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(*Rel*)-1β,2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β, 4α-di-(4-methoxyphenyl)-cyclobutane and other flavonoids from the aerial parts of *Goniothalamus gardneri* and *Goniothalamus thwaitesii*

Veronique Seidel [a], François Bailleul [b], Peter G. Waterman [a,*]

[a] *Phytochemistry Research Laboratories, Department of Pharmaceutical Sciences, Strathclyde Institute for Biomedical Sciences, Glasgow, G4 0NR, UK*

[b] *Laboratoire de Pharmacognosie, Faculté de Pharmacie, B.P.83, 59006 Lille Cédex, France*

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Abstract

The aerial parts of *Goniothalamus gardneri* (Annonaceae) has yielded the known flavonoids 2′-hydroxy-4,4′,6′-trimethoxychalcone (flavokawain A), 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone, 4,2′,4′-trihydroxy-6′-methoxydihydrochalcone, 5,7,4′-trimethoxyflavanone (naringenin trimethyl ether) and 7-hydroxy-5,4′-dimethoxyflavanone (tsugafolin) together with three novel compounds, the dimer characterised as (*rel*)-1β,2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β,4α-di-(4-methoxyphenyl)-cyclobutane, 2′,4′-dihydroxy-4,6′-dimethoxychalcone and 2′-hydroxy-4,4′,6′-trimethoxydihydrochalcone. The last two have previously been synthesised but appear to be new natural products. A similar study of the aerial parts of *G. thwaitesii* led only to the isolation of the known flavonoids myricetin 4′-*O*-methyl ether-3-*O*-α-L-rhamnopyranoside (mearnsitrin) and myricetin-3-*O*-methyl ether (annulatin), together with the triterpenes friedelinol, friedelin and betulinic acid. All compounds were identified by spectroscopic analysis and, for known compounds, by comparison with published data.

Keywords: *Goniothalamus gardneri*; *Goniothalamus thwaitesii*; Annonaceae; Chalcone dimer; (*rel*)-1β,2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β,4α-di-(4-methoxyphenyl)-cyclobutane; Chalcone; Dihydrochalcone; Flavanone; Flavonol glycoside; Flavonol-3-*O*-methyl ether; Friedelane and lupane triterpenes

1. Introduction

The genus *Goniothalamus* Hook. f. & Thoms. (Annonaceae) comprises 50–100 species ranging through Indomalesiana (Mabberley, 1997). They are widely employed in traditional medicine, alone or as part of herbal mixtures, as post-partum protective remedies, abortifacients and insects repellents (Perry, 1980). About 20 species have been investigated and the predominant isolates have been acetogenins (Zafra-Polo et al., 1998), styryl-lactones (Bermejo et al., 1998) and isoquinoline-derived alkaloids (Omar et al., 1992) with significant cytotoxic, insecticidal and antimicrobial activities. To date only a single flavonoid has been reported from the genus (Talapatra et al., 1985).

In a continuation of our studies on the chemistry of the Annonaceae we have examined the aerial parts of *Goniothalamus thwaitesii* Hook. f. & Thoms, a medium-sized tree, and *G. gardneri* Hook. f. & Thoms., a shrub, both endemic to the mid-altitude rain forests of Sri-Lanka (Dassanayake and Fosberg, 1985). Apart from the report of a linear acetogenin from *G. gardneri* (Seidel et al., 1999) this constitutes the first account of metabolites from either species.

2. Results and discussion

The petrol and EtOAc extracts of the aerial parts of *G. gardneri*, through a series of chromatographic fractionations, afforded the flavonoids **1–8**. Compounds **1–**

* Corresponding author at current address: Centre for Phytochemistry, Southern Cross University, PO Box 157, Lismore, NSW 2048, Australia. Tel.: +61-2-6620-3544; fax: 61-2-6622-3459.

E-mail address: pwaterma@scu.edu.au (P.G. Waterman).

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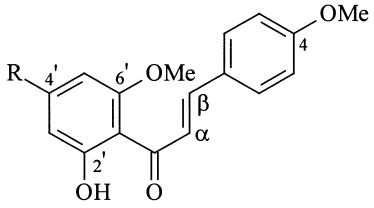
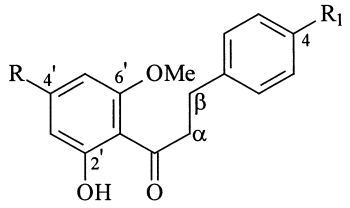
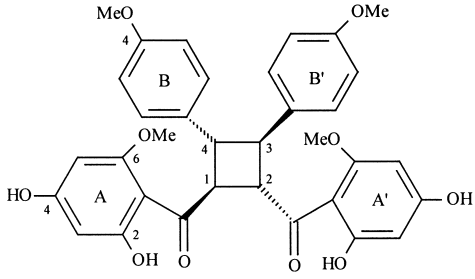


1 R = OMe
2 R = OH



3 R = R_1 = OMe
4 R = OH; R_1 = OMe
5 R = R_1 = OH



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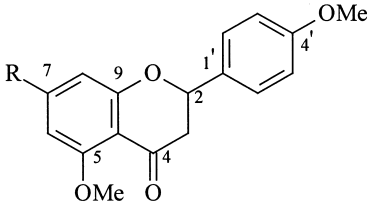
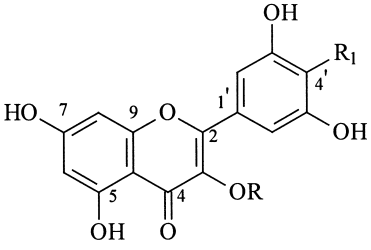


7 R = OMe
8 R = OH



9 R = rhamnose; R_1 = OMe
10 R = Me; R_1 = OH

5, **7** and **8** were shown to be known flavonoids by spectroscopic analysis but in each case unambiguous ^{1}H and ^{13}C NMR assignments, established by HC-COBI, HMBC and NOESY experiments, required some correction in previously published data.

Compounds **1** and **2** were chalcones. Compound **1** (flavokawain-A) was identified by comparison with literature data (Lam and Wrang, 1975). The ^{1}H NMR assignments are reported here for the first time and C-2′, C-4′ and C-6′ resonances are respectively corrected at δ 168.6, 166.2 and 162.7 compared to a previous report (Duddeck et al., 1978). A second chalcone was identifed as 2′,4′-dihydroxy-4,6′-dimethoxychalcone (**2**) for which very limited spectroscopic data were available (Bhardwaj et al., 1982). The structure was confirmed from HMBC correlations and NOE interactions between both H-3′, H-5′ and the 4′-OH, and between H-5′ and the 6′-OMe (δ 3.94). The placements of the methoxyl and hydroxyl groups on the A-ring were further supported with the fact that ^{1}H and ^{13}C NMR data revealed two distinctive chemical shifts for positions 3′ and 5′. If the only methoxyl on the A-ring had been located in position 4′, then signals for 3′ and 5′ would have been equivalent because of a symmetrical A-ring. ^{1}H and ^{13}C NMR assignments are reported for the first time.

Compounds **3–5** showed the spectral characteristics of dihydrochalcones. Comparison of the mp, IR, UV and MS of **3** agreed with published data for 2′-hydroxy-4,4′,6′-trimethoxydihydrochalcone (Braz Filho et al., 1980; Bhardwaj et al., 1982). Unambiguous ^{1}H NMR assignments for methoxyl resonances and ^{13}C NMR assignments are reported for the first time. The spectral data obtained for 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone (**4**) were in close agreement with that

reported (Conserva et al., 1990; Kawanishi et al., 1990) except for H-2′ and H-5 ′, which were reversed. Similarly data obtained for 2′,4,4′-trihydroxy-6′-methoxydihydrochalcone (**5**) were in close agreement with those published (Mizuno et al., 1987) except for H-3′ and H-5′, which were reversed. ^{13}C NMR assignments are reported for the first time.

Naringenin trimethyl ether (**7**) was identified by comparison of spectral data with that published (Lam and Wrang, 1975; Duddeck et al., 1978). ^{1}H NMR assignments for methoxyl resonances and the H-6 and H-8 methines are reported for the first time. ^{13}C NMR assignments agreed with published data except for the C-5, C-6, C-8 and C-9 resonances, which are corrected. Tsugafolin (**8**) was characterised in the same manner. NMR data agreed with those reported (Tanaka et al., 1989), except for H-3′ and H-5′, which were reversed, and for the two methoxyl resonances in the ^{13}C NMR, which were likewise reversed.

Compound **6** exhibited a UV spectrum similar to dihydrochalcones. A bathochromic shift (band II/ca. 35 nm) on addition of alkali, accompanied by an increase in peak intensity in the presence of NaOMe, indicated a 2,4-dihydroxyl system on the A-ring (Mabry et al., 1970). The FABMS yielded a quasi-molecular ion at *m/z* 601, which was consistent with the molecular formula $C_{34}H_{32}O_{10}$, requiring a dimeric structure. HREIMS showed a typical chalcone fragmentation (Mabry and Markham, 1975) with a major fragment ion at *m/z* 300 (chalcone monomer, $C_{17}H_{16}O_5$). Further fragments were observed at *m/z* 167 and 140 (two hydroxyls and one methoxyl on A and A′-rings) and *m/z* 161, 134 and 121 (one methoxyl on the B and B′-rings) (Fig. 1).

The ^{1}H NMR and ^{13}C NMR spectra were relatively simple so requiring a plane of symmetry in the dimer. The chemical shifts were similar to those for chalcone **2** and dihydrochalcone **4**, both isolated from this species. The *J*-modulated ^{13}C NMR spectrum revealed a carbonyl resonance within the normal range of dihydrochalcones (δ 204.1), a pair of aliphatic methines (δ 55.4 and 45.3) and two relatively shielded methoxyl resonances at δ 55.9 and 55.4, requiring their placement adjacent to at least one free *ortho* position (Panichpol

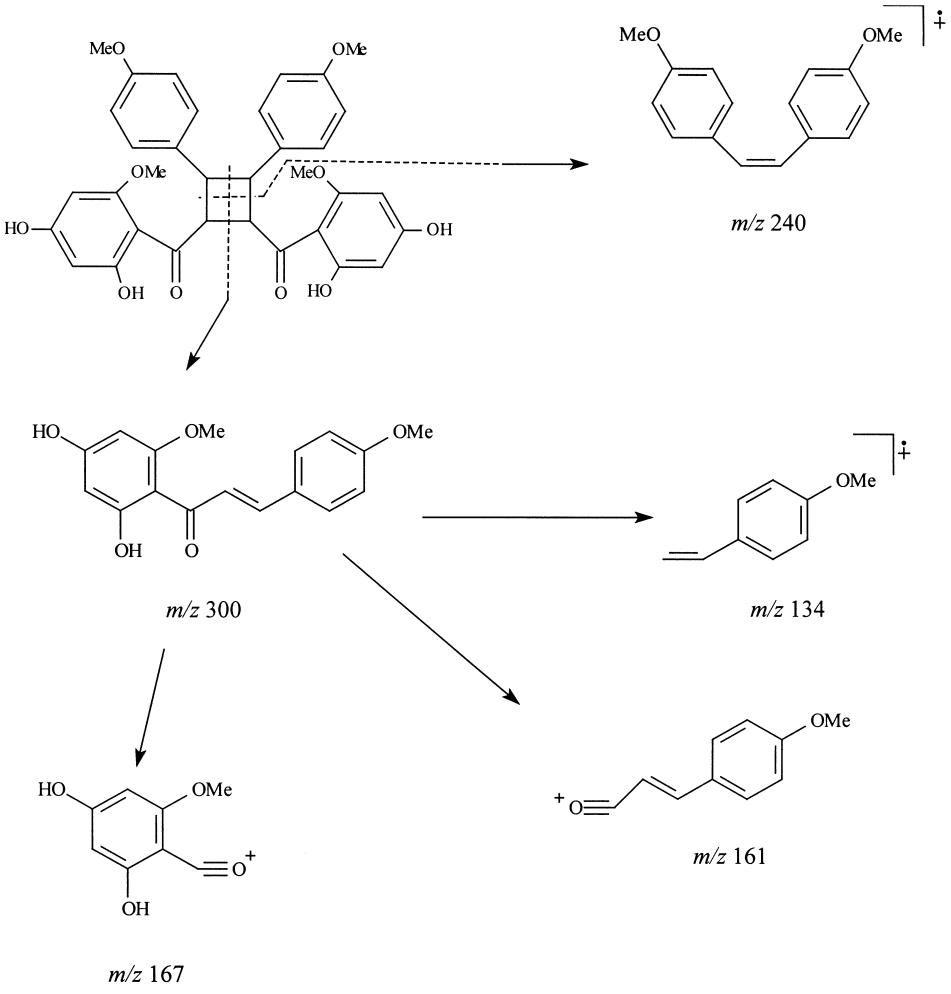


Fig. 1. Fragment ions observed in the EI-mass spectrum of **6**.

and Waterman, 1978). The calculated degrees of unsaturation were satisfied by the presence of a cyclobutane ring symmetrically substituted by identical A/A′-rings on one side, and identical B/B′-rings on the opposite side. This was further confirmed in the HREIMS with the fragment ion at *m/z* 240, attributable to the loss of the B/B′ system (Fig. 1).

The structure of (*rel*)-1β,2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β,4α-di-(4-methoxyphenyl)-cyclobutane was established by a series of HMBC and NOESY experiments. The HMBC spectrum displayed a number of important correlations (Fig. 2):

1. ^3J couplings between both the methoxyls at δ 3.53 and H-2/6B/B′ and the oxygenated carbons at δ 158.7, thus establishing the assignments of the methoxylated C-4B/B′ positions.
2. ^3J interactions between the remaining methoxyls at δ 3.47 and the oxygenated carbons at δ 164.1.
3. ^2J interactions between H-3A/A′ and δ 167.1 and 168.6, and ^2J interactions between H-5A/A′ and δ 167.1 and 164.1, thus allowing the assignments of the hydroxylated C-4A/A′ positions at δ 167.1. Consequently, the methoxylated C-6A/A′ positions were established at δ 164.1, and the remaining hydroxylated C-2A/A′ positions at δ 168.6.
4. a ^3J coupling between the methines at δ 5.14 and C-1B/B′.
5. a ^3J coupling between the methines at δ 4.52 and both C-2/6B/B′ and the carbonyl.

The NOESY experiment revealed enhancement between H-5A/A′ and methoxyls at δ 3.47, thus confirming the assignment of the 6A/A′-OMe. The presence of methoxyl groups in C-4B/B′ was confirmed by a NOE interaction between H-3/5B/B′ and the resonances at δ 3.53. Furthermore, a strong NOE interaction between H-1/2 and H-2/6B/B′ required the placement of the B and B′ rings *trans* respectively to the A and A′ rings, so establishing the relative stereochemistry of **6** as *rel*-(1β,4α). Another NOE interaction between H-1/2 and H-3/4 indicated the relative stereochemistry at C-2/C-3 to be *rel*-(2α,3β).

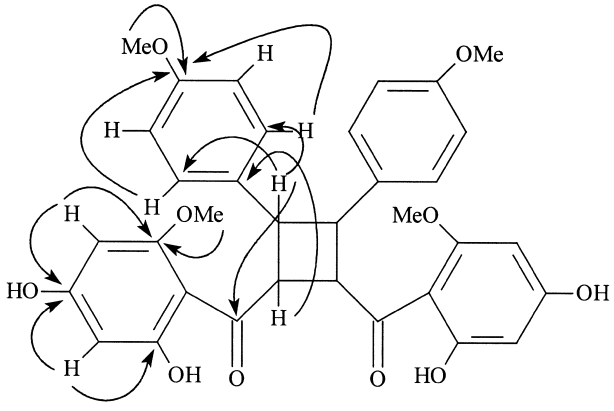


Fig. 2. Some significant correlations in the HMBC spectrum of **6**.

A series of chromatographic fractionations of the aerial parts of *G. thwaitsii* led to the isolation of the flavonoids **9** and **10**, and the triterpenes **11–13**.

The spectrophotometric and spectroscopic data recorded for **9** and **10** were typical of 3-substituted flavonols and allowed them to be characterised as mearnsitrin or myricetin 4′-*O*-methyl ether-3-*O*-α-L-rhamnopyranoside (**9**) (Mackenzie, 1969; cf. Nicollier and Thompson, 1983 — data for myricitrin) and annulatin or myricetin-3-*O*-methyl ether (**10**) (Markham and Whitehouse, 1984). The unambiguous ^{1}H and ^{13}C NMR assignments are reported for the first time for both of these compounds (see Section 3).

The mass fragmentation and NMR data of **11** and **12** suggested they were friedelane triterpenes. A combination of COSY, HC-COBI, HMBC and NOESY experiments allowed unambiguous assignments. Compound **11** was identified as the known friedelan-3α-ol or friedelinol. ^{13}C NMR assignments showed good agreement with published data (Patra and Chaudhuri, 1987), except for C-19, C-21, C-16 and C-22 resonances which were corrected respectively to δ 36.0, 33.6, 36.9 and 39.9. Triterpenes **12** and **13** were identified, respectively, as friedelan-3-one or friedelin and betulinic acid.

The occurrence of flavonoids in *Goniothalamus* is of interest since only one flavanone appears to have been previously isolated from the genus (Talapatra et al., 1985). The composition of the flavonoids found in *G. gardneri* were reminiscent of those reported previously in our laboratory from an African species of Annonaceae, *Monanthotaxis* (*Popowia*) *cauliflora* (Panichpol and Waterman, 1978). Both **2** and **3** have previously been synthesised but this appears to be their first record as natural products. The dimer (**6**) is, despite the presence of some optical activity, thought likely to be an artefact. It is of interest to note that neither styryl-lactones nor alkaloids were found in either of these species in the present study.

3. Experimental

3.1. General

Mps (uncorrected) were determined using a Gallenkamp apparatus. $[\alpha]_D$ were measured on a Bellingham & Stanley model ADP 220 polarimeter. IR spectra were recorded using a Mattson Galaxy 5000 FTIR spectrometer with samples as KBr discs or liquid films. UV spectra were recorded in MeOH on a Unicam UV 4-100 UV/Visible spectrophotometer. Shift reagents were powdered NaOAc and H_3BO_3, and $AlCl_3$, NaOMe and HCl solutions (Mabry and Markham, 1975). HREI and

positive-ion FABMS (nitrobenzoyl alcohol matrix) were run on a JEOL JMS-AX505HA spectrometer. NMR spectra were recorded at 400 MHz (^{1}H) and 100.56 MHz (^{13}C) on a Bruker AMX-400, using the residual solvent peaks as int. standards. COSY, NOESY, HC-COBI and HMBC were performed using standard microprograms.

3.2. Plant material

Aerial parts of *G. gardneri* and *G. thwaitesii* were purchased from the plantation of Lalani Botanicals (Sri Lanka) in May 1997. Voucher specimens are kept by Lalani Botanicals, Colombo, Sri Lanka.

3.3. Extraction and purification of compounds from G. gardneri

Dried powdered aerial parts (510 g) were Soxhlet extracted successively with petroleum ether (b.p. 40–60°C), EtOAc and MeOH. The petrol (12 g) and EtOAc extracts (21 g) were concd in vacuo and fractionated by VLC on silica gel 60H, eluting with *n*-hexane–EtOAc mixtures of increasing polarity. Fractionation of the petrol extract, eluting with *n*-hexane–EtOAc (6:4), followed by column chromatography over silica gel 60 (*n*-hexane–EtOAc, 95:5), afforded **3** (32.9 mg). The fraction obtained on eluting with EtOAc was concentrated in vacuo and the residue was subjected to gel filtration on Sephadex LH-20. The sub-fraction eluted with CHCl3 was then submitted to column chromatography and gave **1** (9.5 mg) (*n*-hexane–EtOAc, 3:1) and **7** (101.6 mg) (*n*-hexane–EtOAc, 1:1). The sub-fraction eluted with 10% MeOH in $CHCl_3$ was also subjected to column chromatography (petrol–EtOAc–EtOH, 14:5:1) to give **4** (31.4 mg).

Fractionation of the EtOAc extract, eluting with *n*-hexane–EtOAc (2:8), followed by gel filtration ($CHCl_3$), yielded **2** (104.1 mg). The fraction eluted with EtOAc was also followed by gel filtration. The sub-fraction eluted with $CHCl_3$ afforded **8** (105.5 mg), while the sub-fractions eluted with 5% MeOH and 8% MeOH in $CHCl_3$ afforded **6** (17.4 mg) and **5** (41.5 mg), respectively.

3.3.1. 2′-Hydroxy-4,4′,6′-trimethoxychalcone (flavokawain-A) (*1*)

Yellow plates (*n*-hexane/EtOAc), m.p. 112°C (Lit. m.p. 114–115°C, (Hänsel et al., 1963)). IR, UV and MS data in agreement with those published (Birch and Hextall, 1955; Hänsel et al., 1963; Lam and Wrang, 1975). ^{1}H NMR ($CDCl_3$) δ 14.40 (1H, *s*, 2′-OH), 7.82 (1H, *d*, J = 15.6 Hz, H-α), 7.78 (1H, *d*, J = 15.6 Hz, H-β), 7.57 (2H, *d*, J = 8.8 Hz, H-2/6), 6.94 (2H, *d*, J = 8.8 Hz, H-3/5), 6.12 (1H, *d*, J = 2.4 Hz, H-3′), 5.97 (1H, *d*, J = 2.4 Hz, H-5′), 3.92 (3H, *s*, 6′-OMe), 3.86 (3H, *s*, 4-OMe), 3.84 (3H, *s*, 4′-OMe). ^{13}C NMR ($CDCl_3$) δ 192.8 (*s*, C=O), 168.6 (*s*, C-2′), 166.2 (*s*, C-4′), 162.7 (*s*, C-6′), 161.6 (*s*, C-4), 142.7 (*d*, C-β), 130.3 (*d*, C-2/6), 128.6 (*s*, C-1), 125.3 (*d*, C-α), 114.6 (*d*, C-3/5), 106.6 (*s*, C-1′), 94.0 (*d*, C-3′), 91.5 (*d*, C-5′), 56.0 (*q*, 6′-OMe), 55.8 (*q*, 4′-OMe), 55.6 (*q*, 4-OMe).

3.3.2. 2′,4′-Dihydroxy-4,6′-dimethoxychalcone (*2*)

Yellow plates (MeOH). m.p. 161–163°C (Lit. m.p. 158–159°C, Bhardwaj et al., 1982): IR ν_{max}^{KBr} cm^{-1}: 3434 (OH), 3193 (OH), 3002, 2981, 2937, 2910, 2836, 1623 (C=O), 1604, 1573, 1511, 1342, 1292, 1259, 1222, 1209, 1168, 1114, 1024, 970, 831. UV λ_{max}^{MeOH} nm: 237 (sh), 363. UV $\lambda_{max}^{AlCl_3}$ nm: 239, 334 (sh), 397. UV λ_{max}^{NaOAC} nm: 237 (sh), 372. UV λ_{max}^{NaOMe} nm: 246 (sh), 332 (sh), 390. ^{1}H NMR ($CDCl_3$) δ: 14.26 (1H, *s*, 2′-OH), 7.81 (1H, *d*, J = 15.6 Hz, H-α), 7.77 (1H, *d*, J = 15.6 Hz, H-β), 7.57 (2H, *d*, J = 8.8 Hz, H-2/6), 6.94 (2H, *d*, J = 8.8 Hz, H-3/5), 6.04 (1H, *d*, J = 2.4 Hz, H-3′), 5.96 (1H, *d*, J = 2.4 Hz, H-5′), 5.42 (1H, brs, 4′-OH), 3.94 (3H, *s*, 6′-OMe), 3.87 (3H, *s*, 4-OMe). ^{13}C NMR ($CDCl_3$) δ 192.9 (*s*, C=O), 168.1 (*s*, C-2′), 163.4 (*s*, C-6′), 162.5 (*s*, C-4′), 161.6 (*s*, C-4), 142.9 (*d*, C-β), 130.4 (*d*, C-2/6), 128.5 (*s*, C-1), 125.3 (*d*, C-α), 114.6 (*d*, C-3/5), 106.8 (*s*, C-1′), 97.0 (*d*, C-3′), 91.2 (*d*, C-5′), 56.1 (*q*, 6′-OMe), 55.6 (*q*, 4-OMe). EI-HRMS *m*/*z* (rel. int.%): 300 (100), 299 (79), 283 (16), 272 (10), 257 (7), 192 (9), 167 (30), 166 (16), 161 (12), 134 (71), 121 (62), 84 (15). Calculated for $C_{17}H_{16}O_5$ 300.0997, found 300.0983.

3.3.3. 2′-Hydroxy-4,4′,6′-trimethoxydihydrochalcone (*3*)

Colourless plates (EtOH). m.p. 108–110°C Lit. m.p. 110–112°C, (Bhardwaj et al., 1982). IR, UV and MS in agreement with published data (Braz Filho et al., 1980; Bhardwaj et al., 1982). ^{1}H NMR ($CDCl_3$) δ: 14.03 (1H, *s*, 2′-OH), 7.17 (2H, d, J = 8.5 Hz, H-2/6), 6.85 (2H, *d*, J = 8.6 Hz, H-3/5), 6.08 (1H, *d*, J = 2.4 Hz, H-3′), 5.93 (1H, *d*, J = 2.4 Hz, H-5′), 3.84 (3H, *s*, 6′-OMe), 3.83 (3H, *s*, 4′-OMe), 3.80 (3H, *s*, 4-OMe), 3.29 (2H, *t*, J = 7.7 Hz, H2-α), 2.95 (2H, *t*, J = 7.7 Hz, H2-β). ^{13}C NMR ($CDCl_3$) δ 204.8 (*s*, C=O), 167.9 (*s*, C-2′), 166.1 (*s*, C-4′), 162.9 (*s*, C-6′), 158.1 (*s*, C-4), 133.9 (*s*, C-1), 129.5 (*d*, C-2/6), 114.0 (*d*, C-3/5), 106.0 (*s*, C-1′), 93.9 (*d*, C-3′), 91.0 (*d*, C-5′), 55.8 (*q*, 6′-OMe), 55.7 (*q*, 4′-OMe), 55.4 (*q*, 4-OMe), 46.2 (*t*, C-α), 30.0 (*t*, C-β).

3.3.4. 2′,4′-Dihydroxy-4,6′-dimethoxydihydrochalcone (*4*)

Colourless needles (MeOH). m.p. 171°C Lit. m.p. 175–176°C, (Bharwaj et al., 1982; Garzon et al., 1987). IR, UV and MS in agreement with published data (Braz Filho et al., 1980; Bharwaj et al., 1982; Conserva et al., 1990; Kawanishi et al., 1990). ^{1}H NMR (C_5D_5N) δ: 14.64 (^{1}H, *s*, 2′-OH), 7.31 (2H, *d*, J = 8.6 Hz, H-2/6), 6.97 (2H, *d*, J = 8.6 Hz, H-3/5), 6.51 (1H, *d*, J = 2.2 Hz, H-3′), 6.28 (1H, *d*, J = 2.2 Hz, H-5′), 3.69 (3H, *s*, 6′-OMe), 3.67 (3H, *s*, 4-OMe), 3.41 (2H, *t*, J = 7.7 Hz, H2-

α), 3.08 (2H, *t*, $J=7.7$ Hz, H_2-β). ^{13}C NMR (C_5D_5N) δ: 205.0 (*s*, C=O), 168.7 (*s*, C-2′), 167.2 (*s*, C-4′), 164.5 (*s*, C-6′), 159.0 (*s*, C-4), 134.8 (*s*, C-1), 130.3 (*d*, C-2/6), 114.9 (*d*, C-3/5), 106.0 (*s*, C-1′), 97.6 (*d*, C-3′), 92.7 (*d*, C-5′), 56.0 (*q*, 6′-OMe), 55.6 (*q*, 4-OMe), 46.8 (*t*, C-α), 30.8 (*t*, C-β).

3.3.5. 4,2′,4′-Trihydroxy-6′-methoxydihydrochalcone (**5**)

Pale amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3332 (OH), 3008, 2923, 2854, 1631 (C=O), 1610, 1590, 1509, 1375, 1265, 1209, 1197, 1168, 1110, 842, 823. UV λ_{max}^{MeOH} nm: 286, 314 (sh). UV $\lambda^{AlCl_3}{}_{max}$ nm: 304, 345. UV λ_{max}^{NaOAC} nm: 324. UV λ_{max}^{NaOMe} nm: 325. MS in agreement with published data (Mizuno et al., 1987). ^{1}H NMR (C_5D_5N) δ: 14.67 (1H, *s*, 2′-OH), 7.32 (2H, *d*, $J=8.3$ Hz, H-2/6), 7.17 (2H, *d*, $J=8.3$ Hz, H-3/5), 6.50 (1H, *d*, $J=2.4$ Hz, H-3′), 6.27 (1H, *d*, $J=2.4$ Hz, H-5′), 3.68 (3H, *s*, 6′-OMe), 3.43 (2H, *t*, $J=7.7$ Hz, H_2-α), 3.12 (2H, *t*, $J=7.7$ Hz, H_2-β). ^{13}C NMR (C_5D_5N) δ: 205.3 (*s*, C=O), 168.8 (*s*, C-2′), 167.2 (*s*, C-4′), 164.6 (*s*, C-6′), 157.7 (*s*, C-4), 133.2 (*s*, C-1), 130.6 (*d*, C-2/6), 116.9 (*d*, C-3/5), 106.0 (*s*, C-1′), 97.6 (*d*, C-3′), 92.8 (*d*, C-5′), 56.1 (*q*, 6′-OMe), 47.2 (*t*, C-α), 31.0 (*t*, C-β).

3.3.6. Rel-(1β,2α)-di-(2,4-dihydroxy-6-methoxybenzoyl)-(3β,4α)-di-(4-methoxyphenyl)-cyclobutane (**6**)

Pale amorphous solid. $[\alpha]_D^{23.5}+17.2°$ ($CHCl_3$, *c* 0.29). IR ν_{max}^{Film} cm^{-1}: 3409 (OH), 2935, 2836, 1623 (C=O), 1596, 1513, 1361, 1247, 1214, 1170, 1114, 1033, 829. UV λ_{max}^{MeOH} nm: 288, 335 (sh). UV $\lambda_{max}^{AlCl_3}$ nm: 308, 365 (sh). UV λ_{max}^{NaOAC} nm: 324. UV λ_{max}^{NaOMe} nm: 323. ^{1}H NMR (C_5D_5N) δ: 14.52 (2H, *s*, 2-A/A′-OH), 7.29 (4H, *d*, $J=8.4$ Hz, 2/6-B/B′), 6.89 (4H, *d*, $J=8.4$ Hz, 3/5-B/B′), 6.49 (2H, *d*, $J=2.4$ Hz, 3-A/A′), 6.23 (2H, *d*, $J=2.4$ Hz, 5-A/A′), 5.14 (2H, *d*, $J=6.4$ Hz, H-1/2), 4.52 (2H, *d*, $J=6.4$ Hz, H-3/4), 3.53 (6H, *s*, 4-B/B′-OMe), 3.47 (6H, *s*, 6-A/A′-OMe). ^{13}C NMR (C_5D_5N) δ: 204.1 (*s*, C=O), 168.6 (*s*, 2-A/A′), 167.1 (*s*, 4-A/A′), 164.1 (*s*, 6-A/A′), 158.7 (*s*, 4-B/B′), 134.3 (*s*, 1-B/B′), 130.1 (*d*, 2/6-B/B′), 114.3 (*d*, 3/5-B/B′), 105.6 (*s*, 1-A/A′), 97.7 (*d*, 3-A/A′), 92.5 (*d*, 5-A/A′), 55.9 (*q*, 6-A/A′-OMe), 55.4 (*q*, 4-B/B′-OMe), 55.4 (*d*, C-1/2), 45.3 (*d*, C-3/4). HREIMS *m*/*z* (rel. int.%): 300 (100), 299 (58), 240 (36), 167 (63), 161 (52), 140 (42), 134 (38), 121 (55). HR-FABMS: calculated for $C_{34}H_{32})_{10}$ $[M+H]^+$ 601.1995, found 601.2074.

3.3.7. Naringenin trimethyl ether (7)

Prisms (*n*-hexane/EtOAc). m.p. 124°C Lit. mp. 123.5 124.5°C (Kaufmann and LAm, 1967). $[\alpha]_D^{23.1}-4.3°$ ($CHCl_3$, *c* 0.234). IR, UV and mS in agreement with published data (Kaufmann and Lam, 1967; Lam and Wrang, 1975). ^{1}H NMR ($CDCl_3$) δ: 7.37 (2H, *d*, $J=8.7$ Hz, H-2′/6′), 6.93 (2H, *d*, $J=8.7$ Hz, H-3′/5′), 6.13 (1H, *d*, $J=2.3$ Hz, H-8), 6.08 (1H, *d*, $J=2.3$ Hz, H-6), 5.34 (1H, *dd*, $J=13.1$, 2.8 Hz, H-2), 3.88 (3H, *s*, 5-OMe), 3.81 (3H, *s*, 4′-OMe), 3.80 (3H, *s*, 7-OMe), 3.02 (1H, *dd*, $J=16.5$, 13.1 Hz, H-3_{ax}), 2.75 (1H, *dd*, $J=16.5$, 2.8 Hz, H-3_{eq}). ^{13}C NMR ($CDCl_3$) δ: 189.3 (*s*, C-4), 165.9 (*s*, C-7), 165.0 (*s*, C-9), 162.2 (*s*, C-5), 159.8 (*s*, C-4′), 130.8 (*s*, C-1′), 127.6 (*d*, C-2′/6′), 114.1 (*d*, C-3′/5′), 105.9 (*s*, C-10), 93.5 (*d*, C-8), 93.0 (*d*, C-6), 56.0 (*s*, 5-OMe), 55.5 (*s*, 4′-OMe), 55.3 (*s*, 7-OMe), 78.8 (*d*, C-2), 45.3 (*t*, C-3).

3.3.8. Tsugafolin (**8**)

Prisms (EtOH). m.p. 203°C Lit. m.p. 208–210°C, Tanaka et al., 1989)). $[\alpha]_D^{19.9}-32.2°$ (C_5H_5N, *c* 0.310) [Lit. $[\alpha]_D^{23}+7.0°$ (C_5H_5N, *c* 0.46), Tanaka et al., 1989)]. IR, UV and MS in agreement with published data (Tanaka et al., 1989). ^{1}H NMR (C_5D_5N) δ: 7.52 (2H, *d*, $J=8.6$ Hz, H-2′/6′), 7.01 (2H, *d*, $J=8.6$ Hz, H-3′/5′), 6.52 (1H, *d*, $J=2.0$ Hz, H-8), 6.47 (1H, *d*, $J=2.0$ Hz, H-6), 5.52 (1H, *dd*, $J=12.8$, 2.8 Hz, H-2), 3.81 (3H, *s*, 5-OMe), 3.67 (3H, *s*, 4′-OMe), 3.22 (1H, *dd*, $J=16.2$, 12.8 Hz, H-3_{ax}), 2.92 (1H, *dd*, $J=16.2$, 2.8 Hz, H-3_{eq}). ^{13}C NMR (C_5D_5N) δ: 188.6 (*s*, C-4), 166.6 (*s*, C-7), 165.9 (*s*, C-9), 163.9 (*s*, C-5), 160.7 (*s*, C-4′), 132.4 (*s*, C-1′), 128.8 (*d*, C-2′/6′), 114.9 (*d*, C-3′/5′), 106.2 (*s*, C-10), 97.3 (*d*, C-8), 95.0 (*d*, C-6), 56.3 (*s*, 5-OMe), 55.7 (*s*, 4′-OMe), 79.7 (*d*, C-2), 46.6 (*t*, C-3).

3.4. Extraction and purification of compounds from G. thwaitesii

Dried powdered aerial parts (495 g) were Soxhlet extracted sequentially with *n*-hexane, EtOAc and MeOH. The *n*-hexane (9.5 g) and EtOAc extract (13 g) were concentrated in vacuo and submitted to vacuum liquid chromatography, eluting with *n*-hexane–EtOAc mixtures of increasing polarity. From the *n*-hexane extract, eluting with *n*-hexane–EtOAc (7:3), afforded fractions containing **11** and **12**. Subsequent gel filtration using Sephadex LH-20, eluting with $CHCl_3$–*n*-hexane (95:5), yielded **11** (17.7 mg). The remaining fractions were pooled, concentrated in vacuo and the residue was subjected to column chromatography. Elution with *n*-hexane–EtOAc (95:5) afforded 12 (17.6 mg). Vacuum liquid chromatography of the EtOAc extract over silica gel, eluting with *n*-hexane–EtOAc (4:6), followed by gel filtration with Sephadex LH-20 ($CHCl_3$) gave **13** (40.1 mg).

The MeOH extract (28 g) was partitioned between EtOAc, *n*-BuOH and water. The EtOAc residue (1.7 g) was subjected to gel filtration with Sephadex LH-20. The fractions containing **9**, eluted with MeOH, were pooled and concentrated in vacuo and the residue submitted to column chromatography on silica gel. Elution with 10% MeOH in $CHCl_3$ afforded **9** (30.7 mg). The fractions containing **10**, also eluted with MeOH, were similarly treated and the residue was purified by repeated gel filtration on Sephadex LH-20. Elution with acetone–EtOH (9:1) yielded **10** (18.8 mg).

*3.4.1. Mearnsitrin (**9**)*

Amorphous solid. $[\alpha]_D^{23.2}$ −76.8° (MeOH, *c* 0.69). IR ν_{max}^{KBr} cm^{-1}. 3427 (OH), 2964, 2935, 2850, 1654 (C=O), 1610, 1506, 1444, 1384, 1303, 1203, 1166, 1056, 1024. UV in agreement with published data (Mackenzie, 1969). ^1H NMR (C_5D_5N) δ: 13.25 (1H, *s*, 5-OH), 7.58 (2H, *s*, H-2′/6′), 6.69 (1H, *d*, $J=2.0$ Hz, H-6), 6.63 (1H, *d*, $J=2.0$ Hz, H-8), 6.22 (1H, *d*, $J=1.0$ Hz, H-1″), 5.07 (1H, *dd*, $J=3.2$, 1.0 Hz, H-2″), 4.61 (1H, *dd*, $J=8.6$, 3.2 Hz, H-3″), 4.30 (1H, *t*, $J=8.6$ Hz, H-4″), 4.28 (1H, *m*, H-5″), 4.08 (3H, *s*, 4′-OMe), 1.52 (3H, *d*, $J=5.2$ Hz, 6″-Me). ^{13}C NMR (C_5D_5N) δ: 179.6 (*s*, C-4), 166.5 (*s*, C-7), 163.5 (*s*, C-5), 158.6 (*s*, C-2), 158.3 (*s*, C-9), 153.0 (*s*, C-3′/5′), 140.1 (*s*, C-4′), 137.1 (*s*, C-3), 127.3 (*s*, C-1′), 110.2 (*d*, C-2′/6′), 106.1 (*s*, C-10), 104.7 (*d*, C-1″), 100.3 (*d*, C-6), 95.1 (*d*, C-8), 73.8 (*d*, C-4″), 73.0 (*d*, C-3″), 72.6 (*d*, C-5″), 72.5 (*d*, C-2″), 60.8 (*q*, 4′-OMe), 18.9 (*q*, 6″-Me). EIMS *m/z* (rel. int.%): 478 (3), 332 (78), 317 (85), 297 (60), 218 (100), 142 (30), 91 (85). HR-EIMS (70 eV): calculated for $C_{22}H_{22}O_{12}$ $[M]^+$ 478.1111. Found 478.1146.

*3.4.2. Annulatin(**10**)*

Yellow amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3313 (OH), 3068, 2948, 2917, 2848, 1654 (C=O), 1623, 1602, 1511, 1378, 1315, 1205, 1164, 1027. UV and MS in agreement with published data (Markham and Whitehouse, 1984). ^1H NMR (C_5D_5N) δ: 13.22 (1H, *s*, 5-OH), 8.14 (2H, *s*, H-2′/6′), 6.72 (1H, *d*, $J=2.0$ Hz, H-6), 6.70 (1H, *d*, $J=2.0$ Hz, H-8), 4.11 (3H, *s*, 3-OMe). ^{13}C NMR (C_5D_5N) δ: 178.0 (*s*, C-4), 166.3 (*s*, C-7), 162.9 (*s*, C-5), 158.0 (*s*, C-9), 152.9 *s*, C-3′/5′), 147.4 (*s*, C-2), 139.4 (*s*, C-3), 139.3 (*s*, C-4′), 128.6 (*s*, C-1′), 109.3 (*d*, C-2′/6′), 104.9 (*s*, C-10), 99.8 (*d*, C-6), 94.8 (*d*, C-8), 60.8 (*q*, 3-OMe).

*3.4.3. Friedelinol (**11**)*

Needles (*n*-hexane/$CHCl_3$). m.p. 268–270°C [Lit. m.p. 292–301°C, Shoppee et al., 1962)]. $[\alpha_D^{21.9}+22.7°$ ($CHCl_3$, *c* 0.176) (Lit. $[\alpha]_D^{20}+18°$ ($CHCl_3$, *c* 1.0) (Shoppee et al., 1962). HR-EIMS (70 eV): calculated for $C_{30}H_{52}O$ $[M]^+$ 428.4018, found 428.4021.

*3.4.4. Friedelin (**12**)*

Needles (*n*-hexane/$CHCl_3$). m.p. 261–262°C [Lit. m.p. 261–262°C, Sainsbury, 1970)]. $[\alpha]_D^{19.1}$ −28.4° ($CHCl_3$, *c* 0.176) [Lit. $[\alpha]_D$ −22.5° ($CHCl_3$, *c* 1.0), Klass et al., 1992)]. IR, MS, ^1H and ^{13}C NMR in agreement with published data (Sainsbury, 1970, Klass et al., 1992; Ageta et al., 1995).

*3.4.5. Betulinic acid (**13**)*

Prisms (EtOH). m.p. 279–284°C [Lit. m.p. 275–278°C, Robinson and Martel, 1970)]. $[\alpha_D^{23.3}+8.4°$ (C_5H_5N, *c* 0.83) [Lit. $[\alpha]_D^{23}+7.9°$ (C_5H_5N, *c* 0.57) (Robinson and Martel, 1970)]. IR, MS, ^1H and ^{13}C NMR in agreement with published data (Robinson and Martel, 1970; Mahato and Kundu, 1994; Siddiqui et al., 1988).

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