Antimicrobial Dialkylresorcinols from *Pseudomonas* sp. Ki19

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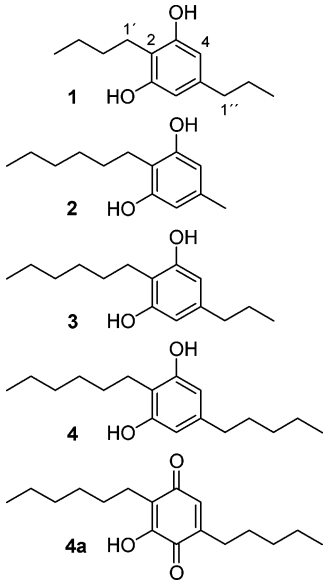
Four dialkylresorcinols (**1**−**4**) were isolated from a liquid culture of *Pseudomonas* sp. Ki19. Compounds **1** and **2**, 2-butyl-5-propylresorcinol and 2-hexyl-5-methylresorcinol, respectively, have not previously been isolated from biological sources, whereas **3** and **4**, 2-hexyl-5-propylresorcinol (DB-2073) and 2-hexyl-5-pentylresorcinol (resorstatin), both have been found in biological systems. The compounds inhibited *Staphyllococcus aureus* at concentrations ≤ 10 μg/mL as well as the fungi *Aspergillus fumigatus* and *Fusarium culmorum* at 50 μg/mL. The formation of possible antimicrobial quinone oxidation products was investigated under bioassay conditions, and they were not found to be responsible for the main antimicrobial activity.

Microbial biocontrol agents (BCAs) show great potential in controlling fungal pathogens in agriculture, and several have been launched as commercial products.[1,2] In comparison with synthetic antifungals, they may be applied fewer times per growth season if BCA colonization and cell cycling is successful, and they may be less prone to resistance buildup if they work by multiple modes of action. A BCA could keep a pathogen at an acceptable level by several means, e.g., by competing for nutrients and space, by inducing the host plant's own defense system, by interfering with the pathogens' cell signaling, or by producing antibiotics.

During screening of microorganisms for biocontrol of fungal pathogens problematic to agriculture, the isolate *Pseudomonas* sp. Ki19 (Pseudomonadaceae) was found to strongly suppress seed-borne net blotch of barley caused by *Drechslera teres* on spring wheat (*Triticum aestivum* L.) in a greenhouse bioassay.[3] The spore germination of a range of fungal pathogens was also inhibited by its concentrated cell-free culture filtrate as well as by a hydrophobic fraction after solid-phase extraction. This prompted us to investigate if the strain produced any antifungal metabolites, as pseudomonads produce a variety of bioactive compounds[4−6] and have been shown to exhibit biological control of soil-borne pathogenic fungi.[7] One type of compounds isolated from *Pseudomonas* are 2,5-dialkylresorcinols, e.g., 2-hexyl-5-propylresorcinol (DB-2073, **3**) and 2-hexyl-5-pentylresorcinol (resorstatin, **4**). This group of compounds has been reported as being moderately antibacterial and antifungal[8,9] and cytotoxic[10] and as radical scavengers.[11] Other variations of the dialkylresorcinol skeleton include 5-heptyl-2-hexylresorcinol, isolated from *Pseudomonas aurefaciens*,[12] and the antibacterial and antifungal 2-butyl-5-pentylresorcinol (stemphol), isolated from the anamorphic fungus *Stemphylium majusculum*[13] and *Pleospora herbarum* (anamorphic species *Stemphylium botryosum*).[14] This paper presents the isolation, structure elucidation, and investigation of antimicrobial activity of four dialkylresorcinols from the *Pseudomonas* sp. Ki19.

Results and Discussion

The isolate *Pseudomonas* sp. Ki19 was grown in liquid culture on defined medium, and bioassay-guided fractionation with SPE and gradient HPLC identified one antifungal and antibacterial fraction. Screening of this and neighboring fractions by ^{1}H NMR aided in isolating four related compounds after an additional HPLC step: the more polar **1** and **2**, compound **3**, which was found in the active fraction, and the less polar **4**.



Compound **1** exhibited a pseudomolecular ion at *m*/*z* 209.1 [M + H]$^+$ in ESIMS and at *m*/*z* 209.1594 [M + H]$^+$ in HRFABMS, which established the molecular formula as $C_{13}H_{20}O_2$. ^{1}H NMR of compound **1** in CH_3OH-d_4 revealed a singlet resonance at δ 6.13 with an integral corresponding to two protons, and upfield signals representative of two *n*-alkyl chains at δ_H 0.92−2.55 with an integral of 16 protons in total. In the COSY spectrum, one alkyl spin system was assigned to be an *n*-propyl moiety and the other alkyl chain to be an *n*-butyl. This was in agreement with the ^{13}C spectrum, which revealed seven alkyl carbons. There were four possible ^{13}C aryl resonances, two with low intensity at δ_C 114.8 and 142.1, and two with high intensity at δ_C 108.0 and 157.2. The carbon resonance at δ_C 108.0 correlated with the two-proton singlet in HSQC as well as in HMBC. In HMBC, there was also a cross-peak between the propyl α-methylene protons and δ_C 142.1 and between the butyl α-methylene protons and δ_C 114.8. Assignment of the remaining alkyl carbons and protons was possible from correlations in the HMBC spectrum. The chemical shifts of the butyl-substituted carbon at δ_C 114.8 and the methines at δ_C 108.0 were in agreement with an *ortho* relation to hydroxy groups. The only possible substitution pattern in accord with the NMR data was a benzene ring carrying two hydroxy groups at C-1 and C-3, together with a butyl chain at C-2 and a propyl chain at C-5. The structure of **1** was proposed to be 2-butyl-1,3-dihydroxy-5-propylbenzene, or in short-hand 2-butyl-5-propylresorcinol.

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Table 1. Minimal Inhibitory Concentrations (μg/mL) for Compounds **1**−**4** and **4a**[a]

	compound					standard		
pathogen	**1**	**2**	**3**	**4**	**4a**	A[b]	F[c]	S[d]
1. *Aspergillus fumigatus*	(50)	50 (10)	50 (10)	(50)	*e*	40	5	
2. *Candida albicans*	(50)	(50)	50	(50)	100	40	*e*	
3. *Drechslera sorokiniana*	(50)	50	(50)	(50)	*e*	4	0.1	
4. *Fusarium culmorum*	50	50 (10)	50 (10)	50	*e*	40	5	
5. *Pseudomonas sevastanoi*				(50)	*e*	*e*	*e*	*e*
6. *Staphyllococcus aureus*	5	2 (1)	3 (2)	7 (6)	100		*e*	10

[a] Numbers refer to concentrations achieving total inhibition of cell growth/spore germination, while numbers in parentheses refer to concentrations showing detectable inhibition of growth. All compounds were tested up to 100 μg/mL. Pathogens 1 and 3−5 are agricultural pathogens, and 1, 2, and 6 are human pathogens. [b] Amphotericin B. [c] Fungazil A. [d] Streptomycin. [e] Not determined.

The HRFABMS spectrum of compound **2** exhibited a pseudomolecular ion at *m*/*z* 209.1534 $[M + H]^+$, indicating a molecular formula identical to that of **1** ($C_{13}H_{20}O_2$). The ^{13}C spectrum revealed similar shifts for the aryl signals, except for C-5, which was positioned 5 ppm upfield at δ 137.1. In analogy with **1**, there were seven alkyl signals, although only one with a typical shift for an *n*-alkyl methyl, at δ 14.6. In ^1H NMR, there was a singlet resonance at δ_H 2.13 (δ_C 21.4) corresponding to an integral of three protons, which exhibited cross-peaks in HMBC to δ_C 137.1 (C-5) and 108.6 (C-4 and C-6). This suggested that C-5 was substituted with a methyl group. The remaining six alkyl carbons were identified as an *n*-hexyl chain by correlations in the COSY and HMBC spectra. The hexyl α-protons at δ_H 2.53 (δ_C 24.1) showed correlations to 157.2 (C-1 and C-3) and 114.5 (C-2), which positioned the hexyl moiety at C-2. Compound **2** was thus proposed to be 2-hexyl-5-methylresorcinol.

Compound **3**'s MS data and NMR data in $CDCl_3$ were found to be in all parts comparable with data reported for DB-2073 (2-hexyl-5-propylresorcinol).[9] For compound **4**, data were comparable with those reported for resorstatin (2-hexyl-5-pentylresorcinol).[11,15] The positions and nature of the alkyl substituents of **3** and **4** were corroborated by H2BC[16] and HMBC $^1H-^{13}C$ correlations.

Methanol extraction of the freeze-dried bacterial cells of *Pseudomonas* sp. Ki19 also yielded compounds **1**−**4** in approximately the same relative amounts as the supernatant. The isolated compounds all have C-2 alkyl groups of even and C-5 alkyl groups of odd number of carbons. This is in line with investigations of dialkylresorcinol biosynthesis in *Pseudomonas*, which have proposed the key step to be a condensation of two β-ketoacyl thioesters, derived from fatty acids. The critical enzymes were found to accept substrate variation, allowing production of a variety of dialkylresorcinols with even C-2 and odd C-5 alkyl groups.[17]

Minimal inhibitory concentrations (MICs) were determined for compounds **1**−**4** against agricultural pathogens and human pathogens (Table 1). Activities against fungi were moderate, with full inhibitions down to 50 μg/mL. The Gram-positive bacterium *Staphyllococcus aureus* was completely inhibited at 2 μg/mL, with detectable inhibition at 1 μg/mL (compound **2**), while *Pseudomonas sevastanoi* was only partly inhibited at 50 μg/mL, indicating that the isolated compounds do not have a strong self-inhibitory effect on the genus level. Overall, compounds **2** and **3** were slightly more potent than compounds **1** and **4**.

In the original article[8] describing **3**, the compound was reported to turn yellow upon exposure to light and air. When drying the crude fractions of **1**−**4**, they had a faint yellow color, and when **4** was allowed to stand in $CDCl_3$ in an NMR tube for 56 h, the solution turned bright yellow and the ^1H NMR spectrum revealed the formation of an additional species. It has been shown that 5-alkylresorcinols induce DNA cleavage in vitro in the presence of O_2 and Cu^{2+} at alkaline pH. The mechanism has been proposed to involve initial oxidation of the 5-alkylresorcinols to 6-alkyl-1,2,4-trihydroxybenzene and subsequent oxidation to 2-alkyl-6-hydroxybenzo-1,4-quinone and oxygen radicals, which are thought to be responsible for DNA strand damage. In addition, the formed quinone is capable of DNA alkylation.[18,19] An analogous biodegradation pathway has been shown for resorcinol in the bacterium *Azoarcus anaerobius*.[20] The yellow NMR sample was thus investigated for additional bioactive compounds. Bioassay-guided isolation yielded **4** and a second fraction containing yellow species **4a**, which exhibited antimicrobial activity. After evaporation, **4a** was obtained as a yellow powder. Investigation with ESIMS in negative ion mode showed a pseudomolecular ion at *m*/*z* 277.1 $[M - H]^-$, and GC-EIMS of the trimethylsilyl derivative yielded a molecular ion at *m*/*z* 350. The mass difference of 73 amu between negative ion mode ESIMS and EIMS indicated that the compound had one functional group available for silylation. The molecular ion in HREIMS at 278.1884 suggested the molecular formula to be $C_{17}H_{26}O_3$. The ^1H NMR spectrum revealed resonances similar to those of compound **4** in the alkyl region, but instead of the original aryl singlet at δ 6.14, there was a methine triplet ($J = 1.3$ Hz) at δ 6.41 (δ_C 134.4). Taken together with the molecular formula, these findings indicated **4a** to be the result of oxidation of the aromatic ring of **4**. Moreover, HMBC data showed correlations to two signals at δ_C 184.5 and 189.3, indicating the presence of two keto-functions, suggesting **4a** to be a dialkyl-substituted benzoquinone. COSY experiments afforded a cross-peak between the signal at δ_H 6.41 and a methylene signal at δ_H 2.41 (the α-protons of the pentyl group), which would fit a four-bond coupling. Aided by HMBC data the structure of **4a** was subsequently suggested to be 2-hexyl-3-hydroxy-5-pentylbenzo-1,4-quinone, which was supported by comparison with NMR data for similar 2,5-dialkyl-3-hydroxybenzo-1,4-quinones.[21,22] The compound was found to inhibit growth of *S. aureus* and *Candida albicans* at 100 μg/mL (Table 1). If oxidation of the dialkylresorcinols took place during bioassay incubation, there was a possibility that the bioassay detected the activity of any resulting quinones rather than the supposed dialkylresorcinols.

To investigate if dialkylhydroxyquinones could be formed under MIC conditions, the resorcinols were incubated with and without pathogens. Compound **4** was incubated for 72 h under different pH, temperature, light, and solvent conditions and analyzed by analytical HPLC. There were no signs of buildup of the corresponding quinones. To expose the dialkylresorcinols to pathogens, standard bioassay tests on *S. aureus* cells and *Fusarium culmorum* spores were performed for compounds **1**−**4** at the 50 μg/mL level. After determining inhibition, the material in the sample wells was centrifuged to sediment the pathogen cells and spores, and the supernatants were evaporated under reduced pressure. Each residue was derivatized by BSTFA and subjected to GC-MS analysis. Quinones were not detected in any of the samples. In the *S. aureus* samples, only compounds **3** and **4** were detected, indicating that **1** and **2** were metabolized or tightly bound to the cells. The *F. culmorum* samples showed dialkylresorcinol peaks comparable to control samples without pathogens. Although these tests do not rule out the possibility of metabolic formation of antimicrobial compounds from the dialkylresorcinols, it suggests that compound **4a** and possible homologues are not responsible for the activity in the MIC bioassay. This was also supported by the higher MIC values obtained for **4a** against *S. aureus* than those for compounds **1**−**4**.

Experimental Section

General Experimental Procedures. UV spectra were recorded in H_2O/CH_3OH (1:1) on a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. ^1H and ^{13}C NMR data were acquired on a Bruker DRX400 MHz NMR spectrometer equipped with a 5 mm QNP probe ($^1H/^{13}C/^{31}P/^{15}N$). All NMR experiments were recorded at 30 °C. For complete structure elucidation, 1D ^1H NMR, COSY, TOCSY, DEPT-HSQC, H2BC, HMBC, and 1D ^{13}C NMR were applied. Pulse sequences were applied as provided by the manufacturer, with the addition of H2BC, which was performed as described by Nyberg et al.[16] Chemical shifts were determined relative to internal $CHCl_3$ (δ_C 77.23; δ_H 7.27) or CH_3-OH-d_3 (δ_C 49.15; δ_H 3.31). HRFABMS was performed on a four-sector tandem mass spectrometer (JEOL SX/SX102A) with glycerol as matrix and PEG as internal standard. HREIMS was done on the same spectrometer with PFK as internal standard. Positive and negative ion mode ESI mass spectra were obtained on a Bruker Esquire ion-trap MS with CH_3OH (gradient grade) as solvent. LC-MS was done with the same mass spectrometer coupled to an analytical HP1100 LC system (Hewlett-Packard) and GC-MS on a HP5890/5970 GC-MS (Hewlett-Packard). SPE was performed with 1 g or 10 g prepacked columns or columns packed in-house with bulk C_{18} material [all Isolute C18 (EC), International Sorbent Technology, Hengoed, UK]. Preparative HPLC was run on a Gilson system at a flow of 10 mL/min with UV monitoring at 210 or 254 nm. Fractions were collected in polypropylene 2 mL 96-well plates. For the mobile phase CH_3CN of HPLC gradient grade and deionized filtered H_2O were used.

Isolate Origin and Identity. *Pseudomonas* sp. Ki 19 was isolated as previously described (referred to as NB21) by Hökeberg,[23] from the roots of a crowberry plant [*Empetrum nigrum* L., (Empetraceae)] collected nearby Kiruna in northern Sweden. The strain was identified to the genus *Pseudomonas*, RNA group I, on the basis of physiological tests and RFLP (restriction fragment length polymorphism) analysis of 16S rDNA[3] and was found to be closely related, but not identical, to *Pseudomonas chlororaphis*. Sequencing of the whole 16S rDNA revealed that it belongs within the various species of the *Pseudomonas fluorescens* group according to the classification proposed by Anzai et al.[24] The strain is deposited at the bacterial culture collection at MASE Laboratories, Uppsala, Sweden, and the 16S rDNA sequence at GenBank (accession no. DQ390584).

Production of Bacterial Cultures. Cultures of the isolate Ki19 were grown on Mineral Medium (MM) for *Pseudomonas*[25] modified by adding 125 mg of citric acid, 12.5 mg of $Na_2MoO_4 \times 2\ H_2O$, and 250 mg of trichloroacetic acid per 1000 mL of the medium with 0.1% glycerol as the sole carbon source. The cultures were started by transferring 20 mL of 24-h-old inocula grown in half-strength Vegetable Peptone Broth (15 g VPB in 1000 mL of deionized H_2O) to each 1000 mL of MM. The cultures were incubated on a rotary shaker (120 rpm) for 68−72 h at 20−25 °C. Cells were removed by centrifugation (11 000 rpm, 15 min, 4 °C), and cell-free supernatants were immediately fractionated by SPE or stored at −70 °C before being processed.

Sample Workup and Isolation Procedures. The cell-free supernatant (3.5 L) was fractionated on a 120 g SPE column. The column was packed and activated with 500 mL of CH_3CN and equilibrated with 500 mL of H_2O before sample loading. Hydrophilic components were washed out with 500 mL of aqueous 5% CH_3CN followed by 500 mL of 20% CH_3CN in H_2O before the lipophilic fraction was eluted with 500 mL of aqueous 95% CH_3CN. The lipophilic eluate was evaporated under reduced pressure and fractionated by gradient preparative HPLC (20% to 100% CH_3CN in H_2O in 10 min with an 8 min hold at 100%) by automatic collection in 2 mL 96-well plates. Fractions 57−60 showed an inhibitory effect in the bioassay. The remaining fractions from 50 to 65 were screened with ^1H NMR for structurally related compounds. A final HPLC step was performed for isolation of compounds **1**−**4**. Isocratic HPLC was run at 40% CH_3CN in H_2O to yield compounds **1** (4.2 mg) and **2** (0.6 mg), at 50% CH_3CN in H_2O to yield **3** (14.2 mg), and at 60% CH_3CN in H_2O to yield **4** (10.0 mg). The column for preparative gradient and isocratic HPLC was a Reprosil-pur C_{18} (100 × 20 mm with guard column 30 × 20 mm, 5 μm). Extraction of cells was done by sonication in 2 × 110 mL of CH_3OH. The methanol extract was diluted with 5% H_2O and run through an activated and equilibrated 10 g SPE column to remove material that binds irreversibly to C_{18}. The column was eluted with 35 mL of aqueous 98% CH_3CN, and the eluate was pooled with the CH_3-OH extract. After evaporation, the material was subjected to preparative HPLC as described above.

In Vitro Bioassay and MIC Determination. Detection of antimicrobial fractions during chromatographic isolation and determination of MIC values were performed using a previously developed bioassay, based on inhibition of spore germination or cell growth in microtiter plates.[26−28]

The pathogen isolates of *Aspergillus fumigatus* Fresen. (Trichocomaceae) and *Staphylococcus aureus* Rosenbach (Staphylococcaceae) were provided by the Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden, and the isolates of *Fusarium culmorum* (W. G. Sm.) Sacc. (Nectriaceae) and *Drechslera sorokiniana* (Sacc.) Subram. & B. L. Jain (Pleosporaceae) by the Plant Pathology and Biocontrol Unit, Swedish University of Agricultural Sciences, Uppsala, Sweden. The isolate of *Candida albicans* (C. P. Robin) Berkhout (Saccharomycetaceae) was provided by the Laboratory of Clinical Microbiology, Centre of Laboratory Medicine, Uppsala University Hospital, Uppsala, Sweden. The isolate of *P. sevastanoi* pv. *sevastanoi* CCM 3580 (Pseudomonadaceae) was purchased from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic. All isolates were maintained as described by Levenfors et al.[26] The strains are deposited at the bacterial and fungal culture collection at MASE Laboratories, Uppsala, Sweden.

For detection of antimicrobial fractions, *C. albicans*, *F. culmorum*, and *S. aureus* were used as test organisms. Replicates of the 2 mL 96-well plates from preparative HPLC were done by transferring aliquots corresponding to 40 mL of culture filtrate to 96-well microtiter plates, from which the solvents were evaporated in a fume hood overnight. To each well was added 100 μL of spore suspension (*F. culmorum*) at 10^4 spores/mL or cell suspension (*C. albicans* and *S. aureus*) at 10^4 cells/mL in appropriate culture media. As peaks in HPLC could elute over one or several wells in the 96-well plates, the transferred sample amount corresponded to 100−400 times higher sample concentration in the bioassay than in the culture supernatant.

Spore/cell suspensions were used as positive controls, and sterile medium was used as negative control. For both detection of antimicrobial fractions and MIC determinations, bioassay plates were incubated in darkness at 27−28 °C (*P. sevastanoi* pv. *sevastanoi* 24 h, *D. sorokiniana* 48 h, *F. culmorum* 72 h, and *A. fumigatus* 96 h) or at 37 °C for 24 h (*C. albicans* and *S. aureus*). Results were evaluated visually according to the following scale: 3, full inhibition of growth comparable to negative control; 2, intermediate inhibition of growth; 1, detectable inhibition of growth; 0, no detectable inhibition of growth, comparable to positive control. All MIC tests were performed in triplicate and repeated once. For the standards amphotericin B, Fungazil A, and streptomycin, MIC values were determined using identical bioassay conditions and pathogen strains, except for the exclusion of *P. sevastanoi* pv. *sevastanoi*. These results have in part been published previously.[29]

Incubation of 4. Buffer solutions of 20 mM were prepared for pH 5.0 (acetic acid), pH 7.0 (NaH_2PO_4), and pH 9.0 (H_3BO_3), and pH was adjusted with 0.5 M HCl or NaOH. The buffers were diluted to 10 mM by an equal volume of CH_3OH or CH_3CN. Triplicate samples consisting of 30 μg of **4** dissolved in 500 μL of each of the solvent mixtures were prepared in HPLC vials and incubated for 72 h in 25 °C, ambient laboratory light; 25 °C, darkness; and 75 °C, darkness. After incubation, the samples were diluted to 1.1 mL with 50% aqueous CH_3OH or 50% aqueous CH_3CN and submitted to HPLC analysis. The samples were analyzed on a Reprosil-pur C_{18} (150 × 4.6 mm, 5 μm) with a gradient run (50% CH_3CN in H_2O to 70% CH_3CN in H_2O in 15 min, then to 100% CH_3CN in 1 min with a hold for 5 min) monitored at 210 nm. A control sample of **4** eluted at t_R 16.36 min and **4a** at t_R 19.67 min. For the incubated samples, a peak eluted at t_R 16.31−16.38 min, but no peak corresponding to **4a** was found for any sample. The pH 9 and heat-treated samples exhibited a pronounced void absorbance and a smaller area for the **4** peak (<10% compared to pH 5 and pH 7 samples).

Analysis of Bioassay Incubation. Compounds **1**−**4** were subjected to bioassay with *S. aureus* and *F. culmorum* in duplicate wells at 50 μg/mL. For *S. aureus*, the incubation was stopped at both 72 h and 7 days. *F. culmorum* was incubated for 7 days. Control wells, containing only growth medium and resorcinols, were incubated for 7 days. The wells containing 100 μL of spore or cell suspension and 5 μg of resorcinol were diluted with 200 μL of CH_3CN and transferred to Eppendorf tubes and evaporated under reduced pressure. The residue

was dissolved in 500 μL of aqueous 50% CH_3CN and centrifuged at 4000 rpm for 10 min to sediment spores and cells. Of the solution, 450 μL was transferred to a new Eppendorf tube and evaporated under reduced pressure. The dried samples were treated with 50 μL of pyridine and 50 μL of Sylon BFT (BSTFA + TMCS, 99:1). The mixture was allowed to stand for 30 min at 55 °C, after which it was diluted with 50 μL of EtOAc. The samples were analyzed by GC-MS on a fused silica column (HP-5MS; 0.25 μm, 30 m × 0.25 mm, Agilent Technologies) using a temperature gradient (170 °C for 5 min, 4 °C/min to 240 °C, 240 °C for 5 min) with the MS detector scanning *m*/*z* 50−550. The injector was held at 240 °C and the GC-MS interface at 260 °C. Samples (1 μL) were injected in splitless mode, and He was used as carrier gas at 1 mL/min. For control samples in liquid medium, compound **1** eluted at t_R 10.22 min with diagnostic ions at *m*/*z* 352 (18, $[M]^+$), 337 (3, loss of CH_3), 310 (41, McLafferty at C-2 side chain), 309 (86, tropylium ion); **2** eluted at t_R 11.13 min with diagnostic ions at 352 (19, $[M]^+$), 337 (2, loss of CH_3), 282 (31, McLafferty at C-2 side chain), 281 (81, tropylium ion); **3** eluted at t_R 14.00 min with diagnostic ions at *m*/*z* 380 (10, $[M]^+$), 365 (2, loss of CH_3), 309 (51, tropylium ion); and **4** eluted at t_R 17.85 min with diagnostic ions at *m*/*z* 408 (2, $[M]^+$), 393 (0.5, loss of CH_3), 337 (10, tropylium ion). A derivatized pure sample of compound **4a** eluted at t_R 18.49 min with diagnostic ions at *m*/*z* 350 (27, $[M]^+$), 335 (9, loss of CH_3), 279 (7, α-cleavage of C-6 alkyl chain), 265 (100, α-cleavage of C-3 alkyl chain).

2-Butyl-5-propylresorcinol (1): white powder; UV (CH_3OH/H_2O, 1:1) λ_{max} (log ϵ) 207 (2.81), 271 (0.42), 280 (sh) (0.35) nm; ^1H NMR (CH_3OH-d_4, 400 MHz) δ 6.13 (2H, s, H-4, H-6), 2.55, (2H, t, J = 7.6 Hz, H-1′), 2.38 (2H, t, J = 7.6 Hz, H-1″), 1.58 (2H, tq, J = 7.6, 7.3 Hz, H-2″), 1.48 (2H, m, H-2′), 1.36 (2H, m, H-3′), 0.922 (3H, t, J = 7.3 Hz, H-4′), 0.917 (3H, t, J = 7.3 Hz, H-3″); ^{13}C NMR (CH_3OH-d_4, 100 MHz) δ 157.2 (C, C-1, C-3), 142.1 (C, C-5), 114.8 (C, C-2), 108.0 (CH, C-4, C-6), 39.1 (CH_2, C-1″), 32.8 (CH_2, C-2′), 25.7 (CH_2, C-2″), 24.1 (CH_2, C-3′), 23.9 (CH_2, C-1′), 14.7 (CH_3, C-4′), 14.3 (CH_3, C-3″); diagnostic HMBC connectivities, H-4→C-1, C-2, C-6, C-1″; H-6→C-2, C-3, C-4, C-1″; H-1′→C-1, C-2, C-3, C-2′, C-3′; H-1″→C-4, C-5, C-6, C-2″, C-3″; HRFABMS *m*/*z* 209.1594 (calcd for $C_{13}H_{21}O_2$ 209.1542).

2-Hexyl-5-methylresorcinol (2): white powder; UV (CH_3OH/H_2O, 1:1) λ_{max} (log ϵ) 207 (2.75), 271 (0.31), 280 (sh) (0.26) nm; ^1H NMR (CH_3OH-d_4, 400 MHz) δ 6.12 (2H, s, H-4, H-6), 2.53, (2H, t, J = 7.7 Hz, H-1′), 2.13 (3H, s, H-1″), 1.48 (2H, m, H-2′), 1.34 (2H, m, H-3′), 1.32 (4H, m, H-4′, H-5′), 0.89 (3H, t, J = 6.9 Hz, H-6′); ^{13}C NMR (CH_3OH-d_4, 100 MHz) δ 157.2 (C, C-1, C-3), 137.1 (C, C-5), 114.5 (C, C-2), 108.6 (CH, C-4, C-6), 33.3 (CH_2, C-4′), 30.7 (CH_2, C-3′), 30.5 (CH_2, C-2′), 24.1 (CH_2, C-1′), 23.9 (CH_2, C-5′), 21.4 (CH_3, C-1″), 14.6 (CH_3, C-6′); diagnostic HMBC connectivities, H-4→C-1, C-2, C-6, C-1″; H-6→C-2, C-3, C-4, C-1″; H-1′→C-1, C-2, C-3, C-2′, C-3′; H-6′→C-4′, C-5′; H-1″→C-4, C-5, C-6; HRFABMS *m*/*z* 209.1534 (calcd for $C_{13}H_{21}O_2$ 209.1542).

2-Hexyl-3-hydroxy-5-pentylbenzo-1,4-quinone (4a): amorphous yellow powder; UV (CH_3OH/H_2O, 1:1) λ_{max} (log ϵ) 268 (0.78), 403 (0.05) nm; ^1H NMR (CH_3OH-d_4, 400 MHz) δ 6.41 (1H, t, J = 1.3 Hz, H-6), 2.41 (2H, t, J = 7.6 Hz, H-1″), 2.39 (2H, t, J = 7.6 Hz, H-1′), 1.53 (2H, m, H-2″, 1.44 (2H, m, H-2′), 1.36 (2H, m, H-3″), 1.35 (2H, m, H-3′), 1.32 (4H, m, H-4′, H-4″), 1.31 (2H, m, H-5′), 0.93 (3H, t, J = 7.5 Hz, H-5″), 0.89 (3H, t, J = 6.9 Hz, H-6′); ^{13}C NMR (CH_3OH-d_4, 100 MHz) δ 189.3 (C, C-1), 184.5 (C, C-4), 155.2 (C, C-3), 154.7 (C, C-5), 134.4 (CH, C-6), 121.8 (C, C-2), 32.8 (CH_2, C-3′), 32.50 (CH_2, C-3″), 32.49 (CH_2, C-4′), 29.43 (CH_2, C-1″), 29.35 (CH_2, C-2′), 28.9 (CH_2, C-2″), 23.4 (CH_2, C-1′), 23.2 (CH_2, C-5′), 23.1 (CH_2, C-4″), 14.5 (CH_3, C-5″), 14.5 (CH_3, C-6′); diagnostic HMBC connectivities, H-6→C-1″, C-4; H-1′→C-1, C-2, C-3, C-4, C-2′; H-1″→C-1, C-5, C-6, C-2″; ESIMS *m*/*z* 277.1 $[M - H]^-$; HREIMS *m*/*z* 278.1884 (calcd for $C_{17}H_{26}O_3$ 278.1882).

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Supporting Information Available: Gradient HPLC chromatogram of SPE eluate, NMR table for **1** and **2**, physical and spectroscopic data for **3** and **4**, ^1H NMR spectra for **1**−**4** and **4a** in CH_3OH-d_4. This material is available free of charge via the Internet at http://pubs.acs.org.

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