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PHYTOCHEMISTRY

Two novel prenylated flavones from the aerial parts of *Melicope micrococca*

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Abstract

Two novel prenylated flavones, 3,5,4′-trihydroxy-8,3′-dimethoxy-7-(3-methylbut-2-enoxy)flavone and 3,5,8,4′-tetrahydroxy-7,3′-dimethoxy-6-(3-methylbut-2-enyl)flavone have been isolated from the aerial parts of *Melicope micrococca* (Rutaceae) together with the known compounds, quercetin 3-*O*-β-D-glucopyranoside, isorhamnetin 3-*O*-rutinoside, the acridone alkaloid arborinine, sitosterol 3β-*O*-glucopyranoside (daucosterol) and 5-*O*-methyl-*myo*-inositol (sequoyitol). The structures of all compounds were elucidated by spectroscopic methods.

Keywords: Melicope micrococca; Rutaceae; Flavones; 3,5,4′-trihydroxy-8,3′-dimethoxy-7-(3-methylbut-2-enyloxy)flavone; 3,5,8,4′-tetrahydroxy-7,3′-dimethoxy-6-(3-methylbut-2-enyl)flavone

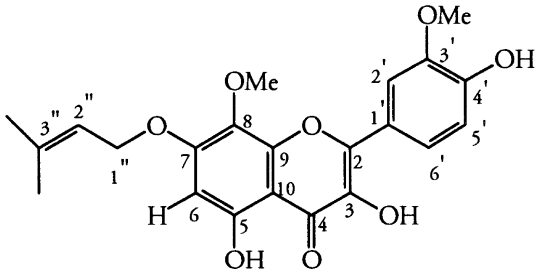
1. Introduction

Melicope micrococca (F. Muell) T. Hartley (Rutaceae), formerly known as *Euodia micrococca* F. Muell. (Hartley, 1990), is a small tree about 5–10 m high, occurring in the rain forests of northern New South Wales and Queensland. Previous studies on this species (as *E. micrococca*) yielded the two common lignans pinoresinol dimethyl ether and sesamin (Cameron & Sutherland, 1961).

As part of our collaboration with the Australian National Herbarium we have undertaken a further phytochemical investigation on this species. This has led to the isolation and structural elucidation of the two novel flavones 3,5,4′-trihydroxy-8,3′-dimethoxy-7-(3-methylbut-2-enoxy)flavone (**1**) and 3,5,8,4′-tetrahydroxy-7,3′-dimethoxy-6-(3-methylbut-2-enyl)flavone (**2**).

2. Results and discussion

A petroleum ether (bp 40–60°C) extract of the aerial parts of *M. micrococca* yielded the novel flavone (**1**) and the acridone alkaloid arborinine. Further extraction with ethyl acetate yielded a second new flavone (**2**), daucosterol and sequoyitol. Finally, the ethyl acetate soluble portion of the methanol extract gave two known flavone glycosides, quercetin-3-*O*-β-D-glucopyranoside and iso-



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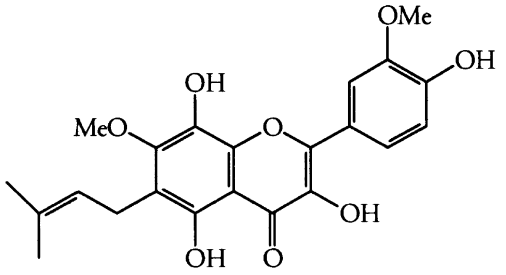

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rhamnetin-3-*O*-rutinoside. All known compounds were identified by comparison of their spectral properties with those reported in the literature (see Section 3).

Novel flavone **1** gave an HREI mass spectrum $[M]^+$ ion that solved for $C_{22}H_{22}O_8$ and a base peak at m/z 346 due to the loss of $[C_5H_8]^+$, which is typical for the loss of an *O*-substituted 3-methylbut-2-enyl group substituent. The ^{1}H NMR spectrum (Table 1) revealed resonances for two methoxyl groups, three phenolic hydroxyl protons of which one (δ 12, 24) was strongly hydrogen bonded, four aromatic protons including three in the form of an ABD spin-system and a 3-methylbut-2-enyloxy group. Analysis of the UV spectrum utilising shift reagents (Mabry, Markham, & Thomas, 1970; Harborne, 1998) indicated the presence of hydroxyl groups at C-3, C-5 and C-4′.

These data suggested that **1** was a hexahydroxyflavone oxygenated at 3,5,7,3′,4′ and either 6 or 8 and with two methoxyl and one prenyloxy substituent. The ^{13}C NMR spectrum Table 1 showed one methoxyl carbon resonated at δ 55.53 that meant that neither or only one of the *ortho* positions were substituted and the other at δ 60.83, indicating that both positions *ortho* to the methoxyl were substituted (Panichpol & Waterman, 1978). The positions of substituents were confirmed by long-range heteronuclear correlation studies using HMBC (Bax & Summers, 1986) (Table 1) and through phase-sensitive NOESY.

In the HMBC spectrum ^2J and ^3J couplings from the readily identified H-bonded 5-hydroxyl group identified C-6 as the unsubstituted position on the A-ring. Comparable couplings from H-6 identified C-10 and C-5 (also identified by 5-OH) and C-7 and C-8 as resonances at δ157.0 and 128.5, respectively, the latter being relatively shielded for an O-bearing carbon because it is flanked by two other O-bearing carbons. The prenyloxy substituent could then be assigned to C-7 because of a ^3J coupling between the C-1′ methylene protons and C-7 and the methoxyl (δ 3.83) to C-8 because of a similar interaction to that carbon. The second methoxyl (δ 3.85) showed a ^3J correlation to one of the two *ortho* oxygenated positions of ring-B and this was confirmed as being C-3′ because of the nOe interaction observed between that methoxyl and H-2′. Compound (**1**) was therefore unambiguously identified as the novel flavone 3,5,4′-trihydroxy-8,3′-dimethoxy-7-(3-methylbut-2-enyloxy) flavone.

Compound (**2**) analysed by HREI mass spectrum for M^+ 414.1281 indicating an empirical formula of $C_{22}H_{22}O_8$. UV and IR spectra were very similar to **1**. The ^{1}H NMR spectrum (Table 2) again showed a 3′,4′-disubstituted B-ring, two methoxyls and a 3-methylbut-2-enyl. However, in this case there was no A-ring proton and the prenyl methylene resonated at δ 3.28 requiring a C-prenyl rather than an *O*-prenyl substituent. The ^{13}C NMR spectrum (Table 2) revealed chemical shifts that suggested the same 3,5,7,8,3′,4′-pattern on a flavone nucleus. One of the methoxyl groups could be placed at C-3′ by direct analogy to **1** and through nOe. An HMBC study (Table 2) once more allowed placement of A-ring

Table 1
NMR spectral data for compound **1** (400 MHz, δ values in DMSO-d_6)

	^{1}H	^{13}C	^2J	^3J
2		146.9		
3	9.54 (s, OH)	135.9		146.9
4		176.2		
5	12.24 (s, OH)	155.6	155.6	96.2, 103.3
6	6.58 (s)	96.2	155.6, 157.0	103.3, 128.5
7		157.0		
8		128.5		
9		147.7		
10		103.3		
1′		122.1		
2′	7.79 (d, J=2.1 Hz)	111.3	122.1	121.7, 146.9, 148.9
3′		147.4		
4′	9.79 (s, OH)	148.9	148.9	115.7, 147.4
5′	6.97 (d, J=8.4 Hz)	115.7	121.7, 148.9	122.1, 147.4
6′	7.75 (dd, J=2.1, 8.4 Hz)	121.7	122.1	111.3
1″	4.68 (d, J=6.7 Hz)	65.7	119.2	138.3, 157.0
2″	5.47 (t, J=6.7 Hz)	119.2		18.0, 25.4
3″		138.2		
3″-Me	1.77 (s)	25.4		
3″-Me	1.74 (s)	18.1		
8-OMe	3.83 (s)	60.8		128.5
3′-OMe	3.85 (s)	55.5		147.4

Table 2
NMR spectral data for compound **2** (400 MHz, δ values in DMSO-d_6)

	^{1}H	^{13}C	^2J	^3J
2		146.3		
3	9.46 (s, OH)	135.8		
4		176.0		
5	12.41 (s, OH)	152.6	152.6	102.5, 110.4
6		110.4		
7		154.3		
8	10.24 (s, OH)	127.1	127.1	
9		149.4		
10		102.5		
1′		122.2		
2′	7.78 (d, J=2.0 Hz)	111.2	122.2	121.6, 146.3, 148.8
3′		147.4		
4′	9.48 (s, OH)	148.8		
5′	6.98 (d, J=8.4 Hz)	115.7	121.6, 148.8	122.1, 147.4
6′	7.73 (dd, J=2.0, 8.4 Hz)	121.6	115.7, 122.2	146.3
1″	3.28 (d, J=7.2 Hz)	21.6	110.4	130.7
2″	5.18 (t, J=7.2 Hz)	122.1		
3″		130.7		
3″-Me	1.74 (s)	25.5		
3″-Me	1.63 (s)	17.7		
7-OMe	3.85 (s)	61.3		154.3
3′-OMe	3.86 (s)	55.6		147.4

substituents. The ^3J couplings from the 5-OH proton identified C-6 as the δ 110.4 resonance and this was the established as the position of the prenyl substituent because of a ^2J interaction for the H-1″ to C-6. The placement of the second methoxyl at C-7 was also established by the HMBC experiment which showed coupling between the methoxyl protons and the carbon at δ 154.3. On this basis **2** was characterised as 3,5,8,4′-tetrahydroxy-7,3′-dimethoxy-6-(3-methylbut-2-enyl)flavone.

The two quercetin glycosides, the acridone alkaloid arborinine and daucosterol were all characterised by analysis of NMR spectra and comparison with published data. A simple mono-methyl myo-inositol derivative isolated from the EtOAc extract was identified as the 5-*O*-methyl ether, sequoyitol. The ^{1}H and ^{13}C NMR spectra are reported in full in Section 3, the assignments being supported by HC-COBI-dec (^1J) and HMBC (^2J, ^3J) heteronuclear coupling experiments.

The synthesis of polyoxygenated *O*-prenyl flavonoids and acridones is one of the major traits that has been observed in the chemistry of *Melicope sensu lato* (Ng et al., 1987) so that chemically *M. micrococca* can be viewed as typical of the genus. The permethylated derivative of **1** has previously been reported from the related species *Melicope triphylla* (Higa, Miyagi, Yogi, & Hokama, 1987).

3. Experimental

Mps: uncorr. UV: MeOH. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.56 MHz) run in DMSO-d_6 unless otherwise stated. EIMS at 70 eV. Vacuum liquid chromatography (VLC) and column chromatography (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 PTLC 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).

3.1. Plant material

The aerial parts were collected from a tree 10 m high at the margin of the rainforest about 12 km west of Bellinger, north-east New South Wales. A voucher specimen (TGH 15158) has been deposited at the Australian national herbarium.

3.2. Isolation of compounds

The dried, ground plant material (242 g) was extracted in a Soxhlet separately and successively with petroleum ether (40–60°C), EtOAc and MeOH. Extracts were concentrated by rotary evaporation under vacuum at a maximum temperature of 40°C. The MeOH extract was subsequently partitioned between EtOAc and water. The petroleum ether extract (10.62 g) was fractionated by VLC over silica gel using solvents of increasing polarity. The fraction obtained with 25–35% EtOAc in *n*-hexane was subsequently subjected to gel filtration (Sephadex

LH-20) eluting with $CHCl_3$ and then $CHCl_3$–MeOH mixtures, to give **1** (100 mg). The VLC eluate 45–55% EtOAc in n-hexane was further fractionated by silica gel CC. A fraction obtained by elution of the column with 4–10% MeOH in $CHCl_3$ was finally purified by prep. TLC (solvent 5% MeOH in $CHCl_3$) to give arbornine (5 mg).

The EtOAc extract (11.63 g) was fractionated by VLC and the eluate obtained from 45% EtOAc in *n*-hexane was first treated by gel filtration (Sephadex LH-20) eluting with $CHCl_3$ and then by CC over silica gel to give a fraction which was finally purified by prep. TLC (10% MeOH in $CHCl_3$) to give **2** (8 mg). The VLC fraction eluted with 1–4% MeOH in EtOAc was further treated by gel filtration and CC in the same manner to yield daucosterol (30 mg). The VLC fraction eluted with 15–20% MeOH in EtOAc was purified by CC over silica gel to give sequoyitol (18 mg).

VLC of the EtOAc soluble part of the MeOH extract (2.85 g) was carried out as previously described. The fraction eluted with 10–25% MeOH in EtOAc was further treated by CC to give two compounds from 40–80% Me_2CO in $CHCl_3$ and 1–5% MeOH in Me_2CO, respectively. These were then separately treated by CC on Sephadex LH-20, eluting with $CHCl_3$, followed by MeOH to give quercetin-3-*O*-*β*-D-glucopyranoside (50 mg) and isorhamnetin-3-*O*-rutinoside (35 mg).

*3.3. 3,5,4′-Trihydroxy-8,3′-dimethoxy-7-(3′-methylbut-2′-enoxy)flavone (**1**)*

Amorphous yellow solid. Found: M^+ 414.1467; $C_{22}H_{22}O_8$ requires 414.1315. UV λ_{max} (EtOH) nm: 259, 387; (+2M NaOH): 260, 354, 438; (+5% $AlCl_3$), 271, 369, 403; (+NaOAc) 259, 391; (+NaOAc–H_3BO_3) 259, 387. IR ν_{max} (KBr) cm^{-1}: 3482, 3313, 1648, 1620, 1602, 1560, 1513, 1322, 1162, 1128, 1035, 995, 879, 784, 754. EIMS *m*/*z* (rel. int.) 414 (31), 0.346 (100) $[M–C_6H_8]^+$, 331 (91), 317 (39). ^1H NMR, ^{13}C NMR (DMSO-d_6), see Table 1.

*3.4. 3,5,8,4′-Tetrahydroxy-7,3′-dimethoxy-6-(3″-methylbut-2″-enyl)flavone (**2**)*

Amorphous yellow powder. Found: M^+ 414.1281; $C_{22}H_{22}O_8$ requires 414.1315. UV λ_{max} (EtOH) nm: 259, 276, 345, 379; (+2M NaOH): 284, 333, 429; (+5% $AlCl_3$), 269, 376, 444 (+NaOAc), 259, 343, 379; (+NaOAc–H_3BO_3): 259, 344, 379. IR ν_{max} (KBr) cm^{-1}: 3401 (br), 1648, 1623, 1567, 1513, 1442, 1384, 1328, 1272, 1272, 1207, 1184, 1037, 873, 798. EIMS *m*/*z* (rel. int.): 414 (100), 399 $[M–CH_3]^+$ (28), 371 (59), 359 (93), 343 (98), 315 (20). ^1H NMR, ^{13}C NMR (DMSO-d_6); see Table 2.

3.5. Quercetin 3-O-β-D-glucopyranoside

Amorphous yellow solid. UV, ^1H NMR, ^{13}C NMR, FAB-MS in agreement with literature data (Mabry et al., 1970; Agarwal, 1989; Markham, Ternai, Stanley, Geiger, & Mabry, 1978).

3.6. Isorhamnetin 3-O-rutinoside

Amorphous yellow solid. UV, ^1H NMR, ^{13}C NMR, FAB-MS in agreement with literature data (Mabry et al., 1970; Agarwal, 1989; Markham et al., 1978; Breitmaier & Voelter, 1987).

3.7. Arborinine (1-hydroxy-2,3-dimethoxy-10-methylacridone)

Yellow amorphous solid, mp 172–174°C (Ref. (Banerjee, Chakravati, Chakravati, Fales, & Klayman, 1961) 175°C). UV, IR, ^1H NMR, ^{13}C NMR, EIMS data in agreement with authentic sample.

3.8. Daucosterol

White amorphous solid. UV, IR, ^1H NMR, ^{13}C NMR data as agreement with authentic sample.

3.9. Sequoyitol

Needles from 90% EtOH, mp 232–234°C (Ref. (Anderson, DeLuca, Bieder, & Post, 1957) 241–242°C). ^1H NMR (400 MHz, D_2O): δ 4.00 (1H, t, $J=2.9$ Hz, H-2), 3.66 (2H, t, $J=9.9$ Hz, H-4, H-6), 3.56 (3H, s, 5-OMe), 3.50 (2H, dd, $J=9.9$, 2.9 Hz, H-1, H-3), 3.03 (1H, t, $J=9.6$ Hz, H-5). ^{13}C NMR (100.56 MHz, D_2O) δ 85.3 (C-5), 73.1 (C-2), 72.8 (C-4, C-6), 72.2 (C-1, C-3), 60.8 (OMe).

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