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Lignans and coumarins metabolites from *Melicope hayesii*

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Abstract

Two novel compounds, the lignan, (+)-2-(3,4-dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane, and the coumarin, (+)-7-(3-methyl-4-carboxybutanoxy)umbelliferone methyl ester, have been isolated from the aerial parts of *Melicope hayesii*. The known compounds, eudesmin, kobusin, *N-p*-coumaroyltyramine, *N*-methyl-2-pyrrolidinone, umbelliferone and 7-(3-methylbut-2-enoxy)umbelliferone were also obtained. Their structures were determined by extensive NMR studies.

Keywords: Melicope hayesii; Rutaceae, lignan; (+)-2-(3,4-Dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane, coumarin; (+)-7-(3-Methyl-4-carboxybutanoxy)umbelliferone methyl ester

1. Introduction

Melicope hayesii is a newly identified species that occurs in the coastal rainforests of southeast Queensland and northeast New South Wales in the altitudinal range 140–900 m (Hartley, 1990). Our phytochemical investigation on the aerial parts of this species has led to the isolation of three structurally related lignans, (+)-2-(3,4-dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo-[3,3,0]octane (**1**), (+)-eudesmin (**3**) and (+)-kobusin (**4**), three simple coumarins, (+)-7-(3-methyl-4-carboxybutanoxy)umbelliferone methyl ester (**2**), umbelliferone (**7**) and 7-(3-methylbut-2-enoxy)umbelliferone (**8**), and two amides, *N-p*-coumaroyltyramine (**5**) and *N*-methyl-2-pyrolidinone (**6**). Compounds **1** and **2** are novel and **6** is reported for the first time from a natural source, while compound **7** may be an artefact.

2. Results and discussion

Extraction of the leaves and stems led to the isolation of **1** and **5**, while **2**, **4** and **6**–**8** were obtained from the wood. Compound **3** was isolated from both extracts. Compounds **3** (Pelter, 1967; Pelter, Ward, Venkata Rao, & Sastry, 1976), **4** (Iida, Nakano, & Ito, 1982), **5** (Atta-ur-Rahman, Bhatti, Akhtar, & Choudhary, 1992), **6** (Fieser & Fieser, 1967), **7** (Chang & Floss, 1977) and **8** (Lassak & Southwell, 1972) were identified by comparison with literature data.

Compound **1** was observed on TLC under UV (254 and 366 nm) as a dark quenching spot. The HREI mass spectrum gave an $[M]^+$ that solved for $C_{20}H_{22}O_6$, in agreement with that required for a 2,6-diaryl-3,7-dioxabicyclo[3,3,0]octane lignan, with two hydroxyl and two methoxyl substituents. The ^{1}H NMR spectrum showed resonances confirming a 2,6-diaryl-3,7-dioxabicyclo [3,3,0]octane lignan (Pelter et al., 1976), with two methoxyl groups and six aromatic protons, suggesting the presence of two 3,4-substituted aryl substituents. The resonances for the protons of the fused difuran system showed equivalence for H-1/H-5, H-2/H-6 and H_2-4/H_2-8, so requiring a symmetrical substitution stereochemistry for the aryl substituents (Pelter et al., 1976). Furthermore, the chemical shift observed for the benzylic protons (H-2/H-6; δ 4.71, d, $J=4.3$ Hz) was in accordance with that reported where the two aryl substituents are equatorial (Pelter et al., 1976).

However, the ^{13}C NMR spectrum exhibited 20-carbon resonances, instead of the 10 anticipated for a completely symmetrical lignan, such as pinoresinol (see Table 1) (Pelter et al., 1976; Vermes, Seligman, & Wagner, 1991) and the ^{1}H NMR spectrum also showed non-equivalent chemical shifts for two series of aromatic protons. This suggested different substituents on the two aryl rings at C-2 and C-6, resulting in magnetic non-equivalence of signals.

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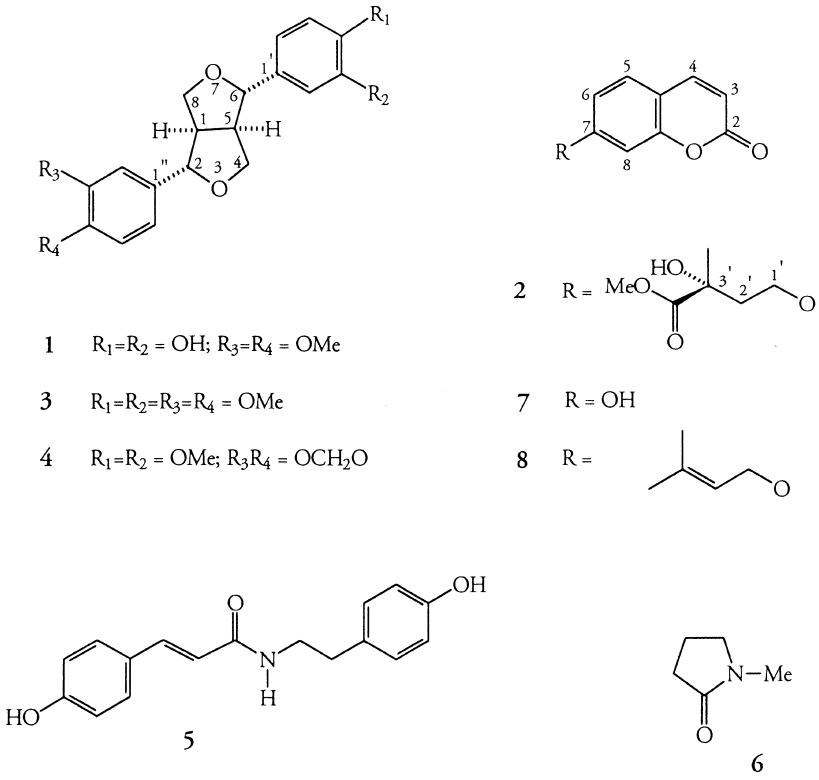

Structure 1

Table 1
^{13}C NMR shift data for lignans **1**, **3** and **4**

C	**1**	**3**[b]	**4**[b]
1/5	54.17/54.21	54.40	54.93
2/6	85.96/86.08	86.01	86.57
4/8	71.83/91.95	71.95	71.92
1′/1″	133.52/133.69	133.79	135.70
2′/2″	109.48/113.60	109.5	106.40
3′/3″[a]	143.72/148.89	148.88	147.80
4′/4″[a]	144.08/149.90	149.45	148.80
5′/5″	111.29/115.59	111.31	109.30
6′/6″	118.55/118.87	118.47	119.30
OMe	56.16/56.18	56.16/56.18	56.10
–O–Me–O			101.20

[a] Signals assigned to C-3 and C-4 of the aryl groups are not definitely assigned in all cases.

[b] All signals, except the methylenedioxy carbon in **4**, represent two equivalent carbons.

A bathochromic shift ($\lambda = 13$ nm) was observed in the UV spectrum of **1** in the presence of $AlCl_3$ and H_3BO_3, indicating that 3,4-dihydroxy substitution in a benzene ring was part of the structure (Mabry, Markham, & Thomas, 1970) and so implying the presence of a 3,4-dimethoxyphenyl group as the other substituent. The latter was confirmed by means of nuclear Overhauser enhancement difference experiments, in which irradiation of the two methoxyl resonances caused enhancement of H-2 (*meta*-coupled doublet) and H-5 (*ortho*-coupled doublet) of the same ABD-spin system. The asymmetric nature of the substituents was further supported by the fragmentation pathway observed in the HR-EI mass spectrum, which showed ions corresponding to dimethoxyphenyl (m/z 137) and dihydroxyphenyl (m/z 109) fragments. Compound **1** is therefore the novel lignan 2-(3,4-dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane. On the basis of its dextrorotatory nature, the absolute configuration must be as depicted (Pelter et al., 1976). Two other lignans were also isolated, eudesmin (**3**) and kobusin (**4**) and they exhibited the same absolute stereochemistry.

Compound **2** was observed on TLC as a bright blue fluorescent spot under UV light (266 nm). The HR-EI mass spectrum suggested an $[M]^+$ solving for $C_{15}H_{16}O_5$, with a base peak at m/z 115 $(C_6H_{11}O_2)^+$ and a major fragment at m/z 162 (162 = umbelliferone). This suggested a substituted 7-oxycoumarin structure, which was supported by the UV spectrum (λ_{max} 313–330 nm), which is attributable to a 7-oxycoumarin (Murray, Mendez, & Brown, 1982).

The ^{1}H NMR spectrum confirmed the presence of a 7-oxygenated coumarin. The remaining signals for the 7-*O*-substituent were observed as two multiplets centred at δ 1.91 and δ 2.20 for the two non-equivalent protons of a methylene coupled to a downfield multiplet at δ 4.05 (2H) for another methylene group attached to the oxygen at C-7. An upfield doublet (3H) at δ 1.25 could be

assigned to a secondary methyl coupled to a methine at δ 2.72 which, in turn, coupled to the non-equivalent methylene protons. A singlet at δ 3.70 (3H) had to be attributed to a methoxyl.

The ^{13}C NMR spectrum showed 15 carbon resonances of which nine could be assigned to umbelliferone (Murray et al., 1982). The remaining resonances were in close agreement with those reported for a 3-methyl-4-carboxybutanoxy substituent (McCormick, McKee, Cardellina, & Boyd, 1996), so that **2** could be identified as 7-(3-methyl-4-carboxybutanoxy)umbelliferone methyl ester. The fragmentation pathway obtained from HR-EI mass spectrum supported the assignment of structure (**2**). Coumarin (**2**) was accompanied by two other known coumarins, 7-(3-methylbut-2-enoxy)coumarin (**8**) and umbelliferone (**7**). The stereochemistry at C-3 in **2** is assumed to be (*S*), because the optical rotation is positive and comparable to the same prenyloxy substituent in the furoquinoline alkaloid, roxiamine-A (McCormick et al., 1996). The corresponding free acid, which has been isolated from another species of Rutaceae (Lassak & Southwell, 1972) is also reported to be (*S*) but has a small negative rotation.

In addition to the lignans and coumarins, two nitrogenous compounds were isolated, *N*-methyl-2-pyrrolidone (**6**) and *N*-*p*-coumaroyltyramine (**5**). No trace of the acridone and furoquinoline alkaloids, highly oxygenated flavonoids or acetophenones that have been found in a number of other species of *Melicope* (Ng et al., 1987) were detected in our study. Similar lignans have been isolated from *M. micrococca*, as *Euodia micrococca*, by Cameron and Sutherland (1961). This species is regarded by Hartley (1990) as probably being the most closely related to *M. hayesii*. However, the most striking chemical similarity for *M. hayesii* is with another sympatric taxon, *M. vitiflora*, which under its previous name, *Euodia vitiflora*, was reported to contain the free acid form of **2** (Lassak & Southwell, 1972).

3. Experimental

3.1. General

M.p.'s: uncorr. UV: MeOH. ^1H (400 MHz) and ^{13}C NMR (100.56 MHz) run in $CDCl_3$, unless otherwise stated. EIMS at 70 eV. Vacuum liquid chromatography (VLC) and CC were performed on Merck (7736) silica gel 60 H (0.04–0.005 mm) and Merck (7734) silica gel (0.063–0.2 mm), respectively. Analytical TLC and prep. TLC were performed on precoated Merck F_{254} silica gel plates and visualised by spraying with anisaldehyde–H_2SO_4. Gel filtration chromatography (GFC) was performed on Sephadex LH-20 (0.25–1 mm).

3.2. Plant material

Melicope hayesii T.G. Hartley was collected at Lamington National Park, Whian Whian State Forest and Bellangry State Forest, Australia. Leaves and stems were separated from the wood. A voucher specimen, TGH 15157 has been deposited at the Australia National Herbarium.

3.3. Extraction and isolation

Dried ground material from plant parts was subjected to sequential Soxhlet extraction with petrol (b.p. 40–60°C), EtOAc and MeOH. Concentrated extracts were initially subjected to VLC with solvents of increasing polarity.

Fr. 8 (2.97 g) obtained by VLC of the EtOAc extract of leaves and stems with 10%–30% EtOAc in hexane yielded (**3**) (59.7 mg). The fr. (1.09 g) eluted with 60% EtOAc in hexane showed on TLC (hexane–EtOAc, 7:3), two dark spots under UV light. Further purification by GFC ($CHCl_3$–MeOH) yielded frs containing the novel impure lignan **1** and **5** (3.1 mg). The lignan fr. was further purified by prep. TLC (EtOAc) to give **1** (4.2 mg).

From the VLC of the EtOAc extract of wood, a fr. eluted with 10–25% EtOAc in hexane showed several bright blue fluorescent compounds under UV (366 nm). This fr. was subjected to GFC ($CHCl_3$–MeOH) and yielded **7** (4.9 mg) and a fr. containing several bright blue fluorescing compounds. These were separated by CC eluting with hexane containing increasing amounts of $CHCl_3$ to give **2** (6.9 mg), **8** (4.2 mg), further **3** (5.3 mg), **4** (5.1 mg) and **6** (1.7 mg).

3.4. (+)-2-(3,4-Dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo [3,3,0] octane (*1*)

Amorphous solid. $[\alpha]_D$ +29° ($CHCl_3$, 0.05). Yield: 1.7×10^{-3}% rel. to plant material. Found: $[M]^+$ m/z 358.1443; $C_{20}H_{22}O_6$ requires 358.1416. UV λ_{max} nm: 206, 217, 279. IR ν_{max} cm^{-1} ($CHCl_3$): 2947, 2831, 2518, 2225, 1450, 1238, 1114, 1039, 1020, 439. ^1H NMR: δ 3.10 (2H, m, H-1/H-5), 3.87 (2H, m, H-4_{ax}/H-8_{ax}), 3.89, (6H, s, 2×OMe), 4.23 (2H, dd, J=6.9. 9.1 Hz, H-4_{eq}/H-8_{eq}), 4.71 (2H, d, J=4.3 Hz, H-2/H-6), 6.75-6.90 (6H, m, Ar–H). ^{13}C NMR: Table 1. EIMS m/z (rel. int.): 358 $[M]^+$ (100), 343 (3), 327 (14), 219 (8), 191 (28), 192 (22), 177 (46), 166 (73), 165 (2), 151 (35), 137 (33), 109 (15).

3.5. (+)-7-(3-Methyl-4-carboxybutanoxy)umbelliferone methyl ester (*2*)

Oil. $[\alpha]_D$ +14° ($CHCl_3$, 0.07). Yield: 1.1×10^{-3}% rel. to plant material. Found: $[M]^+$ m/z 276.0996; $C_{15}H_{16}O_5$ requires 276.0998. UV λ_{max} nm: 248, 256, 325. IR ν_{max} cm^{-1} ($CHCl_3$): 3020, 1730, 1614, 1519, 1425. ^1H NMR: δ 6.24 (1H, d, J=9.5 Hz, H-3), 7.62 (1H, d, J=9.5 Hz, H-4), 7.35 (1H, d, J=8.5 Hz, H-5), 6.80 (1H, dd, J=8.5, 2.3 Hz, H-6), 6.83 (1H, d, J=2.3 Hz, H-8), 1.91/2.20 (2H, m, H-2′), 2.72 (1H, m, H-3′), 4.05 (2H, m, H-1′),

1.25 (3H, d, Me), 3.70 (3H, s, OMe). ^{13}C NMR: δ 17.47(Me-3′), 32.89 (C-2′), 36.47 (C-3′), 51.97 (OMe-4′), 66.47 (C-1′), 101.67 (C-8), 112.80 (C-4a), 113.01 (C-3), 113.35 (C-6), 128.96 (C-5), 143.56 (C-4), 156.09 (C-8a), 161.37 (C═O-2), 162.22 (C-7), 176.61 (C═O-4′). EIMS m/z (rel. int.): 276 $[M]^+$ (11), 245 (14), 217 (10), 175 (21), 162 (23), 134 (47), 115 (100).

3.6. (+)-Eudesmin (**3**)

Amorphous solid. $[\alpha]_D$ +60° ($CHCl_3$, 1), lit. (Iida et al., 1982) +61°. Yield: 7.2×10^{-3}% rel. to plant material. (UV, IR, ^{1}H NMR and EIMS, in agreement with lit. (Iida et al., 1982). ^{13}C NMR: Table 1.

3.7. (+)-Kobusin (**4**)

Colourless oil. $[\alpha]_D$ +58° ($CHCl_3$, 0.03). lit. (Iida et al., 1982) +59°. Yield: 7.8×10^{-4}% rel. to plant material. Found: $[M]^+$ m/z 370.1396; $C_{21}H_{22}O_6$ requires 370.1416. (UV, IR, ^{1}H NMR and EIMS, in agreement with lit. (Iida et al., 1982)). ^{13}C NMR: Table 1

3.8. N-p-Coumaroyltyramine (**5**)

Amorphous solid. Yield: 1.3×10^{-3}% rel. to plant material. Found: $[M]^+$ m/z 283.1170; $C_{17}H_{17}NO_3$ requires 283.1208. UV, IR, NMR and EIMS, in agreement with lit. (Atta-ur-Rahman et al., 1992).

3.9. N-Methyl-2-pyrolidinone (**6**)

Brown oil. Yield: 2.6×10^{-4}% rel. to plant material. Found: $[M]^+$ m/z 99.0689; C_5H_9NO requires 99.0684. UV λ_{max} nm: 203, 223, 283. IR ν_{max} cm^{-1} ($CHCl_3$): 3307, 2943, 1659. ^{1}H NMR: δ 1.97 (2H, qn, J=7.6 Hz, H-4), 2.33 (2H, t, J=8.1 Hz, H-3), 2.80 (3H, s, NMe), 3.34 (2H, t, J=7.04 Hz, H-5). ^{13}C NMR ($CDCl_3$): δ 17.73 (C-4), 29.70 (Me), 30.75 (C-3), 49.57 (C-5), 175.34 (C═O). EIMS (70 eV) m/z (rel. int.): 99 $[M]^+$ (100), 86 (100), 84 (100).

3.10. Umbelliferone (**7**)

Colourless needles, m.p. 223–225°C, lit. (Murray et al., 1982) 223–224°C. Yield: 7.5×10^{-4}% rel. to plant material. Found: $[M]^+$ m/z 162.1446; $C_9H_6O_3$ requires 162.0317. UV, IR, ^{1}H NMR, ^{13}C NMR and EIMS, in agreement with those of authentic sample.

3.11. 7-(3-Methylbut-2-enoxy)umbelliferone (**8**)

Needles from *n*-hexane, m.p. 77–78°C, lit. (Lassak & Southwell, 1972) 77–78°C. Yield: 6.4×10^{-4}% rel. to plant material. Found: $[M]^+$ m/z 230.0948; $C_{14}H_{14}O_3$ requires 230.0943. UV, IR, ^{1}H NMR and EIMS in agreement with lit. (Lassak & Southwell, 1972; Gray, 1981). ^{13}C NMR ($CDCl_3$): δ 17.47, 26.03 (2×Me-3′), 65.67 (C-1′), 118.87 (C-2′), 139.50 (C-3′), 101.80 (C-8), 113.21 (C-4a), 113.46 (C-3), 114.61 (C-6), 128.90 (C-5), 143.64 (C-4), 156.12 (C-8a), 161.37 (C═O-2), 162.37 (C-7). EIMS (70 eV) m/z (rel. int.) 230 $[M]^+$ (5), 182 (12),162 (100), 134 (47), 105 (11).

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