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Myristicyclins A and B: Antimalarial Procyanidins from *Horsfieldia spicata* from Papua New Guinea

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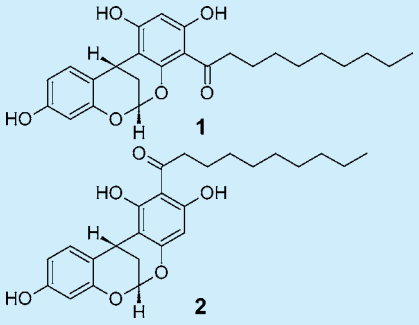
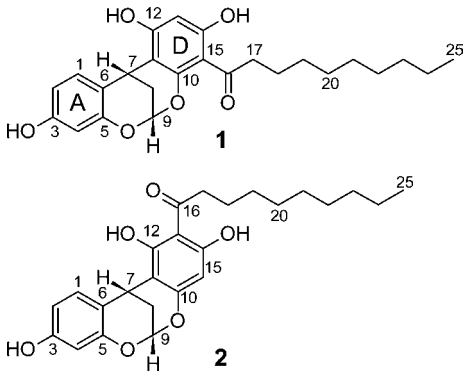
Supporting Information

ABSTRACT: An antimalarial screen for plants collected from Papua New Guinea identified an extract of *Horsfieldia spicata* as having activity. Isolation of the active constituents led to the identification of two new compounds: myristicyclins A (**1**) and B (**2**). Both compounds are procyanidin-like congeners of myristinins lacking a pendant aromatic ring. Myristicyclin A was found to inhibit the ring, trophozoite, and schizont stages of *Plasmodium falciparum* at similar concentrations in the mid-μM range.



The spread of drug resistant malaria in the tropical countries that host a wide diversity of indigenous flora poses a strong threat to the health of the people living there. The International Cooperative Biodiversity Group for Papua New Guinea (ICBG-PNG) has as one of its focus areas the discovery of compounds that have therapeutic relevance for PNG and demonstrate the benefits of preserving biodiversity. ICBG funded biodiversity surveys of endemic and indigenous PNG plants resulted in the collection of 727 distinct samples from an estimated 650 native plants that were screened for antimalarial activity using a previously reported method.[1] Of these, fractions from 38 were identified as active, (>70% inhibition parasite growth) and not toxic (<30% inhibition of human T cell replication). Extracts from 20 of these plants were further analyzed by the method of Grimberg and co-workers,[2] which confirmed activity in all but three of the retested samples. *Horsfieldia spicata* (Rosb.) J. Sinclair (Myristicaceae) was one of a few that exhibited potent activity against ring stage erythrocytic parasites (in addition to inhibition of the trophozoite and schizont stages). Within the genus *Horsfieldia* only *H. irya* has been reported in the literature to be used medicinally in PNG. It is used in Bougainville for stomach ache and diarrhea.[3] The only traditional uses reported for *H. spicata* (a minor hardwood tree) are for dress and wood.[4] Following up on this activity, extracts of mixed wood, twigs, and leaves from *H. spicata* were fractionated leading to the isolation and identification of myristicyclins A (**1**) and B (**2**) whose structures and activity we report here.



Myristicyclin A (**1**)[5] was found to have the molecular formula $C_{25}H_{30}O_6$ (11 units of unsaturation) on the basis of HRESIMS and NMR data. The ^{1}H NMR spectrum of **1** in pyridine-d_5 (Table S1) exhibited signals for a 1,3,4-trisubstituted benzene ring (H-1, δ_H 7.68 ppm, d (8.3 Hz); H-2, δ_H 6.84 ppm, dd (8.3, 2.3 Hz); H-3, δ_H 6.99 ppm, d (2.3 Hz)), a singlet aromatic proton (δ_H 6.494 ppm), two aliphatic

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methine groups (δ_H 4.67, 6.487 ppm), a triplet methyl group (δ_H 0.86 ppm), and also numerous signals for methylene protons (δ_H 1.2−3.2 ppm). Long range and one bond heteronuclear coupling experiments revealed the presence of one carbonyl carbon (δ_C 205.4 ppm), 12 aromatic/olefinic carbons (corresponding to two aromatic rings), two aliphatic methine carbons, one methyl carbon, and numerous methylene carbons. Chemical shift data indicated that five of the aromatic ring carbons are oxygenated (δ_C 158.6, 152.2, 154.3, 162.6, 165.5 ppm). Combined, these data account for 9 degrees of unsaturation, requiring two additional rings to be present. $^1H-^1H$ homonuclear scalar coupling correlation experiments identified two additional spin systems. The first comprises a linear alkyl chain terminated at one end with a methyl group (H_2-17−H_3-25) and at the other end with a ketone based on HMBC correlations from H_2-17 and H_2-18 to C-16 (δ_C 205.4 ppm). The second spans from H-7 to H-9 which, combined with ^{13}C NMR shifts for the corresponding carbons, suggested a $CHCH_2CH$ system with C-9 bearing two oxygen atoms (δ_H 6.487 ppm, δ_C 92.8 ppm). In all, the NMR data suggested the presence of eight carbon−oxygen bonds. Based on the molecular formula and constraints on connectivities among the various substructures, C-7−C-9 were deduced to be involved in a bicyclic system that bridges the two aromatic rings and that incorporates two ether groups.

The presence of this bicyclic system was confirmed using long-range heteronuclear correlation data. H-7 Exhibited HMBC correlations to carbons in both aromatic rings (C-1, C-5, and C-6 in ring A; C-10, C-11, and C-12 in ring D; Figure 1 and Table S1). Although H-7 has a low field chemical shift

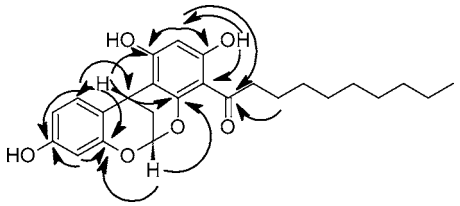
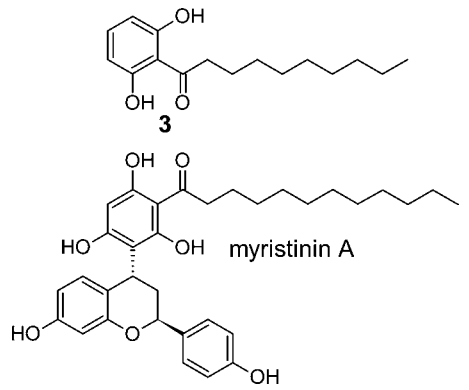


Figure 1. Key HMBC correlations observed for myristicyclin A (**1**).

(δ_H 4.67 ppm), the high-field shift of C-7 (δ_C 23.7 ppm) indicates that C-7 must be attached directly to carbon atoms in both aromatic rings. The low field shift of H-7 is consistent with its being constrained within the deshielding field of two aromatic systems. As previously mentioned, C-9 bears two oxygen atoms and H-9 exhibits HMBC correlations to oxygenated carbons in both aromatic rings (C-5 and C-10, respectively), both of which were also correlated with H-7. Combined, the data established a [3,3,1]-bicyclic system connecting the two aromatic rings. The presence of W-coupling between H-7 and H-9 and the universally small vicinal coupling constants observed among H-7, H-8a, H-8b, and H-9 indicated that H-7 and H-9 were both located quasi-equatorially, establishing the relative configurations of C-7 and C-9. All that remained was to establish the substitution patterns of the three hydroxyl and one acyl substituents for the two aromatic rings.

Ring A corresponded to the previously mentioned 1,3,4-trisubstituted aromatic ring. Attachment of C-7 *ortho* to H-1 was established by HMBC correlations from H-1 to C-7, from H-7 to C-1 (Figure 1), and by a ROESY correlation between H-1 and H-7. Thus, one of the hydroxyl groups was placed *meta* to H-1 at C-3 (δ_C 158.6 ppm), which was also consistent with HMBC correlations from both H-1 and H-4 to C-3 (Figure 1). The high field shifts observed for the three nonoxygenated carbons in ring D (δ_C 97.3, 104.5, and 108.1, for C-13, C-15, and C-11, respectively) suggested a 1,3,5-oxygenation pattern, leaving two possible locations for the acyl chain substituent. Attachment of the acyl chain *ortho* to C-10 was suggested by a weak ROESY correlation between H-9 and H_2-17, thus leading to structure **1**. Further support for this structure comes from a long-range correlation from H-13 to C-16 (Figure 1), by the apparent sharpening and low-field shift of H-14OH (14.46 ppm) presumably due to intramolecular hydrogen bonding to the adjacent ketone oxygen, and the fact that H-13 exhibits HMBC correlations to every carbon in ring D except C-10 which is located *para* to C-13 (Table S1). To provide further confirmation of the proposed substitution pattern of ring D, the per-methyl ether of **1** was synthesized. The presence of ROESY correlations from H-13 to two *O*-methyl groups confirmed location of the acyl chain at C-15 (Figure S22).

Myristicyclin B (**2**)[6] was found to be an isomer of **1** on the basis of HRESIMS and NMR data. The ^1H and ^{13}C NMR data were very similar between **1** and **2**. The correlations observed by COSY and HMBC were also nearly identical between **1** and **2**, suggesting that the same carbon−carbon connectivities are present in both. The most substantive differences are located in ring D. The singlet aromatic proton in ring D exhibited HMBC correlations to all other carbons in the ring except C-12, an oxygenated carbon not involved in the cyclized ether (Table S3). Due to the proximity of the ^1H NMR signals for H-9 and H-15 in pyridine-d_5, an HMBC spectrum was collected in CD_3OD where the signals are better resolved, confirming that both hydrogens correlate to C-10 (Table S4). Moreover, no ROESY correlation was observed between H-9 and H_2-17. Taken together, the data suggested that **2** differed from **1** by exchanging the locations of the acyl chain and the aromatic hydrogen in ring D. Per-methylation of **2** was attempted to provide further confirmation of the structural assignment. However, even under strong conditions (dimethyl carbonate/DBU, 90 °C), the major product exhibited only mono-methylation of **2** at position 3. A possible explanation for this result is the potential for the ketone oxygen to intramolecularly hydrogen bond to the adjacent hydroxyl groups at C-12 and C-14. In either case, the intramolecular interaction orients the ketone group such that the acyl chain will sterically hinder access to the other hydroxyl group.



Four additional compounds that have been previously reported were isolated and characterized from the extract.

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Cubebin,[7−9] dihydrocubebin,[10] and horsfieldin[11] are neolignans, the latter two of which have been previously reported from *Horsfieldia iryaghedhi*.[11] 1-(2,6-Dihydroxyphenyl)-1-decanone (**3**), originally isolated from the wood of *Knema austrosiamensis*,[12] was also purified. A previous report described the dodecanone analogue of **3** as occurring in *Horsfieldia irya*.[13]

The similarities between **1** and **2** suggest that the two compounds have a common biosynthetic precursor. Previous reports have identified the compound myristinin A, which bears striking similarities to the compounds reported here, as occurring in *Horsfieldia* and other genera within the family Myristicaceae.[14] Common features include fused rings A and B from **1** as well as attachment of a *C*-acylated phloroglucinol via a carbon−carbon bond to the equivalent of C-7. Compound **1** can be formally derived from a myristinin A analog bearing decanone rather than dodecanone by forming ring C via ether bond formation with the equivalent of C-9 and cleaving the bond to the aromatic ring. Formation of similar bicyclic systems via ethers are well-known among the type-A proanthocyanidins. However all naturally occurring compounds containing the polycyclic core of **1** reported to date (such as procyanidin A1) also bear an aromatic ring at the equivalent of C-9, making the carbon skeleton found in **1** and **2** unique.

The antimalarial activities of all isolated compounds were examined using an assay that determines IC_{50} values against the ring, trophozoite, and schizont stages of *Plasmodium falciparum*.[15] Finding agents capable of selectively killing the dormant ring stage parasites holds promise as a means of combating drug resistance.[15] Myristicyclin A (**1**) exhibited IC_{50} values of 35, 43, and 54 μM against ring, trophozoite, and schizont stages, respectively. Thus **1** is equally effective against the various stages. Myristicyclin B (**2**) was found to cause hemolysis at 230 μM in addition to its antiparasitic activity (10, 6.6, and 7.9 μM against ring, trophozoite, and schizont stages, respectively), thus limiting its potential utility as a therapeutic. Compound **3** was also found to be hemolytic at 380 μM whereas its activity against *P. falciparum* showed a less favorable profile at 90, 25, and 9.2 μM against ring, trophozoite, and schizont stages, respectively. None of the cubebin and horsfeldin analogues isolated in this study exhibited activity against *P. falciparum*. It is interesting to note that **2**, which somewhat resembles cholesterol with respect to the placement of its side chain relative to the polycyclic core, would be more disruptive of membrane integrity than **1** despite their structures otherwise being identical.

A-type procyanidins, which incorporate the polycyclic core of **1** and **2**, have been associated with a wide variety of activities including antioxidant, anticancer, and antibacterial adhesion.[16−22] Interestingly, A-type proanthocyanidins have also been reported to have antiparasitic activity.[23] Studies have shown that compounds modulating mammalian cell signaling pathways have also shown activity against *P. falciparum*.[15,24−26] Myristinin A has been reported to cause DNA damage and to inhibit DNA polymerase β at concentrations comparable to the IC_{50} of **1**.[27] Malabaricone A, which contains a 2-acyl-1,3-dihydroxyphenyl moiety, was isolated from *Knema glauca* and has been reported to have activity against *P. falciparum*.[28] It is as yet unclear which structural features of the myristicyclins are most important for their antimalarial activities aside from the observation of higher hemolytic toxicity for **2** compared to **1**. The ability of proanthocyanidins to form and quench radicals in oxidative environments such as erythrocyte interiors where *P. falciparum* spends much time might disrupt essential pathways such as methemoglobin metabolism in the parsite.[29] Such reactive oxygen species (ROS) could also be involved in the DNA damage caused by myristinin A[27] or disruption of cell signaling observed for other related compounds.[16,17,19] A comparison of the antimalarial, antioxidant, ROS-forming, and hemolytic activities of the myristicyclins and the myristinins could yield insights into potential mechanisms of action.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details; NMR data and assignments for **1** and **2** in pyridine-d_5 and CD_3OD; NMR data for the permethyl derivative of **1**; antimalarial activity of **1**, **2**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(5) Myristicyclin A (**1**): colorless oil; $[\alpha]_{20}^{D}$ −21 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.30) nm, 238 (3.82) nm, 288 (3.75) nm, 336 (1.03) nm; IR (film) ν_{max} 2919.70, 2845.45, 1635.34, 1623.77, 1558.20, 1540.84, 1363.43, 1088.62, 827.31 cm^{-1}; ^1H and ^{13}C NMR data Tables S1 and S2; HRESIMS *m*/*z* 427.2138 $[M+H]^+$ (calcd for $C_{25}H_{31}O_6$, 427.2121).

(6) Myristicyclin B (**2**): colorless oil; $[\alpha]_{20}^{D}$ +17 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.17) nm, 240 (3.68) nm, 288 (3.45) nm, 340 (0.83) nm; IR (film) ν_{max} 2920.66, 2850.27, 1633.41, 1616.05, 1559.16, 1541.81, 1362.21, 1089.58, 826.35 cm^{-1}; ^1H and ^{13}C NMR data Tables S3 and S4; HRESIMS *m*/*z* 427.2138 $[M+H]^+$ (calcd for $C_{25}H_{31}O_6$, 427.2121).

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