Phenolic Glycosides with Antimalarial Activity from *Grevillea* "Poorinda Queen"

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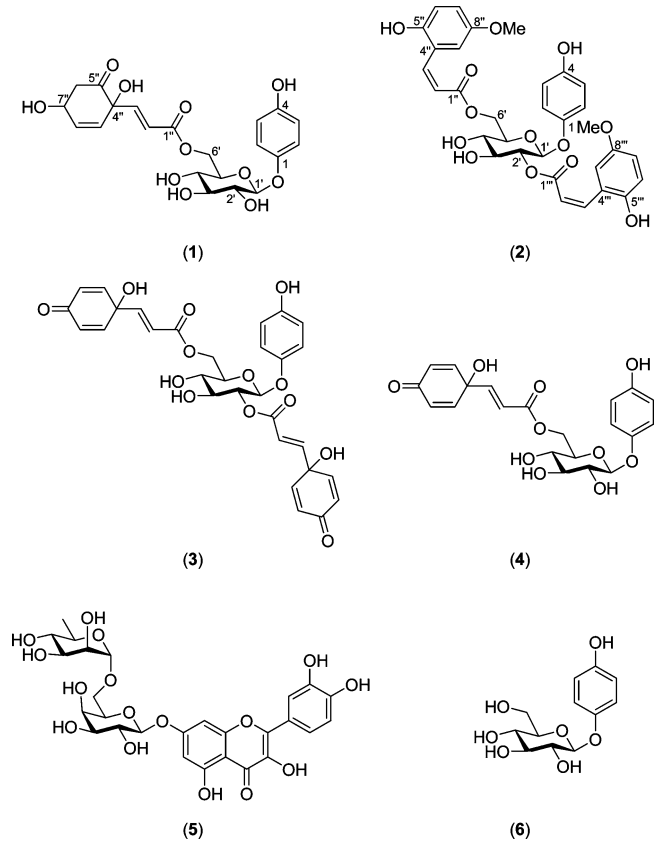
In search of new antimalarial compounds, three new phenolic glycosides, robustasides E (**1**), F (**2**), and G (**3**), in addition to the known compounds robustaside D (**4**) and quercetin-7-*O*-[α-L-rhamnopyranosyl(1→6)-β-D-galactopyranoside] (**5**), were identified during chemical investigations of the MeOH extract from the leaves and twigs of *Grevillea* "Poorinda Queen". The chemical structures of the new compounds were elucidated through 2D NMR spectroscopy, while the absolute configuration of the sugar was elucidated through chemical degradation and comparison with an authentic standard. Discussed in detail are the isolation and structure elucidation of **1**−**3**, as well as the associated *in vitro* anitmalarial activities for **1**−**5**. Also discussed are the *in vivo* anitmalarial and *in vitro* cytotoxic activities for **1**, **3**, and **4**.

Malaria continues to be one of the most debilitating and devastating diseases to humans. Each year 350−500 million malaria cases occur worldwide with more than 1 million deaths from falciparum malaria.[1] In an effort to delay the development and spread of multiple drug-resistant falciparum malaria, artemisinin-based combination treatments (ACTs) are now recommended for first-line treatment of uncomplicated *Plasmodium falciparum* worldwide.[2] An artemisinin-based compound rapidly kills the parasites in the infected person, while the unrelated and more slowly eliminated partner drug persists in the blood to prevent recurrences of malaria.[3] However, there is genuine concern that drug resistance against one of the most efficacious ACTs, artesunate-mefloquine,[4,5] is starting to appear on the Thai−Cambodian border. Thus, there is a significant need for the discovery of new antimalarial drugs with novel structures that have different modes of action from the currently available drugs.

While screening a library of terrestrial plant and marine invertebrate extracts for their *in vitro P. falciparum* activity, an extract of a *Grevillea* "Poorinda Queen" displayed significant antimalarial activity. Subsequent bioassay-guided fractionation of this extract allowed for the identification of three new compounds, robustasides E (**1**), F (**2**), and G (**3**), and the known compounds robustaside D (**4**)[6] and quercetin-7-*O*-[α-L-rhamnopyranosyl(1→6)-β-D-galactopyranoside] (**5**).[7] The structures of the known compounds were elucidated through comparison with literature data.[6,7] Discussed below in detail is the structure elucidation and determination of the absolute configuration of the sugar moiety for **1**−**3**, as well as the antimalarial and cytotoxic activities for **1**, **3**, and **4**.



Results and Discussion

Robustaside E (**1**) was isolated as a brown oil with a pseudomolecular ion ($[M + Na]^+$ obs *m/z* 475.1214; calc *m/z* 475.1211; $\Delta = 0.7$ ppm) suggestive of the molecular formula $C_{21}H_{24}O_{11}$, which equated to 10 double-bond equivalents. Immediately apparent in the NMR data for **1** (Table 1) were resonances consistent with an anomeric methine (^{13}C: δ 104.1; ^1H: δ 4.71: H-1′). Furthermore, COSY correlations from H-1′ to δ 3.39 (H-2′), from δ 3.34 (H-4′) to δ 3.63 (H-5′), and from H-5 to δ 4.30 (H_b-6′) in addition to gHMBC correlations from δ 3.42 (H-3′) to δ 72.2 (C-4′) and δ 75.7 C-5′ and from H-4′ to δ 78.3 C-2′ readily identified a pyranose moiety. The magnitude of the ^1H NMR chemical shifts for H-2′ to H-4′ was indicative of hydroxy substitution at these positions.

COSY correlations from δ 6.68 (H-2/6) to δ 6.91 (H-3/5), along with the value of the coupling constant (8.9 Hz), indicated that these protons were part of a 1,4-disubstituted aromatic ring system. Observed gHMBC correlations from H-2 to δ 117.1 (C-6) and 154.4 (C-4), from H-6 to δ 117.1 (C-2) and C-4, from H-3 to δ 120.0 (C-5) and δ 152.7 ppm (C-1), and from H-5 to δ 120.0 ppm (C-3) and C-1 established the aromatic spin system as a *p*-disubstituted aromatic ring. Furthermore, the ^{13}C NMR chemical shifts of C-1 and C-4 were indicative of aromatic carbons bearing oxygen. An observed gHMBC correlation from H-1′ to C-1 allowed for the *p*-disubstituted aromatic ring to be located at C-1′ of the pyranose ring.

Further analysis of the NMR data for **1** (Table 1) identified a carbonyl carbon at δ 167.9 (C-1″) that was indicative of C-1″ being part of an ester moiety. Furthermore, a COSY correlation from δ 6.24 (H-2″) to δ 7.03 (H-3″), in addition to gHMBC correlations

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Table 1. NMR Data (500 MHz, methanol-d_4) for **1**

no.	δ_C, mult.	δ_H (J in Hz)	COSY	gHMBC[b]	NOESY
1	152.7, C				
2, 6	117.1, CH	6.68, dd (8.9, 1.8)	H-3, H-5	1, 2, 4, 6	
3, 5	120.0, CH	6.91, dd (8.9, 2.4)	H-2, H-6	1, 3, 4, 5	
4	154.4, C				
1′	104.1, CH	4.71, d (7.3)	H-2′	1	H-3′, H-5′
2′	78.3, CH	3.39, m	H-1′	1′	H_2-6′
3′	75.4, CH	3.42, t (7.3)		4′, 5′	H-1′
4′	72.2, CH	3.34, m	H-5′	2′	H_2-6′
5′	75.7, CH	3.63, br t (7.0)	H-4′, H_b-6′		H-1′
6′	65.5, CH_2	4.50, dd (12.0, 2.4)	H_b-6′	1″	H-2′, H-4′
		4.32, dd (12.0, 7.0)	H-4′, H_a-6′		H-2′, H-4′
1″	167.9, C				
2″	123.7, CH	6.24, d (15.9)	H-3″	1″, 4″	
3″	150.6, CH[a]	7.03, d (15.9)	H-2″	1″, 4″	
4″	74.2, C				
5″	199.9, C				
6″	43.7, CH_2	2.68, d (4.2)	H-7″, H-8″	4″, 5″	
7″	74.1, CH	4.03, ddd (4.2, 2.1, 2.1)	H-6″, H-9″		
8″	130.9, CH	6.06, dd (10.2, 2.1)	H_2-6″, H-9″	4″	
9″	150.7, CH[a]	6.63, ddd (10.2, 2.1)	H-7″, H-8″	5″, 7″	

[a] Signals may be interchanged. [b] gHMBC correlations from ^{1}H to indicated ^{13}C.

from H-2″ and H-3″ to C-1″, established that C-1″ to C-3″ were in fact part of an α,β-unsaturated ester. Also evident from Table 1 was a ^{13}C NMR resonance consistent with a ketone carbonyl at δ 199.9 (C-5″) and resonances consistent with a hydroxy methine (^{13}C: δ 73.4; ^{1}H: δ 4.03). COSY correlations from δ 4.03 (H-7″) to δ 2.68 (H-6″), with allylic couplings (J = 2.1 Hz) from H-6″ to δ 6.06 (H-8″), and from H-7″ to δ 6.63 (H-9″) established a 2-hydroxy-3-butene spin system. Observed gHMBC correlations from H-6″ to both a quaternary carbon bearing oxygen at δ 74.2 (C-4″) and C-5″, from H-8″ to C-4″, and from H-9″ to C-5″ and C-7″ allowed for a 2-hydroxycyclcohexen-3-one moiety to be established, with additional oxygenated functionality at C-4″. Finally, H-2″ and H-3″ each showed additional gHMBC correlations to C-4″. This positioned the α,β-unsaturated ester moiety at C-4″. The observation of a gHMBC correlation from H_2-6′ to C-1″ allowed for the α,β-unsaturated hydroxycyclohexenone ester moiety to be substituted on the pyranose ring at C-6′. These data consumed all the double-bond equivalents and all atoms aside from one proton. The only remaining point of attachment for the proton was the oxygen at C-4″, allowing for the formation of a tertiary hydroxy group. Hence the gross structure of **1** was elucidated as the acylated sugar shown.

The geometry of $\Delta^{2''}$ was determined to be *trans* by the value of $J_{2''\text{-}3''}$ (15.9 Hz). The relative configuration of the pyranose ring was also determined through both NOESY correlations and coupling constants. NOESY correlations were observed from H-1′ to both H-3′ and H-5′. These data, in conjunction with the observation of large coupling constants for H-1′, H-2′, and H-5′, indicated that all of the hydroxy groups were equatorial, indicative of β-glucose. Subsequent acid hydrolysis of **1** and comparison of the $[\alpha]_D$ of the acid hydrolysate with an authentic standard of D-glucose (obs +39; std +48) established the sugar as D-glucose. The configurations at C-4″ and C-7″ remain unassigned.

Robustaside F (**2**) was isolated as a brown oil with a pseudomolecular ion ($[M + Na]^+$ obs m/z 647.1766; calc m/z 647.1735; Δ = 4.8 ppm) suggestive of the molecular formula $C_{32}H_{32}O_{13}$, which equated to 17 double-bond equivalents. Analysis of the NMR data for **2** (Table 2) indicated significant symmetry through the presence of duplicated aromatic and olefinic NMR resonances. Comparison of the NMR data for **2** with **1** (Table 1) readily identified **2** as having the same *p*-disubstituted aromatic ring substituted at C-1′ of a pyranose moiety as present on **1**. The magnitude of the coupling constants for H-1′ to H-6′ (large J values, see Table 2) were again indicative of axial−axial couplings and established a β-glucose moiety. However, a difference was noted in the ^{1}H NMR chemical shift for H-2′ of **2** compared to **1**. The ^{1}H NMR chemical shift for

Table 2. NMR Data (500 MHz, methanol-d_4) for **2**

no.	δ_C, mult.[a]	δ_H (J in Hz)	COSY	gHMBC[b]
1	151.9, C			
2, 6	116.9, CH	6.62, d (8.8)	H-3, H-6	1, 3, 4, 6
3, 5	119.9, CH	6.86, d (8.8)	H-2, H-5	1, 2, 4, 5
4	153.7, C			
1′	102.4, CH	4.95, d (8.0)	H-2′	1, 2′
2′	75.1, CH	5.09, t (8.0)	H-1′, H-3′	1′, 3′, 1″
3′	76.1, CH	3.70, m	H-2′, H-4′	2′, 5′
4′	71.9, CH	3.54, t (9.0)	H-3′, H-5′	5′, 6′
5′	75.6, CH	3.74, m	H-4′, H_a-6′, H_b-6′	
6′	64.7, CH_2	4.58, d (11.2)	H-5′, H_b-6′	1‴
		4.39, dd (11.2, 6.6)	H-5′, H_a-6′	5′, 1‴
1″	168.3, C			
2″	117.0, CH	6.61, d (10.7)	H-3″	4″
3″	142.7, CH	7.98, d (10.7)	H-2″	1″, 2″, 5″, 9″
4″	123.0, C			
5″	152.8, C			
6″	118.0, CH	6.78, d (9.1)	H-7″	4″, 8″
7″	119.8, CH	6.82, dd (9.1, 2.1)	H-6″, H-9″	5″, 9″
8″	154.4, C			
9″	113.5, CH	7.02, d (2.1)	H-7″	3″, 5″, 7″
8″-OMe	49.5, CH_3	3.76, s		8″
1‴	169.2, C			
2‴	117.0, CH	6.61, d (10.7)	H-3‴	4‴
3‴	142.3, CH	8.03, d (10.7)	H-2‴	1‴, 2‴, 5‴, 9‴
4‴	123.0, C			
5‴	152.8, C			
6‴	118.0, CH	6.78, d (9.1)	H-7‴	4‴, 8‴
7‴	119.8, CH	6.82, dd (9.1, 2.1)	H-6‴, H-9‴	5‴, 9‴
8‴	154.4, C			
9‴	113.5, CH	7.02, d (2.1)	H-7‴	3‴, 5‴, 7‴
8‴-OMe	49.5, CH_3	3.74, s		8‴

[a] Chemical shifts taken from gHMQC and gHMBC experiments. [b] gHMBC correlations from ^{1}H to indicated ^{13}C.

H-2′ (δ 5.09) in **2** was indicative of ester substitution at C-2′. This was further supported through the observation of a gHMBC correlation from H-2′ to a carbonyl carbon at δ 168.3 (C-1″).

COSY correlations from δ 6.78 (H-6″/6‴) to δ 6.82 (H-7″/7‴) and from H-7″/7‴ to δ 7.02 (H-9″/9‴), in addition to the magnitude of the coupling constants for H-6″/6‴ (9.1 Hz), H-7″/7‴ (9.1, 2.1 Hz), and H-9″/9‴ (2.1 Hz), established that **2** contained two 1,2,4-trisubstituted aromatic ring systems. Furthermore, the ^{13}C NMR chemical shifts of C-6″/6‴ (δ 118.0), C-7″/7‴ (δ 119.8), and C-9″/9‴ (δ 113.5) were indicative of them being *alpha* to oxygenated aromatic carbons. This was confirmed through gHMBC correlations from H-6″/6‴ to δ 154.4 (C-8″/8‴) and from H-7″/7‴ and H9″/9‴ to δ 152.8 (C-5″/5‴). Further gHMBC correlations from δ 3.76 (8-OMe″) and δ 3.74 (8-OMe‴) to C-8″ and C-8‴, respectively, located methoxy groups at C-8″ and C-8‴. An additional gHMBC correlation from H-6″/6‴ to δ 123.0 (C-4″/4‴) indicated carbon substitution at C-4″/4‴.

COSY correlations from δ 6.61 (H-2″) to δ 7.98 (H-3″) and from δ 6.61 (H-2‴) to δ 8.03 (H-3‴), in addition to gHMBC correlations from H-3″ to (C-1″) and from H-3‴ to δ 169.2 (C-1‴), established two α,β-unsaturated carbonyl moieties. Further gHMBC correlations from H-2″/2‴ to C-4″/4‴ and from H-3″/3‴ to C-5″/5‴ and C-9″/9‴ identified that both α,β-unsaturated carbonyl functionalities were part of two coumaroyl ester spin systems and substitution of the 1,2,4-trisubstituted aromatic rings at C-4″/4‴. The magnitude of the coupling constant between H-2″/2‴ and H-3″/3‴ (J = 10.7 Hz) established a *cis* geometry for the $\Delta^{2''}$ and $\Delta^{2'''}$ double bonds. The previously observed gHMBC correlation from H-2′ to C-1″ positioned one of the coumaroyl esters at C-2′. The observation of a further gHMBC correlation of δ 4.58 and δ 4.39 (H_2-6′) to C-1‴ positioned the remaining coumaroyl ester at C-6′. These observations therefore allowed for the gross structure of **2** to be elucidated as the acylated sugar shown.

Due to a lack of material, determination of the absolute configuration of **2** via acid hydrolysis could not be performed. However, it is tentatively assigned as D-glucose on biosynthetic considerations.

Table 3. *In Vitro* Biological Activities (IC_{50}, μM[a]) of **1**−**5** and Chloroquine against a Panel of *Plasmodium falciparum* Lines with Varying Resistance Profiles

compound	D6	3D7	Dd2	K1	TM90-C2B	TM93-C1088
1	55.5	77.4 ± 14.4	55.5 ± 3.0	29.4 ± 20.3	36.2 ± 3.6	25.3 ± 4.3
2	14.7					
3	4.7	5.5 ± 1.7	4.4 ± 2.0	3.9 ± 0.02	2.8 ± 0.03	2.1 ± 0.2
4	5.1	5.5 ± 1.7	3.0 ± 1.1	3.0 ± 0.65	5.3 ± 0.35	4.1 ± 0.48
5	>500					
chloroquine	0.01	0.02 ± 0.00	0.98 ± 0.01	0.4 ± 0.01	0.28 ± 0.07	0.62 ± 0.08

[a] Values are means ± SD, with $n \geq 2$ observations.

Robustaside G (**3**) was isolated as a brown oil with a pseudomolecular ion ($[M + Na]^+$ obs *m/z* 619.1429; calc *m/z* 619.1422; $\Delta = 1.1$ ppm) suggestive of the molecular formula $C_{30}H_{28}O_{13}$, which equated to 17 double-bond equivalents. Comparison of the NMR data of **3** with **2** showed significant similarity. Evident in **3** was an identical acylated β-glucose moiety to that present in **2**, with α,β-unsaturated ester moieties again substituted at C-2′ and C-6′. Also present in **3** was a *p*-disubstituted aromatic system, which was identical to that present in both **1** and **2**, and again substituted at C-1′. These data suggested that the only difference between **3** and **2** was the absence in **3** of the two 1,2,4-trisubstituted aromatic rings, with alternative unsaturated ring systems substituted at C-3″/3‴.

COSY correlations from δ 6.20 (H-6″/8″ and H-6‴/8‴) to δ 6.83 (H-5″/9″ and H-5‴/9‴), with corresponding gHMBC correlations from H-5″/9″ and H-5‴/9‴ to a ketone carbonyl at δ 187.4 (C-7″ and C-7‴), and from H-6″/8″ and H-6‴/8‴ to δ 70.9 (C-4″/4‴) allowed for the formation of two cyclohexa-2,5-dien-1-one spin systems. The ^{13}C chemical shift of C-4″/4‴ was indicative of an oxygenated functionality substituted at this position. Finally, gHMBC correlations from H-5″/9″ to δ 148.7 (C-3″) and from H-5‴/9‴ to δ 149.0 (C-3‴) allowed the α,β-unsaturated ester moieties to be substituted for the cyclohexa-2,5-dien-1-one moieties at C-4″ and C-4‴. These observations accounted for all double-bond equivalents and all atoms aside from two protons. The only remaining point of attachment for these protons was the formation of tertiary hydroxy groups at C-4″/4‴. Hence the gross structure of **3** was determined as shown.

The magnitude of the coupling constant between H-2″/2‴ and H-3″/3‴ ($J = 15.6$ Hz) established a *trans* geometry for $\Delta^{2''}$ and $\Delta^{2''}$. The β-glucose was again determined to be D-glucose through acid hydrolysis of **3** and comparison of the $[\alpha]_D$ with an authentic standard (obs +42; std +48).

The *in vitro* antimalarial activities (IC_{50} values) of **1**−**5** against several *P. falciparum* lines, with different levels of drug susceptibility, are shown in Table 3. All were compared to the existing therapeutic chloroquine. Initial assessment of the five compounds against the chloroquine-sensitive D6 line showed **1** and **2** to have only moderate *in vitro* activity when tested against sensitive lines D6 (IC_{50} of 55.4 and 14.7 μM), and **5** was inactive. No further assaying of **2** and **5** was undertaken. In contrast to **1**, both **3** and **4** were markedly more active against the 3D7 line, with IC_{50} values of 5.5 μM. However, when compared to chloroquine, **3** and **4** were 275-fold less active. Both **3** (3.9 μM) and **4** (3.0 μM) were slightly more active against the chloroquine-resistant K1 strain compared with the chloroquine-sensitive 3D7. Similar to K1, the same trend of higher antimalarial activity of **3** and **4** was seen for the Dd2 line compared with 3D7. When tested against the multidrug-resistant TM93-C1088 and TM90-C2B strains, the mean IC_{50}'s of **3** and **4** ranged between 2.1 and 5.3 μM. However, overall, they were still markedly less active than chloroquine.

The cytotoxicity of **1**, **3**, and **4** and the reference drugs, artesunate and chloroquine, against HEK-293 and HepG2 was determined. The results are summarized in Table 4. Due to limited drug availability, the highest concentration of **1** used in the cytotoxicity assessment was 690 μM. At this concentration, 50% of human cell growth inhibition was not observed for either the HEK-293 or HepG2 cell lines. However, cell growth was inhibited by approximately 20% in each cell line (data not shown). The HEK-293 cells were more susceptible to **3** than **4** (161 μM versus 195 μM), whereas the HepG2 cells were more susceptible to **4** than **3** (221 μM versus 340 μM). HEK-293 was more susceptible to **3** and **4** than HepG2 cells. Overall, both **3** and **4** were considerably less toxic than artesunate and chloroquine.

Table 4. *In Vitro* Cytotoxicity (IC_{50}, μM[a]) of **1**, **3**, and **4** against HEK-293 and HepG2 and the Calculated Selectivity Index (SI) Values for Human Cell Lines against the 3D7 Line of *Plasmodium falciparum*

compound	HEK-293	SI	HEP-G2	SI
1	>690	>10	>690	>10
3	161 ± 18	27	340 ± 6	58
4	195 ± 30	33	221 ± 66	37
artesunate	2.9 ± 2.6	150	12.7 ± 9.0	374
chloroquine	12.0 ± 7.1	600	8.3 ± 3.1	415

[a] Values are means ± SD, with $n \geq 2$ observations.

The selectivity index was marginally lower for **3** compared with that for **4** in HEK-293 cells (Table 4). In contrast, **3** had a 2-fold higher selectivity index in HepG2 cells compared with **4**. Although the selectivity index could not be determined for **1**, it can be estimated to be >10. What is obvious from a comparison of the structures is that **3** and **4** contain at least one hemiquinone moiety, while **1** and **2** have this moiety absent. At this point in time it is suspected that the hemiquinone is the functionality responsible for the order of magnitude increase in antimalarial activity. It is prudent to point out that compounds **1**−**4** also contain a phenolic group, which is a functionality present in many compounds with reported antimalarial activity.[8] This may explain why, even without the hemiquinone functionality, **1** and **2** still retain some biological activity.

Finally, the *in vivo* antimalarial activity of **3** and **4** against *P. berghei* was determined. Due to the limited supply of **3** and **4**, the maximum dosage assessed for tolerability in healthy mice was limited to a 32 mg/kg dose for 4 days. At this dose, no observable physical stress such as ruffled coat, extreme pallor, weight loss, shaking, and reduced mobility in the mice was observed. Preliminary assessment of the antimalarial activity of **3** and **4** using the murine malaria model showed that **4** at 32 mg/kg daily for 4 days suppressed *P. berghei* infection by 21% at D+4 following postinfection relative to the control group. At the same dosage, **3** was devoid of antimalarial activity. By doubling the dose to 32 mg/kg twice a day for 4 days, **4** suppressed *P. berghei* infection by 95% after subcutaneous administration relative to the control group (Figure 1). For comparison purposes, chloroquine, the positive control, suppressed *P. berghei* infection by 58% and 97% at 1 and 2 mg/kg given daily for 4 days, respectively, which was in accord with previous findings.[9]

The are several literature reports of phenolic glycosides belonging to the arbutin (**6**) structure class[10−14] having been isolated from several different genera of plants, including *Vaccinium dunalianum*,[10] *Grevillea robusta*,[6,11] *Hakea saligna*,[11] *Viburnum dilatatum*,[12] *Veronica turrilliana*,[13] and *Bacopa procumbens*.[13] Only one of these reports, however, documents biological activity, with both **6** and the 6′-caffeoyl derivative of **6** having weak radical scavenging activity.[12] No biological activity has been recorded for

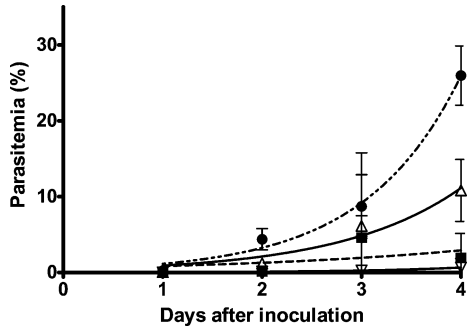

Figure 1. Mean *Plasmodium berghei* parasitemia (±SD) versus time profiles of robustaside D (**4**) (32 mg/kg, bid, ■), chloroquine (1 mg/kg, △, and 2 mg/kg, ▽), and vehicle control (●) after subcutaneous administration on days 0, 1, 2, and 3 in the Peters 4-day test.

4.[6] Alternatively, while no biological activity has been recorded for **5**,[7] the authors state that the BuOH fraction containing **5** had antibacterial activity.[7] This is the first time antimalarial activity has been ascribed to the arbutin structure class. The preliminary *in vitro* and *in vivo* antimalarial activity of **4** is encouraging. However, for this structure series to progress further, mechanism of action studies need to be conducted to determine if the hemiquinone is, as expected, responsible for the observed differences in antimalarial activity.

Experimental Section

General Experimental Procedures. The solvents used (MeOH, MeCN, H_2O, and HCO_2H) were HPLC grade and obtained from Merck. Optical rotations were measured on a Jasco Dip-1000 digital polarimeter, while infrared spectra were acquired on a Bio-Rad FTS-165 Fourier transform infrared spectrometer. NMR data was collected on a Bruker Avance 500 MHz spectrometer using deuterated NMR solvents supplied by Cambridge Isotopes. Spectra were referenced to residual ^{1}H and ^{13}C in the deuterated solvents. LC-MS data were collected on an Agilent LC/MSD Trap XCT mass spectrometer connected to an Agilent 1100 series LC system comprising an in-line degasser, binary pump, autoinjector, column heater, and diode array detector, equipped with Agilent ChemStation LC for 3D software (Rev.A.09.03). Samples were eluted at 0.5 mL/min through a Phenomenex Gemini 5 μm, 50 mm ×2.0 mm C18 HPLC column, using gradient elutions from 5% MeOH in H_2O (+0.05% HCO_2H) to 100% MeOH + 0.05% HCO_2H over 35 min. High-resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) data were collected on a Bruker Apex Ultra FTMS, equipped with a 9.4 T magnet and an ESI source. Compounds were dissolved in MeOH (+1% HCO_2H) and directly infused into the mass spectrometer via a syringe pump. Calibration was applied externally using a cluster from methanolic NaOH/trifluoroacetate solution. Solid phase (C18 and polyamide) for column chromatography was obtained from Phenomenex. High-pressure liquid chromatography (HPLC) was performed on an Agilent 1100 series LC system comprising an in-line degasser, binary pump, autoinjector, column heater, diode array detector, and fraction collector. Spectroscopic data were collected using the Agilent ChemStation LC for 3D software (Rev.A.09.03).

Plant Material. Leaves and twigs of a specimen of *Grevillea* "Poorinda Queen" (Proteaceae) were collected from a garden bed on site at the Defence Science and Technology Organisation at Fishermans Bend, Victoria, Australia, in April 2007 (S 37°49.453′, E 144°54.843′). A voucher specimen (no. RDS 2088) has been lodged with the National Herbarium of Victoria.

Extraction and Isolation. Bulk plant material (100 g) was freeze-dried, ground, and extracted three times with 500 mL of MeOH. The combined MeOH extracts were concentrated *in vacuo* and resuspended in approximately 20 mL of MeOH. The solution was treated with polyamide to remove any polyphenolic compounds and eluted off the column with approximately 500 mL of MeOH. The MeOH eluant was concentrated *in vacuo*, then subjected to a reversed-phase C18 flash vacuum column with gradient elution from 0% to 20%, 50%, 70%, 90%, and 100% MeOH in H_2O. Subsequent bioassay located the activity in the 70% MeOH fraction. This fraction was then further fractionated using C18 semipreparative HPLC (4 mL/min, gradient elution from 5:95 MeCN/H_2O (+0.1% HCO_2H) in H_2O (+0.1% HCO_2H) to 62:38 MeCN/H_2O (+0.1% HCO_2H) over 18 min, through a reversed-phase Phenomenex Luna C18 150 mm × 10 mm, 5 μm HPLC column) to yield the new arbutin derivatives robustasides E (**1**, 20.7 mg), F (**2**, 3.4 mg), and G (**3**, 69.7 mg) and the known compounds robustaside D (**4**, 142.3 mg)[6] and quercetin-7-*O*-[α-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside] (**5**, 400 mg).[7]

Robustaside E (1): brown oil; $[\alpha]_D = -30$ (*c* 0.9, MeOH); UV λ_{max} (PDA) 235, 280 nm; IR (MeOH) ν_{max} 1730, 1671 cm^{-1}; ^{13}C and ^{1}H NMR data (500 MHz, methanol-d_4) see Table 1; HRESIMS *m/z* 475.1214 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_{11}Na$, 475.1211).

Robustaside F (2): brown oil; $[\alpha]_D = -6$ (*c* 0.3, MeOH); UV λ_{max} (PDA) 225, 285 nm; IR ν_{max} (MeOH) 1670 cm^{-1}; ^{13}C and ^{1}H NMR data (500 MHz, methanol-d_4) see Table 2; HRESIMS *m/z* 647.1766 $[M + Na]^+$ (calcd for $C_{32}H_{32}O_{13}Na$, 647.1735).

Robustaside G (3): brown oil; $[\alpha]_D = -25$ (*c* 1.6, MeOH); UV λ_{max} (PDA) 235, 280, 330 nm; IR (MeOH) ν_{max} 1729, 1671 cm^{-1}; ^{1}H NMR data (500 MHz, methanol-d_4) δ 6.83 (4H, d, J = 10.0 Hz, H-5″/5‴, H-9″/H-9‴), 6.78 (2H, d, J = 9.0 Hz, H-3/5), 6.75 (2H, d, J = 15.3 Hz, H-3‴/3″), 6.65 (2H, d, J = 9.0 Hz, H-2/6), 6.31 (1H, d, J = 15.3 Hz, H-2‴), 6.28 (1H, d, J = 15.3 Hz, H-2″), 6.21 (2H, d, J = 10.0 Hz, H-6‴/8‴), 6.19 (2H, d, J = 10.0 Hz, H-6″/8″), 4.97 (1H, t, J = 8.2 Hz, H-2′), 4.94 (1H, d, J = 8.2, H-1′), 4.51 (1H, dd, J = 11.9, 2.1 Hz, H_a-6′), 4.33 (1H, dd, J = 11.9, 6.6 Hz, H_b-6′), 3.67 (1H, ddd, J = 9.1, 6.6, 2.1 Hz, H-5′), 3.64 (1H, t, J = 9.1, H-3′), 3.45 (1H, t, J = 9.3 Hz, H-4′); ^{13}C NMR data (125 MHz, methanol-d_4) δ 187.4 (s, C-7″), 187.4 (s, C-7‴), 167.0 (s, C-1″), 167.6 (s, C-1‴), 154.7 (s, C-4), 152.3 (s, C-1), 151.6 (d, C-5‴), 151.6 (d, C-9‴), 151.5 (d, C-5″), 151.5 (d, C-9″), 149.0 (d, C-3‴), 148.7 (d, C-3″), 129.2 (d, C-6″), 129.2 (d, C-6‴), 129.2 (d, C-8″), 129.2 (d, C-8‴), 123.2 (d, C-2″), 123.2 (d, C-2‴), 120.0 (d, C-3), 120.0 (d, C-5), 117.2 (d, C-2), 117.2 (d, C-6), 102.1 (d, C-1′), 76.3 (d, C-3′), 75.9 (d, C-2′), 75.9 (d, C-5′), 72.3 (d, C-4′), 70.9 (s, C-4″), 70.9 (s, C-4‴), 65.3 (t, C-6′); HRESIMS *m/z* 619.1429 $[M + Na]^+$ (calcd for $C_{30}H_{28}O_{13}Na$, 619.1422).

Robustaside D (4): brown oil; spectroscopic data identical in all respects to the literature.[6]

Quercetin-7-*O*-[α-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside] (5): yellow crystals; spectroscopic data identical in all respects to the literature.[7]

Acid Hydrolysis of 1 and 3. To approximately 5 mg of **1** and **3** was added 500 μL of 1 N HCl, and the reaction mixture heated at 90 °C for 2 h. The reaction mixture was cooled to room temperature and extracted three times with 100 μL of EtOAc, and the aqueous layer was dried *in vacuo*. ^{1}H NMR analysis and comparison with an authentic standard of β-glucose confirmed that the aqueous layer contained β-glucose. Further comparison of the optical rotations for each hydrolyzed product of **1** and **3** with an authentic standard confirmed that the β-glucose was D-glucose.

Parasites and Preparation of Natural Products. Six laboratory-adapted *P. falciparum* lines were used in this study: D6 and 3D7 are chloroquine and pyrimethamine sensitive, K1 is chloroquine and pyrimethamine resistant, Dd2 is chloroquine, pyrimethamine, and mefloquine resistant, and TM93-C1088 and TM90-C2B are atovaquone, chloroquine, and quinine resistant. All isolates were maintained in continuous culture in RPMI-1640-LPLF (Gibco, Invitrogen Corporation, CA, USA), supplemented with 5.97 g/L HEPES buffer, 2.0 g/L D-glucose, 0.05 g/L hypoxanthine, and 40 mg/L gentamycin, containing 10% human plasma and 4% O(+) red blood cells in special gas mixture (5% O_2, 5% CO_2, and 90% N_2) at 37 °C.[15] For *in vitro* antimalarial activity assessment, all samples for testing were dissolved in DMSO to a concentration of 20−25 mg/mL, with subsequent dilutions made in culture media without hypoxanthine.

***In Vitro* Antimalarial Activity of Natural Products.** Parasite susceptibility testing of the natural products was carried out by measuring the inhibition of the radioactive [^{3}H]-hypoxanthine uptake.[16] Briefly, highly synchronous ring stage parasite cultures produced by several rounds of D-sorbitol selection[17] were exposed to 10 2-fold dilutions of the compounds (in triplicates) with concentrations ranging from 125 to 244 ng/mL in 96-well flat bottom microtiter plates at 37 °C for a total of 48 h. Chloroquine was used as a reference drug. Each well contained 100 μL of parasite culture at 2% hematocrit and 1% parasitemia in a culture media prepared as described above but lacking

hypoxanthine. A 20 μL (0.2 μCi) portion of [^{3}H]-hypoxanthine was added to each well 24 h after commencement of the assay. Parasite DNA was harvested onto glass fiber filters, and radioactivity was counted to generate compound concentration−response (counts per minute) curves (GraphPhad Prism V5.0, GraphPad Software, Inc., CA, USA). IC_{50} and IC_{90} values were defined as the concentrations producing 50% and 90% inhibition of uptake of [^{3}H]-hypoxanthine by parasites when compared with compound-free plasma samples (controls).

***In Vitro* Cytotoxicity Assay.** Selected compounds were tested for *in vitro* cytotoxicity against two human cell lines: HEK-293 (embryonic kidney) and HepG2 (liver carcinoma) by the Alamar Blue (Invitrogen Corporation, CA, USA) assay.[18] Cell cultures were maintained in RPMI 1640 media (Sigma, MO, USA) containing 10% fetal bovine serum and 0.03%% L-glutamine (termed complete media) in 75 cm^2 flasks at 37 °C with media changed twice weekly.

Cells from 60−80% confluent flasks were trypsinised, washed in complete media, and plated at 5×10^3 cells per well in 135 μL of complete medium in 96-well flat-bottom plates for 24 h at 37 °C prior to the addition of the compounds. The compounds were assayed from 312 to 0.6 μg/mL, while reference drugs artesunate (Central Pharmaceutical Factory No. 1, Hanoi, Vietnam) and chloroquine (Sigma, MO, USA) were assayed from 120 μM to 60 nM. Triplicate 15 μL aliquots of drug were added, mixed gently, and reincubated for 72 h. Controls included compound-free wells with DMSO (vehicle) as positive controls and cell-free wells with media only, which were used for background subtraction.

Following incubation for 72 h, the culture medium was removed and 50 μL of 10% Alamar Blue in complete medium was added to each well for a further 2 h. The plates were read using a GENios Plus microplate reader and XFluor4 software, using a 530 nm excitation filter and a 595 nm emission filter. Data were obtained from at least two independent experiments for each cell line and analyzed using GraphPad Prism V5.0. The selectivity index was calculated using the IC_{50} values derived from mammalian cells divided by the IC_{50} values obtained against the *P. falciparum* 3D7 line.

Antimalarial Studies against *P. berghei* in Mice. The *in vivo* efficacy of the compound was determined using the Peters 4-day test.[9] This test measures the suppressive activity of blood schizontocides over 4 days at a tolerated dose that does not cause physical stress in healthy mice. Briefly, compound-treated and control female mice ($n = 6$) were inoculated intraperitoneally with 20×10^6 *P. berghei*-infected erythrocytes (ANKA strain). Compounds were dissolved in Tween 80/ethanol (7:3, v/v), diluted 10-fold in distilled H_2O, and administered subcutaneously at about 2 and 6 h after parasite inoculation (D0) and then twice daily for 3 consecutive days. Thin blood smears were made on D+1, D+2, D+3, and D+4 and stained with Giemsa. The degree of infection (parasitemia expressed as percentage of infected erythrocytes) was determined microscopically and analyzed using GraphPad Prism V5.0. Chloroquine was used as a reference drug and was prepared in water.

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Supporting Information Available: Copies of all NMR spectra for new compounds are available free of charge via the Internet at http://pubs.acs.org.

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