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Methylenedioxy- and methoxyflavones from *Melicope coodeana* syn. *Euodia simplex*

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Abstract

Three new natural products, 3,8-dimethoxy-5,7-dihydroxy-3′,4′-methylenedioxyflavone, 3,6,8-trimethoxy-5,7-dihydroxy-3′,4′-methylenedioxyflavone and 3,6,8,3′,4′-pentamethoxy-5,7-dihydroxyflavone were isolated from *Melicope coodeana* syn. *Euodia simplex* (Rutaceae) along with 3,6,3′-trimethoxy-5,7,4′-trihydroxyflavone and 3,3′-dimethoxy-5,7,4′-trihydroxyflavone. The structural assignments are based on ^{1}H and ^{13}C NMR data, including discussion of the chemical shifts of C-2 in 3,5-dihydroxy- and 3-methoxy-5-hydroxyflavones. The presence of highly methoxylated and methylenedioxyflavones is characteristic of the genus *Melicope*, and the present findings support the recent transfer of *Euodia simplex* to *Melicope*.

Keywords: *Euodia simplex*; *Melicope coodeana*; Rutaceae; Methylenedioxyflavones; Methoxyflavones; ^{13}C NMR; Chemotaxonomy; Réunion Island

1. Introduction

In a recent taxonomic revision of *Euodia* J. R. & G. Forst. and of a part of *Melicope* J. R. & G. Forst. (Rutaceae), all *Euodia* species from the Malagasy region were transferred to *Melicope* (Hartley, 2001). As a part of this revision, the name of *Euodia simplex* Frappier ex Cordem., a species endemic to the Réunion Island, was changed to *Melicope coodeana* T. G. Hartley. *Melicope* species are characterized by the presence of highly methoxylated flavonoids, some of them containing the rare methylenedioxy group (Murphy et al., 1974; Fauvel et al., 1981; Jong and Wu, 1989; Higa et al., 1990; Hou et al., 1994). It was, therefore, of interest to establish the flavonoid pattern of *M. coodeana*. No previous phytochemical studies of this species are known.

2. Results and discussion

Extraction of leaves of *M. coodeana* with CH_2Cl_2–MeOH and fractionation of the extract on silica gel afforded five flavonoids. Two major flavonoids contained one methylenedioxy group each, as shown by a characteristic two-proton singlet at δ 6.11 and a methylene carbon resonance at δ 102.5. Both compounds showed spin patterns of a 1,3,4-trisubstituted aromatic ring. NOEs from the methylene group to H-2′ and H-5′ placed the methylenedioxy group in ring B. Chemical shifts of proton and carbon resonances of the B ring in **1** and **2**, identical within experimental error, were closely similar to those reported for similarly substituted flavonoids (Calvert et al., 1979). Compound **1** had a total of two methoxy groups and two hydroxy groups, whereas compound **2** had three methoxy and two hydroxy groups. Both flavonoids showed a low-field proton resonance (δ 13) attributable to a chelated phenol group at C-5.

In a long series of 3-methoxyflavones having a free hydroxy group at C-5, the resonance of C-2 appears in a narrow chemical shift range (δ 154.4–158.3), usually close to δ 156 (Calvert et al., 1979; Roitman and James, 1985; Barbera et al., 1986; Wang et al., 1989; Ahsan et al., 1994; Horie et al., 1995; Boers et al., 1998; Dong et al., 1999). Only a few compounds reported in the literature seem to violate this regularity and their assignments, not established rigorously, appear to be incorrect. Thus,

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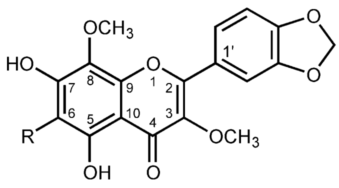
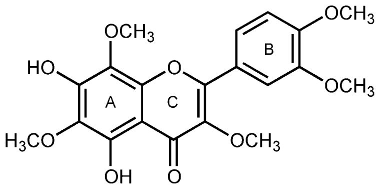


1 R = H
2 R = OCH3



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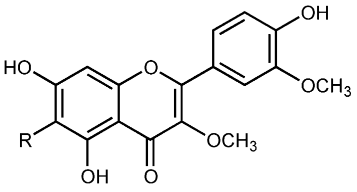

4 R = OCH3
5 R = H

the reported assignments of C-2 (δ 148.1) and C-4′ (δ 157.4) in 3,7,4′-trimethoxy-5,3′-dihydroxyflavone (Dong et al., 1999) should be interchanged. The reported chemical shifts of C-2 (δ around 151.5) in five 3,6,7-trimethoxy-5-hydroxyflavones differing in the substitution pattern of ring B (Calvert et al., 1979; Wang et al., 1989) are in conflict with those reported for a series of 6,7-dioxygenated and 6,7,8-trioxygenated 3-methoxy-5-hydroxyflavones (Roitman and James, 1985; Ahsan et al., 1994). In contrast to the 3-methoxy-5-hydroxyflavones, the chemical shift of C-2 in a large number of 3,5-dihydroxyflavones was found at δ 145.4–147.3 (Markham et al., 1982; Breitmaier and Voelter, 1990; Ashan et al., 1994; Lichius et al., 1994; Dong et al., 1999). Erroneous assignments have to be excluded also here; for example, the resonance at δ 153.7 assigned to C-2 in 3,5-dihydroxy-6,7,8-trimethoxy-3′,4′-methylenedioxyflavone (Hou et al., 1994) presumably belongs to C-5, and the chemical shift of C-2 is apparently δ 145.4. The chemical shift of C-2 thus appears to be one of the best conserved in flavonoids, being expected to be affected appreciably only by the nature of the substituent at C-3 and by a hydrogen bond between 5-OH and the carbonyl group at C-4. *O*-Methylation of the 5-hydroxy group in 3-hydroxy- and 3-methoxyflavones shifts the resonance of C-2 upfield by 3–4 ppm (Calvert et al., 1979; Breitmaier and Voelter, 1990; Dong et al., 1999).

According to the rules outlined above, the presence of a C-2 resonance close to δ 155 proves the 3-methoxyflavone structure of **1** and **2**. Accordingly, one of the methoxy groups present in these compounds exhibited NOEs to H-2′ and H-5′, as expected for a 3-methoxy group. A NOE between the proton of the 5-hydroxy group and the single aromatic hydrogen observed in the ^{1}H NMR spectrum of **1** demonstrated that the 6-position is free. Thus, C-7 and C-8 bear a hydroxy and a methoxy group. Carbon resonances of aromatic methoxy groups flanked by two *ortho* substituents appear around δ 60, whereas those flanked by only one ortho substituent around δ 55 (Roitman and James, 1985). Since both methoxy resonances of **1** were close to δ 60, the second methoxy group is placed at C-8, and hence the hydroxy group is at C-7. All observed HMBC connectivites were in agreement with this structure of **1**. Thus, H-6 showed correlations to C-8 and C-10, 5-OH to C-4, C-5, C-6 and C-10, methoxy protons to the respective carbons C-3 and C-8, and a full coupling network was observed within ring B, including connectivities from H-2′ and H-6′ to C-2.

Compound **2** has a fully substituted A ring. In 5,6,7,8-tetraoxygenated flavonol derivatives with a free hydroxy group at C-5, the resonance of C-5 and C-7 appear near δ 150, and those of C-6 and C-8 near δ 130 (Calvert et al., 1979; Lichius et al., 1994; Horie et al., 1995). In a HMBC spectrum of **2**, the 3-methoxy group correlated with the C-3 resonance at δ 138.9, and the remaining two methoxy groups with carbons at δ 129.0 and 132.8. These two methoxy groups must thus be placed at C-6 and C-8.

NMR spectra of compound **3** showed the presence of five methoxy groups. Analysis of the NMR data similarly as described above showed that **3** differs from **2** by the presence of two methoxy groups instead of the methylenedioxy group in ring B. The compounds **1**–**3** are new natural products. Their NMR data are collected in Tables 1 and 2. Compound **1** has previously been synthesized from 7-prenyloxy-5-hydroxy-3,8-methoxy-3′,4′-methylenedioxyflavone isolated from *M. triphylla* (Higa et al., 1990). Compound **3** has been mentioned in several patents but no description of its preparation, properties or isolation was given. Two last

Table 1
^{1}H NMR (400 MHz) data for flavonoids **1**, **2** and **3**

Hydrogen	**1**[a]	**2**[a]	**3**[b]
6	6.80 (*s*)	–	–
2′	7.84 (*d*)	7.85 (*d*)	7.79 (*d*)
5′	7.08 (*d*)	7.09 (*d*)	7.02 (*d*)
6′	7.92 (*dd*)	7.93 (*dd*)	7.83 (*dd*)
3-OCH$_3$	3.98 (*s*)	3.99 (*s*)	3.98 (*s*)[d]
6- OCH$_3$	–	4.02 (*s*)[c]	3.88 (*s*)[d]
8- OCH$_3$	3.95 (*s*)	4.03 (*s*)[c]	3.97 (*s*)[d]
3′- OCH$_3$	–	–	4.00 (*s*)[d]
4′- OCH$_3$	–	–	4.05 (*s*)[d]
OCH$_2$O	6.11 (*s*)	6.11 (*s*)	–
5-OH	13.01 (*s*)	13.15 (*s*)	12.58 (*s*)
Coupling constants	$^3J_{5',6'} = 8.3$ Hz $^4J_{2',6'} = 1.8$ Hz	$^3J_{5',6'} = 8.3$ Hz $^4J_{2',6'} = 1.8$ Hz	$^3J_{5',6'} = 8.6$ Hz $^4J_{2',6'} = 1.9$ Hz

[a] In pyridine-d_5.

[b] In chloroform-*d*.

[c,d] Assignments marked with the same superscript are interchangeable.

Table 2
^{13}C NMR (100 MHz) data for flavonoids **1**, **2** and **3**

Carbon	**1**[a]	**2**[a]	**3**[b]
2	155.1	155.2	155.6
3	139.2	138.9	138.6
4	179.2	179.5	179.3
5	157.9	152.9[e]	151.5
6	100.2	132.8[f]	130.4[h]
7	158.2	[c,e]	148.0[i]
8	128.7	129.0[f]	127.1[h]
9	[c]	145.6	144.9
10	105.4	104.7	105.1
1′	124.9	125.0	123.1
2′	108.7	108.7	111.0[j]
3′	148.6	148.6	148.6[i]
4′	150.1[d]	150.3[d]	148.8[i]
5′	109.0	109.0	111.1[j]
6′	124.0	124.1	122.2
3-OCH_3	61.3	60.1	–
6-OCH_3	–	61.3[g]	60.2[k]
7-OCH_3	–	–	61.1[k]
8-OCH_3	60.1	60.5[g]	61.7[k]
3′-OCH_3	–	–	55.9[k]
4′-OCH_3	–	–	50.0[k]
OCH_2O	102.5	102.5	–

[a] In pyridine-d_5.

[b] In chloroform-d.

[c] Hidden under the solvent resonance (δ about 149).

[d] Hidden under the solvent resonance, δ value determined from HMBC spectrum.

[e–k] Assignments marked with the same superscript are interchangeable.

flavonoids isolated from *M. coodeana* were identified as **4** and **5** by NOESY experiments and by comparison of their spectral data with those previously reported (Roitman and James, 1985; Wang et al., 1989; Miles et al., 1993).

In addition to the plants belonging to *Melicope*, highly methoxylated and methylenedioxyflavones have so far been isolated from two species of the Rutaceae, *Zieridium ignambiensis* (Elavumoottil et al., 1991) and *Pelea barbigera* (Higa and Scheuer, 1974). In a taxonomic revision by Hartley and Stone (Hartley and Stone, 1989), *Pelea* A. Gray is put in the synonymy with *Melicope*. *Zieridium*, a small genus with only three species all from New Caledonia, belongs to Rutoideae, the same subfamily as *Melicope*. The isolation of **1**–**5** described in the present work fully supports the recent re-classification of *E. simplex* as *M. coodeana* (Hartley, 2001).

3. Experimental

3.1. General

NMR spectra were recorded on a Bruker AMX400 spectrometer at 25 °C, using TMS as internal standard. Mass spectra were obtained with a Jeol JMS-AX505W double-focusing mass spectrometer operating in positive-ion EI mode. UV spectra were obtained from HPLC runs with a Shimadzu SPD-M6A diode array detector. The HPLC system also included two LC-6A pumps and a SCL-6A controller. A 125×4.6 mm Nucleosil 100 C18 (5 μm) column was eluted with MeCN–H_2O containing 0.03% TFA at 1.2 ml/min (linear MeCN gradient from 30 to 50% in 20 min). Preparative HPLC separations were conducted on a Shimadzu system consisting of two LC-10AS pumps, a SCL-10A controller, and a SPD-10A diode array detector, using a 250×12.5 mm column of Nucleosil 100 C18 (5 μm), operated isocratically with 37% MeCN in H_2O with 0.03% TFA. MPLC separations were performed on a 300×30 mm Bæckström column of Merck silica gel 60 (0.040–0.063 mm). VLC and open-column LC separations were performed using Merck silica gel 60 H and Merck silica gel 60 (0.063–0.200 mm), respectively. For TLC, Merck silica gel 60 F_{254} plates were used with toluene–EtOAc–MeOH (30:8:1) as the eluent.

3.2. Plant material

Leaves of *Melicope coodeana* T. G. Hartley (syn. *Euodia simplex* Frappier ex Cordem.) were collected in February 2000 at Plaine de Chicots, Réunion Island, and identified by the authors. The plant material was dried in an air-conditioned room at about 25 °C. Voucher specimen (DFHAA2) was deposited in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen).

3.3. Extraction and isolation

Dried and pulverised plant material (265 g) was extracted with 3×2.8 l of CH_2Cl_2–MeOH (1:1) for one hour on an ultrasonic bath. The combined extracts (57 g) were fractionated by VLC using a step-gradient of EtOAc in heptane, to give a flavonoid-rich fraction (5.4 g) eluting with heptane–EtOAc (1:2). Further fractionation by MPLC and stepwise gradient elution with heptane–EtOAc (4:1 to 1:1), neat EtOAc, and then with EtOAc–MeOH (1:1) yielded fractions 1–7. Compounds **1** (15 mg) and **2** (80 mg) were obtained from fraction 5 (1.5 g, eluted with heptane–EtOAc 1:1) by further fractionation by LC with a step gradient of EtOAc in heptane. Fraction 4 (0.6 g, eluted with heptane–EtOAc 2:1) was subjected to LC twice, using heptane–EtOAc (3:1) and toluene–EtOAc–MeOH (40:8:1), respectively, and **5** (1 mg) was finally purified by prep. HPLC. Compounds **3** and **4** were isolated from fraction 6 (0.1 g, eluted with neat EtOAc) using three successive LC columns eluted with heptane–EtOAc–MeOH (7:3:1), toluene–EtOAc–MeOH (40:6:1) and toluene–EtOAc–MeOH (30:8:1), respectively. Final purification by prep. HPLC yielded pure **3** (8 mg) and **4** (2 mg).

*3.4. 3,8-Dimethoxy-5,7-dihydroxy-3′,4′-methylenedioxyflavone (**1**)*

Yellow crystals (15 mg); EI HRMS *m/z* 358.0689 (M$^{+\cdot}$), $C_{18}H_{14}O_8^{+\cdot}$ requires 358.0683; for NMR data see Tables 1 and 2; UV λ_{max} (HPLC-DAD) 261, 272, 355 nm; TLC R_f 0.55; HPLC k′ 6.5.

*3.5. 3,6,8-Trimethoxy-5,7-dihydroxy-3′,4′-methylenedioxyflavone (**2**)*

Yellow crystals (80 mg); EI HRMS *m/z* 388.0783 (M$^{+\cdot}$), $C_{19}H_{16}O_9^{+\cdot}$ requires 388.0789; for NMR data see Tables 1 and 2; UV λ_{max} (HPLC-DAD) 262, 277, 348 nm; TLC R_f 0.49; HPLC k′ 6.8.

*3.6. 3,6,8,3′,4′-Pentamethoxy-5,7-dihydroxyflavone (**3**)*

Yellow crystals (8 mg); EI HRMS *m/z* 404.1170 (M$^{+\cdot}$), $C_{20}H_{20}O_9^{+\cdot}$ requires 404.1102; for NMR data see Tables 1 and 2; UV λ_{max} (HPLC-DAD) 260, 278, 348 nm; TLC R_f 0.35; HPLC k′ 6.2.

*3.7. 3,6,3′-Trimethoxy-5,7,4′-trihydroxyflavone (**4**)*

Yellow powder (2 mg); UV λ_{max} (HPLC-DAD) 258, 351 nm; TLC R_f 0.25; HPLC k′ 4.1.

*3.8. 3,3-Dimethoxy-5,7,4′-trihydroxyflavone (**5**)*

Yellow powder (1 mg); UV λ_{max} (HPLC-DAD) 258, 349 nm; TLC R_f 0.57; HPLC k′ 7.4.

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