73.1 (CH, C-5″), 97.8 (CH, C-1″); terminal rhamnose 17.1 (CH_3, C-6‴), 66.2 (CH, C-5‴), 68.4[a] (CH, C-2‴), 70.6 (CH, C-4‴), 72.3[a] (CH, C-3‴), 97.8 (CH, C-1‴); 19−21 and 168−171 (6 sugar acetyl groups), [a]interchangeable.

A mixture of **7** (0.633 g, 0.75 mmol), BTMA.ICl_2 (0.263 g, 0.75 mmol), and $CaCO_3$ (0.54 g) in CH_2Cl_2−MeOH, 5:2 (35 mL), was stirred at room temperature for 24 h. The reaction mixture was taken up in water and extracted at pH 6 with CH_2Cl_2. Standard workup of the organic layer provided an amorphous residue of compound **8** [TLC on silica gel (CH_2Cl_2−MeOH, 98:2): homogeneous blue spot with $FeCl_3$] (0.719 g, 99%).

5-Hydroxy-6-iodohexaacetyllinarin (8): yellowish amorphous powder; ^{1}H NMR ($CDCl_3$) δ [aglycone moiety] 3.90 (3H, s, OCH_3-4′), 6.66 (2H, s, H-3 and H-8), 7.04 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.86 (major) and 8.01 (minor) (2H, d, J = 8.8 Hz, H-2′ and H-6′), 13.90 (major) and 14.05 (minor) (1H, s, OH-5); [sugar moiety] 1.15 (3H, d, J = 6.4 Hz, H-6‴), 3.7−5.4 (12 sugar protons); 1.84−2.10 (18H, 6s, 6 sugar acetyles); ^{13}C NMR ($CDCl_3$) δ [aglycone moiety] 55.5 (CH_3, OCH_3-4′), 71.0 (C, C-6), 93.9 (CH, C-8), 104.4 (CH, C-3), 106.2 (C, C-10), 114.2 (CH, C-3′ and C-5′), 123.1 (CH, C-2′), 128.0 (CH, C-2′ and C-6′), 157.4 (C, C-9), 161.0 (C, C-5), 162.5 (C, C-4′), 164.5 (C, C-2), 181.5 (C, C-4); [sugar moiety] inner glucose 65.9 (CH_2, C-6″), 69.0 (CH, C-4″), 70.6 (CH, C-3″), 72.5 (CH, C-2″), 73.1 (CH, C-5″), 98.9 (CH, C-1″); terminal rhamnose 17.0 (CH_3, C-6‴), 66.1 (CH, C-5‴), 67.3[a] (CH, C-2‴), 68.8[a] (CH, C-3‴), 70.4 (CH, C-4‴), 97.7 (CH, C-1‴); 20−22 and 167−172 (6 sugar acetyl groups), [a]interchangeable; ESIMS *m/z* 993 $[M + Na]^+$.

A solution of **8** (0.194 g, 0.2 mmol) in a mixture of THF−0.1 N aqueous NaOH (1:2) (15 mL) was stirred at room temperature for 3 h. THF was removed under vacuum, then the medium was adjusted to pH 4 with 1 N aqueous HCl. To the reaction mixture was then added naringinase (Sigma no. 1385) (45 mg) and stirred at 40 °C for 5 days. The resulting suspension was centrifuged, then the supernatant extracted by EtOAc. The centrifugate and the organic phase were evaporated under vacuum to dryness (96 mg). A solution of this dried residue in THF (5 mL) was diluted with H_2O (100 mL) and then extracted with CH_2Cl_2 (5 × 30 mL) and then EtOAc (3 × 30 mL). The separate standard workup of both organic phases and crystallization of each residue from EtOH provided **10** (43 mg, 52%) and **11** (7 mg, 6%) as pale yellow crystals from the CH_2Cl_2 and EtOAc extracts, respectively.

6-Iodoacacetin (10): mp 254−256 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (3H, s, OCH_3-4′), 6.68 (1H, s, H-8), 6.94 (1H, s, H-3), 7.10 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 8.04 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 14.0 (1H, s, OH-5); ^{13}C NMR (DMSO-d_6) δ 55.0 (CH_3, OCH_3-4′), 69.3 (C, C-6), 92.7 (CH, C-8), 102.5 (CH, C-3), 103.0 (C, C-10), 113.9 (CH, C-3′ and C-5′), 121.9 (C, C-1′), 127.7 (CH, C-2′ and C-6′), 156.8 (C, C-9), 160.6 (C, C-5), 161.8 (C, C-4′), 162.1 (C, C-7), 162.8 (C, C-2), 181.0 (C, C-4); EIMS *m/z* (%) 410 (M^+) (100).

6-Iodoacacetin 7-*O*-glucoside (11): mp 239−240 °C; ^{1}H NMR (DMSO-d_6) δ 3.1−3.7 (6 sugar protons), 3.84 (3H, s, OCH_3-4′), 4.5−5.3 (4 sugar hydroxyls), 5.02 (1H, d, J = 7 Hz, H-1″), 6.99 and 7.04 (2H, 2s, H-3 and H-8), 7.10 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 8.04 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 13.9 (1H, s, OH-5); ESIMS *m/z* 595 $[M + Na]^+$; HRESIMS *m/z* 595.0068 (calcd for $C_{22}H_{21}N_4O_{10}NaI$, 595.0077).

Acacetin (9) from Linarin (3). Linarin (118 mg, 0.2 mmol) in aqueous 11 N HCl (10 mL) was stirred between 50 and 55 °C for 1.5 h and left for 2 h at room temperature. The resulting suspension was filtered, washed several times with water, and dried with P_2O_5 under vacuum to yield a crude residue of acacetin, which was crystallized from MeOH (40 mg, 70%).

Acacetin (9) from Diosmetin (2). To a mixture of diosmetin (3 g, 10 mmol) and $KHCO_3$ (1.1 g, 11 mmol) in DMF (40 mL) was added benzyl chloride (1.38 mL, 12 mmol) and the mixture stirred under nitrogen for 2.5 h at 120 °C. The reaction mixture was cooled, filtered, and evaporated to dryness. The dried residue was purified by flash chromatography (silica gel, CH_2CH_2−MeOH, 99:1) to provide pure 7-benzyldiosmetin (**12**) (2.58 g, 66%) as yellow crystals and 7,3′-dibenzyldiosmetin (0.575 g, 12%).

7-Benzyldiosmetin (12): mp 213−215 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (3H, s, OCH_3-4′), 5.23 (2H, s, CH_2 of the benzyl group), 6.45 (1H, s, H-6), 6.79 (1H, s, H-3), 6.82 (1H, s, H-8), 7.08 (1H, d, J = 8.5 Hz, H-5′), 7.33−7.48 (6H, m, 5H of the benzyl group and H-2′), 7.55 (1H, d, J = 8.5 Hz, H-6′), 9.43 (1H, s, OH-3′), 12.9 (1H, s, OH-5); ^{13}C NMR (DMSO-d_6) δ 55.9 (CH_3, OCH_3-4′), 70.1 (CH_2, benzylic carbon), 93.5 (CH, C-8), 98.7 (CH, C-6), 103.8 (CH, C-3), 105.0 (C, C-10), 112.2 (CH, C-5′), 113.2 (CH, C-2′), 118.9 (CH, C-6′), 123.1 (C, C-1′), 127.9−128.2−128.6 (CH, 5C of the benzyl group), 136.3 (C, 1C of the

benzyl group), 146.9 (C, C-3′), 151.4 (C, C-4′), 157.3 (C, C-9), 161.4 (C, C-5), 164.0 (C, C-2), 164.2 (C, C-7), 181.9 (C, C-4).

To a mixture of **12** (2.340 g, 6 mmol) and $KHCO_3$ (0.6 g, 6 mmol) in DMF (25 mL) was added 5-chloro-1-phenyl-1*H*-tetrazole (1.134 g, 6.3 mmol), and the mixture was stirred under nitrogen for 1 h at 120 °C. The reaction mixture was cooled, filtered, and evaporated to dryness. Flash chromatography (silica gel, CH_2CH_2−MeOH, 98:2) of the dried residue followed by crystallization from MeOH afforded pure 7-benzyl-3′-phenyltetrazolyldiosmetin (**13**) (2.14 g, 67%) as yellow crystals.

7-Benzyl-3′-phenyltetrazolyldiosmetin (13): mp 210−214 °C; ^{1}H NMR ($CDCl_3$) δ 3.89 (3H, s, OCH_3-4′), 5.12 (2H, s, CH_2 of benzyl), 6.45 (1H, s, H-6), 6.53 (1H, s, H-3), 6.58 (1H, s, H-8), 7.14 (1H, d, J = 8.5 Hz, H-5′), 7.36−7.88 (12H, m, 5H of benzyl, 5H of phenyltetrazole, H-2′ and H-6′), 12.66 (1H, s, OH-5).

A suspension of **13** (2.100 g, 3.94 mmol) in MeOH (100 mL) was added to ammonium formate (1.5 g, 24 mmol) and 10% Pd−C (0.5 g) and the mixture stirred at reflux under nitrogen for 2.5 h. The mixture was cooled, and the catalyst was filtered off and washed with MeOH, then THF (150 mL), until the filtrate was colorless. Evaporation to dryness of the mixed filtrates followed by crystallization from MeOH gave pure acacetin (**9**) (mp 267−269 °C) (0.762 g, 68%) as pale yellow crystals. ^{1}H and ^{13}C NMR spectra of **9** were in accordance with the literature.[15]

7,3′-Dimethyl-6-iodoapigenin (15) from Acacetin (9). Methylation of **9** into 7,4′-di-*O*-methylapigenin (**14**) (95%) was performed according to a classical procedure (K_2CO_3, MeI, DMF, rt),[16] then iodination to 7,3′-dimethyl-6-iodoapigenin (**15**) (73%) as previously described.[12]

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