PERGAMON

Phytochemistry 55 (2000) 965–970

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

C-Methyl-flavonoids from the leaf waxes of some Myrtaceae

Eckhard Wollenweber [a,*], Rüdiger Wehde [a], Marion Dörr [a], Günter Lang [b], Jan F. Stevens [c]

[a] *Institut für Botanik der Technischen Universität, Schnittspahnstrasse 3, D-64287 Darmstadt, Germany*
[b] *Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität, Universitätsstraße 1, D-40225 Düsseldorf, Germany*
[c] *Department of Bio-Organic Chemistry, Vrije Universiteit, De Boelelaan 1083, NL-1081 HV Amsterdam, The Netherlands*

Received 3 May 2000; received in revised form 17 July 2000

Abstract

The thin waxy coatings on leaves of nine species of *Callistemon,* two of *Melaleuca* and one species of *Metrosideros*, have been studied for the occurrence of leaf surface flavonoids. The *Callistemon* species and *Metrosideros robusta* exhibit only C-methylated flavonoids, while *O*-methyl flavonoids were detected in *Melaleuca huegelii*. The new natural C-methyl flavonol, 5,7-dihydroxy-3,8,4′-trimethoxy-6-C-methylflavone, was isolated from *Metrosideros robusta*. The leaf wax of *Callistemon coccineus* contains the novel C-methylflavonoid, 5,4′-dihydroxy-8-C-methyl-7-methoxy flavanone.

Keywords: *Callistemon*; *Melaleuca*; *Metrosideros*; Myrtaceae; Lipophilic leaf exudates; C-Methylated flavones; Flavonols; Flavanones

1. Introduction

The genus *Eucalyptus* was the first in the family Myrtaceae that was found to exhibit exudate flavonoids. As early as 1963, Horn and Lamberton reported the C-methylated flavone eucalyptin (5-hydroxy-7,4′-dimethoxy-6,8-di-C-methyl-flavone) as leaf wax constituent of three *Eucalyptus* species, and three further C-methylated flavonoids were found later from the same source (Wollenweber and Kohorst, 1981, and refs. therein). Courtney et al. (1983) reported eucalyptin and 8-desmethyl-eucalyptin from the leaf wax coatings not only for several *Eucalyptus* species, but also for *Syncarpia glomulifera, Lophostemon confertus* and an *Angophora* hybrid. They suggested that C-methylated flavonoids might be distinctive of the family Myrtaceae. Such compounds were also found in *Eugenia biflora* and *Myrcia citrifolia* (Gottlieb et al., 1972), in *Agonis spathulata* (Cannon and Martin, 1977), in two *Leptospermum* species (Mayer, 1990, 1993; Wollenweber et al., 1996) and in *Syzygium alternifolium* (Rao and Rao, 1991). In the scope of our continuing studies on surface flavonoids of higher plants, we analyzed the lipophilic materials coating the leaves of some additional Myrtaceae. In the present paper, we report the exudate flavonoid patterns of members of three genera of this family and the structure elucidation of two new natural C-methyl flavonoids.

2. Results and discussion

A large proportion of the leaf waxes of a selection of Myrtaceae consisted of triterpenoids. Flavonoid aglycones were detected as minor components in these waxes, and, when possible, identified by direct TLC comparisons with authentic samples. Two novel flavonoids were isolated and identified by spectroscopic techniques, i.e. 5,4′-dihydroxy-8-C-methyl-7-methoxy flavanone (**2**) from *Callistemon coccineus* and 5,7-dihydroxy-3,8,4′-trimethoxy-6-C-methylflavone (**4**) from the leaf exudate of *Metrosideros robusta* (Fig. 1).

2.1. Identification of flavonoids

Compound **1** was isolated from the leaf exudate of *C. coccineus*. Its molecular weight (M_r 300 by APCI–MS) was consistent with $C_{17}H_{16}O_5$. MS–MS fragmentation of the $[MH]^+$ ion with m/z 301 resulted in retro Diels–Alder

* Corresponding author. Tel.: +49-6151-163602; fax: +49-6151-166878.
E-mail address: wollenweber@bio.tu-darmstadt.de (E. Wollenweber).

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00348-4

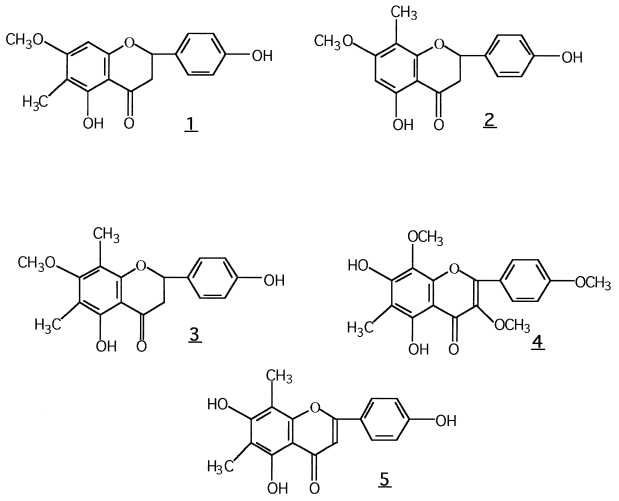

Fig. 1. C-Methylated flavonoids from leaf waxes of some Myrtaceae.

cleavage of the γ-pyranone ring, thereby yielding a B-ring ion, *m/z* 147 $[O{\equiv}C{-}CH{=}CH{-}PhOH]^+$, and an A-ring ion with *m/z* 181, indicating 5,7-dihydroxylation combined with two methyl substituents (cf. isomer **2** in Fig. 2). ^1H NMR analysis revealed the presence of two free hydroxyls, a *para* substituted B-ring, one aromatic A-ring proton, one methoxy group, and one C-methyl subsituent. A flavanone skeleton was evident from the coupling pattern of the C-ring protons (Table 1). In flavanones, the shift values of the H-6 and H-8 protons are often very similar (Markham and Geiger, 1994), and therefore the identity of the aromatic proton was determined by ^1H–^{13}C HMBC correlation. In the HMBC spectrum, the OH-5 proton signal at δ_H 12.23 showed interactions with carbon signals at δ_c 102.2, 104.0, and 159.4, which were assigned to C-6, C-10, and C-5, respectively. The aromatic A-ring proton (δ_H 6.22 s) interacted with all A-ring carbon atoms except C-5 (δ_c 159.4), which indicates that this proton is located *para* to C-5, that is, at C-8. A C–H functionality at position 8 was also clear from the HMQC spectrum, because none of the proton signals shared a cross-peak with C-6 while the aromatic proton showed a $^1J_{H-C}$ coupling with C-8 at δ_C 91.2. Interactions between the C-methyl protons and carbons 5, 7, 8, and C-10 confirmed the position of the C-methyl function at C-6. In addition to OH-5, another OH signal was observed (δ_H 9.59) which showed cross peaks with carbons 3′ and 5′ in the HMBC spectrum. The δ_H 9.59 resonance was therefore identified as OH-4′. These assignments left the *O*-methyl substituent to be located at C-7, in agreement with interactions between C-7 and H-8, and between C-7 and the C-methyl and *O*-methyl protons. Finally, the assignments of carbons 2,3, 8, 2′/6′, 3′/5′, and the C–Me and OMe carbons were aided by ^1H–^{13}C HMQC correlation spectroscopy (Table 1). Flavanone **1** was thus identified as 5,4′-dihydroxy-6-C-methyl-7-methoxy flavanone. This product (poriol 7-methyl ether, or 6-methylnaringenin-7-Me) was reported only once before, as a heartwood constituent of *Pseudotsuga wilsoniana* (Hsieh et al., 1998).

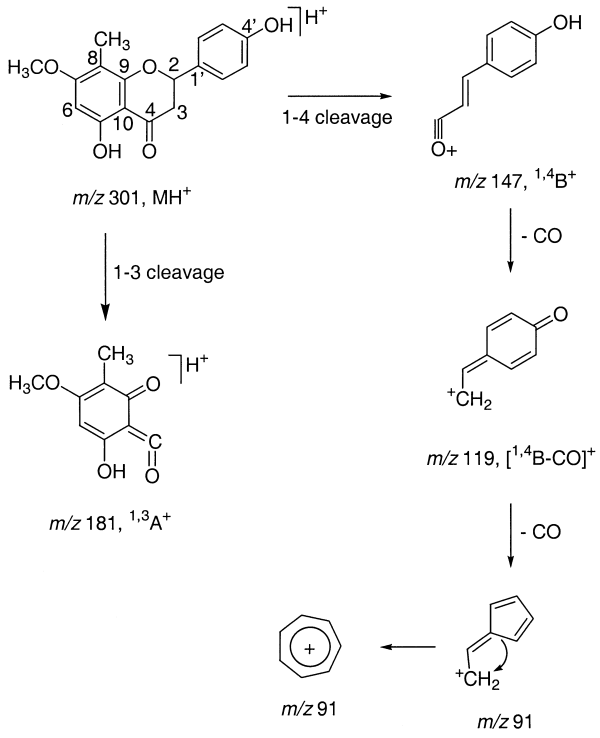

Fig. 2. APCI–MS–MS fragmentation of flavanone **2**. For acronyms and nomenclature of daughter ions, see note under Section 3.4.

The isomeric flavanone **2** was also isolated from *C. coccineus*. The mass of its M^+ ion found by HREIMS was consistent with $C_{17}H_{16}O_5$. The structure of the compound followed from APCI–MS–MS and ^1H NMR data. The daughter-ion spectrum of the $[MH]^+$ ion (*m/z* 301) was virtually identical with that of compound **1** (Fig. 2). Its ^1H spectrum was also very similar to that of compound **1** (Table 1), with only the aromatic proton of **2** resonating at a slightly lower frequency. This was used to assign this resonance to H-6, leaving the C-methyl to be located at carbon-8. Flavanone **2** was thus determined to be 5,4′-dihydroxy-8-C-methyl-7-methoxy flavanone (8-methytnaringenin-7-Me), a new natural flavanone.

Flavanones **1** and **2** were accompanied by compound **3** (M_r 314 by APCI–MS) in the leaf wax of *C. coccineus*. The ^1H spectrum of compound **3** differed from flavanones **1** and **2** by the absence of an aromatic proton resonance and the presence of two C-methyl resonances (Table 1). This left no doubt that this flavanone carried C-methyl substituents at both C-6 and C-8. Compound **3** was thus identified as the flavanone analogue of sideroxylin, 5,4′-dihydroxy-6,8-di-C-methyl-7-methoxy flavanone. This product is known as angophorol, isolated from the resin (“kino”) of *Angophora lanceolata,* where it occurs together with 6,8-dimethylnaringenin (Birch et al., 1960).

Table 1
NMR data [δ in ppm, multiplicity, (J in Hz)]

Atom no.	Compd. **1** (δ_C)	Compd. **1** (δ_H)	Compd. **2** (δ_H)	Compd. **3** (δ_H)	Compd. **4** (δ_C)	Compd. **4** (δ_C)
2	78.6	5.47 *dd* (13.0, 2.9)	5.47 *dd* (12.5, 2.9)	5.47 *dd* (12.8, 2.9)	155.0	
3	41.8	ax 3.31 *dd* (17.1, 13.0) eq 2.72 *dd* (17.1, 2.9)	ax 3.25 *dd* (17.1, 12.5) eq 2.78 *dd* (17.1, 2.9)	ax 3.29 *dd* (17.1, 12.8) eq 2.81 *dd* (17.1, 2.9)	138.1	
4	197.1				178.2	
5	159.4	12.23 *s*(OH)	12.21 *s*(OH)	12.16 *s*(OH)	153.6	12.61 *s*(OH)
6	102.2		6.20 *s*		107.2	
7	165.2				153.6	
8	91.2	6.22 *s*			127.4	
9	161.2				146.5	
10	104.0				103.8	
1′	128.8				122.6	
2′/6′	128.4	7.33 *d* (8.5)	7.32 *d* (8.5)	7.33 *d* (8.5)	130.0	8.04 *d* (9.4)
3′/5′	115.1	6.79 *d* (8.5)	6.80 *d* (8.5)	6.81 *d* (8.5)	114.6	7.17 *d* (8.8)
4′	157.7	9.59 *s* (OH)	9.57 *s* (OH)	9.58 *s* (OH)	161.5	
6-Me	6.7	1.90 *s*		2.00 *s*[a]	7.9	2.03 *s*
8-Me			1.88 *s*	1.97 *s*[a]		
3-OMe					60.0	3.80 *s*
7-OMe	56.1	3.82 *s*	3.84 *s*	3.68 *s*		
8-OMe					61.6	3.83 *s*
4′-OMe					55.6	3.87 *s*

[a] Assignments interchangeable.

Flavonol **4** was isolated from *M. robusta*. HREIMS showed a molecular ion peak at m/z 358.1042 which was consistent with $C_{19}H_{18}O_7$. The ^1H spectrum of compound **4** showed a low-field singlet (OH-5), two doublets typical of an AA′BB′-system (*para* substitued B-ring) and four three-proton singlets attributable to one C-methyl substituent (δ_H 2.03) and three *O*-methyl groups (δ_H 3.80, 3.83, and 3.87) (Table 1). The identification of C-5, C-6, and C-10 followed from their interactions with the OH-5 proton (δ_H 12.61) in the HMBC spectrum. Carbon-6 correlated also with the C-methyl protons at δ_H 2.03, suggesting that C-6 carries the C-methyl substituent. The *O*-methyl group resonating at δ_H 3.87 was located at C-4′, because the carbon atom that interacted with these OMe protons also showed correlations with the 2′/6′ and 3′/5′ protons. The second OMe resonance (δ_H 3.80) showed a cross peak with the carbon signal at δ_C 138.1 which was assigned to C-3 by comparison with ^{13}C data reported for methylated flavonols (Agrawal, 1989). The remaining methoxy singlet showed a cross peak with a carbon signal at δ_C l27.4 in the HMBC spectrum. This correlation is only consistent with 7-hydroxy-8-methoxy substitution, because C-7 atoms (either with an OH or OMe substituent) give rise to signals in the range 145–165 ppm (Agrawal, 1989). Compound **4** was therefore identified as 5,7-dihydroxy-3,8,4′-trimethoxy-6-C-methyl flavone, a novel natural product. Until now only two products with similar substitution patterns were known, namely 5,8,4′-trihydroxy-3,7-dimethoxy-6-C-methyl flavone from the leaf exudate of the fern, *Pityrogramma triangularis* var. *triangularis* (Wollenweber et al., 1985), and 5,4′-dihydroxy-3,7,8-trimethoxy-6-C-methyl flavone from the culture filtrate of the fungus, *Colletotrichum dematium* (Abou-Zaid et al., 1997).

Submilligram amounts of compound **5** were detected in the exudate of several *Callistemon* species (Table 2). The identity of the compound, 6,8-dimethylapigenin, was established by direct TLC comparisons with an authentic sample, obtained by demethylation of sideroxylin (6,8-dimethylapigenin-7-methyl ether) with pyridinium hydrobromide (Howard and Mabry, 1970). 6,8-dimethylapigenin (**5**) was reported only once so far, namely from leaves of *Syzygium alternifolium* (Rao and Rao, 1991).

2.2. Flavonoid patterns

The flavonoid patterns of the nine *Callistemon* species studied are presented in Table 2. Sideroxylin (6,8-dimethylapigenin-7-diMe) and 8-desmethylkalmiatin (6-methyl apigenin-7-Me) are produced by each of these species, although in slightly varying proportions. Eucalyptin (6,8-dimethylapigenin-7,4′-diMe) and 8-desmethyleucalyptin are also quite abundant. The flavonoids, 6,8-dimethyl apigenin (**5**) and 8-desmethyllatifolin (6-methyl-kaempferol-3,7-diMe), occur in a smaller number of species and in much smaller amounts. In the leaf surface

Table 2
Exudate flavonoid patterns of *Callistemon* species[a,b]

	1	2	3	4	5	6	7	8	9
C-Methyl-flavones									
6-Methytapigenin-7-Me (8-Desmethylkalmiatin)	XX	X	XX	XX	X	X	X	(X)	X
6-Methyiapigenin-7,4′-diMe (8-Desmethyleucalyptin)			XX		XX	XX	XX	X	XX
6,8-Dimethylapigenin (**5**)	(X)		(X)		(X)	X	(X)		
6,8-Dimethyiapigenin-7-Me (Sideroxylin)	XX	X	XX	XX	X	X	X	XX	X
6,8-Dimethylapigenin-7,4′-diMe (Eucalyptin)			X		X	X	XX	XX	
C-Methyl-flavonols									
6-Methylkaempferol-3,7-diMe (8-Desmethyllatifolin)		(X)			(X)	(X)		X	
6-Methylquercetin-3,7,3′-triMe (Pinoquercetin-3,7,3′-triMe)						X			
C-Methyl-flavanones									
6-Methylnaringenin-7-Me (**1**)		(X)							
8-Methylnaringenin-7-Me (**2**)		(X)							
6,8-Dimethylnaring.-7-Me (**3**)		(X)							
Unknown			x		(X)	x	(X)		

[a] 1, *Callistemon acuminatus*; 2, *C. coccineus*; 3, *C. juniperinus*; 4, *C. macropunctatus*; 5, *C. rigidus*; 6, *C. salignus*; 7, *C. salignus* var. *viridiflorus*; 8, *C. speciosus*; 9, *C. teretifolius*.

[b] XX, X and (X) roughly indicate relative amounts within one species.

material of *C. speciosus* we found traces of pinoquercetin-3,7,3′-triMe (6-methylquercetin-3,7,3′-triMe). *C. coccineus* is exceptional in that it produces three C-methyl flavanones, 6-methylnaringenin-7-Me, 8-methylnarigenin-7-Me and 6,8-dimethylnaringenin-7-Me. The exudates of *C. juniperinus* and of *C. salignus* contain an additional unidentified compound which is also thought to be a C-methylated flavonoid. Traces of this product are also observed in *C. rigidus* and *C. salignus* var. *viridiflorus*.

All *Callistemon* species studied and *Metrosideros robusta* contain C-methylated flavonoids as leaf wax constituents. *O*-Methylated flavonoids are lacking among the exudate flavonoids of these species, as was reported earlier for *C. lanceolata* (Huq and Misra, 1997). *Melaleuca huegelii,* on the other hand, produces *O*-methylated flavones and flavonols: apigenin (trace), apigenin-7-Me (trace), ap-7,4′-diMe; luteolin (trace), lut-7,3′-diMe; kaempferol, kae-3-Me, kae-3,7-diMe, kae-3,7,4-triMe; quercetin, qu-3-Me, qu-3,7-diMe, qu-3,7,3′-triMe, and quercetin-3,7,4′-trimethyl ether (trace). *Metrosideros styphelioides* is exceptional in that it seems to accumulate the 3-*O*-rhamnoside and the 3-*O*-glucoside of both kaempferol and quercetin, while aglycones are lacking. The phenomenon of flavonoid glycosides being deposited externally on leaf surfaces has been discussed earlier (Wollenweber et al., 1997). The leaf exudates of both *Melaleuca* species are devoid of C-methylated flavonoids.

3. Experimental

3.1. Plant material

Leafy twigs of all species analyzed were collected in the Botanischer Garten der Universität Darmstadt: *Callistemon acuminatus* Cheel, *C. coccineus* F. Muell. (coil. Sept. 1997), *C. juniperinus* Heynh. (coll. Oct. 1999), *C. macropunctatus* (Dum.-Cours.) Court (coll. Oct. 1999), *C. rigidus R. Br.* (coll. Oct. 1995), *C. salignus* (Sm.) DC (coll. Oct. 1995), *C. salignus* var. *viridiflorus* (Sims) Sweet (coll. Oct. 1999), *C. speciosus* (Sims) DC (coll. Oct. 1999), *C. teretifolius* F. Muell. (coll. Oct. 1999), *Melaleuca huegelii* Endl. (coll. May 1999), *Melaleuca styphelioides* Sm. (coll. Sept. 1997), *Metrosideros robusta* A. Cunn. (coll. Sept. 1995).

3.2. Isolation and chromatographic procedures

Fresh plant material was briefly rinsed with acetone to dissolve the waxy coatings from the leaves. In the case of *C. acumunatus, C. juniperinus, C. macropunctatus, C. rigidus,* and *C. teretifolius* only small samples were available. Their exudates were, therefore, analyzed directly by TLC comparisons. Bulk material was available of the other species, yielding exudate amounts between 2 and 11 g. After evaporation of the solvent, the residues were redissolved in a small amount of boiling MeOH, cooled to -10° and centrifuged to separate the MeOH soluble flavonoids from precipitated material (apparently triterpene acids dominating). The supernatants were subsequently chromatographed on Sephadex LH-20 with MeOH as the eluting solvent. Fractions were monitored by TLC on polyamide (DC 11, Macherey-Nagel) with solvents A (petrol$_{100-140}$–toluene–MeCOEt–MeOH 12:6:1:1), B (toluene–petrol$_{100-140}$–MeCOEt–MeOH 12:6:2:1) and C (toluene–MeCOEt–MeOH 12:5:3) and on silica with solvents D (toluene–MeCOEt 9:1) and E (toluene–dioxane–HOAc 18:5:1). Chromatograms were viewed under UV (366 nm) before and after spraying with "Naturstoffreagenz A" (a 1% methanolic solution of diphenyl–boric acid–ethanolamine complex). Terpenoids were visualized by spraying silica plates with $MnCl_2$ reagent, followed by heating (Jork et al., 1989). Fractions of similar flavonoid composition were combined and, in the case of *C. coccineus, C. salignus* and *M. robusta*, rechromatographed over polyamide SC-6, eluted with toluene and increasing amounts of MeCOEt and MeOH. Some fractions of *C. coccineus* as well as of *M. robusta* were further subjected to prep TLC on silica (toluene–MeCOEt 9:1) to yield 1–10 mg of compounds **1**–**3** (*C. coccineus*) and **4** (*M. robusta*). Identification of known flavonoids was achieved by co-TLC with authentic markers available in E.W.'s lab.

HPLC analysis was carried out with a 5 μm Alltima RP-18 column (100 × 2.1 mm) at 0.4 ml min^{-1} using a

linear solvent gradient starting from 10 to 100% MeCN (solvent B) in 1% aq. HCOOH (solvent A) over 30 min. The column oven was set at 25°C. Flavonoids were detected with a photo-diode array detector (220–600 nm). The retention time of kaempferol was 18.05 mm under these conditions. The retention times of compounds **1**–**4** were calculated relative to the R_t of kaempferol (see Sections 3.5–3.8).

3.3. NMR spectrometry

^1H (500 or 600 MHz) and ^{13}C (125 or 150 MHz) NMR data were recorded in DMSO-d_6 at room temperature. The DMSO resonances at δ_H 2.50 and δ_C 39.51 were used as internal shift references. ^1H–^1H COSY and ^1H–^{13}C HMQC/HMBC experiments were performed using standard pulse sequences.

3.4. Mass spectrometry

We decided to follow Claeys' proposed nomenclature for MS–MS fragments of flavonoids (Ma et al., 1997). In this nomenclature system, adapted from MS–MS fragmentation of carbohydrates (Domon and Costello, 1988), A and B ring fragments are labelled $^{i,j}A^+$ and $^{i,j}B^+$ where i and j indicate the cleavage positions in the C-ring. For instance, $^{1,3}A^+$ represents the A ring fragment produced by retro Diels–Alder cleavage of the **1**–**2** and **3**–**4** bonds (see also Stevens et al., 1999).

Atmospheric pressure chemical ionization (APCI) mass spectra were run on a PE Sciex API III Plus triple quadrupole instrument. Samples were introduced by direct injection via the heated nebulizer interface kept at 500°C which heats the column effluent to ca. 120°C. Ionization of the analyte vapour mixture was initiated by a corona discharge needle at ca. 6 kV and a discharge current of ca. 3 μA. The orifice plate voltage was set at 55 V in the positive ion mode. Collision-induced dissociation (CID) experiments were performed with Ar–N_2 (9:1) as target gas at a thickness of ca. 1.9×10^{14} atoms cm^{-2}. The collision energy was 30 V. Other operating conditions were standard.

3.5. Compound **1** (5,4′-dihydroxy-7-methoxy-6-C-methyl flavanone)

APCI-MS data [m/z, (% rel. int.)], 301 $[MH]^+$ (100); MS–MS, 301 (7), 181 $[^{1,3}A]^+$ (100), 147 $[^{1,4}B]^+$ (73), 119 $[^{1,4}B\text{-}CO]^+$ (59), 91 (27). UV λ_{max} (MeOH) 291, 339 nm. HPLC, RR_t (kaempferol) 1.29.

3.6. Compound **2** (5,4′-dihydroxy-7-methoxy-8-C-methyl flavanone)

HREIMS (probe) 70 eV, m/z (rel. int.) 300.0990 M^+ ($C_{17}H_{16}O_5$ requires 300.0998) (100). APCI–MS data [m/z, (%rel. int.)], 301 $[MH]^+$ (100); MS–MS, 301 (7), 181 $[^{1,3}A]^+$ (100), 147 $[^{1,4}B]^+$ (79), 119 $[^{1,4}B–CO]^+$ (59), 91 (27). UV λ_{max} (MeOH) 291, 339 nm. HPLC, RR_t (kaempferol) 1.26.

3.7. Compound **3** (5,4′-dihydroxy-7-methoxy-6,8-di-C-methyl flavanone)

APCI–MS data [m/z, (% rel. int.)], 315 $[MH]^+$ (100); MS–MS, 315 (9), 195 $[^{1,3}A]^+$ (100), 147 $[^{1,4}B]^+$ (79), 119 $[^{1,4}B–CO]^+$ (53), 91 (27). UV λ_{max} (MeOH) 282, 358 nm. HPLC, RR_t (kaempferol) 1.34.

3.8. Compound **4** (5,7-dihydroxy-3,8,4′-trimethoxy-6-methyl flavone)

HREIMS (probe) 70 eV, m/z (rel. int.) 358.1042 M^+ ($C_{19}H_{18}O_7$ requires 358.1053) (61), 343 $[M-CH_3]^+$ (100). UV λ_{max} (MeOH) 278, 326 nm. HPLC, RR_t (kaempferol) 1.36.

Acknowledgements

EW wishes to thank Mr. Helmut Groh (Botanical Garden Darmstadt) for the plant material. JFS is grateful to Ms. Monika Ivancic and Professor Victor Hsu (Oregon State University) for help with recording some of the NMR data.

References

Abou-Zaid, M., Dumas, M., Chauret, D., Watson, A., Thompson, D., 1997. C-Methyl flavonols from the fungus *Colletotrichum dematium* f.sp. *epilobii*. Phytochemistry 45, 957–961.

Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids. Studies in Organic Chemistry 39. Elsevier, Amsterdam.

Birch, A.J., Pettit, D.G., Ryan, A.J., Speake, R.N., 1960. Flavanones in *Angophora lanceolata*. Journal of the Chemical Society, 2063–2066.

Cannon, J.R., Martin, P.F., 1977. The Flavanones of *Agonis spathulata* (Myrtyceae). Australian Journal of Chemistry 30, 2099–2101.

Courtney, J.L., Lassak, E.V., Speirs, G.B., 1983. Leaf wax constitutents of some Myrtaceous species. Phytochemistry 22, 947–949.

Damon, B., Costello, C.E., 1988. A systematic nomenclature for carbohydrate fragmentations in FAB-MS MS spectra of glycoconjugates. Glycoconjugate Journal 5, 397–409.

Gottlieb, O.R., Da Silva, M.L., Maia, J.G.S., 1972. Eucalyptin from *Eugenia* and *Myrcia* species. Phytochemistry 11, 1185.

Horn, D.H.S., Lamberton, J.A., 1963. Nuclear magnetic resonance study of a new flavonoid. Chemistry & Industry 1963, 691.

Howard, G., Mabry, T.J., 1970. Myricetin 3-*O*-methyl ether 3′-*O*-β-D-Glucoside, the major flavonoid of *Oenothera speciosa* (Onagraceae). Phytochemistry 9, 2413–2414.

Hsieh, Y.-L., Fang, J.-M., Cheng, Y.-S., 1998. Terpenoids and flavononoids from *Pseudotsuga wilsoniana*. Phytochemistry 47, 845–850.

Huq, F., Misra, L.N., 1997. An alkenol and C-methylated flavones from *Callistemon lanceolatus* leaves. Planta Medica 63, 369–370.

Jork, H., Funk, W., Fischer, W., Wimmer, H., 1989. Dünnschichtchromatographie, Vol. 1a. Verlag Chemie, Weinheim.

Ma, Y.L., Li, Q.M., van den Heuvel, H., Claeys, M., 1997. Characterization of flavone and flavonol aglycones by collision-induced dissociation tandem mass spectrometry. Rapid Communications in Mass Spectrometry 11, 1357–1364.

Markham, K.R., Geiger, H., 1994. ^{1}H Nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In: Harborne, J.B. (Ed.), The Flavonoids–Advances in Research since 1986. Chapman and Hall, London.

Mayer, R., 1990. Flavonoids from *Leptospermum scoparium*. Phytochemistry 29, 1340–1342.

Mayer, R., 1993. A β-hydroxychalcone from *Leptospermum scoparium*. Planta Medica 59, 269–271.

Rao, J.R., Rao, R.S., 1991. Syzalterin, a new 6,8-di-C-methylflavone from *Syzygium alternifolium* leaves. Indian Journal of Chemistry 30B, 66–67.

Stevens, J.F., Wollenweber, E., Ivancic, M., Hsu, V.L., Sundberg, S., Deinzer, M.L., 1999. Leaf surface flavonoids of *Chrysothamnus*. Phytochemistry 51, 771–780.

Wollenweber, E., Kohorst, G., 1981. Epicuticular leaf flavonoids from *Eucalyptus* species and from *Kalmia latifolia*. Zeitschrift f. Naturforschung 36c, 913–915.

Wollenweber, E., Dietz, V.H., Schilling, G., Favre-Bonvin, J., Smith, D.M., 1985. Flavonoids from chemotypes of the goldback fern, *Pityrogramma triangularis*. Phytochemistry 24, 965–971.

Wollenweber, E., Mann, K., Roitman, J.R., 1996. C-Methyl flavones from the leaf wax of *Leptospermum laevigatum* (Myrtaceae). Zeitschrift f. Naturforschung 51c, 8–10.

Wollenweber, E., Dörr, M., Fritz, H., Valant-Vetschera, K.M., 1997. Exudate flavonoids in several Asteroideae and Cichorioideae (Asteraceae). Zeitschrift f. Naturforschung 52c, 137–143.