# Unusual Coumarin Patterns of *Pelargonium* Species Forming the Origin of the Traditional Herbal Medicine Umckaloabo

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The coumarin patterns of *Pelargonium sidoides* DC. and *Pelargonium reniforme* CURT., forming the origin of the herbal medicine "umckaloabo", were analysed and compared for therapeutic equivalence. For both species, members of tri- and tetraoxygenated coumarins almost exclusively were present in the respective metabolic pools. However, the roots of *P. sidoides* and *P. reniforme* expressed conspicuously distinct coumarin variations, with umckalin, its 7-*O*-methyl ether, 7-acetoxy-5,6-dimethoxycoumarin, 6,8-dihydroxy-7-methoxycoumarin, 6,8-dihydroxy-5,7-tetramethoxycoumarin, artelin and three unique coumarin sulfates as uncommon metabolites of this class of secondary products of *P. sidoides*. Furthermore, the highly oxygenated but known coumarins fraxinol, isofraxetin and fraxidin were associated with the new 8-hydroxy-5,6,7-trimethoxycoumarin as representatives of *P. reniforme*. Of the twelve identified coumarins only the two species shared the ubiquitous scopoletin and the unique 6,7,8-trihydroxycoumarin. From the oxygenation patterns it is evident that the majority of these *Pelargonium* coumarins match the recently established basic structural requirements for marked antibacterial activity, i.e. the presence of a methoxy function at C-7 and an OH group at either the C-6 or C-8 position. The current data on the coumarin profiles of each *Pelargonium* species also indicate a previous erroneous identification of the plant material claimed to be *P. reniforme*. Absence and presence of umckalin and its 7-*O*-methyl ether defines *P. reniforme* and *P. sidoides*, respectively.

## Introduction

The popular herbal medicine, umckaloabo, has a long tradition of use in the treatment of gastrointestinal disorders, hepatic complaints, and respiratory tract infections including tuberculosis (Watt and Breyer-Brandwyk, 1962; Bladt, 1977; Helmstädter, 1996). Root extracts from these species are highly valued by traditional healers and the native population in areas of southern Africa for its curative properties. Botanically, umckaloabo originates from *Pelargonium sidoides* DC. and *Pelargonium reniforme* CURT (Kolodziej and Kayser, 1998). Nowadays ethanolic preparations using the *Pelargonium* species medicinally (Umckaloabo®, Iso-Arzneimittel, Ettlingen, Germany) are successfully employed to treat ENT and respiratory tract infections. Recent studies demonstrated that *Pelargonium*-containing products have potential therapeutic benefits in these conditions (Haidvogl *et al.*, 1996; Dome and Schuster, 1996) and that coumarins and polyphenolic metabolites are of particular interest, representing the alleged biologically active substances (Kolodziej and Kayser, 1998). Thus, a comparison of the coumarin patterns of both *Pelargonium* species was made for a better understanding of the therapeutic benefits of either species. Due to taxonomic ambiguity (Kolodziej and Kayser, 1998) only limited and ambiguous information on the precise chemical nature of the coumarin pattern of *P. reniforme* was available (Wagner and Bladt, 1975). This prompted the present comparative study with botanically defined plant material available through cultivation.

## Materials and Methods

### *Plant material*

The plant material of *P. sidoides* and *P. reniforme* was kindly provided by Dr Willmar Schwabe, Karlsruhe, Germany. The two species of *Pelargonium* were grown in the nearby experi-

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mental garden (greenhouse) of this company at Staffort, Germany. A voucher specimen of each plant species is deposited at the Institut für Pharmazie, Pharmazeutische Biologie, Freie Universität Berlin.

*Extraction and isolation of coumarins from P. sidoides*

General experimental and extraction procedures including the spectroscopic data of compounds **1–4**, **7**, **8**, **10** and **12** obtained from *P. sidoides* are described in details elsewhere (Kayser and Kolodziej, 1995).

HPLC separation (RP-18; 20x250 mm; flow rate 12 ml/min$^{-1}$) of the residue of the remaining aqueous phase using a linear gradient system of $H_2O$–MeOH (9:1 to 3:7 v/v; 40 min) afforded the coumarin sulfates (**13–15**).

*5,6-Dimethoxycoumarin 7-sulfate* (**13**). $R_t$ 15.2–16.1. FAB-MS *m/z* (rel. int.%): 301 $[M-H]^-$ (100), 221 $[(M-H)-SO_3]^-$ (74), 207 (13). $^1$H NMR ($CD_3OD$): δ 3.87, 3.91 (each s, 2 × $OCH_3$), 6.15 (d, *J*=9.5 Hz, H-3), 6.56 (s, H-8), 8.02 (d, *J*=9.5 Hz, H-4). $^{13}$C NMR: $\delta_C$ 61.3, 62.0 (2 × $OCH_3$), 107.2 (C-4a), 112.7 (C-8), 114.8 (C-3), 141.0 (C-4), 146.5 (C-6), 148.6 (C-7), 150.7 (C-5), 151.2 (C-8a), 162.2 (C-2).

*6-Hydroxy-5,7-dimethoxycoumarin 8-sulfate* (**14**) + *8-Hydroxy-5,7-dimethoxy-coumarin 6-sulfate* (**15**). $R_t$ 14.1–15.2. FAB-MS *m/z* (rel. int.%): 317 $[M-H]^-$ (100), 301 (26), 237 $[(M-H)-SO_3]^-$ (74), 221 (33), 207 (27). $^1$H NMR ($CD_3OD$): δ 3.88, 4.00 (each s, 2 × $OCH_3$) 6.20 (d, *J*=9.5 Hz, H-3), 8.03 (d, *J*=9.5 Hz, H-4).

*Extraction and isolation of coumarins from P. reniforme*

The work-up procedure of the plant material followed that of *P. sidoides* (*vide supra*). The ethyl acetate soluble portion was initially chromatographed on Sephadex LH-20 with gradient solvent systems of $H_2O$–MeOH (9:1 → 0:1 v/v). The content of fractions 316–430 was rechromatographed and the subfractions 65–130 were further separated by prep. HPLC with a $H_2O$–MeOH gradient system (9:1→3:7 v/v, flow rate 4 ml/min) to afford (**6**) at $R_t$ 16.0 min.

*5,6-Dihydroxy-7-methoxycoumarin* (**5**). EI-MS (rel. int.%): *m/z* 208 (100) $[M]^+$. $^1$H NMR ($CD_3OD$): δ 3.91, 3.96 (each s, 2 × $OCH_3$), 6.23 (d, *J*=9.4 Hz, H-3), 6.83 (s, H-8), 7.86 (d, *J*=9.4 Hz, H-4).

The *n*-butanol soluble portion was similarly separated on Sephadex LH-20 with gradient solvent systems of $H_2O$–MeOH. Subsequent HPLC separation ($H_2O$–MeOH gradient system 9:1→3:7 v/v, flow rate 4 ml/min) of distinct subfractions yielded compounds **6**, **7**, **9** and **11**.

*6-Hydroxy-5,7-dimethoxycoumarin* (**5**). From fractions 221–260, $R_t$ 18.3 min. EI-MS (rel. int.%): *m/z* 222 (100) $[M]^+$. 1H NMR ($CD_3OD$): δ 3.89 (s, $OCH_3$), 6.20 (d, *J*=9.4 Hz, H-3), 6.71 (s, H-8), 7.83 (d, *J*=9.4 Hz, H-4).

*6,7,8-Trihydroxycoumarin* (**7**). From fractions 113–155, $R_t$ 9.7 min. EI-MS (rel. int.%): *m/z* 194 (100) $[M]^+$. $^1$H NMR data corresponded to those in the literature (Kayser and Kolodziej, 1995).

*8-Hydroxy-6,7-dimethoxycoumarin* (**9**). From fractions 221–260, $R_t$ 18.9 min. EI-MS (rel. int.%): *m/z* 222 (100) $[M]^+$. $^1$H NMR ($CD_3OD$): δ 3.88 (s, 2 × $OCH_3$), 6.32 (d, *J*=9.4 Hz, H-3), 6.72 (s, H-5), 7.86 (d, *J*=9.4 Hz, H-4).

*8-Hydroxy-5,6,7-trimethoxycoumarin* (**11**). From fractions 156–220, $R_t$ 24.5 min. EI-MS (rel. int.%): *m/z* 252 (100) $[M]^+$. $^1$H NMR ($CD_3OD$): δ 3.87, 3.91, 3.95 (each s, 3 × $OCH_3$), 6.32 (d, *J*=9.4 Hz, H-3), 6.72 (s, H-5), 7.86 (d, *J*=9.4 Hz, H-4). $^{13}$C NMR: $\delta_C$ 61.3, 61.6, 62.3 (3 × $OCH_3$), 107.2 (C-4a), 112.7 (C-3), 138.2 (C-4), 141.3 (C-5), 145.6 (C-7), 146.2 (C-6, C-8), 146.7 (C-8a), 161.9 (C-2).

## Results and Discussion

Although *Pelargonium sidoides* and *Pelargonium reniforme* can readily be distinguished by the shape of leaves and the colour of flowers (Van der Walt and Vorster, 1988), the existence of gradual variation between both species adds to general problems of taxonomic classification (Dreyer *et al.*, 1992). Initially, only *P. reniforme* has been suggested to form the plant source of the traditional herbal medicine umckaloabo (Bladt, 1977), but, according to present evidence, the origin of umckaloabo is strongly associated with the morphologically closely related species *P. sidoides* (Kolodziej and Kayser, 1998). Current evidence points also to erroneous identification of the plant material claimed to be *P. reniforme* in an earlier investigation (Wagner and Bladt, 1975). With bo-

tanically defined plant materials available through cultivation, studies on the individual coumarin patterns of each *Pelargonium* species were possible for the first time.

Systematic examination of the aqueous acetone extract of the roots of *P. sidoides* has revealed a wealth of highly oxygenated simple coumarins, including four new (**3**, **7**, **8** and **10**) and three rarely found analogues (**2**, **4** and **12**) (Kayser and Kolodziej, 1995). Reinvestigation of the same material, with its high concentration of remarkable structural variants, has additionally yielded three novel coumarin sulfates (**13–15**), obtained from polar fractions. This group of analogues has hitherto been restricted to three examples reported from a single plant source, *Seseli libanotis* (Lemmich and Shibata, 1984).

The $^1$H NMR spectrum of **13** was superposable with that of 7-hydroxy-5,6-dimethoxycoumarin (**2**) (Kayser and Kolodziej, 1995). However, the conspicuously low mobility in acidic solvents of metabolite (**13**) contrasted with that of (**2**), suggesting structural differences. This conjecture found support by FAB mass spectral analysis, showing the $[M-H]^-$ peak at *m/z* 301 under negative ion conditions, in agreement with the empirical formula $C_{11}H_{10}SO_8$. The presence of a sulfate group was clearly indicated by the characteristic ion *m/z* 221, corresponding to the $[(M-H)-SO_3]^-$ species. Location of the sulfate ester function at C-7 was evident by comparison of the $^{13}$C NMR data of (**13**) with those of the parent coumarin (**2**), indicating an upfield shift of $\Delta\delta$ 7.4 ppm for the *ipso* carbon and downfield displacements of $\Delta\delta$ 8.4 and 13.1 ppm of the *ortho* related carbons C-6 and C-8, respectively. Collectively, these spectroscopic data defined the structure of the new natural coumarin **13**.

Extensive HPLC analysis showed the presence of an inseparable mixture comprised of compound (**14**) and (**15**). Close structural similarity of these constituents and 6,8-dihydroxy-5,7-dimethoxycoumarin (**10**) followed tentatively from the general congruence of $^1$H resonances. Again, the chromatographic behaviour of this homogeneous sample was completely at variance with that of the authentic specimen. An abundant deprotonated molecular ion at *m/z* 317 occurred in the negative ion FAB mass spectrum for (**14/15**) and the loss of 80 mass units indicated again the presence of a sulfate group. The remaining ions in the spectrum could readily be related to the fragmentation pattern of the parent coumarin (**10**). Owing to signal complexity, no useful $^{13}$C NMR data could be obtained for the mixture (**14/15**). Despite this limitation, chromatographic and spectroscopic data established (**14/15**) as the 6- and 8-monosulfate of 6,8-dihydroxy-5,7-dimethoxycoumarin (**10**), respectively.

Identification of **13–15** not only extends the unique series of naturally occurring coumarin sulfates but also introduces the first analogues in which sulfation occurred at a phenolic function.

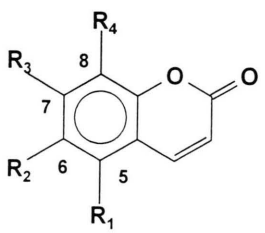
The coumarin pool encountered in the roots of *P. reniforme* significantly differed from that of *P. sidoides*, although it contained similarly highly oxygenated simple coumarins based on tri- and tetraoxygenated substitution patterns. Altogether six coumarins were isolated and their structures identified by means of spectroscopic methods. Known metabolites amongst these analogues included scopoletin (**1**), fraxinol (**5**), isofraxetin (**6**), 6,7,8-trihydroxycoumarin (**7**) and fraxidin (**9**). It should be noted that the natural occurrence of (**7**) has only been demonstrated recently by its isolation from *P. sidoides* (Kayser and Kolodziej, 1995). Differentiation between the various oxygenation patterns was effected by application of $^1$H NOE difference spectroscopy, detection of long range couplings between H-4 and H-8, and $^{13}$C chemical shift data of aromatic methoxy groups (Kayser and Kolodziej, 1995).

In addition to the aforementioned known coumarins, the extract also afforded 8-hydroxy-5,6,7-trimethoxycoumarin (**11**). Its $^1$H NMR spectrum displayed the anticipated three aromatic *O*-methyl resonances ($\delta$ 3.87, 3.91 and 3.95) and a pair of doublets ($\delta$ 6.19 and 8.05, each *d*, $J$ = 9.7 Hz) for the olefinic H-3 and H-4. Absence of an isolated aromatic one-proton singlet readily indicated the 5,6,7,8-tetrasubstitution. Consistent evidence was obtained from mass spectral analysis, indicating the molecular ion at *m/z* 252 and, hence, the presence of an hydroxy function. A prominent $[M-15]^+$ peak localised one of the methoxy groups at C-6 (Wagner and Bladt, 1975). The 5,6,7-arrangement of the *O*-methyl groups was finally confirmed by the NOE association of 5-OMe ($\delta$ 3.95) with H-4 and the 6-OMe function ($\delta$ 3.91), while irradiation of the latter indicated connectivities with both 5-

(δ 3.95) and 7-OMe (δ 3.87). The above evidence, supported by $^{13}C$ NMR data, unambiguously defined the structure of the new coumarin (**11**). It should be noted that tetraoxygenated coumarins represent a rather small group within this class of secondary metabolites (Estévez-Braun and González, 1997).

The picture that emerged from examining the coumarin profiles of both *Pelargonium* species forming the origin of umckaloabo revealed the presence of a remarkable series of simple coumarins in each instance with regard to the high degree of aromatic functionalization including hydroxyl and methoxyl groups. Evidently, two types of trisubstituted coumarins occurred in these species, 5,6,7- and 6,7,8-functionalized analogues. Such combined oxygenation patterns are rarely found in the plant kingdom and the potential exists here for the exploitation of a taxonomically useful set of chemical characters. Notably, the roots of *P. sidoides* and *P. reniforme* express conspicuously distinct coumarin variations (Table II). Of the twelve identified coumarins, the two species share the ubiquitous scopoletin (**1**) and the unique 6,7,8-trihydroxycoumarin (**7**) only, the latter may therefore serve as useful marker of umckaloabo. Although the substitution patterns of the coumarins of the two *Pelargonium* species were very similar, a distinguishing feature appeared to be the presence of a 5,6-dimethoxy arrangement within the group of 5,6,7-trioxygenated members of *P. sidoides* and an unsubstituted 6-hydroxyl function in that of *P. reniforme* (Table I and II). Another discriminating chemical character was the distinct occurrence of coumarin sulfates in *P. sidoides*. Conspicuously, if functionalization of the 8-hydroxy group occurred, then this was sporadically visible at the tetrasubstituted level. It should also be noted that there is much divergence in concentrations (Table II), with generally significantly

Table I. Chemical structures of the coumarins present in *P. reniforme* and *P. sidoides*.




| No | Compound | Trivial | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|---|
| | *6,7-Dioxygenation* | | | | | |
| **1** | 7-Hydroxy-6-methoxycoumarin | Scopoletin | H | $OCH_3$ | OH | H |
| | *5,6,7-Trioxygenation* | | | | | |
| **2** | 7-Hydroxy-5,6-dimethoxycoumarin | Umckalin | $OCH_3$ | $OCH_3$ | OH | H |
| **3** | 7-Acetoxy-5,6-dimethoxycoumarin | | $OCH_3$ | $OCH_3$ | OAc | H |
| **4** | 5,6,7-Trimethoxycoumarin | | $OCH_3$ | $OCH_3$ | $OCH_3$ | H |
| **5** | 6-Hydroxy-5,7-dimethoxcoumarin | Fraxinol | $OCH_3$ | OH | $OCH_3$ | H |
| **6** | 5,6-Dihydroxy-7-methoxycoumarin | Isofraxetin | OH | OH | $OCH_3$ | H |
| | *6,7,8-Trioxygenation* | | | | | |
| **7** | 6,7,8-Trihydroxycoumarin | | H | OH | OH | OH |
| **8** | 6,8-Dihydroxy-7-methoxycoumarin | | H | OH | $OCH_3$ | OH |
| **9** | 8-Hydroxy-6,7-dimthoxycoumarin | Fraxidin | H | $OCH_3$ | $OCH_3$ | OH |
| | *5,6,7,8-Tetraoxygenation* | | | | | |
| **10** | 6,8-Dihydroxy-5,7-dimethoxycoumarin | | $OCH_3$ | OH | $OCH_3$ | OH |
| **11** | 8-Hydroxy-5,6,7-trimethoxycoumarin | | $OCH_3$ | $OCH_3$ | $OCH_3$ | OH |
| **12** | 5,6,7,8-Tetramethoxycoumarin | Artelin | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ |
| **13** | 5,6-Dimethoxycoumarin 7-sulfate | | $OCH_3$ | $OCH_3$ | $OSO_3H$ | H |
| **14** | 6-Hydroxy-5,7-dimethoxycoumarin 8-sulfate | | $OCH_3$ | OH | $OCH_3$ | $OSO_3H$ |
| **15** | 8-Hydroxy-5,7-dimethoxycoumarin 6-sulfate | | $OCH_3$ | $OSO_3H$ | $OCH_3$ | OH |

Table II. Coumarin patterns in *P. reniforme* and *P. sidoides*.

| Compound | *P. sidoides* [a)] | P. reniforme [a)] |
|---|---|---|
| *6,7-Dioxygenation* | | |
| 7-Hydroxy-6-methoxycoumarin | 0.002 | 0.0003 |
| *5,6,7-Trioxygenation* | | |
| 7-Hydroxy-5,6-dimethoxycoumarin | 0.02 | |
| 7-Acetoxy-5,6-dimethoxycoumarin | 0.0004 | |
| 5,6,7-Trimethoxycoumarin | 0.003 | |
| 6-Hydroxy-5,7-dimethoxcoumarin | | 0.0003 |
| 5,6-Dihydroxy-7-methoxycoumarin | | 0.004 |
| *6,7,8-Trioxygenation* | | |
| 6,7,8-Trihydroxycoumarin | 0.0004 | 0.02 |
| 6,8-Dihydroxy-7-methoxycoumarin | 0.0002 | |
| 8-Hydroxy-6,7-dimethoxycoumarin | | 0.002 |
| *5,6,7,8-Tetraoxygenation* | | |
| 6,8-Dihydroxy-5,7-dimethoxycoumarin | 0.01 | |
| 8-Hydroxy-5,6,7-trimethoxycoumarin | | 0.001 |
| 5,6,7,8-Tetramethoxycoumarin | 0.0007 | |
| 5,6-Dimethoxycoumarin 7-sulfate | 0.007 | |
| 6-Hydroxy-5,7-dimethoxycoumarin 8-sulfate | } 0.006 | |
| 8-Hydroxy-5,7-dimethoxycoumarin 6-sulfate | | |

[a)] Percentage related to the dry weight.

higher yields of coumarins in *P. sidoides*. With the exception of the characteristic 6,7,8-trihydroxycoumarin (**7**), the remaining coumarins are only present in small amounts in *P. reniforme*.

Recalling the taxonomic ambiguity of the previously investigated *P. reniforme* (*vide supra*), the reported presence of 7-hydroxy-5,6-dimethoxycoumarin (umckalin) (**2**) and its 7-*O*-methyl ether (**4**) (Wagner and Bladt, 1975) conflicts with current information. Taking into account marked differences in the composition of the essential oils (Kayser *et al.*, 1998), the prevailing chemical evidence strongly suggests revision of the earlier taxonomic identification.

Having established the coumarin profile of each species, it was appropriate to relate this finding to the therapeutic use of umckaloabo. Although the substitution patterns greatly differed by the distribution of oxygen functions, the identified coumarins match the recently established basic structural requirements for marked antibacterial activity, i.e. the presence of a methoxy function at C-7 and an OH group at either the C-6 or C-8 position. Enhancement in potency may be anticipated by favourable structural modifications of these highly oxygenated simple coumarins (Kayser and Kolodziej, 1999). It may therefore be concluded that *P. sidoides* and *P. reniforme* are therapeutically equivalent as far as the antibacterial potential is concerned. Supporting evidence is available from similar antibacterial activities of the crude extracts of *P. sidoides* and *P. reniforme* (Kayser and Kolodziej, 1997).

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