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HIGHLY OXYGENATED COUMARINS FROM *PELARGONIUM SIDOIDES**†

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Key Word Index—*Pelargonium sidoides*; Geraniaceae; roots; coumarins; oxygenation patterns.

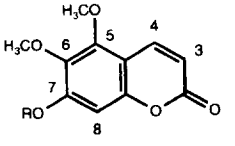
Abstract—The range of natural simple coumarins is extended by discovery of the highly oxygenated representatives 6,8-dihydroxy-7-methoxycoumarin, 6,7,8-trihydroxycoumarin, 6,8-dihydroxy-5,7-dimethoxycoumarin, and 7-acetoxy-5,6-dimethoxycoumarin. They are accompanied in the roots of *Pelargonium sidoides* by the widely occurring scopoletin, associated with the uncommon analogues 5,6,7-trimethoxycoumarin, 7-hydroxy-5,6-dimethoxycoumarin (umckalin), and 5,6,7,8-tetramethoxycoumarin (artelin), these being reported from a plant source for the second time. The structures of these compounds were established from chemical and spectroscopic studies. Differentiation between oxygenation patterns is discussed on the basis of chemical shift data.

INTRODUCTION

Pelargonium species indigenous to areas of southern Africa are traditionally used as an antidiarrhoic and as a general remedy for treatment of colds and infection of lungs in folk medicine [1–3]. Previous studies on *Pelargonium reniforme* Curt revealed the presence of a variety of coumarins and tannins, representing the alleged biologically active principles [4–6]. The hitherto limited information on the precise chemical nature of phenolic constituents of medicinal plants of the genus *Pelargonium* prompted the present more detailed investigation of coumarin metabolites of the related species *Pelargonium sidoides* DC.

RESULTS AND DISCUSSION

The aqueous acetone extract of the roots of *Pelargonium sidoides* DC. was subjected to successive extraction with chloroform, ethyl acetate and *n*-butanol. Subsequent combined fractionation procedures of the individual fractions based on the use of Sephadex LH-20, silica gel and RP-18 material as chromatographic substrates with various solvent systems succeeded in affording a series of tri- and tetra-oxygenated coumarins, (**1**–**7**). Final purification of each compound was achieved by medium pressure liquid chromatography (MPLC) on MCI-gel CHP-20P using a water–methanol gradient system as mobile phase. Amongst these, 7-acetoxy-5,6-dimethoxycoumarin (**3**), 6,8-dihydroxy-7-methoxycoumarin (**4**), 6,7,8-trihydroxycoumarin (**5**), and 6,8-dihydroxy-5,7-dimethoxycoumarin (**7**) represent novel metabolites of the above class of secondary products, while the natural occurrence of the remaining compounds of this group, (**1**, **2** and **6**), are being reported from a plant source for the second time [7].



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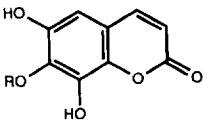

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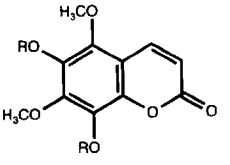

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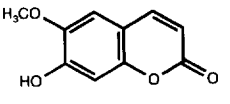

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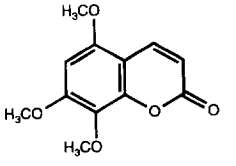

9

*In memory of Professor D. G. Roux.
†Part of the projected Ph.D. thesis of O. Kayser.
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These metabolites were accompanied by the widely distributed scopoletin (**8**), which has been identified by co-chromatography with an authentic specimen and by comparison of the spectroscopic data with those previously published [8]. It is noteworthy that **8** apparently represented the only analogue populating the metabolic pool, which displayed a simpler aromatic oxygenation pattern.

Compounds **1–8** were visualized by the typical fluorescence on TLC plates. Initial identification of these analogues was accomplished by analysis of their ^{1}H NMR data which showed a pair of doublets at *ca* δ6.15 and 7.95 (each 1H, *d*, *J ca* 9.5 Hz), assignable to H-3 and H-4 of an α-pyrone ring system. The number of aromatic methoxy resonances, in conjuction with the presence/absence of an aromatic one-proton singlet in the region δ6.59–6.84, indicated the degree of substitution on the aromatic nucleus. Detections of the broad hydroxyl-absorptions were, however, partly hampered by the fact that these were obscured in the base-line.

The ^{1}H NMR spectra (300 MHz, acetone-d_6) of **1** and **2** exhibited the diagnostic H-3 and H-4 olefinic doublets in the aromatic region (δ 6.16 and 7.95, *d*, J = 9.5 Hz for **1**; δ6.13 and 7.97, *d*, J = 9.7 Hz for **2**). The aromatic region in the spectra additionally displayed a one-proton singlet at δ6.73 and 6.59, respectively, consistent with a trisubstitution pattern on the aromatic ring in each instance. Independent support for the degree of aromatic functionalization was evident from the presence of three aromatic methoxy resonances in the ^{1}H NMR spectrum of **1**, and replacement of one methoxyl signal by the phenol hydroxyl signal at δ9.15 in that of **2**. Mass spectral analysis (EI-MS) of **1** and **2** showed the molecular ions at *m/z* 236 and 222, respectively, corresponding to the respective molecular formulae $C_{12}H_{12}O_5$ and $C_{11}H_{10}O_5$. Detection of long range couplings between the H-4 signal and the aromatic one-proton singlet (J = 0.6 Hz) confirmed that C-8 was unsubstituted and, hence, the 5,6,7-oxygenation pattern for both compounds. NOE experiments additionally indicated association of one of the methoxy groups with H-4 and a single methoxy group in both **1** and **2**. Such a feature was consistent with the anticipated 5,6,7-substitution, but also defined location of the hydroxyl-group at C-7 in **2**. Independent supporting evidence for the arrangement of the methoxyl groups on the coumarin skeleton was available from ^{13}C NMR spectral data [9]. The chemical shifts at δ61.3 and 62.0 (Table 1) of the aromatic methoxy groups in **2** clearly established the presence of substituents at the two *ortho* positions of each of the methoxy groups, consistent with its structure as depicted in **2**. Analogue **1** exhibited the corresponding signals at δ56.7, 61.8 and 62.1, indicating that one methoxyl group (C-7) was flanked by at least one unsubstituted carbon. Furthermore, the downfield shift of C-7 ($\Delta\delta$5.8 ppm) in **1** relative to that in **2** was indicative of the ether linkage. Also, methylation of **2** with Me_2SO_4-K_2CO_3 affording **1**, reflected the same benzene ring oxygenation pattern in both coumarin derivatives. These results defined **2** as umckalin, previously characterized from the related species *P. reniforme* Curt [4–6], and **1** as its 7-*O*-methyl ether. Although the occurrence of the latter in *P. reniforme* has already been claimed as concluded from TLC studies [1], its natural existence is now unequivocally demonstrated by its current isolation from the title plant.

The positive FAB-mass spectrum of **3** showed a $[M + H]^+$ peak at *m/z* 265, consistent with a molecular formula $C_{13}H_{12}O_6$ for the coumarin derivative, and the sodium complex $[M + Na]^+$ at *m/z* 287. Analysis of the ^{1}H NMR spectral data (Table 2) of **3** again revealed the presence of a trisubstituted aromatic nucleus in the coumarin skeleton. Besides two aromatic methoxyl signals (δ3.85 and 4.03), the aliphatic region also displayed the resonance of an aromatic acetoxy function (δ2.35). This observation, taken in conjunction with the presence of an aromatic one-proton singlet (δ6.93), verified the degree of oxygenation in **3**. Owing to insufficient sample quantity, a similar protocol of using NOE experiments and ^{13}C NMR data was precluded. Final structural confimation was, therefore, obtained by acetylation of **2**, which afforded **3**. Noteworthy is the fact that the conversion proved to be unsuccessful under standard procedures (acetic anhydride–pyridine) and required somewhat

Table 1. ^{13}C NMR spectral data for coumarins **1**, **2** and **4–8** (75 MHz; acetone-d_6)

C	1	2	4	5	6	7	8
2	160.8	162.2	160.9	160.9	160.3	160.3	160.9
3	113.0	112.5	113.4	113.3	114.1	112.5	113.3
4	139.4	139.6	144.0	143.9	138.6	138.7	144.6
4a	107.6	107.3	111.7	111.4	109.5	106.4	109.7
5	152.0	155.9	104.4	104.4	142.4	142.5	112.0
6	139.0	138.1	132.9	133.2	136.1	130.0	151.9
7	158.3	152.5	148.9	138.7[a]	144.3[a]	144.9	145.6
8	96.4	99.6	138.9	139.0[a]	145.1[a]	140.4	103.7
8a	150.1	150.4	145.2	145.0	150.7	150.4	151.8
OMe	56.7, 62.1, 61.8	61.3, 62.0	61.2	—	61.5, 61.7, 61.9, 62.0	61.2, 61.4	56.7

[a]Assignments may be interchanged.

Table 2. ^{1}H NMR spectral data for coumarins **1**–**8** [300 MHz; acetone-d_6; J values (Hz) are given in parentheses]

H	1	2	3	4	5	6	7	8
3	6.16 *d* (9.5)	6.13 *d* (9.7)	6.38 *d* (9.7)	6.12 *d* (9.5)	6.12 *d* (9.5)	6.29 *d* (9.9)	6.16 *d* (9.8)	6.20 *d* (9.5)
4	7.95 *d* (9.5)	7.97 *d* (9.7)	8.09 *d* (9.7)	7.74 *d* (9.5)	7.74 *d* (9.5)	7.94 *d* (9.9)	7.99 *d* (9.8)	7.85 *d* (9.5)
5	—	—	—	6.65 *s*	6.65 *s*	—	—	6.84 *s*
8	6.73 *s*	6.59 *s*	6.93 *s*	—	—	—	—	6.59 *s*
OMe	3.82, 3.97, 4.01 (each *s*)	3.86, 4.01 (each *s*)	3.85, 4.03 (each *s*)	3.93 *s*	—	3.90, 3.97, 3.98, 4.04 (each *s*)	3.86, 3.98 (each *s*)	3.86 *s*
OAc	—	—	2.35 *s*	—	—	—	—	—

more drastic conditions (acetylchloride–perchloric acid). The downfield position of H-8 (δ6.93) relative to the same proton in analogues possessing a methoxyl group at the *ortho* position was compatible with the placement of the anisotropic acetoxy substituent at C-7 (see above). This novel coumarin represents the first natural compound known hitherto within this group possessing an acetoxy function.

Close structural similarity of the isomeric groups **1**–**3**, and **4** and **5** followed tentatively from the general congruence of ^{1}H resonances. The key features of the spectra of the latter group were the presence of a typical AB system (δ6.12 and 7.74, J = 9.5 Hz) for the H-3 and H-4 in each instance, and an aromatic singlet (δ6.65) (Table 2), indicating a similar trisubstituted oxygenation pattern on the aromatic nucleus. Differentiation between the 5,6,7- and 6,7,8-oxygenation pattern was effected by a similar protocol of using chemical shift data, NOE experiments and chemical transformations.

The structure of 6,8-dihydroxy-7-methoxycoumarin (**4**) was established by application of ^{1}H NOE difference spectroscopy. Association of the aromatic one-proton singlet (δ6.65) with only the olefinic H-4 signal provided strong evidence for the location of the aromatic proton at C-5, when taken in conjuction with the absence of a long-range coupling of H-4 as observed for **1** and **2** (see above). Owing to the presence of one methoxy resonance (δ3.93), the remaining functionalities in **4** were assumed to be hydroxy groups. This conjecture found support in the mass spectrum of **4**, showing a $[M]^+$ peak at m/z 208, in complete agreement with the expected molecular constitution. Absence of any bathochromic shift in the UV-spectra of **4** upon addition of $AlCl_3$ indicated that the hydroxy functions were devoid of an *ortho* arrangement. These features, supported by ^{13}C NMR spectral data (Table 1), collectively established **4** as 6,8-dihydroxy-7-methoxycoumarin, a novel natural metabolite.

In the EI-mass spectrum of **5**, a $[M]^+$ ion was detected at m/z 194, corresponding to the empirical formula $C_9H_6O_5$. Analysis of its ^{1}H NMR spectrum indicated, in addition to the key features noted above, the absence of methoxyl resonances, conforming to the proposed trihydroxylation pattern. NOE association of H-4 with the single aromatic proton localized the latter at C-5, substantiated by the absence of a long-range Z-coupling of H-4 (see above). Addition of $AlCl_3$ induced a strong bathochromic shift (59 nm) in the UV spectrum relative to that recorded in methanol, indicating the presence of vicinal hydroxy groups in **5**. Collectively these observations facilitated definition of the 6,7,8-trihydroxy arrangement for **5**, another novel coumarin metabolite.

Perusal of the chemical shifts of the remaining aromatic proton and the olefinic H-4 in the group of trioxygenated coumarins, **1**–**5**, revealed diagnostic differences associated with the substitution patterns. Thus, in the 5,6,7-substituted coumarins (**1** and **2**) the absorbance of H-4 exhibited the conspicuous deshielding ($\Delta\delta ca$ −0.23) associated with a γ_{syn}-effect, when compared to 6,7,8-substituted analogues, **4** and **5**. The relatively downfield position of H-4 may, therefore, be taken as indication for oxygenation at C-5 in the coumarin framework, as also indicated by similar chemical shifts in the tetrafunctionalized coumarins **6** and **7** (Table 2). A similar picture emerged from examining the chemical shifts of the remaining aromatic proton. The values for H-5 and H-8, adjacent to methoxy substituents in a series of coumarins (unpublished results), were observed in narrow ranges at *ca* δ6.80 and 6.73, respectively, under the conditions employed (acetone-d_6). In order to evaluate the potential value of these shift criteria, we synthesized 5,7,8-trimethoxycoumarin (**9**) via a Perkin reaction using 2,4-dihydroxy-3,6-dimethoxybenzaldehyde in acetic anhydride–sodium acetate, the chemical shift of H-6 being recorded at δ6.45. A similar observation was made for the corresponding signals in coumarins possessing hydroxy groups at the *ortho* positions. Here, the analogous ^{1}H resonances were located at relatively higher fields ($\Delta\delta$0.14–0.20 ppm). This finding should be of direct value in defining the oxygenation pattern on the benzene ring in simple coumarins. Substituent effects on ^{13}C chemical shifts in coumarin derivatives were previously examined [10, 11], but the applicability to highly substituted analogues appears critical [11].

The 5,6,7,8-tetraoxygenation of the remaining analogues **6** and **7** was readily evident from 'missing' aromatic proton resonances in their ^{1}H NMR spectra. Notable differences included the presence of four and two methoxy resonances, respectively. Collectively the ^{13}C and ^{1}H NMR features (Tables 1 and 2) were compatible with the 5,6,7,8-methoxycoumarin structure for **6** and a dihydroxy-dimethoxycoumarin for **7**. Consistent evidence was obtained from mass spectral analysis, indicating

$[M]^+$ ions at *m/z* 266 and 238, respectively. Conversion of **7** into **6** on treatment with Me_2SO_4–K_2CO_3 provided proof for the same arrangement of substituents and the presence of two hydroxy groups, being replaced by methoxy functions in **6**. The conspicuous absence of any bathochromic shift in the UV spectra of **7** using standard shift reagents, and of NOE association of H-4 with a methoxy group strongly supported the 6,8-dihydroxy-5,7-dimethoxy placing of functionalities in **7**, the natural occurrence of which being reported from a plant source for the first time. Analogue **6**, previously designated as artelin, has originally been obtained from *Artemisia tridentata* [12]. In this context, it should be noted that the structural assessment of the isolate is subject to debate [13, 14] and that unambiguous definition of artelin **6** is hitherto limited to the isolation from *Sapium seberiferum* [14]. Hence, compound **6** has been unequivocally characterized for the second time.

In conclusion, we have identified four (**3**–**5** and **7**) new natural highly oxygenated simple coumarins from *P. sidoides*. In addition, the presence of the uncommon analogues **1**, **2** and **6** of this class in the same plant source is now demonstrated for the first time. This species contains almost exclusively highly oxygenated coumarins and resembles chemically *P. reniforme*.

EXPERIMENTAL

General. Mps: uncorr. NMR spectra (Me_2CO-d_6 with TMS as int. standard) were recorded on a Bruker AC-300 spectrometer at 75 MHz for ^{13}C and 300 MHz for ^1H. EI- and FAB-mass spectra (glycerol/xenon/DMSO) were obtained on a Finnigan MAT CA7A and a CH5 DF mass spectrometer, respectively. HR-mass spectra were run on a MAT 711. HPLC analysis was done with a Knauer instrument system, equipped with a dual pump and a variable UV detector, and computer integrating model (EuroChrom 2000). Prep. TLC plates (Merck, Kieselgel 60 F_{254}, 0.5 mm) were used without activation. Compounds were visualized by exposure to UV and by spraying with $FeCl_3$. All compounds were subjected to final purification on MCI-gel CHP-20P using a H_2O–MeOH gradient system (19:1–2:3) prior to spectroscopic studies.

Methylation of the coumarins (**2**) *and* (**7**). A portion of the coumarins (32 mg and 45 mg for **2** and **6**, respectively) was dissolved in dry Me_2CO (9 ml), followed by addition of K_2CO_3 (2 g) and Me_2SO_4 (1 ml). The reaction mixture was refluxed for 90 min at 40° and subsequently acidified with 10% HCl. After evapn of the Me_2CO and removal of the ppt., the product was extracted using Et_2O to afford **1** (17 mg) and **6** (29 mg), respectively.

Extraction and isolation of compounds. Dried and powdered roots of *P. sidoides* DC. (1.5 kg) were exhaustively extracted with Me_2CO–H_2O (4:1). The combined extracts were reduced in vol. (2 l) and defatted with petrol (20 × 500 ml). The residual aq. phase was successively extracted with $CHCl_3$ (20 × 500 ml), EtOAc (26 × 500 ml), and *n*-BuOH (30 × 500 ml). Evapn of solvent produced a brown residue in each instance (5, 12 and 10.5 g, respectively).

Ethyl acetate phase. The ethyl acetate soluble portion was initially chromatographed on Sephadex LH-20 (40 × 5 cm) with MeOH–H_2O (1:1) as eluant to remove co-occuring oligomeric condensed tannins. Subsequent fractionation of the eluate (4 g) on Sephadex LH-20 (90 × 8 cm) with H_2O containing increasing amounts of MeOH (100:0–50:50) afforded 16 crude subfractions. Following qualitative TLC analysis on silica gel (EtOAc–H_2O–HCO_2H, 18:1:1) appropriate fractions (10 ml) were combined and further resolved as follows:

6,8-*Dihydroxy*-5,7-*dimethoxy*-2H-*benzopyran*-2-*one* (**7**). The content of fractions 290–321 was rechromatographed on Lichroprep RP-18 (40 × 1 cm) under medium pressure (MPLC, 8 bar) with H_2O–MeOH (9:1). Test tubes 48–60 (10 ml fractions) consisted of **7** (53 mg). Mp 144–147° (crystals). EI-MS (rel. int. %): *m/z* 238 $[M]^+$ (57); UV λ_{max}^{MeOH} nm: 262, 331. ^{13}C and ^1H NMR data (see Tables 1 and 2).

7-*Hydroxy*-6-*methoxy*-2H-*benzopyran*-2-*one* (*scopoletin*) (**8**). Repeated chromatography of the fractions 371–421 on Lichroprep RP-18 with H_2O–MeOH (17:3), followed by purification of the subfractions 56–67 using H_2O–MeOH (7:3) as eluant afforded **8** (6 mg). Mp 202–204°. EI-MS (rel. int. %): *m/z* 192 $[M]^+$ (75). UV λ_{max}^{MeOH} nm: 232, 345. ^{13}C and ^1H NMR data (see Tables 1 and 2).

The content of test tubes 422–460 was similarly resolved on Lichroprep RP-18 (H_2O–MeOH, 4:1) to give two distinct subfractions, collected in test tubes 91–95 and 96–110, respectively.

5,6,7-*Trimethoxy*-2H-*benzopyran*-2-*one* (**1**). The former subfraction afforded **1** (13 mg). Mp 73–75° (lit. 74–76° [1]). EI-MS (rel. int. %): *m/z* 236 $[M]^+$ (100). UV λ_{max}^{MeOH} nm: 215, 313. ^{13}C and ^1H NMR data (see Tables 1 and 2).

6-*Hydroxy*-5,7-*dimethoxy*-2H-*benzopyran*-2-*one* (*umckalin*) (**2**). The latter fraction yielded **2** (90 mg). Mp 146–147° (lit. 146–147° [1]). EI-MS (rel. int. %): *m/z* 222 $[M]^+$ (73). UV λ_{max}^{MeOH} nm: 210, 328. ^{13}C and ^1H NMR data (see Tables 1 and 2).

Chloroform phase. The residue of the chloroform extractives was subjected to chromatography on a silica column (80 × 8 cm) using the gradient system $CHCl_3$–MeOH (99:1–4:1). Frs 69–81 were further resolved by prep. TLC (toluene–Me_2CO, 3:2; 3x).

5,6,7,8-*Tetramethoxy*-2H-*benzopyran*-2-*one* (*artelin*) (**6**). The R_f band at 0.30 gave **5** (18 mg). Mp 52–55° (powder) (lit. 96°, needles [13]). EI-MS (rel. int. %): *m/z* 266 $[M]^+$ (100). UV λ_{max}^{MeOH} nm: 214, 329. ^{13}C and ^1H NMR data (see Tables 1 and 2).

Butanol phase. The residue of the *n*-BuOH soluble portion was fractionated on Sephadex LH-20 (90 × 8 cm) using the gradient system H_2O–MeOH (1:0–1:1). After the emergence of fluorescent material (4.5 l), 10 ml fractions were collected.

7-*Acetoxy*-5,6-*dimethoxy*-2H-*benzopyran*-2-*one* (**3**). The content of fractions 71–120 was similarly purified on

Lichroprep RP-18 with H_2O–MeOH (9:1), followed by final HPLC purification of the test tubes 71–79 on Eurospher 100C-18 (25 × 0.8 cm; 5 μm) using H_2O–MeOH (4:1) at a flow rate of 1.5 ml min^{-1} to give **3**, R_t 5.4 (6 mg). Mp 123–125°. FAB-MS (rel. int. %): *m/z* 265 $[M + H]^+$ (29); HR-MS $[M]^+$ *m/z* 264.06364 (calcd for $C_{13}H_{12}O_6$ 264.06339). UV λ_{max}^{MeOH} mn: 230, 328. ^{1}H NMR data (see Table 2).

Conversion of umckalin **3** *into the 7-O-acetyl derivative* **3**. A small sample (10 mg) of **2** was dissolved in toluene (2 ml) and acetylated using acetyl chloride (2 ml) and perchloric acid (3 drops). The reaction mixture was allowed to stand for 24 hr, followed by addition of ice H_2O and extraction with $CHCl_3$ (4 × 50 ml) to afford **3** (4 mg) which proved to be identical (MS, ^{1}H NMR, TLC) with that obtained from *P. sidoides*. Separation of the product **3** from unchanged starting material **2** was effected by prep. TLC (toluene–Me_2CO 3:2; R_f 0.76 and 0.62, respectively). Frs 122–178 consisted of a mixture of **4** and **5**. This was finally resolved by HPLC on Eurospher 100C-18 (25 × 0.8 cm; 5 μm) with H_2O–MeCN (9:1) at a flow rate of 0.8 ml min^{-1} to afford 2 fractions at R_f 7.0 and 7.5, respectively.

6,8-*Dihydroxy*-7-*methoxy*-2H-*benzopyran*-2-*one* (**4**). The latter eluate, R_t 7.5 (3 mg), consisted of **4**. Mp 198–200°. EI-MS (rel. int. %): *m/z* 208 $[M]^+$ (100). UV λ_{max}^{MeOH} nm 209, 305. ^{13}C and ^{1}H NMR data (see Tables 1 and 2).

6,7,8,-*Trihydroxy*-2H-*benzopyran*-2-*one* (**5**). The former fraction, R_t 7.0 (6 mg), yielded **5**. Mp 237–239°. EI-MS (rel. int. %): *m/z* 194 $[M]^+$ (100). UV λ_{max}^{MeOH} nm: 209, 340; + $AlCl_3$ 216, 399; + $AlCl_3$/HCl 216, 373. ^{13}C and ^{1}H NMR data (see Tables 1 and 2).

Synthesis of 5,7,8-*trimethoxy*-2H-*benzopyran*-2-*one* (**9**). 2,4-Dihydroxy-3,6-dimethoxybenzaldehyde (100 mg), NaOAc (300 mg), and Ac_2O (10 ml) were refluxed (180°, 24 hr), and the reaction mixture extracted with $CHCl_3$. Subsequent treatment of the organic phase with 20% HCl (40°, 2 hr), followed by methylation with Me_2SO_4–K_2CO_3 (see above) afforded a crude mixture. Prep. TLC (toluene–Me_2CO, 3:2) afforded a main band at R_f 0.65 to yield **9** (5 mg). ^{1}H NMR: δ3.86, 3.89, 3.94 (each *s*, 3 × OMe), 6.30 (*d*, *J* = 9.6 Hz, H-3), 6.45 (*s*, H-6), 8.02 (*d*, *J* = 9.6 Hz, H-4).

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REFERENCES

1. Bladt, S. (1974) *Dissertation*. Universität München.
2. Bladt, S. (1977) *Dt. Apotheker Ztg* **117**, 1655.
3. Van der Walt, J. J. A. and Vorster, P. J. (1988) *Pelargoniums of Southern Africa*, Vol. 3, pp. 7–9. National Botanic Gardens, Kirstenbosch.
4. Wagner, H., Bladt, S., Abraham, D. J. and Lotter, H. (1974) *Tetrahedron Letters* 3807.
5. Wagner, H. and Bladt, S. (1975) *Phytochemistry* **14**, 2061.
6. Bladt, S. and Wagner, H. (1988) *Dt. Apotheker Ztg* **292**, 292.
7. Buckingham, J., Macdonald, F. M. and Bradley, H. M. (1994) *Dictionary of Natural Products*. Chapman & Hall, London.
8. Sankar, S. S., Gilbert, R. D. and Fornes, R. E. (1982) *Org. Magn. Res.* **19**, 222.
9. Roitman, J. N. and James, L. F. (1985) *Phytochemistry* **24**, 835.
10. Duddeck, H. and Kaiser, M. (1982) *Org. Magn. Res.* **20**, 55.
11. Macias, F. A., Massanet, G. M. and Rodriguez-Luis, F. (1989) *Magn. Res. Chem.* **27**, 892.
12. Brown, D., Asplund, R. O. and McMahon, V. A. (1975) *Phytochemistry* **14**, 1083.
13. Ahluwalia, V. K. and Sunita (1977) *Indian J. Chem.* **15B**, 936.
14. Yang, P. and Kinghorn, A. D. (1985) *J. Nat. Prod.* **48**, 486.