JOURNAL OF NATURAL PRODUCTS

ARTICLE

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Diarylpropanes and an Arylpropyl Quinone from *Combretum griffithii*

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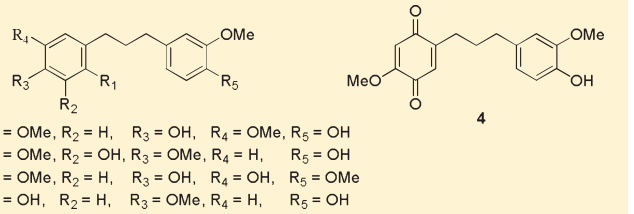
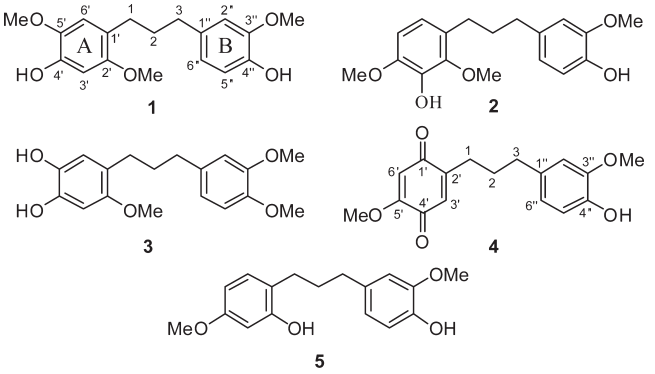
Supporting Information

ABSTRACT: Three new diarylpropanes (**1**−**3**), a new arylpropyl quinone (**4**), and the known 1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)propane (**5**) were isolated from a methanol extract of stems of *Combretum griffithii*. Their structures were elucidated by spectroscopic methods. Compounds **1**, **2**, **4**, and **5** showed cytotoxicity against one or more cancer cell lines (KB, MCF7, and NCI-H187), and compound **5** exhibited activity against *Mycobacterium tuberculosis* (MIC 3.13 μg/mL).



Combretum griffithii Van Heurck & Müll. Arg. (Combretaceae) is a vine, known as "Khamin khruea"[1] in Thai, and a water decoction of the stem has been used traditionally by local people as a treatment for hepatitis.[2] No previous phytochemical study of *C. griffithii* has been reported. However, previous investigations of *Combretum* species resulted in the isolation of triterpenes, triterpene glucosides,[3,4] pentacyclic triterpenes,[5,6] triterpenoids with 9β,19-cyclopropyl-1-en-3-one skeletons,[7] cyclobutane dimers,[8] cycloartane triterpenes,[9,10] and ellagic acid derivatives.[11] As part of our work on bioactive constituents of Thai plants, a MeOH extract of stems of *C. griffithii* showed cytotoxicity against the KB oral human epidermal carcinoma cell line (IC_{50} 2.6 μg/mL). We report herein the isolation, structural elucidation, and bioactivity of three new diarylpropanes (**1**−**3**) and a new arylpropyl quinone (**4**), named griffithanes A−D, together with the known diarylpropane **5** from *C. griffithii*. It should be noted that **5** was the first diarylpropane reported from the family Combretaceae.



RESULTS AND DISCUSSION

Separation of a MeOH extract of *C. griffithii* by silica gel column chromatography (CC) and preparative TLC yielded four new compounds (**1**−**4**) and 1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)propane (**5**).[12] Compound **1** was a viscous liquid, and its molecular formula was deduced as $C_{18}H_{22}O_5$ from HRESITOFMS (observed m/z 319.1548 [M + H]$^+$), indicating eight degrees of unsaturation. The IR spectrum showed absorption bands for OH (3419 cm^{-1}) and aromatic (3000, 1612, and 1512 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra and a DEPT experiment showed 18 carbon signals attributable to three methoxy, three methylene, five methine aromatic, and seven nonprotonated carbons. The ^{1}H NMR spectrum of **1** showed a $-CH_2-CH_2-CH_2-$ unit at δ 2.56 (t, J = 8.0 Hz, H-1), 1.89 (quint, J = 8.0 Hz, H-2), and 2.60 (t, J = 8.0 Hz, H-3), which was confirmed by the COSY spectrum. The two benzene rings showed resonances indicating a 1,2,4,5-tetrasubstituted ring A [δ 6.63 (s, H-6′) and 6.41 (s, H-3′)] and a 1,3,4-trisubstituted ring B [δ 6.69 (br s, H-2″), 6.83 (d, J = 8.4 Hz, H-5″), and 6.68 (br d, J = 8.4 Hz, H-6″)]. Three OCH_3 signals were also observed [δ 3.77 (s), 3.80 (s), and 3.85 (s)]. The ^{13}C NMR spectrum exhibited five resonances at low field [δ_C 147.4, 147.8, 142.9, 146.4, and 143.6] indicating oxygenated ring carbons. The HMBC spectrum demonstrated correlations of H-3′ to C-2′, C-4′, and C-5′; H-6′ to C-5′, C-4′, C-2′, and C-1; methoxy protons at δ_H 3.77 to C-2′; and methoxy protons at δ_H 3.80 to C-5′, consistent with a 1,2,4,5-tetrasubstituted ring A. HMBC correlations of H-2″ to C-4″ and C-6″; H-5″ to C-1″, C-3″, and C-4″; H-6″ to C-2″and C-4″; H-3 to C-1″, C-2″, and C-6″; and methoxy protons at δ_H 3.85 to C-3″ confirmed the connection of ring B. The NOESY spectrum exhibited correlations between H-3′ and methoxy protons at C-2′; H-6′ and methoxy protons at C-5′; and H-2″ and methoxy protons at C-3″. On the basis of the above data, compound **1** was defined as a new compound, 1-(4-hydroxy-2,5-dimethoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)propane, which was named griffithane A.

Received: July 14, 2011
Published: September 16, 2011

Compound **2** was assigned the molecular formula $C_{18}H_{22}O_5$, as deduced from HRESITOFMS data (observed *m/z* 319.1547 $[M + H]^+$). The IR spectrum indicated the presence of OH (3425 cm^{-1}) and aromatic (3000, 1605, and 1511 cm^{-1}) groups. The ^1H and ^{13}C NMR and DEPT spectra of **2** were similar to those of **1**, except that the aromatic protons of ring A showed doublets at δ 6.64 (J = 8.4 Hz, H-5′) and 6.77 (J = 8.4 Hz, H-6′), indicating that ring A of **2** was a 1,2,3,4-tetrasubstituted benzene ring. The HMBC spectrum showed correlations indicating OCH_3 groups at C-2′ and C-4′ and an OH group at C-3′. Thus, compound **2** was a new compound, 1-(3-hydroxy-2,4-dimethoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)propane, and it was named griffithane B.

Compound **3** had the molecular formula $C_{18}H_{22}O_5$ as deduced from HRESITOFMS. Absorption bands in the IR spectrum at 3424, 1605, and 1506 cm^{-1} indicated the presence of OH and aromatic groups. The ^1H and ^{13}C NMR spectra, and a DEPT experiment, revealed that **3** was a 1,3-diarylpropane containing three OCH_3 groups that were similar to those of **1** and **2**, except for the different positions on both benzene rings. An OCH_3 group at C-2′ and OH groups at C-4′ and C-5′ on aromatic ring A were deduced from the HMBC correlations of H-3′ to C-1′, C-2′, and C-4′, and CH_3O-2′ to C-2′, as well as from NOESY correlations between CH_3O-2′ and H-3′ and between OH protons at C-4′ and C-5′. In addition, the ^{13}C NMR resonances showed downfield shifts of C-2′ (δ_C 150.9), C-4′ (δ_C 144.7), and C-5′ (δ_C 139.2), while C-3′ appeared upfield (δ_C 97.4) due to electronic effects from the *ortho* position of methoxy and hydroxy groups. Benzene ring B contained two OCH_3 groups at C-3″ (δ_C 146.3) and C-4″ (δ_C 143.5), which were confirmed by the HMBC correlations of CH_3O-3″ to C-3″ and CH_3O-4″ to C-4″. Thus, **3** was defined as 1-(4,5-dihydroxy-2-methoxyphenyl)-3-(3,4-dimethoxyphenyl)propane, which was named griffithane C.

Compound **4** was obtained as orange crystals and was assigned the molecular formula $C_{17}H_{18}O_5$ from HRESITOFMS (observed *m/z* 325.1047 $[M + Na]^+$), indicating nine degrees of unsaturation. The IR data showed the presence of OH (3450 cm^{-1}) and conjugated dicarbonyl (1673 and 1647 cm^{-1}) groups. The ^{13}C NMR and DEPT data exhibited 17 carbons attributable to two methoxy, three methylene, five methine, and seven nonprotonated (including two carbonyl ketone) carbons. The ^1H and ^{13}C NMR spectra revealed a 1,3-disubstituted propane having an aromatic ring B similar to that of **1**; however, ring A was different. The ^1H NMR spectrum of **4** showed signals corresponding to two olefinic protons at δ_H 6.45 (s, H-3′) and 5.87 (s, H-6′). The ^{13}C NMR spectrum showed the presence of two carbonyl carbons [δ_C 182.2 and 187.4] and four olefinic carbons [δ_C 150.3 (C-2′), 130.5 (C-3′), 158.5 (C-5′), and 107.7 (C-6′)], which indicated a benzoquinone unit. The HMBC spectrum showed correlations of H-3′ to C-1 and C-5′; H-6′ to C-1′, C-2′, C-4′, and C-5′; and methoxy protons to C-5′. NOESY correlations of H-3′ with H-1 and methoxy protons at C-5′ with H-6′ confirmed the 1,4-benzoquinone moiety containing an OCH_3 group at C-5′ and connected to the propyl chain at C-1. Thus, **4** was elucidated as a new compound, 1-[2-(5-methoxy-1,4-benzoquinone)]-3-(4-hydroxy-3-methoxyphenyl)propane, which was named griffithane D.

Compounds **1**, **2**, **4**, and **5** exhibited cytotoxicity against the KB cancer cell line with IC_{50} values of 2.13, 5.67, 1.42, and 2.18 μg/mL, respectively. Compound **4** also showed cytotoxic activity against NCI-H187 and MCF7 cancer cell lines, with IC_{50} values of 1.08 and 6.75 μg/mL, respectively. These assays indicate that the OCH_3 group at C-3″ and OH group at C-4″ (*ortho*-substituted on ring B in **1**, **2**, **4**, and **5**) contribute to the cytotoxic activity against the KB cancer cell line. The benzoquinone of ring A might also play an important role in enhancing cytotoxicity. In addition, compound **5** displayed antimycobacterial activity against *Mycobacterium tuberculosis*, with a MIC of 3.13 μg/mL. Compounds **1**−**5** were tested for antiplasmodial (antimalarial) activity in vitro against *Plasmodium falciparum*, but they were all inactive at 10 μg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. UV spectra were measured on an Agilent 8453 UV−visible spectrophotometer. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ as an internal reference. HRESITOFMS were obtained using a Micromass Q-TOF-Z spectrometer. Column chromatography and preparative TLC were carried out on silica gel 60 (230−400 mesh) and PF_{254}, respectively.

Plant Material. Stems of *C. griffithii* were collected from Khon Kaen Province, Thailand, in June 2009, and were identified by James F. Maxwell, Department of Biology, Chiang Mai University. A voucher specimen (SRITUBTIM 61) was deposited at the Udon Thani Rajabhat University Herbarium, Thailand.

Extraction and Isolation. Air-dried stems of *C. griffithii* (2.7 kg) were ground and extracted successively at room temperature with hexane (3 L × 3), EtOAc (3 L × 3), and MeOH (3 L × 3) to give crude hexane (5.3 g), EtOAc (20.3 g), and MeOH (136.7 g) extracts.

The MeOH extract (61.8 g) was separated initially by silica gel CC, eluted with a gradient system of hexane−EtOAc and EtOAc−MeOH. Each fraction (100 mL) was monitored by TLC, and fractions with similar TLC patterns were combined to yield six fractions (F_1−F_6). Fraction F_1 was subjected to silica gel flash CC, eluted with a gradient of CH_2Cl_2−MeOH to furnish seven subfractions ($F_{1/1}$−$F_{1/7}$). Subfraction $F_{1/2}$ was separated by preparative TLC (EtOAc−hexane, 1:3, three times) to yield **1** (10.0 mg), **2** (79.7 mg), and **4** (46.8 mg). Subfraction $F_{1/3}$ was purified by preparative TLC (EtOAc−hexane, 1:1.5, three times) to afford **5** (79.0 mg) and an additional amount of **2** (41.6 mg). Preparative TLC of subfraction $F_{1/4}$ (EtOAc−hexane, 1:1.5, three times) gave an additional amount of **5** (11.0 mg). Preparative TLC of $F_{1/7}$ (EtOAc−hexane, 1:1.5, three times) afforded **3** (20.0 mg). Fraction F_2 was subjected to flash CC, eluted with MeOH−CH_2Cl_2 (1:50), and further separated by preparative TLC (EtOAc−hexane, 1:1.5) to yield an additional amount of **1** (8.3 mg).

Griffithane A (***1***): viscous liquid; UV (MeOH) λ_{max} (log ε) 204 (4.63), 288 (3.69) nm; IR (neat) ν_{max} 3419, 3000, 2935, 2856, 1612, 1512, 1450, 1413, 1268, 1198, 1151, 1108, 1032, 998, 931, 853, 819 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.83 (1H, br d, J = 8.4 Hz, H-5″), 6.69 (1H, br s, H-2″), 6.68 (1H, d, J = 8.4 Hz, H-6″), 6.63 (1H, s, H-6′), 6.41 (1H, s, H-3′), 3.85 (3H, s, OCH_3-3″), 3.80 (3H, s, OCH_3-5′), 3.77 (3H, s, OCH_3-2′), 2.60 (2H, t, J = 8.0 Hz, H-3), 2.56 (2H, t, J = 8.0 Hz, H-1), 1.89 (2H, quint, J = 8.0 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8 (C-4′), 147.4 (C-2′), 146.4 (C-3″), 143.6 (C-4″), 142.9 (C-5′), 134.2 (C-1″), 120.9 (C-6″), 119.0 (C-1′), 114.2 (C-5″), 113.9 (C-6′), 111.0 (C-2″), 101.0 (C-3′), 56.7 (OCH_3-5′), 55.9 (OCH_3-2′). 55.8 (OCH_3-3″), 35.5 (C-3), 31.8 (C-2), 29.0 (C-1); HRESITOFMS *m/z* 319.1548 $[M + H]^+$ (calcd for $C_{18}H_{22}O_5$ + H, 319.1545).

Griffithane B (***2***): viscous liquid; UV (MeOH) λ_{max} (log ε) 204 (5.11), 288 (4.15) nm; IR (neat) ν_{max} 3425, 3000, 2934, 2842, 1605, 1511, 1451, 1267, 1231, 1199, 1119, 1032, 856, 818 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.82 (1H, d, J = 8.4 Hz, H-5″), 6.77 (1H, d, J = 8.4 Hz, H-6′), 6.68 (2H, m, H-2″ and H-6″), 6.64 (1H, d, J = 8.4 Hz,

H-5′), 3.91 (3H, s, OCH_3-4′), 3.87 (3H, s, OCH_3-3″), 3.82 (3H, s, OCH_3-2′), 2.59 (2H, t, J = 8.0 Hz, H-3), 2.56 (2H, t, J = 8.0 Hz, H-1), 1.86 (2H, quint, J = 8.0 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.7 (C-2′), 147.6 (C-3′), 146.2 (C-3″), 143.5 (C-4″), 139.7 (C-4′), 134.3 (C-1″), 127.5 (C-1′), 124.4 (C-6′), 120.9 (C-6″), 114.1 (C-5″), 111.0 (C-2″), 110.0 (C-5′), 60.6 (OCH_3-4′). 60.4 (OCH_3-2′), 55.8 (OCH_3-3″), 35.3 (C-3), 32.7 (C-2), 29.1 (C-1); HRESITOFMS m/z 319.1547 $[M + H]^+$ (calcd for $C_{18}H_{22}O_5$ + H, 319.1545).

Griffithane C (**3**): viscous liquid; UV (MeOH) λ_{max} (log ε) 204 (4.68), 288 (3.72) nm; IR (neat) ν_{max} 3424, 3001, 2934, 2842, 1605, 1506, 1451, 1371, 1265, 1118, 1030, 853, 816 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (1H, d, J = 8.0 Hz, H-5″), 6.73 (2H, s, H-6′ and H-2″), 6.68 (1H, d, J = 8.0 Hz, H-6″), 6.48 (1H, s, H-3′), 5.45 (1H, s, OH), 5.17 (1H, s, OH), 3.88 (3H, s, OCH_3-3″), 3.87 (3H, s, OCH_3-4″), 3.77 (3H, s, OCH_3-2′), 2.57 (2H, t, J = 8.0 Hz, H-3), 2.55 (2H, t, J = 8.0 Hz, H-1), 1.84 (2H, quint, J = 8.0 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.9 (C-2′), 146.3 (C-3″), 144.7 (C-4′), 143.5 (C-4″), 139.2 (C-5′), 134.6 (C-1″), 123.4 (C-1′), 120.9 (C-6″), 115.9 (C-6′), 114.5 (C-5″), 111.1 (C-2″), 97.4 (C-3′), 56.5 (OCH_3-2′). 55.8 (OCH_3-3″), 55.2 (OCH_3-4″), 35.2 (C-3), 31.9 (C-2), 29.0 (C-1); HRESITOFMS m/z 319.1544 $[M + H]^+$ (calcd for $C_{18}H_{22}O_5$ + H, 319.1545).

Griffithane D (**4**): orange crystals (EtOAc); mp 121−123 °C; UV (MeOH) λ_{max} (log ε) 203 (3.99), 266 (3.63) nm; IR (neat) ν_{max} 3450, 3065, 3017, 2930, 2848, 1673, 1647, 1599, 1513, 1454, 1364, 1269, 1122, 1031, 957, 851, 818 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (1H, d, J = 8.0 Hz, H-5″), 6.65 (1H, d, J = 8.0 Hz, H-6″), 6.64 (1H, s, H-2″), 6.45 (1H, s, H-3′), 5.87 (1H, s, H-6′), 3.84 (3H, s, OCH_3-3″), 3.77 (3H, s, OCH_3-5′), 2.58 (2H, t, J = 7.6 Hz, H-3), 2.43 (2H, t, J = 7.6 Hz, H-1), 1.79 (2H, quint, J = 7.6 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.4 (C-1′), 182.2 (C-4′), 158.5 (C-5′), 150.3 (C-2′), 146.5 (C-3″), 143.8 (C-4″), 133.2 (C-1″), 130.5 (C-3′), 120.9 (C-6″), 114.3 (C-5″), 110.9 (C-2″), 107.7 (C-6′), 56.2 (OCH_3-5′). 55.9 (OCH_3-3″), 35.1 (C-3), 29.8 (C-2), 28.6 (C-1); HRESITOFMS m/z 325.1047 $[M + Na]^+$ (calcd for $C_{17}H_{18}O_5$ + Na, 325.1052).

Antimalarial Assay. Antimalarial activity was evaluated in vitro against the parasite *Plasmodium falciparum* (K1, multi-drug-resistant strain), using the method of Trager and Jensen.[13] Quantitative assessment of malarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[14] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of $[^3H]$-hypoxanthine by *P. falciparum*. The standard compound dihydroartemisinin exhibited an IC_{50} value of 1.0 ng/mL.

Antimycobacterial Assay. Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the microplate Alamar Blue assay (MABA).[15] The standard drug isoniazid showed MIC values of 0.23−0.46 μg/mL.

Cytotoxicity Assay. Cytotoxicity assays against human epidermoid carcinoma (KB), human breast cancer (MCF7), and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method as described by Skehan and co-workers.[16] The reference substance was doxorubicine.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra for **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

Financial support from Khon Kaen University via the Natural Products Research Unit is acknowledged. We thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, for partial support. We are indebted to the Bioassay Research Facility of the National Center for Genetic Engineering and Biotechnology via the Bioresource Research Network (BRN) for bioactivity tests. We thank Dr. S. Sritubtim, Chemistry Program, Udon Thani Rajabhat University, for providing plant material.

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