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Busseihydroquinones A–D from the Roots of *Pentas bussei*

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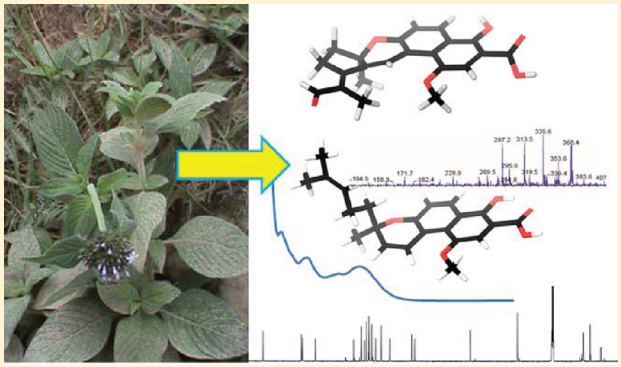
Supporting Information

ABSTRACT: Four new naphthohydroquinones, named busseihydroquinones A–D (**1**–**4**), along with a known homoprenylated dihydronaphthoquinone (**5**), were isolated from the CH_2Cl_2/MeOH (1:1) extract of the roots of *Pentas bussei*. Although the genus *Pentas* is frequently used by traditional healers for the treatment of malaria, only marginal activities against the chloroquine-sensitive (D6) and the chloroquine-resistant (W2) strains of *Plasmodium falciparum* were observed for the crude root extract and the isolated constituents of this plant.



Malaria remains a widespread, potentially fatal parasitic infection causing millions of deaths every year.[1] Most effective antimalarials are unaffordable to the majority of developing countries, where malaria is prevalent, and the efficacy of these preparations is declining rapidly.[2,3] As modern techniques, e.g., genomics, high-throughput screening, and target-oriented drug development strategies, have not yet fulfilled the expectations that appeared promising upon their introduction, chemotherapeutics is still the cornerstone of patient management and will likely remain so for the foreseeable future.[4−6] Rapid resistance development to existing medicines reveals the essential need for drugs with novel modes of action.[6] Since all first-line antimalarials are natural products or their derivatives,[5] evaluation of plant-based indigenous medicines still appears as one of the promising sources of novel antiplasmodial leads.

Originating from its traditional use for the treatment of malaria in Africa and Asia, the interest in the genus *Pentas* has recently increased.[7] *P. longiflora* was recognized as a source of quinones,[8−11] with its pyranonaphthoquinones showing potent antiplasmodial activity.[11] Antiplasmodial anthraquinones were recently isolated from the roots of *P. lanceolata*.[11] The use of *Pentas bussei* for malaria treatment at the Kenyan coast has been reported.[12] The crude extract of its roots showed moderate activity against *Plasmodium* strains.[13] De Kimpe et al. reported the isolation of a novel benzochromene[14] as well as a naphthohydroquinone[15] from the roots of this plant without, however, reporting their biological activities. The isolation, spectroscopic identification, and biological evaluation of four additional naphthohydroquinones named here as busseihydroquinones A–D (compounds **1**–**4**, Figure 1) from the roots of *P. bussei* are reported along with the confirmation of the presence of the previously reported homoprenylated naphthohydroquinone (**5**).[14]

RESULTS AND DISCUSSION

Chromatographic separation of the CH_2Cl_2/MeOH (1:1) root extract of *P. bussei* yielded five compounds, which were characterized by NMR, UV, IR, CD, and MS methods.

Busseihydroquinone A (1). **1** was isolated as needle-like crystals by recrystallization from MeOH. HRMS provided the exact mass of *m*/*z* 309.2300 $[M + H]^+$, compatible with the molecular formula $C_{15}H_{16}O_7$ (calcd 309.2379). In agreement with MS, the ^{13}C NMR spectrum showed 15 carbon signals consisting of a carbonyl, four methyl, and 10 aromatic carbons (Table 1). ^1H NMR data confirmed the presence of four *O*-methyl groups, of which HMBC and HSQC indicated three methoxy functionalities (δ_H 3.91, 3.90, and 3.76) and one methyl ester group (δ_H 3.94), as well as the presence of two aromatic proton singlets. Using dry DMSO-d_6 as solvent permitted the identification of two (δ_H 9.83 and 12.45) intramolecularly hydrogen-bonded hydroxy groups. The HMBC correlations (Supporting Information) of the aromatic proton at δ_H 6.94 to two oxygenated quaternary carbons ($^2J_{CH}$ to δ_C 146.3 and $^3J_{CH}$ to δ_C 154.6), to two quaternary carbons ($^2J_{CH}$ to δ_C 102.2, $^3J_{CH}$ to δ_C 127.1), and to the ester carbonyl

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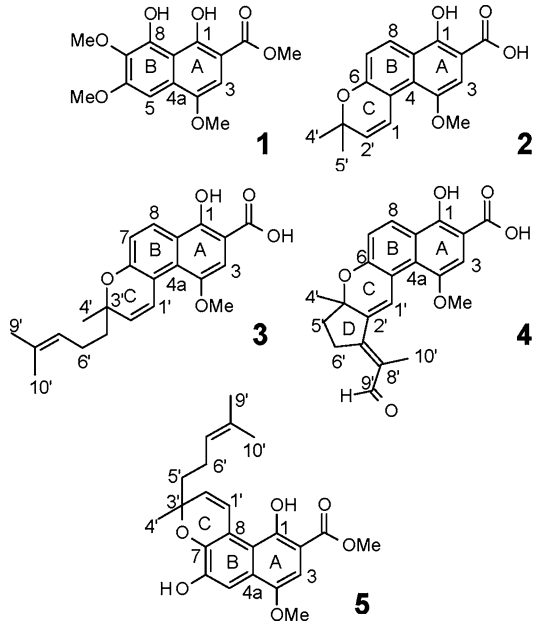

Figure 1. Naphthohydroquinones isolated from the roots *of P. bussei*. The racemic nature of compounds **3**–**5** was revealed by their lack of optical rotation.

carbon ($^3J_{CH}$ to δ_C 170.4) and a further weak correlation to an additional quaternary carbon ($^4J_{CH}$ to δ_C 109.9) allowed its assignment as H-3 of ring A. HMBC data revealed that C-4 of this ring is methoxy substituted ($^3J_{CH}$ δ_H 3.90 to δ_C 146.3), whereas C-1 is hydroxylated. A further weak HMBC correlation of δ_H 6.94 to δ_C 93.5, originating from a $^4J_{CH}$ *W*-coupling, pointed to the B ring and defined the position of its H-5 (HSQC cross-peak of δ_H 7.05 to δ_C 93.5). HMBC correlations of this proton defined the positions of two methoxy ($^2J_{CH}$ to δ_C155.5, $^3J_{CH}$ to δ_C 134.9) and hydroxy groups (weak $^4J_{CH}$ to δ_C 148.8) in addition to confirming its relative position to the bridging carbons ($^2J_{CH}$ to δ_C 127.1, $^3J_{CH}$ to δ_C 109.9) of the naphthalene backbone. The $^3J_{CH}$ HMBC correlation of δ_H 7.05 to δ_C 146.3 confirmed its *peri* position to C-4. The deshielded methoxy signal (δ_C 60.1) bound to shielded C-7 was indicative of being both *ortho* to a hydroxy at C-8 and *ortho* to a methoxy at C-6.[16] NOESY cross-peaks (Supporting Information) between H-3 and 4-OCH_3 as well as H-5 and 6-OCH_3 further confirmed the position of the aromatic protons and the two methoxy groups. In the MS spectrum of **1** the vicinal position of the C-1 hydroxy group and the ester carbonyl was indicated by expulsion of MeOH ($[M + 1]^+ - 32$), resulting in the base peak, by formation of the corresponding characteristic ketene ion ("*ortho* effect"). The new compound was therefore characterized as methyl 1,8-dihydroxy-4,6,7-trimethoxynaphthalene-2-carboxylate (Figure 1) and was given the trivial name busseihydroquinone A.

Busseihydroquinone B (2). **2** was isolated as a powder and showed a molecular ion peak of *m*/*z* 299.0919 $[M - H]^-$, consistent with the molecular formula $C_{17}H_{16}O_5$ (calcd

Table 1. ^{1}H and ^{13}C NMR of Compounds 1–5 (DMSO-d_6, 25 °C)[a]

	1		2		3		4		5	
position	δ_H	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		154.6, C		154.9, C		155.0, C		154.9, C		156.4, C
2		102.2, C		103.0, C		102.9, C		105.4, C		102.6, C
3	6.94, s	100.4, CH	7.07, s	104.2, CH	7.06, s	104.3, CH	7.10, s	103.9, CH	6.88, s	98.8, CH
4		146.3, C		148.5, C		148.5, C		148.2, C		146.0, C
4a		127.1, C		125.9, C		125.9, C		126.4, C		126.2, C
5	7.05, s	93.5, CH		114.4, C		114.3, C		115.6, C	7.41, s	105.4, CH
6		155.5, C		154.2, C		154.4, C		154.3, C		149.2, C
7		134.9, C	7.13, d (8.2)	118.3, CH	7.10, d(8.7)	118.2, CH	7.21, d (9.1)	118.1, CH		142.5, C
8		148.8, C	8.12, d (8.2)	125.0, CH	8.11, d (8.7)	124.8, CH	8.22, d (9.1)	126.9, CH		117.5, C
8a		109.9, C		120.8, C		120.8, C		121.2, C		114.5, C
1′			7.63, d (9.2)	122.1, CH	7.70, d (10.4)	122.5, CH	8.27, s	126.5, CH	7.83, d (10.3)	122.6, CH
2′			5.71, d (9.2)	128.2, CH	5.68,d (10.4)	127.3, CH		137.3, C	5.76, d (10.3)	128.4, CH
3′				75.1, C		77.4, C		82.0, C		77.0, C
4′			1.44, s	27.0, CH_3	1.37, s	25.4, CH_3	1.32, s	21.1, CH_3	1.39, s	24.9, CH_3
5′					1.67, m	39.9, CH_2	2.18, 2.30, m	37.2, CH_2	1.70, m	39.9 CH_2
6′					2.05, m	22.2, CH_2	2.88, 3.39, m	25.6, CH_2	2.08, m	22.2 CH_2
7′					5.05, t (6.8)	123.9, CH		152.6, C	5.08, t (6.8)	124.0 CH
8′						130.9, C		130.0, C		130.9, C
9′					1.49, s	17.4, CH_3	10.0, s	191.8, CH	1.51, s	17.4, CH_3
10′					1.59, s	25.3, CH_3	2.05, s	11.8, CH_3	1.60, s	25.4, CH_3
4-OMe	3.90, s	55.6, CH_3	3.86, s	55.6, CH_3	3.85, s	55.7, CH_3	3.89, s	56.0, CH_3	3.86, s	55.5, CH_3
6-OMe/ OH	3.91, s	55.7, CH_3							9.96, br s	
7-OMe	3.76, s	60.1, CH_3								
8-OH	9.83, br s									
1-OH	12.45, br s		12.40, br s		12.20, br s		12.40, br s		12.29, br s	
2-COOMe		170.4, C		172.5, C		172.6, C		172.3, C		171.5, C
2-COOMe	3.94, s	52.6, CH_3							3.94, s	52.5, CH_3

[a]NMR spectra, including assigned HMBC and NOE correlations, are shown in the Supporting Information.

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299.0953). Its COSY spectrum (Supporting Information) revealed two independent pairs of aromatic or vinylic protons vicinal to each other as well as the presence of a noncoupled aromatic proton and a methoxy group. The HMBC spectrum revealed a substituted naphthalene skeleton with a comparable substitution pattern of its A ring, indicated by similar chemical shifts for C-1−C-4 to those of compound **1** (Figure 1, Table 1). NOE cross-peaks between H-3 and 4-OCH_3 as well as H-1′ and 4-OCH_3 revealed the annulation of ring C (2,2-dimethylpyran) to ring B, forming a 3*H*-benzo[*f*]chromene skeleton. The NOESY and HMBC data (Supporting Information) allowed unambiguous assignment of all functionalities, including determination of the position of the dimethyl substituents of the C ring. Hence, **2** was identified as the new 1-hydroxy-4-methoxy-3′,3′-dimethyl-3*H*-benzo[*f*]chromene-2-carboxylic acid and was given the trivial name busseihydroquinone B. It should be noted that **2** is isomeric to mollugin,[17] a natural product isolated from *Pentas longiflora*[11] and synthetically extensively explored by De Kimpe et al.[18−21]

Busseihydroquinone C (3). **3** was isolated as an amorphous solid and was observed to have the exact mass *m*/*z* 368.1418 $[M - H]^-$, attributable to the molecular formula $C_{22}H_{23}O_5$ (calcd 368.1460). Its ^1H and ^{13}C NMR shifts (Table 1 and Figures S16 and S17) indicated a 3*H*-benzo[*f*]chromene skeleton comparable to that of compound **2**. The close similarity of the chemical shifts of atoms 1−8 suggested identical substitution patterns for rings A and B for **2** and **3**, which was confirmed by HSQC/HMBC analysis. The annulation of the benzochromene ring (Figure 1) was confirmed by $^3J_{CH}$ HMBC magnetization transfers (Supporting Information) between H-8 and C-4a, H-1′ and C-4a, H-7 and C-5, and H-2′ and C-5. A further confirmation was provided by the NOESY cross-peaks of 4-OCH_3 to H-3 and H-1′, whereas no NOE transfer between 4-OCH_3 and H-8 (Figure 2) was

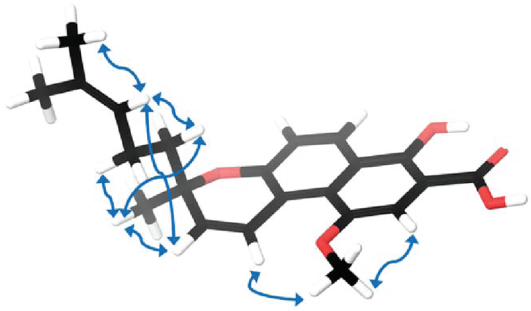

Figure 2. Key NOE correlations observed for compound **3** (mixing time 700 ms, DMSO-d_6, 25 °C, 800 MHz, Supporting Information).

observed. Similar to **2**, cyclization of a geranyl substituent at C-5 resulted in formation of the pyran ring of **3**, revealed by the mutually coupled doublets at δ_H 7.70 and 5.68 and the quaternary carbon at δ_C 77.3. HMBC correlations along with the NOE data (Figure 2) indicated a methyl and a 4-methylpent-3-enyl substituent at C-3′ of the pyran ring. By combination of the above spectroscopic evidence, the third new compound was identified as *rac*-1-hydroxy-4-methoxy-3′-methyl-3′-(4-methylpent-3-enyl)-3*H*-benzo[*f*]chromene-2-carboxylic acid (**3**) and was given the trivial name busseihydroquinone C.

Busseihydroquinone D (4). **4** was isolated as an amorphous solid with exact molecular mass (HRMS) *m*/*z* 379.1182, attributed to $C_{22}H_{20}O_6$ (calcd for $[M - H]^-$ 379.1176). The NMR chemical shifts (Table 1) indicated a 3*H*-benzo[*f*]chromene backbone similar to compounds **2** and **3**, with a hydroxy (δ_H 12.40) at C-1, a carboxylic (δ_C 172.6) at C-2, and a methoxy (δ_H 3.89, δ_C 56.0) substituent at C-4 in ring A, derived by HMBC data (Supporting Information). The presence of a pair of *ortho*-coupled protons (δ_H 7.21 and 8.22, ^3J = 9.1 Hz) indicated disubstitution in ring B. HMBC correlation of the doublet at δ_H 8.22 with δ_C 154.9 (C-1) allowed its assignment to H-8, *peri* to the C-1 hydroxy group. The HMBC cross-peaks of H-8 (δ_H 8.22) to C-6 (δ_C 154.3) and C-4a (δ_C 126.4) along with the NOE between 4-OCH_3 and H-1′ (Figure 3) further confirmed the *syn*-annulation of ring B

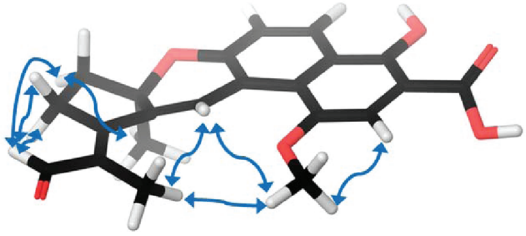

Figure 3. Key NOE correlations observed for compound **4** (mixing time 700 ms, DMSO-d_6, 25 °C, 800 MHz, Supporting Information).

to C. The presence of a chromene ring was indicated by a singlet at δ_H 8.27 (H-1′) showing a HMBC correlation to C-4a (δ_C 126.4), C-5 (δ_C 115.6), and C-6 (δ_C 154.3) of ring B as well as with C-3′ (δ_C 82.0) and the quaternary sp^2-hybridized carbon C-2′ at δ_C 137.3. The multiplicity of H-1′ (δ_H 8.27, s) and the HMBC cross-peak pattern of the aliphatic signals 4′-CH_3, 5′-CH_2, and 6′-CH_2 revealed the presence of a methyl (CH_3-4′) and a pentacyclic ring attached to ring C. In ring D, the COSY spectrum indicates two mutually coupled methylene groups at δ_H 2.30 and 2.18 (δ_C 37.2) and 2.88 and 3.39 (δ_C 25.6) assignable to 5′-CH_2 and 6′-CH_2, respectively. The 1-methyl-2-oxoethylidene substituent attached to C-7′ is indicated by HMBC correlations of 10′-CH_3 (δ_H 2.05) to the carbonyl C-9′ (δ_C 191.8), the quaternary C-8′ (δ_C 130.0), and C-7′ (δ_C 152.6), to which also 5′-CH_2 and 6′-CH_2 showed HMBC correlations. Further confirmation was provided by the HMBC correlations of the H-9′ aldehydic proton (δ_H 10.0) to C-8′ (δ_C 130.0) and CH_3-10′ (δ_C 11.8). NOEs observed between H-9′ and 6′-CH_2 as well as between CH_3-10′ and H-1′ revealed the *E*-geometry of the C7′═C8′ bond. The new compound was characterized as *rac*-(7′*E*)-1-hydroxy-4-methoxy-3′a-methyl-2′-[1-methyl-2-oxoethylidene]-7a,8,1′,2′-tetrahydro-7-oxa-cyclopenta[*b*]phenanthrene-2-carboxylic acid (**4**) and given the trivial name busseihydroquinone D.

Spectroscopic analysis (Supporting Information) of compound **5** (HRMS *m*/*z* 399.2020 $[M + H]^+$, calcd for $C_{23}H_{26}O_6$ 399.1808) confirmed the presence of *rac*-methyl-1,6-dihydroxy-4-methoxy-3′-methyl-3′-[4-methyl-3-pentenyl]-3*H*-benzo[*f*]-chromene-2-carboxylate in the roots of *P. bussei*, as previously reported De Kimpe et al.[14] The annulation of **5**, which is different from that of **2**−**4**, was confirmed by NOEs observed between H-5 and OMe-4 and between H-1′ and OH-1, as shown in Figure 4. Detailed spectroscopic data are given in the Supporting Information. The circular dichroism spectra of compounds **3**−**5** did not show any observable Cotton effects in the 250−600 nm region; neither was significant optical rotation observed (Experimental Section). These indicated that

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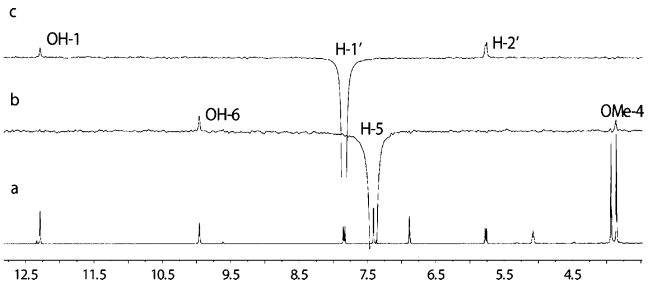

Figure 4. NOE correlations observed for compound **5** indicative of its mode of annulation. (a) Reference spectrum; (b) NOESY1D spectrum, H-5 irradiated; (c) NOESY 1D spectrum, H-1′ irradiated. Spectra were obtained in DMSO-d_6 solution at 25 °C and 500.30 MHz using a mixing time of 700 ms and relaxation delay of 2.5 s.

compounds **3**−**5** occur as racemic mixtures in the analyzed plant sample. For **5**, a +34.8 specific rotation was previously reported,[14] which we are unable to confirm here.

Five natural products possessing the 3*H*-benzo[*f*]chromene heterocyclic system were isolated. In compounds **2**−**4** the bridging atoms of rings B and C are C-5 and C-6, whereas in compound **5** C-7 and C-8, as was confirmed by HMBC and NOESY spectra. The heterocyclic system of **2**−**5** has not been reported from any other organism, and thus further investigation of the genus *Pentas* is likely to provide more novel natural products.

Biological Activity. The crude extract and all isolated compounds have been examined in vitro for their antiplasmodial activity against the D6 and W2 strains of *Plasmodium falciparum* (Table 2). Despite the traditional use of the roots of the plant for malaria, our findings revealed that the compounds showed only marginal activity (IC_{50} 19−36 μM) against the D6 strain with little cytotoxicity (MCF-7 human breast cancer cells, see Experimental Section), while the crude extract was found to be inactive (IC_{50} 49−50 μg/mL). As the use of *P. bussei* for the treatment of bacterial diseases has also been reported,[14,22] compounds **1**−**5** have been investigated for antifungal and antibacterial activities. However, none of them showed significant inhibition of *Aspergillus niger*, *Escherichia coli*, *Candida albicans*, or *Staphylococcus aureus*, in agar diffusion assays.

Phytochemical investigation of *P. bussei* resulted in the isolation of four new natural products. The plant has previously been studied by De Kimpe and co-workers,[14,15] but only two compounds had been identified, of which the presence of one is confirmed in this study. Anthraquinones and pyranonaphthoquinones have been reported from the roots of previously evaluated plants of the genus, such as *P. lanceolata* and *P. longiflora*.[11] Despite the indigenous use of the genus *Pentas* including *P. bussei* against malaria and bacterial infections,[14,22] in our hands neither the crude root extract nor the isolated compounds showed significant antiplasmodial, antibacterial, or antifungal activities, in contrast to the anthraquinones and naphthalene derivatives isolated from two other *Pentas* species.[11] This observation reveals the urgent need for thorough scientific evaluation of indigenous medicines to distinguish myths from helpful practices.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 341 LC polarimeter. IR spectra (KBr disks) were recorded on a Perkin-Elmer 1725 FTIR spectrometer. NMR analysis was carried out on Varian Unity Inova 500, 600, or 800 MHz spectrometers. Structural assignment was performed on the basis of gCOSY,[23] gNOESY,[24] gHSQC,[25] and gHMBC[26] spectra. ESIMS was performed on a Perkin-Elmer PE SCIEX API 150 EX instrument equipped with a Turbolon Ionspray source and a Gemini 5 mm C18 110 Å HPLC column using a H_2O/CH_3CN gradient (80:20 to 20:80). High-resolution mass spectrometric analysis (Q-TOF-MS) was performed at Stenhagen Analyslab AB, Gothenburg, Sweden. Analytical HPLC was run on a Hewlett-Packard Series 1050 HPLC using the software Chromulan (Pikron Ltd.), a Gemini 5 mm C18 110 Å HPLC column, and MeOH/H_2O eluent mixtures. Column chromatography was performed on oxalic acid-impregnated silica gel [the silica gel was deactivated by mixing 2 kg of silica gel 60 (70−230 mesh) with 3% oxalic acid (30 g in 1 L of H_2O) and allowed to stand for 30 min, filtered, and dried in an oven (100 °C) for 45 min]. TLC was done using silica gel 60 F254. An Inotech cell "Harvester 96" on glass fiber filter mats was used to evaluate the bioassays. [G-^{3}H] hypoxanthine uptake was determined using the MicroBeta TriLux liquid scintillation and luminescence counter.

Plant Material. The root of *Pentas bussei* was collected from Mombasa, coastal region of Kenya, on July 14, 2010. The plant material was identified by Mr. Patrick Chalo Motiso, School of Biological Sciences, University of Nairobi, where a voucher specimen under voucher number MEA 2010/003 (*P. bussei*) is deposited.

Extraction and Isolation. The ground roots of *P. bussei* (1.1 kg) were extracted by cold percolation with CH_2Cl_2/MeOH (1:1) three times for 24 h in each case. The marc was then extracted with MeOH as above. The extract was concentrated using a rotary evaporator to yield a brownish crude extract (63 g, 5.72%). A 61 g portion of the CH_2Cl_2:MeOH (1:1) crude extract was subjected to column chromatography (CC, 80 cm length and 80 mm diameter, 400 g of oxalic acid-impregnated silica gel) with an increasing gradient of EtOAc in *n*-hexane. A total of 250 fractions (each ca. 150 mL) were collected. Fractions 15−20 (2% EtOAc in *n*-hexane) were combined

Table 2. In Vitro Antiplasmodial Activity and Cytotoxicity of Crude Extract and Isolated Compounds of *Pentas bussei*

	antiplasmodial activity[a]			SI[c]	
sample	D6 clone	W2 clone	LD_{50}[b] (μM)	D6	W2
root extract	49.04 ± 8.73	49.86 ± 0.00			
1	36.03	144.43	304.13	8.44	2.11
2	27.82	126.36	373.54	13.43	2.96
3	32.65	60.08	>271.62	>8.32	>4.52
4	19.59	80.10	>262.07	>13.43	>3.27
5	30.56	85.04	86.39	2.83	1.02
chloroquine	0.0094	0.0063			
mefloquine	0.029	0.034			

[a]IC_{50} is given in μg/mL for crude and in μM for pure compounds. [b]LD_{50}: cytotoxicity. [c]SI: selectivity index. These values are the mean value of at least 3 (IC_{50}) or at least 6 (LD_{50}) independent experiments. Details are given in the Supporting Information.

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and purified on Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) to give compound **5** (30 mg, yellow solid). Fractions 63 and 64 (7% EtOAc in *n*-hexane) were combined and purified by CC (eluent: increasing gradient of EtOAc in *n*-hexane) to give busseihydroquinone A (**1**, 20 mg, brown crystals). Fractions 75−79 (10% EtOAc in *n*-hexane) were combined and purified by CC (eluent: increasing gradient of EtOAc in *n*-hexane) to give busseihydroquinone D (**4**, 25 mg, orange solid). Fractions 115−118 (15% EtOAc in *n*-hexane) were combined and purified on Sephadex LH-20 to give busseihydroquinone B (**2**, 30 mg, orange powder). Fractions 131−135 (20% EtOAc in *n*-hexane) were combined and purified using CC (eluent: increasing gradient of EtOAc in *n*-hexane) to give busseihydroquinone C (**3**, 15 mg, red solid). The MeOH extract (70 g) was subjected to CC (eluent: increasing gradient of MeOH in CH_2Cl_2). A total of 100 fractions (each ca. 150 mL) were collected. Busseihydroquinones A (**1**, 20 mg, brown crystals) and B (**2**, 40 mg, yellow powder) and compound **5** (15 mg, yellow solid) were reisolated. Compound purity was determined by NMR and HPLC.

*Busseihydroquinone A (**1**):* brown crystals (MeOH), mp 171.5 °C; UV (MeOH) λ_{max} 285, 250 nm; IR (KBr) ν_{max} 3412, 1637, 1618, 1522, 1400 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 309.2300 $[M + H]^+$ (calcd $C_{15}H_{16}O_7$, 309.2379); ESIMS *m*/*z* 309.5 $[M + H]^+$, 277.1 $[M + H]^+$ −MeOH.

*Busseihydroquinone B (**2**):* orange powder; UV (MeOH) λ_{max} 280, 265, 230 nm; IR (KBr) ν_{max} 3547, 3415, 1637, 1618, 1522, 1399 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 299.0919 $[M - H]^-$ (calcd for $C_{17}H_{16}O_5$, 299.0953).

*Busseihydroquinone C (**3**):* red, amorphous solid; $[\alpha]^{20}$ +0.9° (MeOH); UV (MeOH) λ_{max} 305, 245, 210 nm; IR (KBr) ν_{max} 3550, 3413, 3236, 1638, 1617 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 368.1418 $[M - H]^-$ (calcd for $C_{22}H_{23}O_5$, 368.1460).

*Busseihydroquinone D (**4**):* orange, amorphous solid; $[\alpha]^{20}$ +0.9° (MeOH); UV (MeOH) λ_{max} 310, 275, 250 nm; IR (KBr) ν_{max} 3550, 3413, 3236, 1654, 1638, 1618, 1522, 1450, 1400 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 379.1182 $[M - H]^-$ (calcd for $C_{22}H_{20}O_6$, 379.1176).

*Compound **5**:* yellow solid; $[\alpha]^{20}$ +0.6° (MeOH); ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 399.2020 $[M + H]^+$ (calcd for $C_{23}H_{26}O_6$ 399.1808). The NMR and MS data are in agreement with the data given in ref 14.

Antiplasmodial Assay. Two laboratory clones of *Plasmodium falciparum*, the Sierra Leone D6 chloroquine-sensitive and the Indochina W2 chloroquine-resistant, were maintained in continuous culture to attain replication robustness prior to assays. Drug susceptibility was tested by the malaria SYBR Green I-based in vitro assay technique.[27] The reference antimalarial drugs chloroquine and mefloquine, having well-documented IC_{50} values, were tested along with test samples of naphthohydroquinones isolated from the roots of *P. bussei* as described above.

Cytotoxicity Assay. MCF-7 human breast cancer cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in humidified 5% CO_2. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density to ensure exponential growth for the duration of the assay. After a 24 h preincubation, growth medium was replaced with experimental medium containing the appropriate drug concentrations or vehicle controls (0.1% or 1.0% v/v DMSO). After 48 h incubation, cell viability was measured using PrestoBlue cell viability reagent (Invitrogen AB, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as the mean ± SE for six replicates as a percentage of vehicle control (taken as 100%). Experiments were performed independently at least three times. Statistical analyses were performed using a two-tailed Student's *t* test. $p < 0.05$ was considered to be statistically significant.

Antimicrobial Screening. The disk diffusion technique was applied. Initially 1 mg/1 mL stock solutions of the compounds were prepared for each compound, and 100 μL was transferred onto sterile paper disks (0.1 mg/disk) and allowed to dry. Microbial suspensions (100 μL) were inoculated onto Petri dishes dispensed with appropriate media, and dry paper discs impregnated with the sample compounds were transferred onto these plates. This was performed in triplicates under sterile conditions, and the plates were incubated at 37 °C for *E. coli*, *Staph. aureus*, and *C. albicans* and at 25 °C for *A. niger*. Two commercially used antibiotics, streptomycin for bacteria and nystatin for fungi, were used as standards at 0.1 mg/100 μL concentration for comparison of the antimicrobial activities, while solvents used to prepare the samples were used as controls. The antimicrobial activity was determined after 24, 48, and 72 h.

■ ASSOCIATED CONTENT

Supporting Information

Original NMR and MS spectra of all compounds, HPLC chromatograms, confidence intervals for the cytotoxicity tests. 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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