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Flavones and phenylpropenoids in the surface exudate of *Psiadia punctulata*

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Abstract

Three flavones, 5,7-dihydroxy-2′,3′,4′,5′-tetramethoxyflavone, 5,4′-dihydroxy-7,2′,3′,5′-tetramethoxyflavone, and 5,7,4′-trihydroxy-2′,3′,5′ -trimethoxyflavone were isolated from the leaf exudate of *Psiadia punctulata*, together with the previously reported 5-hydroxy-7,2′,3′,4′,5′-pentamethoxyflavone and 5,7,3′-trihydroxy-2′,4′,5′-trimethoxyflavone. The two phenylpropenoids, *Z*-docosyl-*p*-coumarate and *E*-docosyl-*p*-coumarate were also isolated. The structures were determined on the basis of spectroscopic evidence.

Keywords: *Psiadia punctulata*; Compositae; Surface exudates; Flavones; Phenylpropenoids; 5,7-Dihydroxy-2′,3′,4′,5′-tetramethoxyflavone; 5,4′-Dihydroxy-7,2′,3′,5′-tetramethoxyflavone; 5,7,4′-Trihydroxy-2′,3′,5′-trimethoxyflavone; *Z*-Docosyl-*p*-coumarate

1. Introduction

The leaves of *Psiadia punctulata* (DC) Vatke are extensively covered by an exudate, especially when young. The exudate is most likely responsible for the unpalatability (to herbivores) and drought resistance (as we have observed) which is exhibited by this shrub, in a manner comparable to that described for *Larrea* spp. (Rhoades, 1977). The chemistry of the exudate could also explain the use of the species for relief of fever and abdominal pains (Kokwaro, 1976) as well as its use for expunging ectoparasites from cattle (Beentje, 1994).

In an earlier communication we described the presence of kaurene and trachyloban diterpenes in the exudate (Midiwo et al., 1997). We compared these metabolites with the diterpenes reported from *P. arabica* Jaub and Spach, with which *P. punctulata* is sometimes confused (Abou-Zaid et al., 1991), and suggested that there were differences which could be used to delineate them as distinct taxa.

Plant leaf exudates are known to most often contain mixtures of terpenoids and non-polar flavonoids (Wollenweber, 1988). In this paper we report on the isolation and characterisation of five flavones, three of which are new, along with two geometrically isomeric phenylpropenoid docosyl esters, one of which has not been reported before.

2. Results and discussion

Fresh leaves of *P. punctulata* were dipped in ethyl acetate for short periods (ca. 20 s) providing a gummy extract on removal of solvent. We have established (see Experimental) that the average exudate to dry leaf weight is about 1:4. TLC analysis of the extract revealed seven major spots of phenolic nature (254 nm UV light, ferric chloride spray reagent). Silica gel chromatography led to the successful isolation and characterization of five flavones and two phenylpropenoids.

The first compound (**1**) characterised was a flavone. The presence of the flavone moiety was deduced from the UV (λ_{max} 270 and 362 nm), ^{1}H NMR (δ 7.10 for H-3) and ^{13}C NMR (δ 162.7 for C-2, 110.6 for C-3 and 183.3 for C-4) spectra. The ^{1}H NMR spectrum also indicated the presence of a chelated hydroxyl, a free hydroxyl, meta-coupled H-6 and H-8 protons, a single B-ring proton and four methoxyl substituents. In the EIMS, the molecular ion was established as 374 ($C_{19}H_{18}O_8$), and a fragment ion appearing at m/z 153 derived from retro-Diels–Alder fragmentation (Mossa et al., 1992), suggested the presence of two hydroxy groups in ring A, at C-5 and C-7. The four methoxyl

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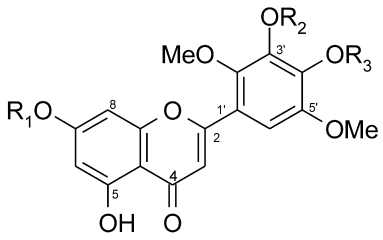

1 $R_1 = H, R_2 = R_3 = Me$
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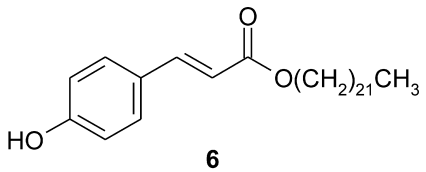

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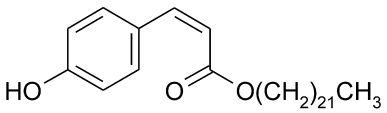

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groups must therefore be placed in ring B, at positions C-2′,-3′,-4′, and -5′. The ^{13}C NMR resonances for the methoxyl carbons (Table 1) confirmed that three were di-*ortho* substituted (resonances > 59.5 ppm) while one had a free *ortho* position (resonance < 58.5 ppm) (Panichpol and Waterman, 1978). In a NOESY experiment the singlet at δ 7.22 (H-6′) was observed to interact with the methoxyl resonance at δ 3.84. On these grounds and comparison with data for 5-hydroxy-7,2′,3′,4′,5′-pentamethoxyflavone (**2**) (Mossa et al., 1992), compound **1** was identified as 5,7-dihydroxy-2′,3′,4′,5′-tetramethoxyflavone.

A second flavone (**3**) was similar to compound **1**, the NMR spectra indicating a tetramethoxyflavone with the same substitution pattern and with EIMS showing M^+ again at 374. However, the EIMS of this compound revealed that it had a methoxyl and a hydroxyl group in ring-A due to the presence of a fragment ion at *m/z* 167, with the hydroxyl group being placed at C-5 (chelated proton at δ 12.78 in the ^1H NMR spectrum). The ^{13}C NMR spectrum indicated the same B-ring oxygenation pattern as in **1** with the chemical shift for the ring-B methoxyls (δ 61.4, 61.3, 56.3 and 56.0) indicating that two of them were di-*ortho* substituted. Thus it was concluded the C-5′ was methoxylated with C-2′, C-3′ and C-4′ bearing two methoxyls and one hydroxyl. This hydroxyl group was deduced to be at the 4′-position because of the 40 nm shift in band I in the UV spectrum on addition of NaOH (Markham, 1982). This requires that compound **3** was 5,4′-dihydroxy-7,2′,3′,5′-tetramethoxyflavone. This assertion was confirmed by NOE interactions between the 4′-hydroxyl proton and two methoxy methyls (δ 3.86 and 4.02) and between one methoxyl (δ 3.89) and H-6 and H-8 and one methoxyl (δ 3.86) and H-6′.

The third flavone (**4**) showed the same oxygenation pattern but with only three methoxyl substituents. The EIMS indicated M^+ at 360 ($C_{18}H_{16}O_8$), with a fragment at *m/z* 153 which places two hydroxyl groups on ring A. The presence of hydroxyl groups at C-5 and C-7 were established by observation of bathochromic shifts for band I at 326 nm on addition of $AlCl_3$ (no change with HCl) and a NaOAc shift of bands II, respectively. This leaves three methoxyls and a further hydroxy group in ring B. The spectroscopic evidence (Experimental and Table 1) indicated that its ring B was identical to that of compound **3**. Compound **4** was therefore designated as 5,7,4′-trihydroxy-2′,3′,5′-trimethoxyflavone.

The other flavones isolated from this exudate were shown to be 5-hydroxy-7,2′,3′,4′,5′-pentamethoxyflavone (**2**) and 5,7,3′-trihydroxy-2′,4′,5′-trimethoxyflavone (**5**) which had previously been isolated from *P. arabica* (El-Feraly et al., 1990; Mossa et al., 1992).

The complexity of the non-polar flavone profile in the *P. punctulata* exudate is much less than that in *P. arabica*. Furthermore the *P. punctulata* flavones appear to be exclusively B-ring tetra-oxygenated while for *P. arabica*

Table 1
^{13}C NMR (125 MHz) chemical shift position for flavones **1**–**5**[a]

C	1	2	3	4	5
2	162.7	161.9	161.9	163.4	163.2
3	110.6	110.3	110.1	110.4	109.5
4	183.3	182.9	182.9	183.4	183.5
4a	105.6	105.8	102.9	105.6	105.5
5	163.7	162.5	162.4	163.7	163.7
6	100.6	98.1	98.2	100.5	100.5
7	166.7	165.7	165.8	166.6	166.4
8	95.5	92.2	92.8	95.5	95.4
8a	159.3	158.1	158.1	159.4	159.3
1′	121.0	120.0	119.8	121.0	115.3
2′	147.9[b]	147.9[b]	141.4[b]	144.0	149.0
3′	147.1[b]	146.8[b]	143.6[b]	146.8	143.5
4′	148.6[b]	147.6[b]	149.0[b]	142.0	146.7
5′	150.7[b]	149.7[b]	139.6[b]	150.6	146.6
6′	107.9	106.5	105.9	103.0	107.6
C-2′-OCH_3	61.6[c]	61.6[c]	61.3[c]	61.2	61.8
C-3′-OCH_3	61.9[c]	61.6[c]	61.4[c]	61.1	
C-4′-OCH_3	61.8[c]	61.5[c]			61.1
C-5′-OCH_3	56.9	56.7[d]	56.3[d]	56.8	57.0
C-7- OCH_3		56.0[d]	56.0[d]		

[a] Compounds **1**, **4** and **5** run in pyridine-d_5; **2** and **3** in $CDCl_3$.
[b,c,d] Values in the same column are interchangeable.

examples are observed of mono-, di-, tri- and tetra-oxygenation in this ring (Abou-Zaid et al., 1991). The differences in flavonoid composition sets the two taxa apart in the same manner as the diterpenoid disparities already noted. Therefore their proposed synonymy (Abou-Zaid et al., 1991) may not be correct.

Together with the flavones two isomeric *p*-coumarate esters were isolated. Compound **6** was identified as E-docosyl *p*-coumarate which is already known from the stems of *Bauhinia manca* (Achenbach et al., 1986). Compound **7** had similar characteristics to **6** and was shown to be its *Z*-isomer, which seems to be reported here for the first time. While the main components of aerial exudates are non-polar flavonoids and terpenes these two non-polar esters indicate that they are sometimes other minor components of the hydrophobic external exudate.

3. Experimental

3.1. General

Mps: uncorr. Analytical TLC: Merck pre-coated silica gel 60 F_{254} plates. CC on silica gel 60 (70–230 mesh). EIMS: direct inlet, 70 eV. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were run on a DRX-500 (Brüker) spectrometer using solvent resonances to calibrate the spectra.

3.2. Plant material

The material used and vouchers have been described previously (Midiwo et al., 1997).

3.3. Extraction

Extraction with ethyl acetate was done as previously described (Midiwo et. al., 1997) to give 494 g of exudate from aerial parts which when dried weighed 1700 g. The crude extract (100 g) was adsorbed onto silica gel (100 g) and added to a silica gel (500 g) column under *n*-hexane. Elution was achieved with *n*-hexane/CH_2Cl_2/MeOH mixtures of increasing polarity to give **1** (2.2 g), **2** (2.0 g), **3** (58 mg), **4** (10 mg), **5** (130 mg), **6** (267 mg), **7** (60 mg).

3.4. Physical and spectral data of isolated compounds

3.4.1. 5,7-Dihydroxy-2′,3′,4′,5′-tetramethoxyflavone (*1*)

Yellow needles (*n*-hexane/CH_2Cl_2). Mp 226–227°. UV λ_{max} (MeOH) nm: 216, 270, 362; NaOMe: 218, 285, 325; NaOAc: 222, 272, 362; NaOAc/H_3BO_3: 222, 272, 362; $AlCl_3$: 216, 285, 363; $AlCl_3$/HCl: 218, 279, 368. ^{1}H NMR (pyridine-d_5): δ 3.84 (OMe-5′), 3.88 (OMe-4′), 3.95 (OM-2′), 3.99 (OMe-3′), 6.79 (1H, *d*, J=2.0 Hz, H-6), 6.85 (1H, *d*, J=2.0 Hz, H-8), 7.10 (1H, *s*, H-3), 7.22 (1H, *s*, H-6′), 13.63 (1H, *s*, 5-OH). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.): 374 (54, $[M]^+$), 359 (5), 333 (6), 301 (4), 257 (7), 153 (8), 149 (14), 129 (12), 115 (64), 69 (64), 61 (100).

3.4.2. 5-Hydroxy-7,2′,3′,4′,5′-pentamethoxyflavone (*2*)

Yellow needles (*n*-hexane/CH_2Cl_2). Mp 127–128° (lit. 124–125° Mossa et al. 1992). UV λ_{max} (MeOH) nm: 216, 268, 330; NaOMe: 220, 281, 364; NaOAc: 221, 270, 330; NaOAc/H_3BO_3: 221, 270, 330; $AlCl_3$: 218, 280, 364; $AlCl_3$/HCl: 220, 280, 362. ^{1}H NMR ($CDCl_3$): δ 3.88 (OMe-4′), 3.89 (OMe-7), 3.92 (OMe-2′), 3.97 (Me-5′), 3.99 (Me-3′), 6.38 (1H, *d*, J=2.2 Hz, H-6), 6.45 (1H, *d*, J=2.2 Hz, H-8) 6.88 (1H, *s*, H-3) 7.03 (1H, *s*, H-6′), 12.81 (1H, *s*, H-5). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.): 388 (100, $[M]^+$), 373 (9), 342 (10), 7 (6).

3.4.3. 5,4′-Dihydroxy-7,2′,3′,5′-tetramethoxyflavone (*3*)

Yellow needles (*n*-hexane/CH_2Cl_2). Mp 164–166°. UV λ_{max} (MeOH) nm: 218, 267, 324; NaOMe: 220, 266, 364; NaOAc: 225, 260, 336; NaOAc/H_3BO_3: 225, 265, 323; $AlCl_3$: 220, 276, 362; $AlCl_3$/HCl: 220, 276, 362. ^{1}H NMR ($CDCl_3$): δ 3.86 (OMe-5′), 3.89 (OMe-7), 3.93 (OMe-2′), 4.02 (OMe-3′), 6.12 (1H, *s*, 4′-OH), 6.39 (1H, *d*, J=2.0 Hz, H-6), 6.46 (1H, *d*, J=2.0 Hz, H-8) 6.85 (1H, *s*, H-3), 6.93 (1H, *s*, H-6′), 12.78 (1H, *s*, 5-OH). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.): 374 (100, $[M]^+$), 359 (8). 167 (6), 57 (8).

3.4.4. 5,7,4′-Trihydroxy-2′,3′,5′-trimethoxyflavone (*4*)

Yellow needles (*n*-hexane/EtOAc). Mp 231–233°.UV λ_{max} (MeOH) nm: 228, 269, 326; NaOMe: 230, 272, 368; NaOAc: 226, 272, 360; NaOAc/H_3BO_3: 226, 266, 368; $AlCl_3$: 228, 274, 360; $AlCl_3$/HCl: 228, 274, 334. ^{1}H NMR (acetone-d_6): δ 3.84(OMe-5′), 3.87(OMe-4′), 3.91 (OMe-2′), 6.27 (1H, *d*, J=2.0 Hz, H-6) 6.49 (1H, *d*, J=2.0 Hz, H-8), 6.49 (1H, *d*, J =2.0, H-8), 6.76 (1H, *s*, H-3), 6.97 (1H, *s*, H-6′) 13.68 (1H, *s*, 5-OH). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.): 360 (100, $[M]^+$), 345 (8), 243 (11), 165 (6), 153 (9).

3.4.5. 5,7,3′-Trihydroxy-2′,4′,5′-trimethoxyflavone (*5*)

Yellow needles (*n*-hexane/EtOAc). Mp 254–255° (lit. 232–233° Mossa et al. 1992) UV λ_{max} (MeOH) nm: 216, 270, 362; NaOMe: 220, 280, 408; NaOAc: 220, 278, 365; NaOAc/H_3BO_3: 220, 268, 362; $AlCl_3$: 216, 276, 408; $AlCl_3$/HCl: 216, 276, 408. ^{1}H NMR (CD_3OD): δ 3.85 (OMe-5′), 3.92 (OMe-3′), 3.98 (OMe-2′), 6.78 (1H, *d*, J=2.1 Hz, H-6), 6.85 (1H, *d*, J=2.1 Hz, H-8), 7.04 (1H, *s*, H-3), 7.18 (1H, *s*, H-6′) 13.78 (1H, *s*, 5-OH). ^{13}C NMR: see Table 1. EIMS *m*/*z* (rel. int.): 360 (100, $[M]^+$), 345 (8).

3.4.6. E-Docosyl-p-coumarate (*6*)

White flakes (*n*-hexane/EtOAc). Mp 84–86°. UV λ_{max} (MeOH) nm: 214, 230, 300, 310, 360. ^{1}H NMR ($CDCl_3$): δ 7.63 (1H, *d*, J=16.0 Hz, H-β) 7.44 (2H, *d*, J=8.7 Hz, H-2/6), 6.82 (2H, *d*, J=8.7 Hz, H-3/5), 6.31 (1H, *d*, J=16.0 Hz, H-α), 4.20 (2H, *t*, OCH_2), 1.27–1.70

(37H, *m*, aliphatic chain), 0.89 (3H, *t*, Me). ^{13}C NMR ($CDCl_3$): δ 173.0 (C=O), 159.0 (C-4), 144.5 (C-α), 131.4 (C-2/6), 127.5 (C-5), 116.1 (C-3/5) 110.1 (C-β), 64.9 (OCH_2), 22.1–33.3 (aliphatic chain), 15.5 (Me).

3.4.7. Z-Docosyl-p-coumarate (7)

White flakes (*n*-hexane/EtOAc). Mp 61–63°. UV λ_{max} (MeOH) nm: 214, 230, 300, 360. ^{1}H NMR ($CDCl_3$): δ 7.64 (2H, *d*, $J=8.6$ Hz, H-2/6), 6.87 (2H, *d*, $J=8.6$ Hz, H-3/5), 6.82 (1H, *d*, $J=12.8$ Hz, H-α), 5.86 (1H, *d*, $J=12.8$ Hz, H-β), 4.15 (2H, *t*, OCH_2), 1.27–1.31 (37H, *m*, aliphatic chain), 0.89 (3H, *t*, Me). ^{13}C NMR ($CDCl_3$): δ 167.1 (C=O), 156.0 (C-4), 143.6 (α-C), 132.0 (C-2/6), 127.7 (C-1) 117.4 (C-3/5), 115.2 (C-β), 64.8 (OCH_2), 22.9–32.2 (aliphatic chain), 14.3 (Me). EIMS *m*/*z* (rel. int.): 472 (6, $[M]^+$), 444 (5), 416 (24), 164 (100), 147 (41), 120 (18), 69 (17), 59 (17).

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