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CYTOTOXIC PRENYLATED FLAVANONES FROM *MONOTES ENGLERI*

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Key Word Index—*Monotes engleri*; Dipterocarpaceae; leaves; prenylated flavanones; human cancer cell lines; cytotoxic activity.

Abstract—From the leaves of *Monotes engleri*, five prenylated flavanones were isolated as constituents that displayed cytotoxic activity against several human cancer cell lines. Three of these substances are novel, namely, 6-(1,1-dimethylallyl)naringenin, 6-(1,1-dimethylallyl)eriodictyol and 3′-*O*-methyl-6-(1,1-dimethylallyl)-eriodictyol, with the other two active substances being the known flavanones, 6,8-diprenyleriodictyol and hiravanone. Additionally, two novel, but non-cytotoxic, biogenetically related flavanones were isolated, 6-[(2*RS*)-hydroxy-3-methyl-3-butenyl]-8-prenyleriodictyol and 5,4′-dihydroxy-4″,4″-dimethyl-5″-methyl-5″*H*-dihydrofurano[2″,3″:6,7]flavanone. The structures of the new compounds were determined by spectral analysis 1D- and 2D-NMR experiments.

INTRODUCTION

The genus *Monotes* belongs to the subfamily Monotoideae of the Dipterocarpaceae; altogether, 36 species are distributed in Africa and Madagascar [1]. While several species of another subfamily, Dipterocarpoideae, of the Dipterocarpaceae, have been reported as sources of triterpenes [2–8], sesquiterpenes [9, 10] and stilbenoids [11–17], plants of the subfamily Monotoideae have not yet been studied in detail. However, preliminary phytochemical tests on the leaves and wood of *M. engleri* using only UV and TLC have been reported [18], and an extract of the bark of *M. engleri* has been found to exhibit anti-candidial activity [19].

As a part of an ongoing collaborative search for novel antineoplastic agents of plant origin, the leaves of *M. engleri* were collected in Zimbabwe and subjected to detailed laboratory investigation, since an ethyl acetate-soluble extract was found to exhibit significant cytotoxicity against several human cancer cell lines. We report herein five prenylated flavanones (**1–5**) that were isolated in this study as cytotoxic constituents of the leaves of *M. engleri*. Three of these compounds, **1–3**, are novel structures, and two novel flavonoids, **6** and **7**, that were non-cytotoxic, are also described. In addition, the cytotoxic potential of **1–7** against a panel of human tumor cell lines is presented.

RESULTS AND DISCUSSION

An ethyl acetate extract of the leaves of *M. engleri* was fractionated utilizing a human glioma cell line (U373) to monitor bioactivity. Five prenylated flavanones (**1–5**) were isolated as active constituents through bioassay-guided chromatographic fractionation techniques, along with two non-cytotoxic flavanones (**6** and **7**).

Compound **1**, obtained as the major constituent, was deduced as having an elemental formula of $C_{20}H_{20}O_5$ by HREI mass spectrometry (obsd *m/z* 340.1312). Compound **1** showed characteristic signals for a flavanone at δ_H 2.72 (1H, *dd*, J = 17.1 and 3.0 Hz, H-3β), 3.19 (1H, *dd*, J = 17.1 and 13.3 Hz, H-3α) and 5.42 (1H, *dd*, J = 13.3 and 3.0 Hz, H-2β), in its ^{1}H NMR spectrum, and at δ_C 43.7 (C-3) and 79.6 (C-2) in its ^{13}C NMR spectrum [20, 21]. The carbonyl group at C-4 was hydrogen-bonded with the C-5 OH group, as evidenced by a signal which appeared as a singlet at δ_H 13.24 in the ^{1}H NMR spectrum of **1**. Two sets of ^{1}H NMR doublet signals at δ_H 6.90 (2H, *d*, J = 8.7 Hz, H-3′ and H-5′) and 7.39 (2H, *d*, J = 8.7 Hz, H-2′ and H-6′), which coupled with the ^{13}C NMR resonances at δ_C 116.1 and 129.0, respectively, in the ^{1}H-^{13}C HETCOR NMR spectrum of **1**, gave evidence

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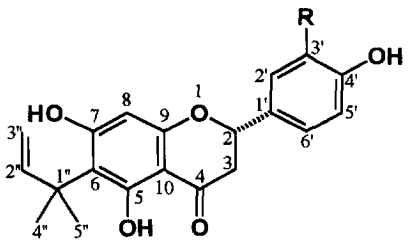

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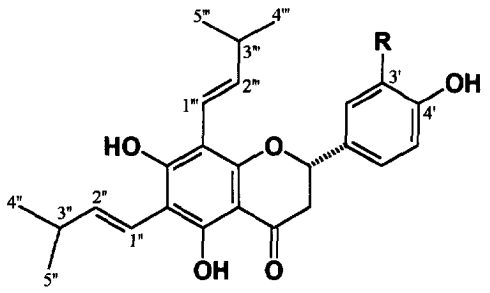
3 R = OCH_3



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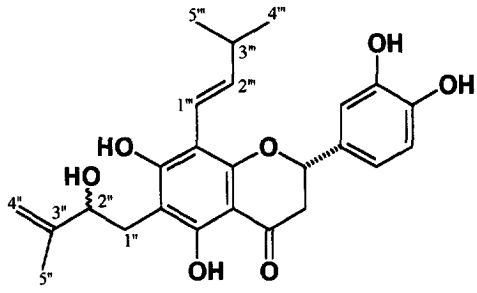

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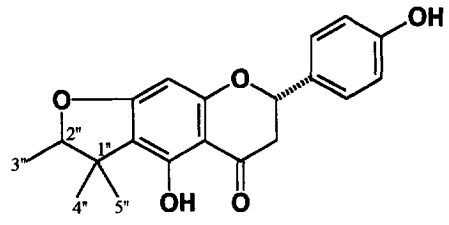

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of a 4′-monosubstituted B-ring. A 1,1-dimethylallyl substituent was apparent from the observed characteristic ^{1}H NMR signals for this functionality [δ_H 1.57 (6H, *s*, H_3-4″ and H_3-5″), 4.83 (1H, *dd*, J = 10.6 and 1.6 Hz, H-3″ *cis*), 4.93 (1H, *dd*, J = 17.5 and 1.6 Hz, H-3″ *trans*), and 6.32 (1H, *dd*, J = 17.5 and 10.6 Hz, H-2″)] [22, 23], which correlated with several ^{13}C NMR signals [δ_C 29.2 (C-4″, interchangeable with C-5″), 29.3 (C-5″, interchangeable with C-4″), 108.5 (C-3″), and 150.9 (C-2″)] in the HETCOR NMR spectrum of **1**. Since an aromatic singlet at δ_H 5.98 (1H) also occurred in the ^{1}H NMR spectrum, the structure of **1** could be proposed as being a flavanone containing a 1,1-dimethylallyl group at C-6 or C-8. The position of this substituent was suggested as being at C-6 on the basis of a previous report which pointed to differences in the chemical shift value of OH-5 (δ_H 13.24 in **1**) in the ^{1}H NMR spectra of flavanones dependent on the presence or absence of C-6 and C-8 substituents [24]. A selective INEPT NMR experiment [25] was used to confirm the position of the 1,1-dimethylallyl group, in which irradiation ($^3J_{CH}$ = 5 Hz) at δ_H 13.24 led to the enhancement of the ^{13}C NMR resonances at δ_C 164.6 (C-5), 113.6 (C-6) and 103.3 (C-10). Similar irradiation of H_3-4″/H_3-5″ (δ_H 1.57), H-2″ (δ_H 6.32) and H-3″ (δ_H 4.83), enhanced the carbon signals at δ_C 150.9 (C-2″), 113.6 (C-6), and 41.1 (C-1″); 113.6 (C-6), 41.1 (C-1″), and 29.2 (C-4″, 5″); and 41.1 (C-1″) and 150.9 (C-2″), respectively. Therefore, the position of substitution of the 1,1-dimethylallyl group was determined unambiguously as being C-6. All ^{1}H and ^{13}C NMR assignments for **1** were performed using appropriate ^{1}H-^{1}H homonuclear decoupling, COSY, APT, HETCOR and selective INEPT NMR experiments. To define the configuration at C-2 in the molecule of **1**, a CD experiment was run, in which a positive Cotton effect was observed at *ca* 330 nm ($n \rightarrow \pi^*$ transition) along with a negative Cotton effect at 280–290 nm ($\pi \rightarrow \pi^*$ transition), consistent with the compound being a 2*S*-flavanone [26]. Thus, the structure of **1** was assigned as the novel compound 6-(1,1-dimethylallyl)naringenin.

The molecular formulae of the novel compounds **2** and **3** were established in turn as $C_{20}H_{20}O_6$ and $C_{21}H_{22}O_6$ by HREI mass spectrometry (obsd m/z 356.1266 and 370.1419, respectively). Analysis of their ^{1}H NMR, ^{13}C NMR and mass spectral data indicated that they were closely related flavanone structures containing a 1,1-dimethylallyl functionality in the same manner as compound **1** but differing only in their B-ring pattern. Compound **2** showed the presence of a 3′,4′-dihydroxyphenyl group, with signals at δ_H 6.66 (2H, *s*, H-5′ and H-6′) and 7.02 (1H, *s*, H-2′) in its ^{1}H NMR spectrum and signals at δ_C 114.7 (C-2′), 116.0 (C-5′) and 119.2 (C-6′), in its ^{13}C NMR spectrum. A mass spectral fragment ion of **2** at m/z 136 provided evidence of a 3′,4′-dihydroxphenyl group as constituting the B-ring of **2** [27]. The B-ring of **3** was

deduced as a 3′-*O*-methyl-4′-hydroxyphenyl moiety [δ_H 3.88 (3H, *s*, OCH_3), 6.87 (1H, *d*, $J = 8.1$ Hz, H-5′), 7.00 (1H, *dd*, $J = 8.1$ and 1.8 Hz, H-6′) and 7.18 (1H, *d*, $J = 1.8$ Hz, H-2′); δ_C 56.3 (OCH_3), 111.1 (C-2′), 115.7 (C-5′), and 120.5 (C-6′)], by comparison with reported data for this functionality [27]. Therefore, compounds **2** and **3** were structurally assigned as 6-(1,1-dimethylallyl)eriodictyol and 3′-*O*-methyl-6-(1,1-dimethylallyl)eriodictyol, respectively.

Compounds **4** ($C_{25}H_{28}O_6$) and **5** ($C_{26}H_{30}O_6$) were identified as the known compounds 6,8-diprenyleriodictyol [27] and hiravanone [28], respectively, by comparison of their spectral data with published values.

The novel compounds **6** and **7** are biogenetically related to the other flavanones obtained in this investigation. Compound **6** exhibited a molecular formula of $C_{25}H_{28}O_7$, as deduced by HREI mass spectrometry (obsd *m/z* 440.1830). The ^1H and ^{13}C NMR spectra of **6** indicated resonances attributable to prenyl substitution [δ_H 1.59 (3H, *s*, H_3-4‴, interchangeable with H_3-5‴, 1.61 (3H, *s*, H_3-5‴, interchangeable with H_3-4‴), 3.23 (2H, *m*, H-1‴) and 5.21 (1H, *m*, H-2‴); δ_C 17.8 (C-4‴, interchangeable with C-5‴), 22.6 (C-1‴), 25.9 (C-5‴, interchangeable with C-4‴), 123.9 (C-2‴) and 130.9 (C-3‴)], which are similar to those of the prenyl unit at C-8 in the structures of **4** and **5**. Another side-chain, a 2-hydroxy-3-methyl-3-butenyl group [δ_H 1.81 (3H, *s*, H_3-5″), 2.73, 2.78 (1H, *dd*, $J = 14.5$ and 9.0 Hz, H-1″a), 2.98, 3.01 (1H, *dd*, $J = 14.5$ and 2.0 Hz, H-1″b), 4.25, 4.27 (1H, *m*, H-2″), 4.76 (1H, *br*, *s*, H-4″a), and 5.01 (1H, *d*, $J = 1.5$ Hz, H-4″b)] was identified by comparison with reported ^1H NMR data for this functionality [20, 29, 30]. These ^1H NMR signals were correlated with the carbons at δ_C 18.5 (C-5″), 29.4 (C-1″), 77.2, 77.3 (C-2″) and 110.3, 110.4 (C-4″) using a HETCOR NMR experiment. It was apparent that the C-6 prenyl group in **4** had been replaced by a 2-hydroxy-3-methyl-3-butenyl group [20]. Compound **6** occurred as a mixture of epimers at the asymmetric centre of the 2-hydroxy-3-methyl-3-butenyl group, which could be separated by HPLC, as described in the Experimental. Although the ^1H NMR assignments for both epimers of **6** could be obtained at 500 MHz, it was not possible to obtain additional spectroscopic data and thus fully solve the structures of these epimers (**6A** and **6B**) because of compound quantity limitations.

The molecular formula of the novel compound **7** was established by HREI mass spectrometry (obsd *m/z* 340.1312) as $C_{20}H_{20}O_5$. Analysis of the ^1H NMR spectrum indicated resonances consistent with the presence of a trimethyldihydrofuran ring [δ_H 1.18 (3H, *s*, H_3-4″, interchangeable with H_3-5″), 1.34 (3H, *d*, $J = 7$ Hz, H_3-3″), 1.42 (3H, *s*, H_3-5″, interchangeable with H_3-4″), and 4.46 (1H, *q*, $J = 7$ Hz, H-2″)] [31], which revealed correlations with the ^{13}C NMR signals at δ_C 21.0 (C-4″; interchangeable with C-5″), 25.5 (C-3″), 14.5 (C-5″; interchangeable with C-4″), and 91.6 (C-2″) in the HETCOR NMR spectrum of **7**. A selective INEPT NMR experiment was employed to afford stronger evidence for the position of the trimethyldihydrofuran functionality in **7**. A soft-proton irradiation ($^3J_{CH} = 5$ Hz) of OH-5 at δ_H 12.4 enhanced the carbon signals at δ_C 104.0 (C-10), 115.3 (C-6) and 160.1 (C-5). Similar irradiations at δ_H 1.19 (H_3-4″; interchangeable with H_3-5″; $^3J_{CH} = 4$ Hz) and at δ_H 4.46 (H-2″; $^3J_{CH} = 6$ Hz) resulted in carbon enhancements at δ_C 43.7 (C-1″), 91.6 (C-2″) and 115.3 (C-6), and at δ_C 21.0 (C-4″; interchangeable with C-5″) and 25.5 (C-3″). Thus, it was apparent that the trimethyldihydrofuran ring of **7** was linked to C-6 and C-7 in the A-ring of **7**. Comparison of the ^1H- and ^{13}C-NMR spectral data of **7** with those of compound **1**, suggested that trimethyldihydrofuran ring in **7** might occur by cyclization of the 1,1-dimethylallyl group with OH-7 in **1**. A chiral centre existing in the trimethyldihydrofuran ring of **7** resulted in the occurrence of epimers and slightly different resonances of these unresolved epimers were observed in the ^1H and ^{13}C NMR spectra of **7**, as presented in the Experimental and Table 1. No attempt was made to separate these epimers because **7** was also isolated in relatively small quantities.

Compounds **1**–**7** were evaluated against a panel of human cancer cell lines [32] and the results are reported in Table 2. Compounds **1**–**5** were broadly cytotoxic (ED_{50} values < 4 μg ml^{-1}) in several cell lines, whereas compounds **6** and **7** were non-cytotoxic. Compounds **4** and **5**, which contain two prenyl side-chains at C-6 and C-8, exhibited stronger activity than compounds **1**–**3**, which contain a 1,1-dimethylallyl group at C-6.

Prenylated flavanones have been found to occur in several families such as the Leguminosae [20, 21, 33–40], Compositae [24, 41], Rutaceae [28, 42], Platanaceae [43], Meliaceae [26, 44] and Velloziaceae [27]. In terms of their biological activity, certain prenylated flavanones from the Velloziaceae have been shown to exhibit antifungal activity [27], and prenylated flavonoids from *Morus* species (Moraceae) were reported as inhibitors of tumor promotion [45]. In addition, some isoflavonoids containing a 1,1-dimethylallyl group exhibited antimutagenic activity [46].

EXPERIMENTAL

Mps: uncorr. IR: film. ^1H and ^{13}C NMR: 300, 360 or 500 MHz instruments with TMS as int. standard. EIMS (70 eV) direct probe.

Plant material. Leaves of *M. engleri* Gilg were collected in a tropical rain forest at Chinhoi, Zimbabwe, in December 1991 and identified by one of the authors (T.E.C.). A voucher specimen (A858) has been deposited in the Field Museum of Natural History, Chicago, Illinois, U.S.A.

Extraction and isolation. Air-dried, milled leaves (250 g) were extracted twice overnight with MeOH (2 × 1.5 l). The resultant extracts were combined, concd and diluted with H_2O to afford an aq. MeOH

Table 1. ^{13}C NMR data of compounds **1–7**

Carbon	Compound 1	2	3	4	5	6	7
2	79.6	79.6	79.9	79.6	79.8	79.63, 79.49	80.07, 80.04
3	43.7	43.7	43.8	43.5	43.7	43.61, 43.45	43.48, 43.35
4	197.5	197.5	197.5	197.8	197.8	197.6	197.8
5	164.6	164.5	164.6	160.0	160.1	160.4	160.18, 160.12
6	113.6	113.6	113.6	108.5	108.6	106.6	115.3
7	166.1	166.4	166.3	162.2	162.2	165.0	168.1
8	96.5	96.6	96.6	107.8	107.7	109.0	91.2
9	161.9	161.9	161.9	158.8	158.8	158.7	164.61, 164,59
10	103.3	103.2	103.2	103.2	103.2	102.8	104.0
1′	130.9	131.6	131.3	131.8	131.6	131.9	130.7
2′	129.0	114.7	111.1	114.5	110.9	114.5	116.1
3′	116.1	145.9	148.4	145.9	148.3	146.0	129.0
4′	158.7	146.4	147.9	146.1	147.7	146.1	158.7
5′	116.1	116.0	115.7	115.9	115.6	115.9	129.0
6′	129.0	119.2	120.5	118.9	120.2	118.9	116.1
1″	41.1	41.3	41.4	21.7	21.7	29.4	43.74, 43.66
2″	150.9	151.0	150.9	123.3*	123.4*	77.35, 77.21	91.6
3″	108.5	108.3	108.4	131.9†	131.9†	147.8	25.5
4″	29.2*	29.2*	29.2*	17.9‡	17.9‡	110.42, 110.36	21.0*
5″	29.3*	29.3*	29.3*	25.8‡	25.8‡	18.5	14.5*
1‴	—	—	—	22.4	22.4	22.6	—
2‴	—	—	—	123.2*	123.3*	123.9	—
3‴	—	—	—	132.0†	132.1†	130.9	—
4‴	—	—	—	17.9§	17.9§	17.8*	—
5‴	—	—	—	25.8§	25.8§	25.9*	—
OCH_3	—	—	56.3	—	56.2		—

*†‡§ Assignments bearing the same superscript are interchangeable.

Table 2. Evaluation of cytotoxic potential of isolates obtained from *M. engleri* with human cancer cell lines

Compound	Cell line*† A431	BC1	Col2	HT	KB	KB-VI	LNCaP	Lu1	Mel2	U373	ZR-75-1
1	1.2	4.6	15.8	>20	>20	15.8	>20	>20	5.9	3.3	6.7
2	13.7	>20	11.9	7.1	>20	>20	12.1	>20	10.3	5.3	15.8
3	7.8	2.6	5.4	7.6	>20	5.7	8.0	8.4	10.2	1.9	6.6
4	10.9	2.6	4.8	5.1	5.4	4.8	1.5	15.7	6.8	3.1	3.4
5	5.5	3.4	9.0	5.9	4.2	10.4	>20	10.4	10.6	2.8	6.0
6	>20	18.3	>20	17.4	>20	>20	19.5	>20	18.4	>20	10.5
7	>20	>20	>20	>20	>20	8.4	12.3	17.1	>20	>20	4.1

* Key: A431 = epidermoid carcinoma; BC1 = breast cancer, Col2 = colon cancer, HT = fibrosarcoma; KB = oral epidermoid carcinoma; KB-V1 = multidrug-resistant KB; LNCaP = prostate cancer, Lu1 = lung cancer; Me12 = melanoma; U373 = glioma; ZR-75-1 = hormone-dependent breast cancer.

† Results are exposed as ED_{50} values ($\mu g\ ml^{-1}$) and were obtained using standard protocols [32].

soln (300 ml), which was washed with hexane (2 × 300 ml). The aq. layer was partitioned with EtOAc (3 × 300 ml). The combined EtOAc layers were evapd to give an extract (16 g), which exhibited cytotoxicity against several human cell lines. The EtOAc extract was sepd into 15 frs by silica gel (500 g) CC using hexane–EtOAc (gradient mixts) for elution with cytotoxicity monitored using a human glioma cell line (U373). Fr. 4 (1 g), which eluted with hexane–EtOAc (7:3), showed significant inhibitory activity (ED_{50} 3.4 $\mu g\ ml^{-1}$) against U373 cells, and was subjected to silica gel H CC using $CHCl_3$–MeOH (100:1) and hexane–EtOAc (10:1), repeatedly, to afford, in turn, compounds **1** (20 mg, 0.008% w/w) and **7** (12 mg, 0.005% w/w). The fifth fr. from this column (1.2 g) (U373; ED_{50} 2.7 $\mu g\ ml^{-1}$) eluted with hexane–EtOAc (1:1) was further fractionated by CC to afford **4** (38 mg, 0.015% w/w) and **6** (13 mg, 0.005% w/w) using $CHCl_3$–MeOH (gradient mixts) for elution, with the final purification of these compounds conducted by prep. TLC using $CHCl_3$–MeOH (15:1 and 10:1, respectively). Compound **6** could be sepd into two

main isomers, **6A** (0.8 mg) and **6B** (0.9 mg) by HPLC using MeOH–MeCN–H_2O (29:6:15). However, due to the small amount of each isomer, only their respective ^1H NMR spectrum at 500 MHz were obtained. Compound **2** (5 mg, 0.002% w/w) was obtained from the more polar fr. 9 of the original CC sepn [hexane–EtOAc (1:9)] and purified by prep. TLC using hexane–EtOAc (5:3). The first fr. of the original CC sepn [hexane–EtOAc (9:1)] was applied onto a further silica gel column to provide **3** (8 mg, 0.003% w/w) and **5** (30 mg, 0.012% w/w) using $CHCl_3$ as eluent, with final purification effected by prep. TLC using $CHCl_3$–MeOH (100:1, R_f 0.4 and 0.5, respectively). Compounds **1–3** showed an orange colour and compounds **4** and **5** exhibited a light yellow colour, using 10% vanillin–H_2SO_4 and heating.

6-(1,1-*Dimethylallyl*)*naringenin* (**1**). Colourless needles (hexane and EtOAc), mp 167–168°. $[\alpha]_D^{25}$ −5.5° (Me_2CO; *c* 0.3). CD: $\Delta\varepsilon_{253}$ +1.4, $\Delta\varepsilon_{289}$ −4.6, $\Delta\varepsilon_{353}$ +0.7 (MeOH; *c* 0.29 mM). UV λ_{max}^{MeOH} nm (log ε): 239 (3.88), 287 (3.90), 336 (3.54). IR ν_{max} (film) cm^{-1}: 3383, 3084, 2966, 1930, 1701, 1634, 1520, 1445, 1342, 1296, 1155, 1101, 897. ^1H NMR (300 MHz, Me_2CO-d_6): δ 1.57 (6H, *s*, H_3-4″ and H_3-5″), 2.72 (1H, *dd*, *J* = 17.1 and 3.0 Hz, H-3β), 3.19 (1H, *dd*, *J* = 17.1 and 13.3 Hz, H-3α), 4.83 (1H, *dd*, *J* = 10.6 and 1.6 Hz, H-3″ *cis*), 4.93 (1H, *dd*, *J* = 17.5 and 1.6 Hz, H-3″ *trans*), 5.42 (1H, *dd*, *J* = 13.3 and 3.0 Hz, H-2β), 5.98 (1H, *s*, H-8), 6.32 (1H, *dd*, *J* = 17.5 and 10.6 Hz, H-2″), 6.90 (2H, *d*, *J* = 8.7 Hz, H-3′ and H-5′), 7.39 (2H, *d*, *J* = 8.7 Hz, H-2′ and H-6′), 13.24 (1H, *s*, 5-OH). ^{13}C NMR (75 MHz; Me_2CO-d_6): Table 1. EIMS (70 eV) *m/z*: $[M]^+$ 340 (59), 325 (45), 297 (8), 220 (12), 205 (100), 192 (27), 179 (12), 165 (12), 120 (17), 111 (17), 107 (11). HREIMS *m/z*: 340.1312 (calcd for $C_{20}H_{20}O_5$, 340.1311).

6-(1,1-*Dimethylallyl*)*eriodictyol* (**2**). Colourless powder (hexane), mp > 300°. $[\alpha]_D^{25}$ 0° (Me_2CO; *c* 0.1). CD: $\Delta\varepsilon_{253}$ +0.4, $\Delta\varepsilon_{289.5}$ −4.7, $\Delta\varepsilon_{357.2}$ +0.2 (MeOH; *c* 0.28 mM). UV λ_{max}^{MeOH} nm (log ε): 238 (3.90), 286 (3.92), 336 (3.52). IR ν_{max} (film) cm^{-1}: 3395, 2959, 2922, 2853, 1636, 1447, 1341, 1288, 1194, 1155, 1111, 1074, 826. ^1H NMR (300 MHz; Me_2CO-d_6): δ 1.57 (6H, *s*, H_3-4″ and H_3-5″), 2.71 (1H, *dd*, *J* = 17.1 and 3.0 Hz, H-3β), 3.14 (1H, *dd*, *J* = 17.1 and 12.8 Hz, H-3α), 4.82 (1H, *dd*, *J* = 10.6 and 1.3 Hz, H-3″ *cis*), 4.92 (1H, *dd*, *J* = 17.5 and 1.3 Hz, H-3″ *trans*), 5.35 (1H, *dd*, *J* = 12.8 and 3.0 Hz, H-2β), 5.98 (1H, *s*, H-8), 6.32 (1H, *dd*, *J* = 17.5 and 10.6 Hz, H-2″), 6.66 (2H, *br s*, H-5′ and H-6′), 7.02 (1H, *s*, H-2′), 13.23 (1H, *s*, 5-OH). ^{13}C NMR (75 MHz, Me_2CO-d_6): Table 1. EIMS (70 eV) *m/z*: $[M]^+$ 356 (67), 341 (32), 234 (13), 221 (16), 220 (12), 219 (11), 205 (100), 193 (13), 192 (33), 179 (17), 177 (13), 165 (19), 163 (15), 136 (19). HREIMS *m/z*: 356.1266 (calcd for $C_{20}H_{20}O_6$, 356.1260).

3′-O-*Methyl*-6-(1,1-*dimethylallyl*)*eriodictyol* (**3**). Colourless powder (hexane), mp > 300° (dec). $[\alpha]_D^{25}$ −6.2° (Me_2CO; *c* 0.58). CD: $\Delta\varepsilon_{253}$ = +0.7, $\Delta\varepsilon_{293}$ = −4.6, $\Delta\varepsilon_{334}$ = +0.7 (MeOH; *c* 0.27 mM). UV λ_{max}^{MeOH} nm (log ε): 235 (3.88), 293 (3.89), 335 (3.31). IR ν_{max} (film) cm^{-1}: 3385, 2967, 2936, 1705, 1634, 1520, 1447, 1362, 1292, 1196, 1155, 1101, 1034, 821. ^1H NMR (300 MHz; Me_2CO-d_6): δ 1.57 (6H, *s*, H_3-4″ and H_3-5″), 2.72 (1H, *dd*, *J* = 17.1 and 3.0 Hz, H-3β), 3.22 (1H, *dd*, *J* = 17.1 and 12.9 Hz, H-3α), 3.88 (3H, *s*, OCH_3), 4.83 (1H, *dd*, *J* = 10.6 and 1.3 Hz, H-3″ *cis*), 4.93 (1H, *dd*, *J* = 17.5 and 1.3 Hz, H-3″ *trans*), 5.41 (1H, *dd*, *J* = 12.9 and 3.0 Hz, H-2β), 5.99 (1H, *s*, H-8), 6.32 (1H, *dd*, *J* = 17.5 and 10.6 Hz, H-2″), 6.87 (1H, *d*, *J* = 8.1 Hz, H-5′), 7.00 (1H, *dd*, *J* = 8.1 and 1.8 Hz, H-6′), 7.18 (1H, *d*, *J* = 1.8 Hz, H-2′), 13.24 (1H, *s*, 5-OH). ^{13}C NMR (75 MHz; Me_2CO-d_6): Table 1. EIMS (70 eV) *m/z*: $[M]^+$ 370 (100), 355 (28), 329 (9), 327 (10), 234 (12), 221 (19), 220 (14), 205 (67), 192 (20), 179 (11), 177 (12), 150 (14). HREIMS *m/z*: 370.1419 (calcd for $C_{21}H_{22}O_6$, 370.1416).

6,8-*Diprenyleriodictyol* (**4**). Yellow oil. UV, ^1H NMR ($CDCl_3$), EIMS comparable with lit. values [27]. CD: $\Delta\varepsilon_{251}$ +0.3, $\Delta\varepsilon_{295}$ −4.9, $\Delta\varepsilon_{347}$ +0.6 (MeOH; *c* 0.24 mM). IR ν_{max} (film) cm^{-1}: 3393, 2961, 2920, 2853, 2361, 1634, 1522, 1449, 1379, 1342, 1287, 1184, 1117, 816. ^1H NMR (300 MHz; Me_2CO-d_6): δ 1.62 (6H, *s*, H_3-4‴ and H_3-5‴), 1.65 (3H, *s*, H_3-5″, interchangeable with H_3-4″), 1.76 (3H, *s*, H_3-4″, interchangeable with $H_3$5″), 2.75 (1H, *dd*, *J* = 17.1 and 3.0 Hz, H-3β), 3.11 (1H, *dd*, *J* = 17.1 and 12.0 Hz, H-3α), 3.30 (1H, *d*-like, H-1‴), 3.33 (1H, *d*-like, H-1″), 5.16 (1H, *t*-like, H-2‴), 5.18 (1H, *t*-like, H-2″), 5.37 (1H, *dd*, *J* = 12.0 and 3.0 Hz, H-2β), 6.88 (2H, *s*, H-5′ and H-6′), 7.05 (1H, *s*, H-2′), 12.48 (1H, *s*, 5-OH). ^{13}C NMR (75 MHz; Me_2CO-d_6): Table 1.

Hiravanone (**5**). Pale yellow oil. ^1H NMR ($CDCl_3$), UV, IR, EIMS comparable with lit. values [28]. CD: $\Delta\varepsilon_{253}$ +0.3, $\Delta\varepsilon_{291}$ −7.6, $\Delta\varepsilon_{357.2}$ +0.8 (MeOH; *c* 0.23 mM). ^1H NMR (Me_2CO-d_6): δ 1.61 (6H, *s*, H_3-4‴ and H_3-5‴), 1.65 (3H, *s*, H_3-5″, interchangeable with H_3-4″), 1.75 (3H, *s*, H_3-4″, interchangeable with H_3-5″), 2.77 (1H, *dd*, *J* = 17.1 and 3.0 Hz, H-3β), 3.17 (1H, *dd*, *J* = 17.1 and 13.0 Hz, H-3α), 3.32 (2H, *m*, H-1‴ and H-1″), 3.89 (3H, *s*, OCH_3), 5.18 (2H, *t*-like, H-2″ and H-2‴), 5.41 (1H, *dd*, *J* = 13.0 and 3.0 Hz, H-2β), 6.88 (1H, *d*, *J* = 8.1 Hz, H-5′), 7.00 (1H, *dd*, *J* = 8.1 and 1.8 Hz, H-6′), 7.19 (1H, *d*, *J* = 1.8 Hz, H-2′), 12.49 (1H, *s*, 5-OH). ^{13}C NMR (75 MHz; Me_2CO-d_6): Table 1.

6-(2-*Hydroxy*-3-*methyl*-3-*butenyl*)-8-*prenyl*-*eriodictyol* (**6**). Pale yellow gum. UV λ_{max}^{MeOH} nm (log ε): 234 (3.84), 295 (3.65), 348 (3.14). IR ν_{max} (film) cm^{-1}: 3383, 2983, 2922, 2859, 1701, 1634, 1522, 1447, 1373, 1287, 1180, 1119, 901, 814. ^{13}C NMR (75 MHz; Me_2CO-d_6): Table 1. EIMS (70 eV) *m/z*: $[M]^+$ 440 (12), 422 (32), 407 (14), 370 (30), 369 (100), 367 (20), 314 (13), 313 (68), 271 (15), 243 (15), 233 (52), 231 (23), 203 (28), 189 (15), 177 (57), 153 (28), 137 (10), 136 (29). HREIMS *m/z*: 440.1830 (calcd for $C_{25}H_{28}O_7$, 440.1835). Two C-2″ epimers of **6** which could not be structurally resolved individually were separated by HPLC [column: YMC-Pack ODS-AQ (250 × 4.6 mm i.d.), solvent: MeOH–MeCN–H_2O (29:6:15), flow rate: 0.8 ml min^{-1}] as **6A** (49 min) and **6B** (55 min).

6A. ^{1}H NMR (500 MHz; Me_2CO-d_6): δ 1.59 (3H, *s*, H_3-4‴, interchangeable with H_3-5‴) 1.61 (3H, *s*, H_3-5‴, interchangeable with H_3-4‴), 1.81 (3H, *s*, H_3-5″), 2.65 (1H, *dd*, J = 17.0 and 3.0 Hz, H-3β), 2.73 (1H, *dd*, J = 14.5 and 9.0 Hz, H-1″a), 3.00 (1H, *dd*, J = 17.0 and 13.0 Hz, H-3α), 3.01 (1H, *dd*, J = 14.5 and 2.0 Hz, H-1″b), 3.23 (2H, *br d*, J = 7.0 Hz, H-1‴), 4.25 (1H, *br d*, J = 9.0 Hz, H-2″), 4.76 (1H, *br s*, H-4″a), 5.01 (1H, *d*, J = 1.5 Hz, H-4″b), 5.21 (1H, *br t*, J = 7.0 Hz, H-2‴), 5.28 (1H, *dd*, J = 13.0 and 3.0 Hz, H-2β), 6.85 (1H, *d*, J = 8.3 Hz, H-5′), 6.87 (1H, *dd*, J = 8.3 and 1.8 Hz, H-6′), 7.04 (1H, *d*, J = 1.8 Hz, H-2′), 12.77 (1H, *s*, 5-OH). **6B**. ^{1}H NMR (500 MHz; Me_2CO-d_6): δ 1.59 (3H, *s*, H_3-4‴, interchangeable with H_3-5‴), 1.61 (3H, *s*, H_3-5‴, interchangeable with H_3-4‴), 1.81 (3H, *s*, H_3-5″), 2.68 (1H, *dd*, J = 17.0 and 3.0 Hz, H-3β), 2.78 (1H, *dd*, J = 15.0 and 8.0 Hz, H-1″a), 2.98 (1H, *dd*, J = 15.0 and 2.0 Hz, H-1″b), 3.00 (1H, *dd*, J = 17.0 and 13.0 Hz, H-3α), 3.23 (2H, *t*-like, J = 6.3 Hz, H-1‴), 4.27 (1H, *br d*, J = 8.0 Hz, H-2″), 4.76 (1H, *br s*, H-4″a), 5.01 (1H, *br s*, H-4″b), 5.21 (1H, *br t*, J = 6.3 Hz, H-2‴), 5.31 (1H, *dd*, J = 12.0 and 3.0 Hz, H-2β), 6.85 (1H, *d*, J = 8.3 Hz, H-5′), 6.87 (1H, *dd*, J = 8.3 and 1.8 Hz, H-6′), 7.04 (1H, *d*, J = 1.8 Hz, H-2′), 12.75 (1H, *s*, 5-OH).

5,4′ - *Dihydroxy* - 4″,4″ - *dimethyl* - 5″ - *methyl* - 5″ - *H-dihydrofurano*[2″,3″:6,7]*flavanone* (**7**). Colourless needles, mp 260–262° (dec). UV λ_{max}^{MeOH} nm (log ε): 219 (4.51), 295 (4.35), 335 (4.26). IR ν_{max} (film) cm^{-1}: 3252, 2963, 2922, 2853, 2361, 1655, 1593, 1524, 1472, 1365, 1287, 1153, 1103, 1063, 841, 762. ^{1}H NMR (300 MHz; Me_2CO-d_6): δ 1.18, 1.20 (3H, *s*, H_3-4″, interchangeable with H_3-5″), 1.34, 1.35 (3H, *d*, J = 7 Hz, H_3-3″), 1.42, 1.43 (3H, *s*, H_3-5″, interchangeable with H_3-4″), 2.72, 2.73 (1H, *dd*, J = 17 and 3 Hz, H-3β), 3.19 (1H, *dd*, J = 17 and 13 Hz, H-3α), 4.46, 4.47 (1H, *q*, J = 7 Hz, H-2″), 5.43, 5.46 (1H, *dd*, J = 13 and 3 Hz, H-2β), 5.89, 5.90 (1H, *s*, H-8), 6.90 (2H, *d*, J = 8 Hz, H-3′ and 5′), 7.39 (2H, *d*, J = 8 Hz, H-2′ and H-6′), 12.43, 12.47 (1H, *s*, 5-OH). ^{13}C NMR (75 MHz, Me_2CO-d_6): Table 1. EIMS (70 eV) *m/z*: [M]$^+$ 340 (51), 326 (18), 325 (83), 206 (12), 205 (100), 120 (12). HREIMS *m/z*: 340.1312 (calcd for $C_{20}H_{20}O_5$, 340.1311).

Cytotoxicity testing. Data for compounds **1**–**7** were obtained using a panel of human cancer cell lines and established protocols [32]. A human glioma cell line (U373) was used to monitor the fractionation of compounds **1**–**7** from MeOH extracts of *M. engleri*.

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