

A Phenol Glucoside, Uncommon Coumarins and Flavonoids from *Pelargonium sidoides* DC

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Structural examination of the metabolic pool of the aerial parts of *Pelargonium sidoides* DC has led to the isolation of two new metabolites, 4-allyl-2,5-dimethoxyphenol-1- β -D-glucopyranoside and 6,7-dihydroxycoumarin-8-sulfate, along with a series of uncommon compounds including (2R,3R)-(+)-dihydroquercetin-3- β -glucopyranoside, (2R,3R)-(+)-dihydrokaempferol-3- β -glucopyranoside, fraxetin-7- β -glucopyranoside, 7-methoxycoumarin-6- β -glucopyranoside, umckalin, orientin-2''-gallate, and isoorientin-2''-gallate. They are accompanied by the widespread isoorientin, orientin, fraxetin, (2R,3S)-epigallocatechin-3-gallate, gallic acid and protocatechuic acid. The structures of the compounds were established from spectroscopic studies. Determination of configurations was achieved by circular dichroism.

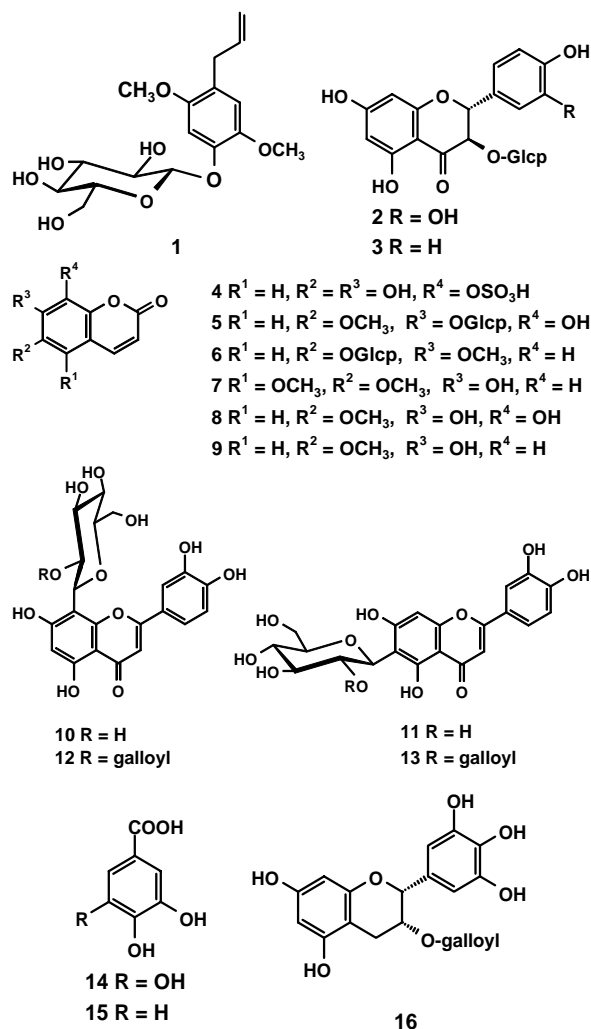
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Introduction

Pelargonium sidoides DC (Geraniaceae) is a herbaceous commonly black-flowered perennial with a tuberous rootstock. This species is predominantly found over large parts of the interior of southern Africa, but also occurs in coastal mountain ranges [1, 2]. The roots of the plant are highly estimated in a number of medical systems of ethnic groups, including Zulu, Bantu, Xhosa and Miengu [3, 4]. Decoctions are often used by traditional practitioners for the treatment of a variety of ailments including gastrointestinal disorders, cough, chest pain, bronchial infection and tuberculosis. The therapeutic importance of this plant is also shown by the employment of an ethanolic fluid extract in modern phytotherapy to cure ear, nose and throat disorders as well as respiratory tract infections [5]. The aerial parts of this *Pelargonium* species are used in wound healing in traditional medicine. In contrast to the roots, little information is available of the constituents of the herbal material. To provide a chemical rationale for the utilisation in wound healing, a compositional study of the aerial parts of *P. sidoides* was initiated.

Results and Discussion

The aqueous acetone extract of the aerial parts of *Pelargonium sidoides* DC was subjected to successive extraction with petroleum ether, dichloromethane, ethyl acetate and 1-butanol. Subsequent combined fractionation procedures of the extractives of the ethyl acetate and 1-butanol phases based on the use of Sephadex LH-20, silica gel and RP-18 material as chromatographic substrates with various gradient systems succeeded in affording compounds (**1**–**16**). Amongst these, the phenol glucoside **1** and the coumarin sulfate **4** represent new metabolites, while the natural occurrence of the *O*-galloyl-*C*-glycosylflavones **12** and **13** has only recently been demonstrated in the morphologically closely related species *P. reniforme* [6]. Noteworthy is also that the dihydroflavonols **2** and **3** as well as the coumarins **4**–**7** represent rarely found metabolites, their occurrence being hitherto confined to a small number of plant species. These metabolites were accompanied by the widely distributed compounds, **8**–**11** and **14**–**16**. Compounds **7** and **9**–**15** were identified as umckalin (**7**), scopoletin (**9**), orientin



(**10**), isoorientin (**11**), 2''-O-galloylorientin (**12**), 2''-O-galloylisorientin (**13**), gallic acid (**14**) and protocatechuic acid (**15**) by direct comparison with authentic specimens [6, 7], and compounds **2** (dihydroquercetin-3-β-D-glucopyranoside), **3** (dihydrokaempferol-3-β-D-glucopyranoside), **8** (fraxetin) and **16** (epigallocatechin-3-gallate) on the basis of their spectral data, identical with those reported previously [8–11]. Structural assessment was achieved by MS analyses and NMR data. Allocation of ¹H and ¹³C signals was facilitated by COSY, HETCOR and HMBC experiments.

Although the structures of the dihydroflavonols **2** and **3** are firmly established [8, 9, 12], the occurrence of stereochemical variants in the plant kingdom [13] prompted definition of the configurations at the chi-

ral C-2 and C-3 of the isolates. The CD spectra of the (2*R*,3*R*)-series of dihydroflavonols exhibit a negative Cotton effect at *ca.* 295 nm and a positive one at about 330 nm [14, 15]. Thus, the negative and positive Cotton effects observed for the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively in these diagnostic wavelength regions, for both compounds unambiguously defined a (2*R*,3*R*) absolute stereochemistry for the dihydroflavonol moiety in both **2** and **3**. While the relative 2,3-*trans* stereochemistry was clearly indicated by ¹H NMR coupling constants ($J_{2,3}$ *ca.* 9.5 Hz in each case), the presence of a β-D-glucopyranosyl moiety in **2** and **3** was evident from the characteristic coupling constant of the anomeric protons (*ca.* $J = 7.5$ Hz) and ¹³C chemical shift data (see Experimental) being in agreement with those reported for β-D-glucopyranoside [16]. Attachment of the hexosyl residue at the 3-hydroxyl group followed from the relatively up-field positions of the anomeric protons (δ *ca.* 3.8) and lowfield shifts of the C-3 signals ($\delta_C = 77$) as compared with those (δ_C *ca.* 73) in the aglycones [9, 12]. It should be noted that the optical rotation [α]_D +41.7° ($c = 0.64$ in MeOH) of **2** agreed well with that reported for the (2*R*,3*R*)-form [13], while measured ([α]_D +35.7°, $c = 0.68$ in MeOH) and given values ([α]_D –17.6°, $c = 0.795$ in MeOH) of **3** [17] significantly differed. However, these authors did not provide any proof for both relative and absolute stereochemistry of the isolated compound and the negative sign of the optical rotation may therefore be indicative of the (2*S*,3*S*) enantiomer. Collectively, these features unambiguously defined **2** and **3** as (2*R*,3*R*)-(+)-dihydrokaempferol-3-β-D-glucopyranoside and (2*R*,3*R*)-(+)-dihydroquercetin-3-β-D-glucopyranoside, respectively.

Compound **1**, obtained as a white solid, was characterized as a new phenol glycoside on the basis of spectroscopic and chemical analyses. Its positive FAB mass spectrum showed an [M+H]⁺ peak at m/z 357 and an [M-hexosyl]⁺ ion at m/z 194, supported by the corresponding fragments at m/z 355 and 193 in the negative mode. Besides the presence of aliphatic proton signals due to a β-glucopyranosyl moiety ($J_{1',2'}$ = 7.9 Hz), analysis of the ¹H NMR spectrum of **1** revealed the presence of two isolated aromatic one-proton singlets at $\delta = 6.78$ (H-3) and 6.90 (H-6) suggesting a *para*-arrangement, two aromatic methoxy resonances at $\delta = 3.77$ and 3.80, and aliphatic proton signals arising from a 2-propenyl side chain (two methylene groups at $\delta = 3.23$, br s, H₂-7 and $\delta = 5.00$, m, H₂-9 as well as a methine proton at $\delta = 5.93$, m, H-8). The ¹³C NMR spec-

trum of **1** exhibited, in addition to signals attributable to a β -D-glucopyranosyl moiety [16], 11 resonances, for $2 \times \text{CH}_3$, $2 \times \text{CH}_2$, $3 \times \text{CH}$ and $4 \times \text{C}$. The chemical shifts at $\delta_{\text{C}} = 56.6$ of the two aromatic methoxy groups clearly established the presence of at least one proton at the *ortho* positions of each of the methoxy functions, consistent with its structure as depicted in **1**. Independent supporting evidence for the arrangement of substituents on the aromatic nucleus was available from NOE experiments, indicating associations of the isolated aromatic protons with just one methoxy group in each instance and *vice versa*. Linkage of the glucosyl moiety to C-1 was evident from NOE connectivity between the anomeric proton signal at $\delta = 4.86$ and H-6 ($\delta = 6.90$), thus unequivocally establishing the substitution pattern on the phenolic nucleus. On acid hydrolysis compound **1** furnished glucose, identified by co-chromatography with an authentic sample, while the D-configuration followed from the optical rotation of the liberated carbohydrate, $[\alpha]_{\text{D}} +14.3^\circ$ ($c = 0.56$ in H_2O). Compound **1** was, therefore, defined as 4-allyl-2,5-dimethoxyphenol-1- β -D-glucopyranoside. This is the first isolation of **1** from a natural source, although the occurrence of its aglycone has been demonstrated in some higher plant species [12, 18–21].

Compounds **4**–**6**, present in the 1-butanol phase, were visualized by the typical fluorescence on TLC plates. Initial identification of these metabolites was accomplished by analysis of their ^1H NMR spectra which displayed a pair of doublets at *ca.* $\delta = 6.0$ – 6.4 and 7.8 – 8.0 (each 1H, d, J *ca.* 9.5 Hz), assignable to H-3 and H-4 of an α -pyrone ring system. A common chromatographic feature of these metabolites was their conspicuously low mobility in acidic solvents, suggesting the presence of a highly polar functionality in the molecules or reflecting a glycosidic nature.

The negative FAB mass spectrum of **4** showed a quasimolecular ion at m/z 273 $[\text{M-H}]^-$, consistent with the empirical formula $\text{C}_9\text{H}_6\text{O}_8\text{S}$. In the HREI mass spectrum, the peak of highest mass was detected at m/z 194.02132, corresponding to the $[\text{M-SO}_3]^{+}$ species (194.02153 calculated for $\text{C}_9\text{H}_6\text{O}_5$), supporting the reading of the FABMS data. Besides the signals for the olefinic H-3 and H-4, the ^1H NMR spectrum revealed the presence of an aromatic one-proton singlet ($\delta = 7.60$) merely, thus suggesting a trisubstituted oxygenation pattern. Assignment of this residual proton to C-5 was facilitated by NOE experiments, indicating a prominent association with H-4. The conspicuous downfield position of H-5 suggested the place-

ment of the sulfate group at either C-6 or C-8. The location of this functional group at C-8 was inferred from the negative response of **4** to Gibbs reagent, indicating the presence of a functionalized hydroxy group in this position. Compound **5** (*vide infra*) and its 8-O- β -D-glucopyranoside analogue (fraxin) were used as positive and negative reference samples, respectively, thus facilitating the reading of colourations. The characterization of **4** not only extends the range of unique natural coumarin sulfates [22], but also introduces the first 6,7,8-trisubstituted analogue in this group of secondary products.

Compound **5** was found to be a coumarin glycoside with the molecular formula $\text{C}_{16}\text{H}_{18}\text{O}_{10}$, as indicated by mass spectral $\{(+)\text{-FAB-MS } m/z \text{ 371 } [\text{M+H}]^+, \text{HREIMS } m/z \text{ 208.03701 calcd. 20803718 for } \text{C}_{10}\text{H}_8\text{O}_5 [\text{M-hexosyl}]^+\}$ and NMR analyses. The key features of the ^1H NMR spectrum of **5** were the presence of a typical AB spin system ($\delta = 5.96$ and 7.79 , $J = 9.0$ Hz) for H-3 and H-4, an aromatic methoxy signal ($\delta = 3.82$), an aromatic one-proton singlet ($\delta = 6.77$, H-5), and signals arising from a O- β -glucopyranosyl residue ($\delta = 4.72$, $J = 7.6$ Hz, H-1'). Consistent evidence for the carbohydrate moiety was obtained from ^{13}C NMR data. NOE association of the aromatic one-proton singlet with both the olefinic H-4 signal and the methoxy resonance provided strong evidence for the location of the single aromatic proton at C-5 and the methoxyl adjacent at C-6, consistent with its ^{13}C NMR chemical shift ($\delta_{\text{C}} = 56.4$). A positive Gibbs test indicated the presence of a free hydroxyl group at C-8, thereby placing the hexosyl residue at O-7. This assumption was confirmed by a notable downfield shift of C-7 ($\Delta\delta = 5.6$) relative to the aglycone ($\delta_{\text{C}} = 141.7$). On the basis of these findings, compound **5** was identified as fraxetin-7- β -D-glucopyranoside (isofraxoside) [23], a rarely found metabolite.

Compound **6** was similarly identified as 7-methoxycoumarin-6- β -D-glucopyranoside (magnolioside) [24, 25]. The position of the methoxy group, assigned to C-7, was unambiguously established through long-range correlations with H-8 in the HMBC experiment. Additional significant HMBC connectivities were between H-5 and both C-4 and C-7. Again, the downfield shift of C-6 ($\Delta\delta = 6.4$) in the ^{13}C NMR spectrum of **6** compared with that of the aglycone ($\delta_{\text{C}} = 145.4$) [26] corroborated the bonding position of the glucosyl moiety in this position and, hence, provided confirmatory evidence for the uncommon

6-hydroxy-7-methoxy arrangement in the aglycone moiety.

Although a wealth of coumarins has so far been isolated from *P. sidoides* [22], compounds **5** and **6** represent the first glycosides of this group of metabolites encountered in this plant source. Since the assignment of signals in this work was achieved by HMBC experiments and the NMR spectral data were not previously recorded in CD₃OD, these are included for comparative purposes (see Experimental Section).

Of the remaining coumarins **7–9**, identified as the exclusive 7-hydrox-5,6-dimethoxycoumarin (umckalin) and the common fraxetin and scopoletin, respectively, the first deserves to be mentioned. While highly oxygenated coumarins represent chemical markers for the traditionally used roots of both *P. sidoides* and *P. reniforme*, the occurrence of **7** is apparently confined to the former species that, according to present evidence, forms the origin of the plant source of umckaloabo [22]. Compound **7** demonstrated to be present also in the aerial parts of *P. sidoides* may thus facilitate the identification of this species growing in natural habitats without harvesting roots.

Experimental Section

General

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and optical rotations were measured on a Perkin Elmer 241 M/MC polarimeter. ¹H NMR (400 MHz) and ¹³C NMR (100.6 MHz) spectra were obtained using a Bruker AC-400 instrument with tetramethylsilane as an internal standard, and chemical shifts are expressed in δ values. EIMS and HREIMS were acquired with a Finnigan MAT CH₇A and a Finnigan MAT 711 spectrometer respectively. FABMS measurements were carried out on a Finnigan MAT CH₅DF model. HPLC separations were done with a Knauer instrument. Analytical TLC analyses were performed on pre-coated Merck Kieselgel 60F₂₅₄ plates with EtOAc-HCOOH-H₂O (18:1:1), unless stated otherwise.

Plant material

The plant material of *P. sidoides* was kindly provided by Dr Willmar Schwabe GmbH & Co., Karlsruhe, Germany. A voucher specimen is deposited at the Institute of Pharmacy, Pharmaceutical Biology, Freie Universität Berlin, Germany.

Extraction and isolation

Dried and ground (4 mm) herbal plant material of *P. sidoides* (2 kg) was exhaustively extracted with petroleum

ether at room temperature to remove lipophilic substances. Subsequent treatment with acetone-water (4:1) yielded, on evaporation of the solvent, a brownish residue (223 g). A portion (150 g) was dissolved in water (300 ml) and the aqueous phase was successively extracted with CH₂Cl₂ (4 × 250 ml; 13 g), EtOAc (8 × 250 ml; 19 g) and 1-BuOH (6 × 250 ml; 39 g).

Ethyl acetate phase

Ethyl acetate soluble extractives (15 g) were chromatographed on Sephadex LH-20 (23 × 75 cm) with a H₂O-MeOH gradient system (1:0 → 0:1). Using TLC analysis on silica gel (EtOAc-HCOOH-H₂O, 18:1:1), appropriate fractions (each 50 ml) were combined to give 22 crude subfractions, of which the following 7 were further fractionated: fraction 2 (H₂O eluate, 200–650 ml; 352 mg), fraction 6 (90% H₂O-MeOH eluate, 2.9–4.15 l; 250 mg), fraction 7 (80% H₂O-MeOH eluate, 4.15–6.45 l; 207 mg), fraction 10 (80% H₂O-MeOH eluate, 8.95–10.1 l; 301 mg), fraction 15 (40% H₂O-MeOH eluate, 22.55–24.85 l; 208 mg), fraction 16 (40% H₂O-MeOH eluate, 24.85–29 l; 144 mg), fraction 18 (40% H₂O-MeOH eluate, 33 l–35.75 l; 622 mg).

Fraction 2 was rechromatographed on Sephadex LH-20 with H₂O-MeOH (1:1). The content of test tubes 1–10 (5 ml fractions; 234 mg) was subsequently subjected to HPLC separation (RP-18; H₂O-MeOH 9:1 → 0:1; 45 min; flow rate 4 ml/min; 40 °C) to yield **1** (*R_f* 27.8 min, 90 mg).

Fraction 6 was purified by chromatography on silica gel with petroleum ether-EtOAc (9:1 → 0:1) to afford **7** in test tubes 80–250 (10 ml fractions; 38 mg). A portion of the content of test tubes 651–800 (16 mg) eluted with petroleum ether-EtOAc (1:1) was subjected to HPLC separation (RP-18; H₂O-MeOH 1:0 → 0:1; 30 min; flow rate 3 ml/min; 40 °C) to yield **8** (*R_f* 7.6 min, 2 mg).

Fraction 7 was further resolved by similar HPLC separation (RP-18; H₂O-MeOH 3:2 → 0:1; 15 min; flow rate 3 ml/min; 40 °C) to give compounds **14** (*R_f* 4.8 min; 12 mg), **15** (*R_f* 6.0 min; 5 mg), **2** (*R_f* 7.7 min; 6 mg) and **3** (*R_f* 8.8 min; 20 mg).

Fraction 10 was subjected to silica gel column chromatography (1.3 × 43 cm) with an EtOAc-MeOH gradient system (1:0 → 0:1), followed by HPLC purification (RP-18, H₂O-MeOH 1:0 → 0:1; 35 min, flow rate 2 ml/min, 40 °C) of the eluate 150–420 ml (gradient system EtOAc-MeOH 3:2) to afford **10** (10 mg). Fractions 28–250 (each 15 ml) yielded a mixture of **10** and **11**, which was resolved by similar reversed-phase HPLC: **10** (*R_f* 12.0 min; 6 mg) and **11** (*R_f* 11.4 min; 4 mg).

Fraction 15 was similarly subjected to silica gel column chromatography using an petroleum ether-EtOAc gradient system (4:1 → 1:0). The content of test tubes 40–280 (each 15 ml) was rechromatographed on Sephadex LH-20 with MeOH. Fractions 7–20 (each 10 ml) gave **12** (30 mg).

Fraction 16 was subjected to similar purification steps including CC on silica gel with petroleum ether-EtOAc (4:1 → 1:0) gradient system. Rechromatography of the fraction 270–430 (each 15 ml) eluted with just EtOAc over Sephadex LH-20 with MeOH yielded **13** in fractions 41–200 (each 10 ml; 20 mg).

Fraction 18 was again resolved by CC on silica gel using petroleum ether-EtOAc (4:1 → 5:6), followed by further purification of the fraction 127–432 (each 15 ml; eluted with 70% petroleum ether-EtOAc) on Sephadex LH-20 with H₂O-MeOH (1:0 → 1:1) yielded **16** (fractions 14–29, each 7 ml; 28 mg).

4-Allyl-2,5-dimethoxyphenol-1-β-D-glucopyranoside (**1**)

A white amorphous solid; $[\alpha]_D -32^\circ$ ($c = 0.5$ in MeOH). UV (MeOH): λ_{\max} nm ($\log \epsilon$) = 288 (3.68), 205 (4.35). ¹H NMR (CD₃OD): $\delta = 3.23$ (s, H₂-7), 3.35–3.49 (m, H-2'-H-5'), 3.66 (dd, $J = 6.3$ and 12.0 Hz, H_a-6'), 3.90 (dd, $J = 2.2$ and 12.0 Hz, H_b-6'), 3.77, 3.80 (each s, 2 × OCH₃), 4.86 (d, $J = 7.9$ Hz, H-1'), 5.93 (m, H-8), 6.78 (s, H-3), 6.90 (s, H-6). ¹³C NMR: $\delta_C = 34.7$ (C-7), 56.6 (2 × OCH₃), 62.7 (C-6'), 71.7 (C-4'), 75.0 (C-2'), 78.0 (C-3'), 78.5 (C-5'), 103.6 (C-6 and C-1'), 115.5 (C-9), 116.8 (C-3), 123.7 (C-4), 138.5 (C-8), 144.4 (C-2), 153.3 (C-1). (-)-FAB-MS m/z : 355 [M-H]⁻, 193 [(M-H)-glucosyl]⁻; EI-MS: m/z (rel. int. %) = 194 (100), 179 (22).

Compound **1** on hydrolysis with 1 N HCl in 50% aqueous MeOH (80 °C, 2 h) gave glucose and an aglycone, extracted with EtOAc. The aqueous layer was examined by TLC with sugar standards, and its optical rotation was determined.

(2R,3R)-(+)-Dihydroquercetin-3-β-D-glucopyranoside (**2**)

A yellow amorphous solid; R_f 0.22; R_t 7.6; $[\alpha]_D +41.7^\circ$ ($c = 0.64$ in MeOH). CD ($c = 7.5 \times 10^{-3}$ M in MeOH) $\theta_{329} +17.58$, $\theta_{292} -40.69$, $\theta_{253} +7.63$. ¹H NMR (CD₃OD): $\delta = 3.00$ (m, H-5''), 3.13 (t-like, $J = 9.0$, H-3''), 3.21 (dd, $J = 7.6$ and 9.0, H-2''), 3.25 (t-like, $J = 9.0$, H-4''), 3.61 (dd, $J = 12.0$ and 5.8 Hz, H_a-6''), 3.78 (dd, $J = 12.0$ and 2.0 Hz, H_b-6''), 3.87 (d, $J = 7.6$ Hz, H-1''), 4.93 (d, $J = 9.6$ Hz, H-3), 5.24 (d, $J = 9.6$ Hz, H-2), 5.90 (d, $J = 2.0$ Hz, H-6), 5.92 (d, $J = 2.0$ Hz, H-8), 6.77 (d, $J = 8.2$ Hz, H-5'), 6.84 (dd, $J = 8.2$ and 1.9, H-6'), 6.96 (d, $J = 1.9$ Hz, H-2'). ¹³C NMR: $\delta_C = 62.7$ (C-6''), 71.4 (C-4''), 74.7 (C-2''), 77.3 (C-3), 77.7 (C-3''), 78.3 (C-5''), 83.6 (C-2), 96.4 (C-6), 97.4 (C-8), 102.6 (C-10/1''), 115.9 (C-2'), 116.3 (C-5'), 121.1 (C-6'), 129.1 (C-1'), 146.5 (C-4'), 147.4 (C-3'), 164.2 (C-5), 165.6 (C-9), 169.3 (C-7), 195.9 (C-4). (+)-FAB-MS: $m/z = 489$ [M+Na]⁺, 467 [M+H]⁺; (-)-FAB-MS: $m/z = 465$ [M-H]⁻; HREIMS: 304.05898 calcd. 304.05831 for C₁₅H₁₂O₇.

(2R,3R)-(+)-Dihydrokaempferol-3-β-D-glucopyranoside (**3**)

Yellow needles; R_f 0.28; R_t 8.6; $[\alpha]_D +35.7^\circ$ ($c = 0.68$ in MeOH). CD ($c = 10 \times 10^{-3}$ M in MeOH) $\theta_{327} +6.73$, $\theta_{296} -11.43$, $\theta_{252} +2.55$. ¹H NMR (CD₃OD): $\delta = 2.98$ (m, H-5''), 3.10 (t-like, $J = 9.0$, H-3''), 3.21 (dd, $J = 7.7$; 9.0, H-2''), 3.25 (t-like, $J = 9.0$, H-4''), 3.59 (dd, $J = 12.0$ and 5.8 Hz, H_a-6''), 3.76 (dd, $J = 12.0$ and 2.0 Hz, H_b-6''), 3.80 (d, $J = 7.7$ Hz, H-1''), 4.98 (d, $J = 10.1$ Hz, H-3), 5.27 (d, $J = 10.1$ Hz, H-2), 5.90 (d, $J = 2.0$ Hz, H-6), 5.92 (d, $J = 2.0$ Hz, H-8), 6.82 (d, $J = 8.5$ Hz, H-3'/5'), 7.36 (d, $J = 8.5$, H-2'/6'). ¹³C NMR: $\delta_C = 62.7$ (C-6''), 71.3 (C-4''), 74.6 (C-2''), 77.2 (C-3), 77.6 (C-3''), 78.3 (C-5''), 83.6 (C-2), 96.4 (C-6), 97.4 (C-8), 102.6 (C-10/1''), 116.3 (C-3'/C-5'), 128.6 (C-1'), 130.5 (C-2'/C-6'), 159.4 (C-4'), 164.3 (C-5), 165.6 (C-9), 169.2 (C-7), 196.2 (C-4). (+)-FAB-MS: $m/z = 473$ [M+Na]⁺, 451 [M+H]⁺; (-)-FAB-MS: $m/z = 449$ [M-H]⁻; HREIMS: 450.11582 calcd. 450.11622 for C₂₁H₂₂O₁₁.

1-Butanol phase

1-BuOH soluble extractives (10 g) were chromatographed over polyamide with H₂O. Fractions 1–13 (each 250 ml; 123 mg) were further purified by prep. TLC using EtOAc-MeOH-H₂O (18:1:1) as developing system. The bands at R_f 0.24 and 0.26 furnished compounds **5** and **6**, respectively.

Fractions 46–50, eluted with H₂O containing 0.1% (NH₄)₂CO₃ from the polyamide column were subsequently fractionated on Sephadex LH-20. The subfractions 3–5 (each 5 ml; 142 mg) were resolved by HPLC (RP-18, H₂O-MeOH 1:0 → 0:1; 25 min, flow rate 3 ml/min) to give compound **4** at R_t 4.5 (30 mg).

6,7-Dihydroxycoumarin-8-sulfate (**4**)

R_f 0.16 in 1-BuOH-HOAc-H₂O (63:27:10). UV (MeOH): λ_{\max} nm ($\log \epsilon$) = 330 (3.00), 207 (3.53). ¹H NMR (CD₃OD): $\delta = 6.41$ (d, $J = 9.5$ Hz, H-3), 7.60 (s, H-5), 7.99 (d, $J = 9.5$ Hz, H-4). ¹³C NMR: $\delta_C = 114.1$ (C-10), 115.4 (C-3), 121.5 (C-5), 130.3 (C-8), 140.2 (C-6), 148.6 (C-4), 148.9 (C-9), 150.6 (C-7), 166.5 (C-2). (-)-FAB-MS: $m/z = 273$ [M-H]⁻, 245 [(M-H)-CO]⁻; HREIMS: 194.02132 calcd. 194.02153 for C₉H₆O₅. EI-MS: m/z (rel. int.%) = 194 (43), 166 (52), 138 (24), 137 (20), 133 (71).

Fraxetin-7-β-D-glucopyranoside (**5**)

R_f 0.22. ¹H NMR (CD₃OD): $\delta = 3.4$ –3.54 (m, H-2'-6'), 3.82 (s, OCH₃), 5.07 (d, $J = 7.3$ Hz, H-1'), 5.96 (d, $J = 9.0$ Hz, H-3), 6.77 (s, H-5), 7.79 (d, $J = 9.0$ Hz, H-4). ¹³C NMR: $\delta_C = 56.4$ (OCH₃), 62.6 (C-6'), 71.1 (C-4'), 75.6 (C-2'), 78.4 (C-3'), 78.6 (C-5'), 102.1 (C-1'), 107.1 (C-3), 105.0 (C-5), 134.8 (C-8), 146.3 (C-4), 147.3 (C-7), 150.8 (C-6), 156.5 (C-9), 165.5 (C-2).

(-)-FAB-MS: $m/z = 369$ [M-H]⁻, 207 [(M-H)-glucosyl]⁻; (+)-FAB-MS: $m/z = 393$ [M+Na]⁺, 209 [(M+H)-glucosyl]⁺; HREIMS: 208.03701 calcd. 208.03718 for C₁₀H₈O₅.

glucosyl]⁺; HREIMS: 192.04218 calcd. 192.04226 for C₁₀H₈O₄.

7-Methoxycoumarin-6-β-D-glucopyranoside (6)

R_f 0.23. ¹H NMR (CD₃OD): $\delta = 3.4 - 3.54$ (m, H-2'-6'), 3.90 (s, OCH₃), 5.07 (d, $J = 7.3$ Hz, H-1'), 6.31 (d, $J = 9.5$ Hz, H-3), 7.18 (s, H-8), 7.21 (s, H-5), 7.90 (d, $J = 9.5$ Hz, H-4). ¹³C NMR: $\delta_C = 57.1$ (OCH₃), 62.5 (C-6'), 71.3 (C-4'), 74.8 (C-2'), 77.9 (C-3'), 78.5 (C-5'), 102.1 (C-1'), 105.3 (C-8), 110.9 (C-5), 114.6 (C-3/10), 145.7 (C-4), 148.3 (C-7), 151.1 (C-9), 151.8 (C-6), 163.6 (C-2). (+)-FAB-MS: $m/z = 377$ [M+Na]⁺, 355 [M+H]⁺, 193 [(M+H)-

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