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Published
2013

Journal Title
Phytochemistry

DOI
https://doi.org/10.1016/j.phytochem.2012.09.019

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Pyridocoumarin, aristolactam and aporphine alkaloids from the Australian rainforest plant *Goniothalamus australis*

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Abstract

Chemical investigation of the \(\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}\) extracts from aerial parts of the Australian plant *Goniothalamus australis* has resulted in the isolation of two pyridocoumarin alkaloids, goniothalines A (1) and B (2) as well as eight known natural products, aristolactam AII (3), enterocarpam II (4), caldensine (5), saurist lactam (6), (-)-anonaine (7), asimilobine (8), altholactone (9) and (+)-goniofufurone (10). The chemical structures of all compounds were determined by extensive spectroscopic and spectrometric analysis. Methylation of 2 using TMS-diazomethane afforded 1, which unequivocally established that both 1 and 2 possessed a novel 10-methyl-2H-pyrano[2,3-f]quinolin-2-one skeleton. These novel pyridocoumarin alkaloids are putatively proposed to arise biosynthetically from an aporphinoid precursor. Compounds 1-10 were evaluated for *in vitro* antimalarial activity against a chloroquine-sensitive *Plasmodium falciparum* line (3D7). Saurist lactam (6) and (-)-anonaine (7) exhibited the most potent antiparasitic activity with IC\(_{50}\) values of 9 and 7 µM, respectively.

Keywords

*Goniothalamus australis*, Annonaceae, alkaloid, pyridocoumarin, aristolactam, aporphine, styryl-lactone, goniothaline.
1. Introduction

The plant genus *Goniothalamus* Hook. f. & Thoms. (Annonaceae) consists of 134 species localised throughout Indomalesiana ([The Plant List, 2010](https://www.theplantslist.org/dwc/terms/)) and sporadically, Oceania ([Saunders and Munzinger, 2007](https://www.theplantslist.org/dwc/terms/)). The endemic *Goniothalamus australis* Jessup, commonly referred to as China Pine, is the only member of *Goniothalamus* documented in Australia ([Jessup, 1986](https://www.theplantslist.org/dwc/terms/)). Indeed, Annonaceae, which is comprised of more than 119 genera and 1756 species globally ([The Plant List, 2010](https://www.theplantslist.org/dwc/terms/)), is largely under-represented in Australia with reports of only 47 species, belonging to 16 genera ([PlantNET, 2012](https://www.theplantslist.org/dwc/terms/)).

*Goniothalamus* species are of significance in Traditional Asian medicine (Perry, 1980) with extracts from various species used for: the treatment of oedema and rheumatism ([Lu et al., 1985](https://www.theplantslist.org/dwc/terms/)); fever([Siti Najila et al., 2002](https://www.theplantslist.org/dwc/terms/)); analgesia ([Srivet and Vatèle, 1998](https://www.theplantslist.org/dwc/terms/)); inflammation and as abortifacients ([Burkill, 1966](https://www.theplantslist.org/dwc/terms/)). To the best of our knowledge *G. australis* was not utilised by the indigenous population.

*Goniothalamus* species are reputed for their production of a series of acetogenins and styryl-lactones as well as 1-benzyltetrahydroisoquinoline and indole-derived alkaloids ([Waterman, 1985](https://www.theplantslist.org/dwc/terms/)). These compounds possess significant cytotoxic ([Blázquez et al., 1999; Wiart, 2007](https://www.theplantslist.org/dwc/terms/)), antibacterial ([Wiart, 2007](https://www.theplantslist.org/dwc/terms/)) and antimalarial activities ([Lekphrom et al., 2009; Noor Rain et al., 2007; Siti Najila et al., 2002](https://www.theplantslist.org/dwc/terms/)). From the ≥ 30 species that have been investigated to date, flavanones, terpenes and phenylpropanoids have also been reported ([Dictionary of Natural Products, 2011; Teruna, 2006; Waterman, 1985; Wiart, 2007](https://www.theplantslist.org/dwc/terms/)).

*G. australis* was selected for this study due to the propensity for *Goniothalamus* species to produce bioactive compounds ([Seidel et al., 2000](https://www.theplantslist.org/dwc/terms/)) and the
limited knowledge of the chemistry of this endemic species.†

This paper describes the isolation and structure elucidation of a novel class of pyridocoumarin alkaloids (1 and 2), four aristolactams (3-6), two aporphine alkaloids (7 and 8) and two styryl-lactones (9 and 10) (Fig. 1) from the aerial parts of G. australis (Fig. 1). Furthermore, the in vitro antimalarial evaluation for all compounds towards a chloroquine-sensitive strain of Plasmodium falciparum (3D7) is reported.

2. Results and discussion

A small quantity of the plant material, comprising of leaf, wood, heartwood, bark and inflorescence samples was sequentially extracted with n-hexane, CH₂Cl₂ and CH₃OH. The CH₂Cl₂ and CH₃OH extracts were combined then analysed by LC-MS. The LC-MS data from the wood sample indicated UV-active compounds that contained prominent ions in the (+)-LRESIMS at m/z 266, 280, and 294 suggesting the presence of alkaloids. Additional ions were also detected in the extract of the bark (m/z 258, 268, 272 and 296), heartwood (m/z 258 and 272), and leaf (m/z 266). The wood, heartwood, bark and leaf material were subsequently selected for large-scale extraction and purification.

The wood of G. australis was sequentially extracted with n-hexane, CH₂Cl₂, and CH₃OH. The CH₂Cl₂/CH₃OH extracts were combined, evaporated then re-suspended in CH₃OH and passed through a polyamide gel (PAG) column to remove tannins. Subsequent fractionation of the CH₃OH eluent using C₁₈ HPLC (CH₃OH-H₂O-0.1% TFA) afforded the known alkaloids aristolactam AII (3), caldensine (5) and sauristolactam (6). Further semi-preparative C₁₈ HPLC separation (CH₃OH-H₂O-0.1% TFA) on several non-alkaloidal fractions yielded the styryl-lactones altholactone

† Brophy and co-workers have previously examined the volatile oil of this species (Brophy et al., 2004)
In the same manner as the wood above, the bark was extracted with CH$_2$Cl$_2$/CH$_3$OH and treated with PAG. The resulting CH$_3$OH eluent was then fractionated using a C$_{18}$ flash column employing a stepwise gradient of CH$_3$OH-H$_2$O-0.1% TFA to obtain four fractions. Purification of selected compounds on the basis of MS and UV data from fraction 4 using C$_{18}$ semi-preparative HPLC (CH$_3$OH-H$_2$O-0.1% TFA) led to the novel pyridocoumarins, goniothalines A (1) and B (2), as well as the known natural products: 3, 4, 6, 8 and 9.

Extraction and purification of the leaf material yielded (-)-anonaine (7), and the furano-pyrones 9 and furano-furone 10. A large-scale extraction of the heartwood was undertaken for the purpose of obtaining larger quantities of compounds 1 and 2 for $^{13}$C NMR and derivatisation studies as discussed below.

Goniothaline A (1) was obtained as a stable light-brown gum. A molecular formula of C$_{15}$H$_{13}$NO$_4$ was assigned to 1 following analysis of both the NMR and (+)-HRESIMS data. The $^1$H NMR spectrum of 1 (Table 1) displayed signals for four methines ($\delta_H$ 8.81, 7.44, 8.28, 6.62), two methoxys ($\delta_H$ 4.01, 4.11) and a C-methyl ($\delta_H$ 2.97). Analysis of the gCOSY spectrum, in combination with the $^1$H-$^1$H coupling constants identified two –HC=CH– spin systems. Furthermore, one of these olefinic moieties ($\delta_H$ 8.81 / 7.44) was shown to be part of a 2,3,4-trisubstituted pyridine system based on gHMBC and ROESY data analysis (Fig. 2). gHMBC correlations from the C-methyl protons at $\delta_H$ 2.97 to carbons resonating at $\delta_C$ 123.7, 145.3 and 115.7, in combination with a strong ROESY correlation between $\delta_H$ 2.97 and the $\beta$-pyridine proton at $\delta_H$ 7.44 established a 4-methylpyridine system. These data were consistent with other natural products such as those belonging to the azafluorenone (Mueller et al., 2009) and azaanthracene (Vallejos et al., 1999) structure classes, which contain para-substituted pyridine moieties. The protons from the other isolated
olefinic system ($\delta_H 8.28 / 6.62$) displayed a large coupling constant ($J = 9.7$ Hz) indicative of a cis configuration. gHMBC correlations from these sp$^2$ protons to a carbonyl resonance at $\delta_C 159.4$, and two quaternary downfield carbons at $\delta_C 111.4$ and 148.4 suggested an $\alpha, \beta$-unsaturated $\delta$-lactone nucleus, which was supported by a strong absorbance at 1713 cm$^{-1}$ in the IR spectrum (Pretsch et al., 2009). The protons of the methoxy groups at $\delta_H 4.11$ and 4.01 exhibited strong $^3J_{CH}$ correlations with downfield carbons at $\delta_C 146.2$ and 142.1, respectively. At this stage all atoms within 1 had been accounted for, however the substructures elucidated could not be linked together. Further analysis of the molecular formula for 1 indicated 10 hydrogen deficiencies. These data indicated that 1 contained a dimethoxylated benzenoid system. A gHMBC correlation from the $\delta$-lactone methine proton at $\delta_H 8.28$ to one of the methoxy substituted carbons at $\delta_C 146.2$ in conjunction with a strong ROESY correlation between $\delta_H 4.11$ and 8.28 (Fig. 2) supported a coumarin motif, however placement of the remaining methoxy unit ($\delta_H 4.01 / \delta_C 61.8$) could not initially be determined. Thus two possible orientations (ortho- or para-) for the methoxy groups existed. In order to unequivocally assign the structure of 1 we performed an additional gHMBC experiment in which the heteronuclear coupling constant was set to 4 Hz in an attempt to observe extra long-range correlations. Fortuitously, two critical $^4J_{CH}$ correlations were identified that included $\delta_H 8.28$ to $\delta_C 115.7$, and $\delta_H 2.97$ to $\delta_C 148.4$ (Fig. 2). These data indicated a 1,2-dimethoxybenzene system was present in 1, and allowed the orientation of the 4-methylpyridine moiety to be determined. Thus the chemical structure of 1 was assigned as 5,6-dimethoxy-10-methyl-2H-pyrano[2,3-\textit{f}]quinolin-2-one, to which we have designated the trivial name goniothaline A.

Goniothaline B (2), was obtained as a stable light-brown gum. The molecular formula of 2 was determined to be C$_{14}$H$_{11}$NO$_4$ on the basis of the NMR and (+)-
HRESIMS data. The $^1$H NMR spectrum of 2 displayed a high degree of homology with 1 (Table 1) however two notable differences were identified; these included the presence of only one methoxy signal ($\delta_H$ 4.04) and an additional downfield exchangeable signal ($\delta_H$ 9.58) in 2. The NMR data in combination with the MS results indicated that one of the methoxy moieties in 1 had been replaced with a hydroxy group in 2. Further confirmation of the presence of a phenol moiety in 2 was supported by the UV spectrum, which underwent a bathochromic shift on addition of base. gHMBC and ROESY data analysis suggested that the hydroxy group ($\delta_H$ 9.58) was substituted at C-6. Methylation of the goniothaline B (2) with TMS-diazomethane in CH$_2$Cl$_2$/CH$_3$OH afforded goniothaline A (1) in moderate yield following C$_{18}$ SPE purification. On the basis of the spectroscopic, spectrometric and synthetic derivatisation data the chemical structure of 2 was assigned to goniothaline B.

Goniothalines A (1) and B (2) are the first members of the 10-methyl-2$H$-pyrano[2,3-f]quinolin-2-one structure class to be reported from a natural source and their biogenesis warrants some discussion. A number of primitive flowering plant families including the Annonaceae and Eupomatiaceae, are known to produce aporphine and modified aporphine alkaloids. Of particular note are the azaanthracene alkaloids such as cleistopholine (Waterman and Muhammad, 1985) and annopholine (Rasamizafy et al., 1987), and the 1-aza-7-oxoaporphine alkaloids sampangine (Rao et al., 1986) and the eupomatadines (Carroll and Taylor, 1991). Taylor proposed that the 1-aza-7-oxoaporphine alkaloids may be formed via an ‘extradiol’ cleavage of a 5,6-dihydroxy-7-oxoaporphine, followed by transamination and oxidative decarboxylation (Taylor, 1984). Tadic et al. subsequently proposed that the azaanthracenes could diverge from the route to the 1-aza-7-oxoaporphine by

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† It is acknowledged that a similar scaffold: 2$H$-pyrano[2,3-f]quinolin-2-one has been reported from synthetic means (Atkins and Bliss, 1978; da-Matta et al., 2000)
degradation of the side chain generated through ‘extradiol’ cleavage of the catechol to yield an aldehyde or its equivalent which upon reduction yields cleistopholine (Tadic et al., 1987).

Since goniothalines A and B possess a 4-methyl-5,8-dioxygenated quinoline moiety in common with annopholine and considering G. australis metabolises a series of aporphinoids it is conceivable that 1 and 2 are also highly modified aporphine alkaloid derivatives. A plausible biogenetic pathway is outlined in Scheme 1. Starting from the tetrahydroxy-7-oxoaporphine (i), 'extradiol' cleavage and further oxidation of the side chain could yield a 8,9-dihydroxycleistopholine derivative (ii). Reduction of the quinone yields the hydroquinone (iii), which could then undergo a further 'extradiol' cleavage to yield a trihydroxylated alkaloid (iv). Decarboxylation to v, followed by lactonisation and methylation could then yield either 1 or 2.

Due to our ongoing interest in antimalarial natural products we evaluated compounds 1-10 in an in vitro radiometric P. falciparum growth inhibition assay (Barnes et al., 2012; Davis et al., 2010; Yang et al., 2010). Prior to screening, all compounds were re-analysed by $^1$H NMR spectroscopy in order to determine both the stability of molecules 1-10 and their purity. All compounds were shown to be >95% pure, and stable. Table 2 shows the in vitro activity of 1-10 against a chloroquine-sensitive P. falciparum line (3D7). (−)-Anonaine (7) and sauristolactam (6) displayed the most significant antiparasitic activity with an IC$_{50}$ of 7 and 9 μM towards P. falciparum respectively. The 3-methoxy analogue of 6, caldensine (5) displayed an IC$_{50}$ of 25 μM. The 2.8-fold difference in activity between 5 and 6 indicates the 3-methoxy substituent moderately reduces biological function. The N-demethyl analogue of 6, aristolactam AII (3), showed an IC$_{50}$ of 28 μM whereas enterocarpam II (4), the 8-methoxy analogue of 3, was inactive. The novel natural products goniothalines A (1) and B (2) displayed no in vitro antiparasitic activity at 50 μM.
Antimalarial data on additional analogues are required before conclusive structure activity relationships can be ascertained, however the present data suggests that methylation of the nitrogen and demethoxylation at C-8 in the aristolactam skeleton is important for *P. falciparum* growth inhibition.

The antimalarial activity for three of the compounds isolated during this study has been reported previously, albeit against different strains of *P. falciparum*. In this regard, aristolactam AII (3) exhibited an EC$_{50}$ of 9.5 µg/mL towards *P. falciparum* T9/94 (*Wirasathien, 1996*); whilst altholactone (9) had an IC$_{50}$ of 2.6 µg/mL against the *P. falciparum* K1 strain whereas (+)-goniofufurone (10) was inactive (*Lekphrom et al., 2009*).

3. Conclusion

Chemical investigations of the CH$_2$Cl$_2$/CH$_3$OH extracts from the aerial parts of *G. australis* resulted in the identification of two novel alkaloids, goniothalines A (1) and B (2), as well as eight previously identified natural products, which included four aristolactam alkaloids (3-6), two aporphine alkaloids (7 and 8) and two styryl-lactones (9 and 10). The identification of the novel skeleton, 10-methyl-2$H$-pyrano[2,3-f]quinolin-2-one, from *G. australis* supports further chemical exploration of the endemic species of Australian Annonaceae. This is the first report of the antimalarial activity of enterocarpam II (4), caldensine (5), sauristolactam (6) and asimilobine (8).

4. Experimental

4.1. General
Optical rotations were recorded on a Jasco P-1020 polarimeter. IR and UV spectra were recorded on a Bruker Tensor 27 spectrophotometer and a Jasco V-650 UV/vis spectrophotometer, respectively. LRESIMS were recorded on a Mariner time-of-flight spectrometer equipped with a Gilson 215 eight probe injector. LC-MS data was generated using a Waters Alliance 2790 HPLC equipped with a Waters 996 photodiode array detector and an Alltech evaporative light scattering detector that was attached to a Water ZQ mass spectrometer. HRESIMS were recorded on a Bruker Apex III 4.7 Tesla Fourier transform ion cyclotron resonance mass spectrometer. NMR spectra were recorded at 30 °C on either a Varian 500 MHz or 600 MHz Unity INOVA spectrometer. The \(^1\)H and \(^{13}\)C chemical shifts were referenced to the solvent peaks for DMSO-\(d_6\) at \(\delta_H\ 2.49\) and \(\delta_C\ 39.5\), respectively. Standard parameters were used for the 2D NMR experiments, which included gHSQC (\(^1J_{CH} = 140\) Hz) and gHMBC (\(^2J_{CH} = 8.0\) or 4.0 Hz). Phenomenex solid phase extraction (SPE) cartridges (10 × 50 mm, nylon frit, packed with Sepra C\(_{18}\) bonded silica, 35-75 μm, 150 Å) were used for small-scale plant extraction and purification of the reaction products. An Edwards Instrument company Bio-line orbital shaker was used for the large-scale plant extractions. HPLC grade solvents (RCI Lab-Scan) and filtered Milli-Q H\(_2\)O (Millipore) were utilised throughout these experiments. Polyamide gel CC6 (PAG) (30 g, 0.05-0.016 mm; Machery Nagel), packed into an open glass column (50 × 50 mm), and preconditioned and eluted with CH\(_3\)OH was used for the removal of tannins/polyphenolics. A stainless steel guard cartridge (Alltech, 10 × 30 mm) was prepacked with plant extracts pre-adsorbed to C\(_{18}\) (Phenomenex end-capped Sepra C\(_{18}\) bonded silica, 35-75 μm, 150 Å). A Waters 600 pump fitted with a 996 photodiode array detector and 717 plus autosampler was used for the semi-preparative and analytical HPLC separations. A Thermo Betasil C\(_{18}\) column (5 μm 143 Å, 21.2 × 150}
mm), a Thermo Betasil phenyl column (5 μm 143 Å, 21.2 × 150 mm) and a Phenomenex Luna C<sub>18</sub> column (5 μm 100 Å, 10 × 250 mm) were used for semi-preparative HPLC. A Phenomenex analytical Luna C<sub>18</sub> column (5 μm 100 Å, 4.6 × 50 mm) was used for LC-MS.

O+ erythrocytes were obtained from the Australian Red Cross Blood Service. Chloroquine (catalogue #C6628, >98%) was purchased from Sigma Aldrich. The 384-well Falcon sterile tissue culture treated plates were from Becton Dickinson.

4.2. Plant material

Leaf, wood, heartwood, bark and inflorescence samples of *Goniothalamus australis* Jessup (Annonaceae) were collected from Timber Reserve 66, Mt. Lewis, Queensland, Australia on the 30<sup>th</sup> of November 1997. A voucher specimen (AQ 604788) has been deposited at the Queensland Herbarium, Brisbane, Australia. Collections were air-dried, ground to a fine powder and stored at room temperature prior to extraction.

4.3. Small-scale plant extraction and LC-MS analysis

The leaf, wood, heartwood, bark and inflorescence material (300 mg) were added to a SPE cartridge, then extracted with n-hexane (7 mL), CH₂Cl₂ (7 mL) and CH₃OH (10 mL). The n-hexane extract was discarded and the CH₂Cl₂/CH₃OH extracts were combined, evaporated to dryness, then re-suspended in CH₃OH (1 mL), prior to LC-MS injection (10 μL). The LC-MS was performed using an analytical Phenomenex Luna column and a gradient from H₂O-CH₃OH-HCOOH (95:5:0.1) to CH₃OH-HCOOH (100:0.1) in 20 min, then isocratic conditions were employed for 5 min at CH₃OH-HCOOH (100:0.1), all at a flow rate of 1 mL/min.
4.4. Large-scale extraction and isolation

In separate extraction processes, the wood (10 g) and leaves (10 g) of *G. australis* was sequentially extracted with *n*-hexane (250 mL), CH$_2$Cl$_2$ (250 mL) and CH$_3$OH (250 mL × 2). The CH$_2$Cl$_2$ and CH$_3$OH extractions were combined and dried under reduced pressure to yield a crude extract (0.66 g wood; 1.85 g leaf). This material was resuspended in CH$_3$OH (150 mL) and loaded onto a PAG column. The resulting CH$_3$OH eluent (0.49 g wood; 1.6 g leaf) was preadsorbed onto C$_{18}$ bonded silica and packed into a stainless steel guard cartridge that was subsequently attached to a semi-preparative C$_{18}$ Betasil HPLC column. Isocratic HPLC conditions of H$_2$O-CH$_3$OH-TFA (90:10:0.1) were employed for the first 10 min, followed by a linear gradient to CH$_3$OH-TFA (100:0.1) over 40 min, then isocratic conditions of CH$_3$OH-TFA (100:0.1) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0 min and subsequently analysed by (+)-LRESIMS.

In the case of the wood, HPLC fractions 40 (m/z 266), 43 (m/z 280) and 48 (m/z 294) contained the ions of interest and subsequent lyophilisation yielded the known alkaloids, aristolactam AII (3, 5.8 mg, 0.058% dry wt), sauristolactam (6, 4.3 mg, 0.043% dry wt) and caldensine (5, 2.2 mg, 0.022% dry wt), respectively. Further analysis of all UV-active fractions from the first HPLC separation by $^1$H NMR spectroscopy and MS identified that fraction 27 contained pure altholactone (9, 11.8 mg, 0.118% dry wt), while fraction 26 (5.0 mg) consisted of a related semi-pure metabolite. The latter fraction was subjected to further semi-preparative HPLC using a Phenomenex Luna C$_{18}$ column. Isocratic HPLC conditions of H$_2$O-CH$_3$OH-TFA (90:10:0.1) were initially employed for the first minute, followed by a linear gradient to H$_2$O-CH$_3$OH-TFA (50:50:0.1) over 50 min at a flow rate of 4 mL/min. Fraction 35
afforded (+)-goniofufurone (10, 1.1 mg, 0.011% dry wt).

In the case of the leaf, (+)-LRESIMS analysis of the sixty HPLC fractions afforded pure (+)-goniofufurone (fraction 26, 10, 7.5 mg, 0.075% dry wt) and altholactone (fraction 27, 9, 4.3 mg, 0.043% dry wt). Fraction 36 was subjected to further semi-preparative HPLC using a Phenomenex Luna C18 column. Isocratic HPLC conditions of H2O-CH3OH-TFA (50:50:0.1) were initially employed for the first minute, followed by a linear gradient to H2O-CH3OH-TFA (40:60:0.1) over 50 min at a flow rate of 4 mL/min. Fraction 21 afforded (-)-anonaine (7, 1.2 mg, 0.012% dry wt).

The bark (50 g) was sequentially extracted with n-hexane (430 mL × 2), CH2Cl2 (430 mL × 2) and CH3OH (830 mL × 2). The CH2Cl2 and CH3OH extractions were combined and dried under reduced pressure to yield a crude extract. This material was resuspended in CH3OH (150 mL) and loaded onto a PAG column. The resulting CH3OH fraction (4.35 g) was fractionated using a C18 flash column, employing a stepwise gradient consisting of CH3OH-H2O (10:90), CH3OH-H2O (30:70), CH3OH-H2O (60:40), and CH3OH to yield 4 fractions. Fraction 4 (570 mg) was adsorbed onto C18 bonded silica, packed into a guard cartridge then fractionated using semi-preparative C18 HPLC. A linear gradient from H2O-CH3OH-TFA (90:10:0.1) to H2O-CH3OH-TFA (10:90:0.1) was run over 90 min, at a flow rate of 9 mL/min. Ninety fractions (90 × 1 min) were collected from time = 0 min then analysed by (+)-LRESIMS. Fraction 37 contained pure asimilobine (8, 1.6 mg, 0.003% dry wt). Further separation of fraction 62 was performed on a Betasil phenyl column using an isocratic gradient of CH3OH-H2O-TFA (60:40:0.1) to yield pure aristolactam AII (3, 3.0 mg, 0.006% dry wt) and enterocarpam II (4, 3.0 mg, 0.006% dry wt). Further purification of fractions 29 to 31 (which contained ions m/z 258 and
272) using a Phenomenex Luna C_{18} column (250 × 10 mm) and employing an isocratic gradient of CH_{3}OH-H_{2}O-TFA (20:80:0.1) yielded goniothalines A (1, 2.5 mg, 0.005% dry wt) and B (2, 1.0 mg, 0.002% dry wt).

The heartwood (50g) was defatted (2 × 430 mL hexane) and extracted with CH_{2}Cl_{2} (2 × 430 mL). The CH_{2}Cl_{2} extract was evaporated (246 mg) and adsorbed to C_{18} and directly fractionated by semi-preparative HPLC over two steps using the same HPLC conditions as stated for the isolation of 1 and 2 from the bark. The heartwood afforded goniothalines A (1, 1.0 mg, 0.002% dry wt) and B (2, 5.1 mg, 0.012% dry wt).

4.4.1. Identification of known compounds

Compounds 3-10 were identified as the previously reported natural products, aristolactam AII (3) (Priestap, 1985), enterocarpam II (4) (Kamaliah et al., 1986), caldensine (5) (Cardozo Júnior and Oliveira Chaves, 2003), sauristolactam (6) (Rao and Reddy, 1990), (-)-anonaine (7) (Guinaudeau et al., 1983; Simas et al., 2001) asimilobine (8) (Guo et al., 2011), altholactone (9) (Loder and Nearn, 1977) and (+)-goniofufurone (10) (Fang et al., 1990) following 1D / 2D NMR (^{1}H, gCOSY, gHSQC, gHMBC, ROESY) and MS data analysis and comparison with literature values. Optical rotations were recorded for compounds 7, 8, 9 and 10 and were shown to match literature data: (Guinaudeau et al., 1983), (Guo et al., 2011), (Enders and Barbion, 2008) and (Prasad and Gholap, 2008) respectively.

4.4.2. Goniothaline A

Stable light-brown gum; UV \( \lambda_{\text{max}} \) (CH_{3}OH) (log \( \varepsilon \)): 226 (4.33), 284 (4.01), 291 (4.00) nm; IR \( \nu_{\text{max}} \) (KBr) 1713, 1644, 1463, 1367, 1204, 1140, 1083, 1037, 908 cm^{-1}; \(^{1}\)H and \(^{13}\)C NMR data, see Table 1; (+)-LRESIMS \( m/z \) (rel. int) 272 (100); (+)-
4.4.3. *Goniothaline B*

Stable light-brown gum; UV $\lambda_{max}$ (CH$_3$OH) (log $\varepsilon$): 226 (4.11), 258 (3.61), 284 (3.65), 291 (3.55), 372 (2.61) nm; UV $\lambda_{max}$ (CH$_3$OH + NaOH) (log $\varepsilon$): 269 (3.36), 293 (3.36), 319 (3.32), 369 (2.73); IR $\nu_{max}$ (KBr) 3355 (br), 1716, 1640, 1566, 1494, 1471, 1403, 1306, 1203, 1144, 1079, 958 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; (+)-LRESIMS m/z (rel. int) 258 (100); (+)-HRESIMS m/z 258.0758 (C$_{14}$H$_{12}$NO$_4$ [M+H]$^+$ requires 258.0761)

4.5 *Methylation of 2 using TMS-diazomethane*

*Goniothaline B* (2, 1.0 mg, 0.0004 mmol) was dissolved in CH$_3$OH-CH$_2$Cl$_2$ (1:1, 200 $\mu$L) at room temperature before TMS-diazomethane (2.0 M in Et$_2$O, 77 $\mu$L, 0.154 mmol) was added dropwise. ([Garfunkle et al., 2009](#)) The reaction was stirred for 20 min at room temperature, evaporated to dryness then adsorbed to C$_{18}$ bonded silica then loaded onto a C$_{18}$ SPE cartridge. The cartridge was sequentially eluted with H$_2$O-CH$_3$OH-TFA (5 mL 90:10:0.1; 5 mL 70:30:0.1; 5 mL 50:50:0.1; 5 mL 30:70:0.1; and 5 mL 10:90:0.1). Lyophilisation of the H$_2$O-CH$_3$OH-TFA (50:50:0.1) eluent afforded pure goniothaline A (1, 0.9 mg, 85% yield).

4.6. *P. falciparum growth inhibition assay*

*Plasmodium falciparum* growth inhibition assays were carried out using an isotopic microtest as previously described ([Andrews et al., 2000](#)). Briefly, ring-stage infected erythrocytes (0.5% parasitemia and 2.5% hematocrit) were seeded into triplicate wells of 96 well tissue culture plates containing serial dilutions of...
the positive control (chloroquine, Sigma Aldrich, catalogue #C6628, >98%) or test compounds and incubated under standard *P. falciparum* culture conditions. After 48 h, 0.5 μCi [%[^3]H]-hypoxanthine was added to each well after which the plates were cultured for a further 24 h. Cells were harvested onto 1450 MicroBeta filter mats (Wallac) and [%[^3]H] incorporation was determined using a 1450 MicroBeta liquid scintillation counter. Percentage inhibition of growth compared to matched DMSO controls (0.5%) was determined and IC\textsubscript{50} values were calculated using linear interpolation of inhibition curves (Huber and Koella, 1993). The mean IC\textsubscript{50} (±SD) was calculated over three independent experiments, each carried out in triplicate.

**Acknowledgments**

The authors would like to thank L. Jessup, G. Guymer and P. Forster from the Queensland Herbarium for sample collection and identification. K. Andrews and H. Vu from Griffith University are acknowledged for the supply of *P. falciparum* strain 3D7 and HRESIMS measurements, respectively. We thank R. Quinn for access to the *G. australis* plant samples, which form part of the Eskitis Institute’s Nature Bank biota library. We also acknowledge the Australian Red Cross Blood Service for the provision of type O+ erythrocytes. The Australian Research Council is acknowledged for support towards the NMR and MS equipment (LE0668477 and LE0237908).

**Supplementary data**

Supplementary data [[^1]H, ^13^C, gCOSY, gHSQC, gHMBC (8 Hz and 4 Hz) and ROESY NMR spectra and data tables for goniothalines A (1) and B (2)] associated with this article can be found, in the online version, at doi:
Figure Legends

**Fig. 1.** Chemical structures for natural products 1-10.

**Fig. 2.** Key $^2$J\textsubscript{CH} (–→) and $^4$J\textsubscript{CH} (→→→) gHMBC and ROESY (←→→) correlations for 1.

Table Legends

**Table 1.** $^1$H and $^{13}$C NMR data for gonothalines A (1) and B (2).

**Table 2.** *In vitro* antimalarial activity for compounds 1-10.

Scheme Legend

**Scheme 1.** Proposed biogenesis of gonothalines A (1) and B (2).

References


Seidel, V., Bailleul, F., Waterman, P. G., 2000. (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. Phytochemistry (Elsevier) 55, 439-446.